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Effects of virginiamycin and monensin plus tylosin on ruminal protein metabolism in steers fed corn-based finishing diets with or without wet corn gluten feed[1,2]

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ABSTRACT: Six ruminally cannulated steers (345 ± 20 kg initial BW) were used in a 6 × 6 Latin square to evaluate effects of diet and antibiotics on ruminal protein metabolism. Two diets and three antibiotic treatments were arranged factorially. One diet contained (DM basis) 72% dry-rolled corn, 12% soybean meal, 10% alfalfa hay, and 4% molasses (SBM), and the other contained 63% dry-rolled corn, 30% wet corn gluten feed, and 5% alfalfa hay (WCGF). Antibiotic treatments included control, virginiamycin (175 mg/d; VM), and monensin/tylosin (250 and 100 mg/d, respectively; MT). Steers were fed at 12-h intervals at a rate of 2.4% of empty BW daily. Each period included 18 d of adaptation and 3 d of ruminal fluid collections. Samples were collected at 0, 2, 4, 6, 8, and 10 h after the morning feeding on d 19 and 20. On d 21, rumens were dosed 2 h after the morning feeding with 350 g of solubilized casein to evaluate in vivo ruminal protease and deaminase activities. Ruminal fluid samples were collected 1, 2, 3, 4, and 6 h after the casein dose. On d 19 and 20, antibiotics had no effect on ruminal pH or concentrations of VFA, lactate, ammonia, ciliated protozoa, α-amino nitrogen (AAN), or peptide N, but VM reduced ($P < 0.01$) the concentration of isovalerate compared to MT and control. After casein dosing (d 21), peptide N concentration was unaffected by antibiotics, but AAN were higher ($P < 0.01$) for VM than MT and control. Relative to MT and control, VM reduced ruminal isovalerate ($P = 0.05$) and increased ruminal propionate ($P < 0.01$) on d 21. Ruminal pH was lower ($P < 0.01$) in steers fed SBM than in steers fed WCGF, but lactate concentrations were unaffected by diet. Steers fed SBM had higher ($P < 0.05$) ruminal concentrations of total VFA and propionate. Ammonia concentrations were lower before feeding and higher after feeding for steers fed WCGF ($P < 0.01$). Steers fed WCGF had higher counts of total ciliated protozoa than steers fed SBM ($P < 0.05$) due to greater *Entodinium* sp. ($P < 0.05$). Steers fed WCGF had higher ($P < 0.01$) ruminal AAN and peptide N concentrations than those fed SBM on d 19 and 20. After casein dosing, ruminal peptide N concentrations were similar, but AAN were lower ($P < 0.01$) for WCGF than SBM. Overall, VM appeared to depress ruminal deaminase activity, and MT had minimal effects on ruminal fermentation products. The protein in WCGF appeared to be more readily degradable than that in SBM.

Key Words: Antibiotics, Cattle, Fermentation, Protein, Protozoa

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Introduction

Virginiamycin is an antimicrobial feed additive approved for use in cattle to improve performance. It is believed to alter ruminal fermentation primarily by changing ruminal microbial populations. The gram-positive bacteria antimicrobial activity and subsequent alterations in ruminal fermentation products are similar to those of monensin (Hedde et al., 1982; Nagaraja et al., 1997), namely an increase in propionate at the expense of acetate and methane. However, effects on ruminal protein metabolism have not been fully investigated for virginiamycin.

Monensin may reduce protein degradation, but it appears mainly to affect deamination of AA in the rumen (Russell and Strobel, 1989). In vitro and in vivo experiments have demonstrated reduced ammonia

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Table 1. Diet compositions, % of DM

Ingredient	Diet	
	SBM	WCGF
Dry-rolled corn	72.1	62.9
Wet corn gluten feed	—	30.0
Soybean meal	12.0	—
Alfalfa	10.0	5.0
Molasses	4.0	—
Limestone	1.3	1.6
Calcium phosphate	0.3	—
Trace mineralized salt[a]	0.3	0.3
Potassium chloride	—	0.2
Vitamin premix[b]	+	+
OM, % of DM[c]	95	95
CP, % of DM[c]	14.2	14.4

[a]Contained (%): NaCl (95.5 to 98.5); Mn (> 0.24); Fe (> 0.24); Mg (> 0.05); Cu (> 0.032); Zn (> 0.032); I (> 0.007); and Co (> 0.004).
[b]Provided 2,200 IU vitamin A, 300 IU vitamin D, and 10 IU vitamin E per kilogram of diet DM.
[c]Calculated from average intakes.

production with greater AA concentrations in response to monensin (Van Nevel and Demeyer, 1977; Poos et al., 1979). Tylosin is commonly used in conjunction with monensin for control of liver abscesses in cattle fed in confinement. Virginiamycin has been shown to reduce deamination in vitro (Van Nevel et al., 1984). We hypothesized that virginiamycin also would reduce deamination in vivo.

The objective of this study was to measure the effects of virginiamycin and monensin plus tylosin on ruminal protein metabolism in steers fed corn-based finishing diets with or without wet corn gluten feed.

Materials and Methods

Animals and Treatments. Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee. Six ruminally cannulated Holstein steers (345 ± 20 kg initial BW) were used in a 6 × 6 Latin square design to evaluate effects of diet and antibiotics on ruminal protein metabolism. Two diets and three antibiotic treatments were arranged factorially. The two finishing diets (Table 1) were (DM basis): 1) 72% dry-rolled corn, 12% soybean meal, and 10% chopped alfalfa hay (**SBM**); or 2) 63% dry-rolled corn, 30% wet corn gluten feed (Sweet Bran, Cargill Corn Milling, Blair, NE), and 5% chopped alfalfa hay (**WCGF**). The primary differences between these diets were the source of CP and the source and level of fiber. Three antibiotic treatments were mixed into the rations just before feeding: 1) control (no antibiotic); 2) monensin and tylosin (250 and 100 mg/d, respectively; **MT**); and 3) virginiamycin (175 mg/d; **VM**). Steers were housed in tie-stalls in a controlled environment room (21°C) with continuous lighting. Each period lasted 21 d with 18 d of adaptation and 3 d of sample collection. Diets were offered every 12 h at 1.2% (DM basis) of empty BW (95% of unshrunk BW).

Sample Collection and Analysis. On d 19 and 20 of each period, ruminal fluid was collected immediately before feeding and at 2, 4, 6, 8, and 10 h after the morning feeding. Rumens were dosed through the cannula with 300 mL of a Cr-EDTA solution after the prefeeding collection on d 19 to estimate ruminal liquid dilution rate, outflow, and volume (Binnerts et al., 1968). At each sampling time, a 500-mL sample of ruminal fluid was collected after thorough mixing of the ruminal contents. Ruminal fluid pH was measured immediately, then samples were strained through four layers of cheesecloth. Strained ruminal fluid (8 mL) was mixed with 2 mL of 25% (wt/vol) metaphosphoric acid and stored frozen for later analysis of NH_3, VFA, and total lactate. Samples of strained ruminal fluid were stored frozen for Cr analysis. For analysis of peptide N, 30 mL of strained ruminal fluid was centrifuged at 500 × *g* for 20 min (4°C) to remove feed particles and protozoa, the supernatant was poured off and centrifuged at 30,000 × *g* for 15 min (4°C) to remove bacteria, and 10 mL of cell-free supernate was mixed with 0.5 mL of 70% (wt/wt) $HClO_4$ and stored frozen for later analysis of free α-amino nitrogen (**AAN**) and peptide N. Unstrained ruminal fluid collected at 0, 4, and 8 h after feeding was fixed with equal weights of 10% formalin for counting ciliate protozoa.

Strained ruminal fluid collected 4 h after feeding was used to determine in vitro protease and deaminase activities (Siddons and Paradine, 1981). For this assay, 2 mL of casein solution containing approximately 6 mg N/mL, 6 mL of McDougall's buffer, and 2 mL of strained ruminal fluid was incubated in duplicate at 39°C for 3 h in 50-mL centrifuge tubes flushed with CO_2 and fitted with gas-release valves. Incubations without casein and without ruminal fluid also were conducted to correct for background. After acidification with 4 mL of 30% (wt/vol) trichloroacetic acid and centrifugation at 20,000 × *g* for 10 min at 4°C,

supernate from each tube was frozen for later analysis of Kjeldahl N and NH_3 concentrations (AOAC, 1990).

On the last day of each period (d 21), steers were fed their usual morning ration, and then, 2 h after feeding, rumens were dosed with 350 g of solubilized casein to determine in vivo protein metabolism. The highly soluble and degradable protein source was added at a high level to disjoint substrate availability from utilization by the ruminal microbial population. Ruminal fluid was collected at 1, 2, 3, 4, and 6 h after casein dosing. Ruminal pH was measured immediately, and samples were acidified, centrifuged, and frozen as before for fermentation products analysis (peptide N, AAN, NH_3, VFA, and total lactate). This procedure is similar to that reported by Siddons and Paradine (1981), but we measured AAN and peptide N instead of non-NH_3 N. By comparing concentrations of ruminal protein fermentation products over time, protease and deaminase activities were evaluated in vivo.

Samples of feed and orts were collected the last 7 d of each period. Orts were collected each day before the morning feeding. Partial DM of feed and orts was determined after drying at 55°C for 48 h and air equilibrating for 24 h before storage. Feed and orts samples were composited by steer within period and ground (1-mm screen) before further analysis for DM (105°C for 24 h), OM (450°C for 5 h), and Kjeldahl N.

Thawed ruminal samples were prepared for analysis by centrifuging at 30,000 × *g* for 15 min at 4°C and collecting the supernatant. Concentrations of VFA were measured using gas chromatography. The procedures of Broderick and Kang (1980) and Barker and Summerson (1941) were used to measure concentrations of NH_3 and total lactate, respectively. Concentrations of ruminal fluid AAN and acid-hydrolyzed ruminal fluid (peptide N plus AAN) were determined using automated trinitrobenzene sulfonic acid analysis (Palmer and Peters, 1969, as described by Wessels et al., 1996). The trinitrobenzene sulfonic acid assay measures primary amines, therefore our estimates of AAN include the N present on the termini of peptides, but our estimates of peptide N would not include the terminal amino groups on peptides. Concentrations of Cr were measured by atomic absorption spectrophotometry. Liquid dilution was calculated for each steer in each period by regressing the natural logarithms of Cr concentrations on time; the negative slope was the liquid dilution rate, and the 0-h intercept (transformed back to Cr concentration) was divided into the Cr dose to determine ruminal volume. Counting of ciliate protozoa was according to Goad et al. (1998).

Statistical Analysis. Intakes and ruminal measures based on Cr-EDTA kinetics were statistically analyzed as a Latin square using the general linear models procedure of SAS (SAS System for Windows Release 6.11, SAS Inst. Inc., Cary, NC). The model included effects of steer, period, diet, antibiotic, and the diet × antibiotic interaction. Ruminal fermentation products, in vitro protease and deaminase activities, and protozoal counts were analyzed using the mixed model procedure of SAS. The ruminal fermentation and protozoal counts for d 19 and 20 were analyzed as a split-split-plot, with the main plot arranged as a Latin square with sampling day as the sub-plot and sampling times (hours after feeding) as the sub-sub-plot. The model included steer, period, diet, antibiotic, d, h, and interactions (diet × antibiotic; diet × d, antibiotic × d; diet × antibiotic × d; diet × h; antibiotic × h; and diet × antibiotic × h). Steer × period × diet × antibiotic and steer × period × diet × antibiotic × d were included as random variables to serve as main-plot and sub-plot error terms, respectively. In vitro protease and deaminase activities were analyzed as a split-plot with the main plot arranged as a Latin square and with sampling day as the sub-plot. The model included steer, period, diet, antibiotic, d, and interactions (diet × antibiotic; diet × d; antibiotic × d; and diet × antibiotic × d). Steer × period × diet × antibiotic was used as a random variable to serve as the main-plot error term. Ruminal fermentation products measured on d 21 were analyzed as a split-plot Latin square with sampling times (h post-infusion of casein) as the sub-plot. The model included steer, period, diet, antibiotic, h, and interactions (diet × antibiotic, diet × h, antibiotic × h, and diet × antibiotic × h). Steer × period × diet × antibiotic was included as a random variable to serve as the main-plot error term. Missing observations occurred for all observations from one steer in one period (MT/SBM) and another steer for h 4 through 10 on d 19, as well as all data related to ruminal volume, liquid dilution rate, and ruminal outflow for that period (control/SBM). Significant effects were $P \leq 0.05$, and tendencies were $0.05 < P < 0.10$. The reported SEM are for n = 5 to reflect the highest SEM among treatments.

Results

Virginiamycin and MT had little effect on DM, OM, or CP intakes (Table 2). Steers fed WCGF consumed 8% more DM and OM as well as 10% more CP than those fed SBM ($P < 0.05$). Ruminal volume was 18.6% larger ($P < 0.05$) for steers fed WCGF than for those fed SBM and was not affected by antibiotics. Neither antibiotic treatment nor diet affected ruminal liquid dilution. Because ruminal volumes were larger for steers fed WCGF and there were no differences in liquid dilution rate, ruminal outflow was greater for steers consuming WCGF than for those fed SBM ($P < 0.05$). Antibiotic and diet tended to interact on ruminal outflow ($P = 0.06$). Steers fed VM and SBM had lower ruminal outflow than steers fed control or MT with SBM, but steers fed VM and WCGF had greater ruminal outflow than those fed control or MT with WCGF.

In vitro protease and deaminase activities (Table 2) were lower in steers fed WCGF ($P < 0.05$) than in steers fed SBM. Steers fed MT tended ($P < 0.08$) to

Table 2. Feed intake, ruminal liquid volume and outflow, and in vitro deaminase and protease activities in steers fed corn-based finishing diets with or without antibiotics

	SBM[a]			WCGF[a]			
Item	Control	MT[a]	VM[a]	Control	MT[a]	VM[a]	SEM[b]
DM intake, kg/d[c]	8.22	7.76	7.68	8.37	8.57	8.51	0.23
OM intake, kg/d[c]	7.81	7.38	7.30	7.96	8.16	8.10	0.22
CP intake, kg/d[c]	1.17	1.09	1.08	1.21	1.22	1.23	0.05
Ruminal volume, L[c]	28.3	31.6	28.6	33.9	33.6	37.6	2.0
Liquid dilution, %/h	7.17	6.81	6.16	7.26	6.92	7.20	0.63
Ruminal outflow, L/d[cd]	49.2	49.8	41.9	56.9	53.6	62.7	3.8
In vitro deaminase activity[ce]	0.15	0.19	0.16	0.13	0.12	0.12	0.020
In vitro protease activity[cfg]	0.77	1.04	0.90	0.68	0.76	0.77	0.078

[a]SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin.
[b]n = 5.
[c]Diet, $P < 0.05$.
[d]Antibiotic × diet, $P = 0.06$.
[e]Activity as mg NH_3-N · h^{-1} · mL^{-1}.
[f]Antibiotic, $P = 0.08$.
[g]Activity as mg nonprotein N · h^{-1} · mL^{-1}.

have greater in vitro protease activity than control steers, with steers fed VM being intermediate. No differences in deaminase activity were attributable to the antibiotics.

Ruminal pH, lactate, and VFA concentrations (d 19 and 20) are summarized in Table 3 and Figure 1. Ruminal pH (Figure 1) was lower ($P < 0.01$) for steers fed SBM than for those fed WCGF. The concentration of total VFA was higher in steers fed SBM than WCGF ($P < 0.05$), and lactate was low at all times and similar between diets. Although the trend was for numerically higher concentrations of individual VFA in steers consuming SBM, only propionate was higher ($P < 0.05$). Acetate concentrations tended to suggest a diet × h interaction ($P = 0.09$). Concentrations were similar at all sampling times except 2 h after feeding when steers fed SBM had higher acetate than those fed WCGF. There was a significant diet × antibiotic × h interaction for butyrate ($P = 0.002$). When steers were fed SBM with MT, concentrations of butyrate were lower at 6 to 10 h after feeding compared to VM and control treatments. However, when WCGF was fed, steers treated with VM had lower concentrations of butyrate than steers treated with MT or control, except at 2 h after feeding. Overall, butyrate concentrations tended to be higher in steers fed SBM. There were significant diet × h interactions ($P < 0.001$) for isobutyrate, isovalerate (Figure 1), and valerate. Steers fed WCGF had lower concentrations before feeding, similar concentrations at 2 and 4 h after feeding, and then lower concentrations at 6 to 10 h after feeding.

For d 19 and 20, ruminal concentrations of nitrogenous fermentation products are summarized in Figure 1. Overall, steers fed WCGF had greater peptide N concentrations than steers fed SBM, and antibiotic treatments had only minor effects, wherein they reduced peptide N concentrations at 2 h after feeding (antibiotic × h, $P < 0.01$). There tended to be an antibiotic × diet interaction for peptide N concentration ($P = 0.07$). When SBM was fed, MT and VM had lower concentrations of peptide N than the control (1.68 and 1.73 vs 2.05 m*M*; SEM = 0.19), but when WCGF was fed, MT resulted in higher peptide N concentrations than VM or control (3.40 vs 2.97 and 2.91 m*M*). Although antibiotic × diet × h interactions were significant for AAN concentrations ($P = 0.003$), the treatment × h interactions ($P < 0.001$) are presented because they adequately describe the data (Figure 1). Concentrations of AAN were similar among antibiotic treatments, except that steers treated with MT had lower concentrations than steers fed VM or control at 2 h after feeding. Steers fed WCGF had much greater AAN concentrations at 2 h after feeding than those fed SBM. Ruminal NH_3 concentrations for WCGF were lower than SBM before feeding, higher 2 h after feeding, and then lower 8 to 10 h after feeding ($P < 0.001$). Antibiotic treatments had little effect on NH_3 concentrations.

Ruminal ciliate protozoal counts (d 19 and 20) are presented in Table 4. Antibiotics had no effect on total counts or generic composition. Steers fed WCGF had higher numbers of *Entodinium* sp. than steers fed SBM ($P < 0.05$); this contributed to greater total ciliate counts because *Enodinium* sp. were the predominate ciliate. *Isotricha* sp. and *Polyplastron* sp. were greater ($P \leq 0.05$) in steers fed SBM than in those fed WCGF.

On the day of casein infusion (d 21, Table 5 and Figure 2), ruminal pH was higher and total VFA were lower ($P < 0.05$) for steers receiving MT than control or VM. The lower total VFA in MT-treated steers was primarily because propionate was lower ($P < 0.05$) in MT-treated steers vs steers receiving VM or control. There tended to be an antibiotic × diet interaction for isovalerate ($P = 0.08$). Steers fed SBM and MT or control had higher concentrations of isovalerate than those receiving VM (4.21 and 3.50 vs 2.66 m*M*; SEM

Table 3. Ruminal VFA and lactate concentrations in steers fed corn-based finishing diets with or without antibiotics

Acids, m*M* (SEM)[c]	Hour[b]	SBM[a]			WCGF[a]		
		Control	MT[a]	VM[a]	Control	MT	VM
Total VFA[df] (5.7)	0	109	105	110	88	90	94
	2	115	118	125	108	103	110
	4	111	115	119	98	101	103
	6	107	102	118	94	96	97
	8	109	104	117	90	92	97
	10	103	103	111	89	91	91
Acetate[fh] (3.7)	0	53.3	53.9	51.3	49.8	50.1	51.0
	2	58.3	61.7	61.3	56.9	52.8	54.5
	4	54.7	59.5	55.6	53.9	53.4	53.4
	6	51.5	52.5	53.3	52.2	51.5	51.1
	8	51.6	53.2	52.2	50.5	48.5	51.2
	10	49.5	53.2	49.4	49.6	48.9	49.4
Propionate[df] (4.6)	0	32.1	27.7	37.0	20.2	22.5	28.0
	2	33.2	31.3	39.5	27.8	26.6	33.8
	4	32.4	32.0	39.1	23.7	26.9	31.6
	6	31.1	29.0	39.3	22.4	25.5	29.9
	8	31.6	29.5	39.7	21.2	24.3	30.4
	10	30.2	28.9	36.9	20.8	24.1	28.2
Butyrate[efgi] (3.3)	0	17.3	16.7	16.9	13.4	13.0	10.8
	2	18.3	18.6	19.0	16.8	16.9	16.2
	4	18.6	17.6	18.9	15.0	15.5	13.3
	6	18.5	15.1	20.0	14.5	14.7	11.7
	8	20.0	15.5	20.5	13.9	14.3	11.2
	10	17.7	15.1	20.1	13.8	13.9	10.0
Isobutyrate[fg] (0.11)	0	1.11	1.16	1.03	0.96	0.96	0.85
	2	0.91	1.08	0.99	1.14	1.09	0.93
	4	0.86	1.04	0.90	1.00	0.98	0.88
	6	0.87	0.96	0.88	0.89	0.85	0.73
	8	0.94	1.00	0.89	0.86	0.86	0.72
	10	0.93	1.06	0.91	0.90	0.91	0.72
Valerate[efg] (0.23)	0	2.04	1.86	2.13	1.37	1.20	1.40
	2	2.24	2.18	2.60	2.43	2.33	2.51
	4	2.12	2.01	2.38	2.33	2.02	2.31
	6	2.01	1.75	2.38	1.89	1.53	1.82
	8	2.10	1.76	2.40	1.62	1.34	1.66
	10	1.82	1.65	2.24	1.45	1.19	1.44
Lactate[f] (0.037)	0	0.35	0.18	0.18	0.17	0.21	0.18
	2	0.24	0.17	0.32	0.25	0.23	0.25
	4	0.18	0.17	0.18	0.21	0.22	0.20
	6	0.17	0.16	0.16	0.19	0.21	0.19
	8	0.18	0.16	0.17	0.18	0.19	0.18
	10	0.17	0.16	0.17	0.17	0.19	0.17

[a]SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin.
[b]After feeding.
[c]n = 5.
[d]Diet, $P < 0.05$.
[e]Diet, $P < 0.10$.
[f]Hour, $P < 0.002$.
[g]Diet × hour, $P < 0.001$.
[h]Diet × hour, $P = 0.09$.
[i]Diet × antibiotic × hour, $P = 0.002$.

= 0.38), but for cattle fed WCGF, both MT and VM were somewhat lower than control (3.06 and 3.04 vs 3.74 m*M*; SEM = 0.38). Overall, isovalerate was lower when VM was fed ($P = 0.05$). Acetate, butyrate, isobutyrate, valerate, and lactate concentrations were not affected by antibiotic treatment. Steers fed WCGF had higher ruminal pH, lower propionate, and lower butyrate concentrations ($P < 0.05$). Isobutyrate concentrations were lower for the first 3 h after casein infusion for steers fed SBM than for those fed WCGF, but were similar between 4 and 6 h after dosing ($P < 0.05$). Ruminal valerate concentrations were similar for both diets for the first 3 h after casein dosing, then valerate declined for steers fed WCGF but continued to in-

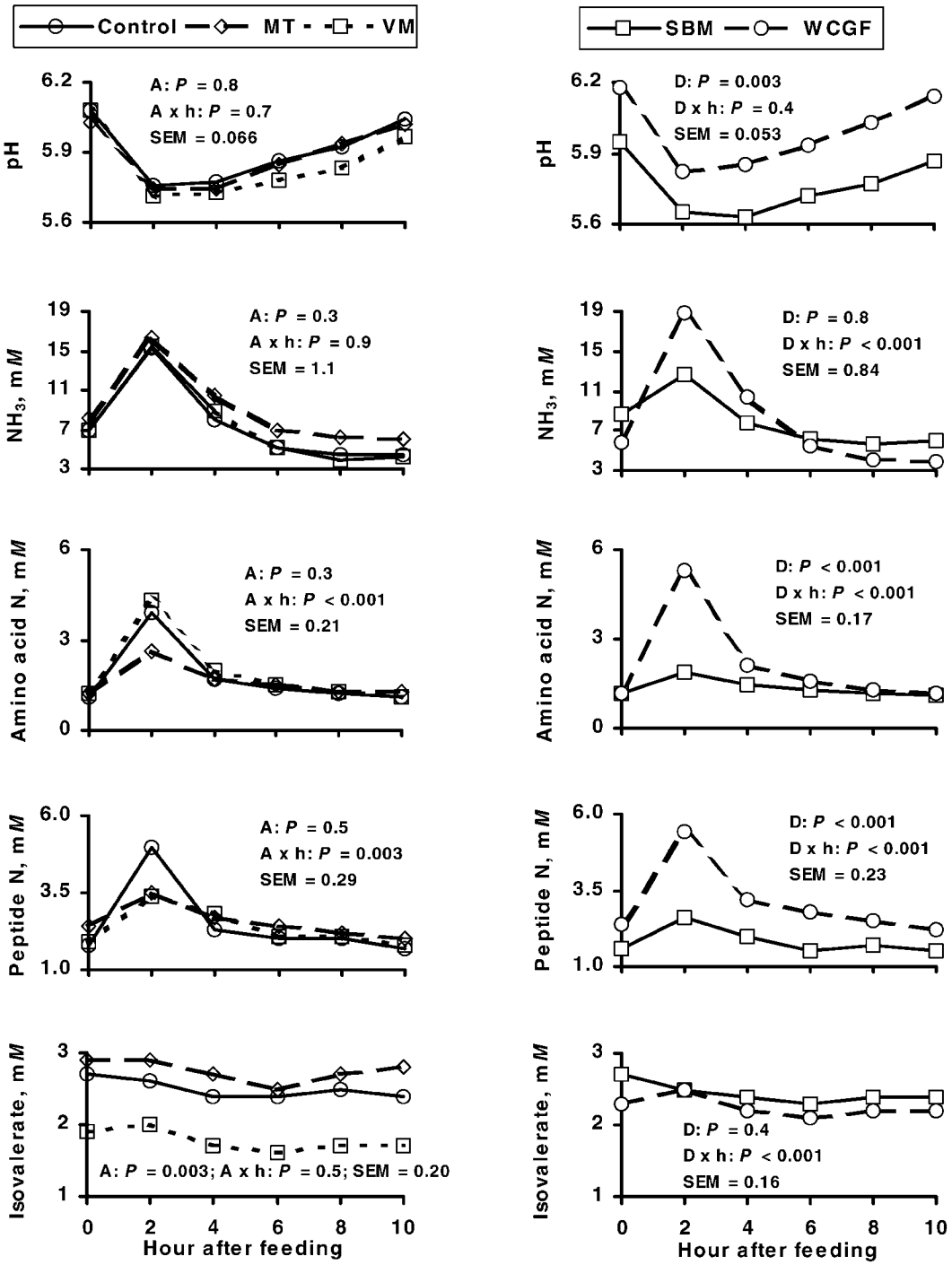

Figure 1. Ruminal pH and fermentation products in steers fed corn-based finishing diets with or without antibiotics. MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin; SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; A = antibiotic; D = diet. SEM for n = 5.

crease over time for those fed SBM ($P < 0.05$). There were no significant differences for acetate, isovalerate, and lactate between diets.

Ruminal nitrogenous fermentation products, after casein dosing, are summarized in Figure 2. No ruminal samples were collected directly prior to casein dosing, but concentrations of peptides and AAN at dosing should have been similar to those at 2 h after feeding on the previous days (Figure 1). Ruminal peptide N and NH_3 concentrations were similar for the antibiotic treatments. Ammonia concentrations were lower for steers fed SBM than for those fed WCGF during the first 3 h after casein infusion, and were similar 4 h after dosing, but were higher for steers fed SBM 6 h

Table 4. Ruminal protozoal populations in steers fed corn-based finishing diets with or without antibiotics

Genera (SEM)[c]	Hour[b]	SBM[a]			WCGF[a]		
		Control	MT[a]	VM[a]	Control	MT[a]	VM[a]
Dasytricha sp.[f]	0	1.6	2.5	2.8	1.6	2.2	4.4
10^3/g	4	0.9	2.2	3.2	0.0	0.7	4.4
(1.4)	8	1.5	0.3	1.3	0.3	2.2	2.8
Isotricha sp.[df]	0	5.9	5.2	3.9	4.4	2.5	1.9
10^3/g	4	1.7	2.1	2.5	1.1	1.4	0.3
(1.0)	8	1.4	2.5	1.1	1.4	1.6	1.6
Entodinium sp.[dfg]	0	0.39	0.54	0.35	1.17	1.17	1.29
10^6/g	4	0.25	0.41	0.22	0.85	1.00	0.97
(0.25)	8	0.26	0.40	0.21	0.97	1.08	1.06
Polyplastron sp.[ef]	0	2.6	0.6	2.2	0.0	0.4	1.0
10^3/g	4	1.0	0.0	1.1	0.0	0.4	0.5
(0.69)	8	1.1	1.3	0.9	0.0	0.4	0.2
Total[dfg]	0	0.40	0.55	0.36	1.18	1.17	1.30
10^6/g	4	0.25	0.41	0.23	0.85	1.00	0.98
(0.25)	8	0.26	0.41	0.21	0.98	1.09	1.07

[a]SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin.
[b]After feeding.
[c]n = 5.
[d]Diet, $P < 0.05$.
[e]Diet, $P = 0.05$.
[f]Hour, $P < 0.05$.
[g]Diet × hour, $P < 0.05$.

after dosing. Steers treated with VM had significantly higher AAN concentrations than MT or control ($P = 0.001$). Peptide N concentrations were similar for the two diets. Steers fed WCGF had lower concentrations of AAN averaged over all collections compared to those fed SBM. Concentrations of AAN increased from 1 to 2 h after dosing for steers fed SBM, then decreased over the next 4 h, whereas steers fed WCGF had a linear decrease in AAN concentrations over time ($P = 0.02$). These results would suggest that there was lower deaminase activity in steers fed SBM but similar protease activity between the two diets.

Discussion

The differences in intake due to diet formulation are consistent with reports by Scott et al. (1997) and McCoy et al. (1997), in which steers were fed a basal finishing diet of dry-rolled corn (83% of DM) and diets that replaced dry-rolled corn with wet corn gluten feed (20 to 45% of DM). In each case, there was a significant increase in DMI when the corn gluten feed was added. The greater intake of our WCGF diet likely contributed to the greater ruminal volume and fluid outflow. Merchen et al. (1986) fed lambs either a high- or low-forage diet at two levels of intake. For both diets, ruminal volume and fluid outflow were greater at the higher level of intake. Kreikemeier et al. (1990) found a 23% greater ruminal liquid fill and passage in steers as intake of a steam-rolled wheat finishing diet increased from two to three times maintenance.

It is unclear why there were differences in ruminal outflow due to VM between the diets (i.e., VM decreased ruminal outflow when fed with SBM, but increased ruminal outflow when fed with WCGF). When VM was fed with SBM, the liquid dilution rate was numerically lower than when MT or no antibiotic was added to the diet. However, with WCGF, the liquid dilution rates were similar among antibiotic treatments and the greater ruminal outflow for VM was attributable to numerically greater ruminal volumes. Because intakes were similar within diets, mechanisms beyond intake likely affected ruminal outflow. Other reports measuring ruminal flow rate with virginiamycin are lacking.

In our study, few differences in pH and VFA due to MT or VM were observed. Others have reported similar results (Zinn, 1987; Morris et al., 1990; Coe et al., 1999). Coe et al. (1999) found no differences in fermentation products as steers were adapted to an all-concentrate diet with either control, virginiamycin (175 or 250 mg/d) or monensin/tylosin (250 and 90 mg/d, respectively). Two experiments reported by Fiems et al. (1990) found no differences in pH and total VFA in wethers treated with virginiamycin (25 or 65 mg/kg of diet). When Horton and Nicholson (1980) fed steers a 60% concentrate diet with either no antibiotics, monensin (33 mg/kg of diet), tylosin (11 mg/kg of diet), or monensin plus tylosin, they found only the steers receiving monensin alone had lower proportions of acetate and butyrate, with greater proportions of propionate compared with controls. Steers receiving

Table 5. Ruminal VFA and lactate concentrations in steers fed corn-based finishing diets with or without antibiotics after dosing rumens with 350 g casein

Item, m*M* (SEM)[c]	Hour[b]	SBM[a]			WCGF[a]		
		Control	MT[a]	VM[a]	Control	MT[a]	VM[a]
Total VFA[def] (5.4)	1	124	114	127	106	93	113
	2	129	118	135	109	97	117
	3	130	124	131	112	100	120
	4	123	120	131	109	92	110
	6	122	105	124	101	89	110
Acetate[f] (3.1)	1	57.4	53.2	50.5	55.1	50.8	57.5
	2	57.8	54.0	52.5	56.5	53.2	59.4
	3	56.4	55.0	49.5	57.4	54.3	60.1
	4	52.8	53.2	48.5	55.9	50.9	54.4
	6	51.5	47.0	44.8	51.7	50.6	55.5
Propionate[def] (4.8)	1	39.7	31.3	50.8	25.9	22.7	34.8
	2	41.5	32.6	54.3	26.2	23.7	35.6
	3	42.3	34.5	52.7	27.1	24.5	36.8
	4	39.5	33.9	52.7	26.9	21.9	34.3
	6	39.8	30.1	51.3	25.6	21.5	34.8
Butyrate[efg] (4.4)	1	19.8	22.3	19.2	17.1	13.0	14.5
	2	21.5	22.8	20.4	17.4	12.9	14.4
	3	22.6	24.5	20.5	17.5	12.6	14.2
	4	22.7	23.3	20.8	17.0	11.4	13.1
	6	23.3	19.5	19.6	16.1	11.3	12.6
Isobutyrate[fg] (0.17)	1	1.18	1.23	0.88	1.48	1.24	1.15
	2	1.43	1.47	1.13	1.81	1.53	1.45
	3	1.69	1.75	1.29	2.02	1.75	1.71
	4	1.78	1.86	1.44	1.94	1.61	1.72
	6	1.53	1.51	1.33	1.58	1.29	1.52
Valerate[fg] (0.54)	1	2.50	2.43	3.34	2.84	2.35	2.67
	2	2.68	2.73	3.80	3.21	2.68	3.04
	3	2.84	3.07	3.86	3.40	2.90	3.24
	4	2.95	3.24	4.13	3.32	2.67	3.25
	6	3.14	2.91	4.17	2.87	1.97	2.88
Lactate (0.058)	1	0.17	0.17	0.20	0.23	0.22	0.22
	2	0.20	0.17	0.19	0.21	0.21	0.20
	3	0.26	0.18	0.28	0.19	0.21	0.21
	4	0.46	0.19	0.28	0.20	0.21	0.20
	6	0.20	0.17	0.30	0.18	0.21	0.19

[a]SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin.
[b]After casein dosing.
[c]n = 5.
[d]Antibiotic, $P < 0.05$.
[e]Diet, $P < 0.05$.
[f]Hour, $P < 0.05$.
[g]Diet × hour, $P < 0.05$.

tylosin or monensin plus tylosin had proportions of acetate and propionate similar to controls with a reduced proportion of butyrate only in the monensin plus tylosin group. Changes in fermentation resulting from MT may not be the same as with monensin alone (Nagaraja et al., 1987).

It is generally accepted that monensin has a "protein-sparing" effect in the rumen through reduced protein, peptide, and AA degradations (Bergen and Bates, 1984; Russell and Strobel, 1989; Nagaraja et al., 1997). In our study, however, we observed no effect of monensin on protein, peptide, or AA degradations. Yang and Russell (1993) fed nonlactating cows timothy hay 12 times daily with or without monensin, and then infused 560 g of trypticase into the rumen to assess the effects of monensin on ammonia accumulation and the rate of disappearance of peptide and amino N from the rumen. They found a lower ammonia concentration and reduced rates of peptide and AA disappearance after casein infusion when monensin was fed. Poos et al. (1979) reported greater feed protein in abomasal contents of steers treated with monensin when fed diets based on corn cobs supplemented with either brewers dried grain or urea. In contrast, in experiments with cattle fed high concentrate diets, monensin had minimal or no protein sparing effect. Steers fed 90% concentrate diets with monensin/tylosin had numerically higher ruminal con-

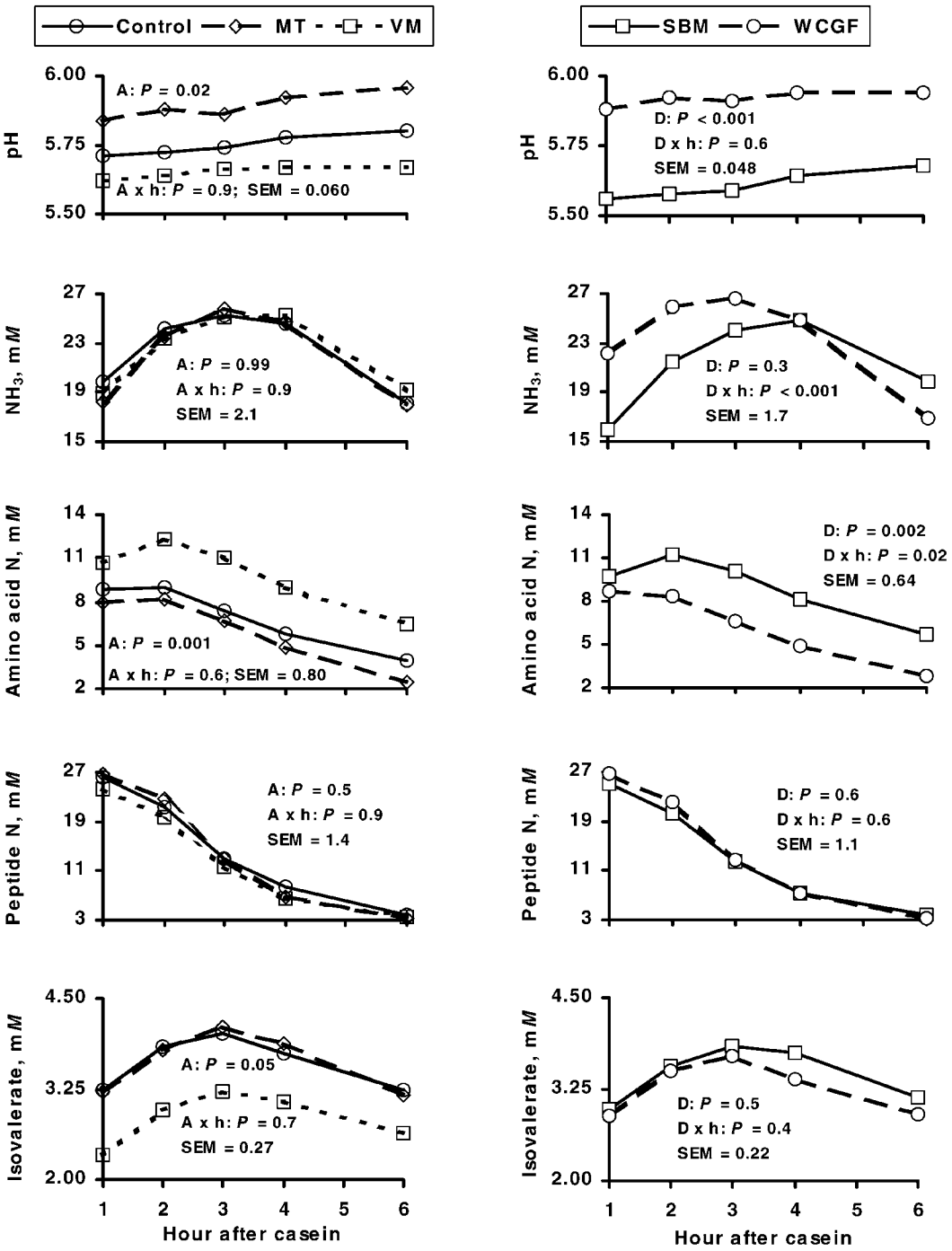

Figure 2. Ruminal pH and fermentation products in steers fed corn-based finishing diets with or without antibiotics after dosing rumens with 350 g solubilized casein. MT = 250 mg/d monensin and 100 mg/d tylosin; VM = 175 mg/d virginiamycin; SBM = diet based on dry-rolled corn with 12% soybean meal; WCGF = diet based on dry-rolled corn with 30% wet corn gluten feed; A = antibiotic; D = diet. SEM for n = 5.

centrations of isobutyrate, isovalerate, and greater ruminal N digestibility compared to controls (Morris et al., 1990). In steers fed a 60% barley-based diet, NH_3 was lower in steers with monensin but not significantly different from controls, and the steers receiving either tylosin or monensin plus tylosin had significantly greater NH_3 levels than control or monensin-treated steers (Horton and Nicholson, 1980). Ruminal AA concentrations were greatest for the control steers, with the monensin-treated steers having lower concentrations and the tylosin- or monensin plus tylosin-treated steers having the lowest concentrations. Zinn et al. (1994) reported a greater amount of feed N escaping ruminal degradation in steers fed 80 to 90% concentrate diets when 28 mg/kg of monensin was added.

The effect of virginiamycin on ruminal N metabolism has been much less studied. In vitro studies by Van Nevel et al. (1984) and Van Nevel and Demeyer (1990) reported that the effects of virginiamycin were similar to those of monensin in vitro (i.e., reduction in casein degradation and ammonia production). Our in vivo results (higher AAN concentrations and reduced isovalerate concentration) suggested that VM had a protein-sparing effect, primarily through reduced deamination of AA. However, our in vitro results did not support this conclusion. Also, we did not observe lower NH_3 concentrations in VM-fed steers. The lower pH in steers fed VM when casein was added may have reduced ammonia absorption from the rumen.

The replacement of a portion of the dry-rolled corn and all of the SBM with WCGF appeared to have a moderating effect on ruminal fermentation. The lower ruminal pH and greater total VFA in steers fed SBM was likely due to greater starch content of this diet compared to WCGF (Merchen et al., 1986). Wet corn gluten feed contains more NDF (42 vs 11%) and fewer nonstructural carbohydrates (23 vs 71%) than dry-rolled corn (Ham et al., 1994). Ham et al. (1994) found no differences in ruminal pH, total VFA, or individual VFA due to feeding wet corn gluten feed. In their study, steers were fed 12 times daily, which may have altered the response to treatments when compared to our study with twice-daily feeding. Differences in ciliated protozoa also may have contributed to differences between diets in pH and VFA concentrations. Nagaraja et al. (1992) found significantly lower pH and greater total VFA concentrations in ciliate-free than in faunated steers. The ciliate-free steers had fourfold greater numbers of total anaerobic bacteria than faunated steers. In our study, there was nearly a threefold greater ciliate population in the steers fed WCGF, which could have reduced the total bacterial concentration and, ultimately, lowered the rate of fermentation. Also, the ability of protozoa to sequester starch and subsequently slow the rate of ruminal fermentation may have contributed to the higher ruminal pH when WCGF was fed. On the other hand, the differences in daily minimum pH may have affected protozoal counts. Purser and Moir (1959) demonstrated a strong positive correlation between minimum daily pH and ciliate counts.

The WCGF diet appeared to offer N sources that were more readily degradable than those in SBM. In spite of a larger ruminal liquid volume and higher pH in steers receiving WCGF, they had greater peptide N, AAN, and NH_3 concentrations 2 h after feeding. Condensed corn-steep water added to WCGF is a source of soluble protein and could provide the readily available source of degradable protein. This is in agreement with Firkins et al. (1984), who found only 27% of the original N in wet corn gluten feed compared with 52% for soybean meal after 2 h of in situ incubation. Although there were similar rates of N disappearances for wet corn gluten feed and soybean meal between 2 to 8 h, there was significantly less residual N for wet corn gluten feed than soybean meal after 8 h due to the rapid release of N from the WCGF before 2 h.

Some of the difference noted in N metabolism may be attributed to the protozoal densities. Jouany (1996) reported that faunated sheep had greater ruminal ammonia concentrations than defaunated sheep. Entodiniomorphid ciliates are able to ingest and digest insoluble feed particles. Faunated sheep with a mixed population produced approximately 25% more ammonia during 6 h of in vitro incubations with SBM than did defaunated sheep. Casein degradation was unaffected by mixed ciliated protozoa due to its soluble nature and lack of utilization by entodiniomorphid ciliates. The less soluble protein found in dry-rolled corn may have been relatively more available in the steers fed WCGF due to the threefold greater ciliate counts and the ability of ciliated protozoa to consume particulate matter and subsequently degrade insoluble proteins. Based on the higher AAN and lower NH_3 concentrations after casein infusion, steers fed SBM had lower ruminal deaminase activity, but protease activity was similar to steers fed WCGF.

Implications

Virginiamycin appeared to have a protein-sparing effect on feed proteins in the rumen of steers fed corn-based finishing diets. Thus, the inclusion of virginiamycin into diets could increase metabolizable protein supply to cattle. Although monensin has been shown to have protein-sparing effects in animals fed high forage diets, our study with steers fed high concentrate diets did not support this conclusion. Inclusion of wet corn gluten feed in a finishing diet provided a more rapidly degradable protein source than dry-rolled corn and soybean meal as well as a moderating effect on ruminal fermentation. Higher pH and reduced organic acid concentrations resulted when wet corn gluten feed was included into the diet, possibly because of higher protozoal concentrations, suggesting that use of wet corn gluten feed could reduce the occurrence of ruminal acidosis in finishing cattle.

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