INFLUENCE OF COTTONSEED MEAL SUPPLEMENTATION ON VOLUNTARY INTAKE, RUMINAL AND CECAL FERMENTATION, DIGESTA KINETICS AND SERUM INSULIN AND GROWTH HORMONE IN MATURE EWES FED PRAIRIE HAY[1,2]

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ABSTRACT

To determine the influence of protein supplementation on intake and fermentation of low-quality hay, six ruminal- and cecal-cannulated Rambouillet ewes (avg wt 43.6 kg) in a crossover design were given ad libitum access to prairie hay with or without 80 g of cottonseed meal (CSM)·head^{-1}·d^{-1}. Voluntary hay intake was measured the last 7 d of each 18-d period. Ruminal, cecal and blood samples were collected at 0, 1 (except cecal), 3, 6, 9, 12, 15, 18, 21 and 24 h post-supplementation on d 14 of each period to measure fluid dilution rate, fermentation characteristics and serum concentrations of insulin and growth hormone. An intraruminal dose of Yb-labeled hay, followed by fecal sampling on d 15 through 18, was used to measure particulate passage rate. Voluntary intake of prairie hay was increased ($P<.04$) from 23.7 to 28.3 g/kg of body weight by CSM supplementation. Particulate passage rate constants did not differ ($P>.15$) between supplemented (3.76%/h) and control (3.72%/h) ewes, and total mean retention time was not altered ($P>.15$) by CSM supplementation. Ruminal retention time of particulates did not differ ($P>.15$) between treatments; however, intestinal transit time was faster ($P<.03$; 18.1 vs 22.6 h) in supplemented than in control ewes. Estimated gastrointestinal dry matter fill was greater ($P<.05$; 14.3 vs 12.9 g/kg body weight) in supplemented ewes. Ruminal fluid volume did not differ ($P>.15$) between treatments; however, supplemented ewes tended to have faster fluid dilution rates ($P<.14$) and fluid outflow rates ($P<.11$) than control ewes. Cecal fluid volume, dilution rate and outflow rate did not differ ($P>.15$) between groups. Ruminal and cecal pH and total volatile fatty acids were similar between treatments. Similarly, cottonseed meal supplementation did not affect ($P>.15$) ruminal or cecal ammonia concentrations. Molar proportions of ruminal and cecal individual fatty acids were not affected ($P>.15$) by CSM supplementation. Feeding cottonseed meal increased ($P<.05$) serum insulin, decreased ($P<.07$) serum growth hormone and increased ($P<.06$) serum free fatty acids, but did not influence ($P>.15$) either serum urea N or glucose concentrations. Cottonseed meal supplementation in ewes fed prairie hay caused increased hay intake but had minimal effects on ruminal and cecal fermentation.

(Key Words: Hay, Cottonseed Oilmeal, Rumen, Cecum, Insulin, Somatotropin.)

Introduction

Forage intake has been increased (Church and Santos, 1981; Kartchner, 1981; McCollum and Galyean, 1985b), decreased or not changed (Rittenhouse et al., 1970; Branine et al., 1985; Judkins et al., 1987) in supplemented as compared with nonsupplemented ruminants consuming low-quality forages or grazing dormant western rangeland. Increased intake as a result of protein supplementation is usually attributed to increased rate of digestion and(or) passage (Ellis, 1978). Thus, evaluation of responses to supplementation necessitates evaluation of digesta kinetics.

In sheep, the hindgut may represent a volume of 15 to 26% of that of the rumen, and contain 14 to 40% of the feed intake (Hoover, 1978). Minimal information is available, however, about the influence of protein supplementation on hindgut fermentation in ruminants fed low-quality forages (Caton et al., 1985).

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TABLE 1. CHEMICAL COMPOSITION OF PRAIRIE HAY AND COTTONSEED MEAL

Item	Prairie hay	Cottonseed meal
	%	
Dry matter	93.9	95.1
Ash[a]	9.3	7.1
Neutral detergent fiber[a]	71.1	30.1
Acid detergent fiber[a]	47.2	21.7
Acid detergent lignin[a]	6.2	9.1
Crude protein[a]	6.3	40.5
Total N[a]	1.0	6.5
Soluble N[a]	.3	.8
Insoluble N[a]	.7	5.7
Insoluble available N[a]	.5	5.4
Acid detergent insoluble N[a]	.2	.3
Forage and supplement dry matter digestibilities[b]		
Control		
Standard in vitro	45.4 ± 1.6	
In situ + acid-pepsin	54.1 ± 1.3	
Supplemented		
Standard in vitro	50.7 ± .9	64.2 ± .9
In situ + acid-pepsin	57.4 ± 1.4	74.7 ± 1.3

[a]Dry matter basis.

[b]Values are means ± standard errors based on six observations/treatment.

The potential for increased duodenal protein flow could alter the metabolic status of protein-supplemented ruminants fed low-quality forages. Abomasal infusion of casein (Johnson et al., 1981) increased serum insulin concentrations in steers fed prairie hay. Oldham et al. (1982) reported increased serum growth hormone concentrations in cows fed formaldehyde-treated vs nontreated protein supplements. Effects of feeding supplemental protein on these metabolic hormones has not been extensively investigated.

This study examined the influence of cottonseed meal supplementation on forage intake, ruminal and cecal fermentation patterns, digesta kinetics, serum insulin, growth hormone, glucose, urea N and free fatty acids in mature ewes fed low-quality prairie hay.

Experimental Procedure

Six mature, nonpregnant, Rambouillet ewes (avg wt 43.6 kg), fitted with permanent ruminal and cecal cannulae, were used in a crossover design. Ewes were individually housed in 1.5- × 3.0-m pens with continuous access to fresh water and trace mineral salt blocks[4]. Each period of the crossover design was 18 d. The first 13 d were for diet adaptation. Prairie hay (table 1) consisting primarily of Andropogon spp (bluestem) chopped through a 2.54-cm screen was offered each morning at 0700 in amounts sufficient to allow ad libitum consumption. Three ewes in each period were offered 80 $g \cdot head^{-1} \cdot d^{-1}$ of mechanically extracted cottonseed meal (CSM; table 1) at 0700. Supplement was offered in the feeder when hay refusals were being weighed back and was consumed within 15 min. Level of CSM supplementation was based on pre-trial consumption of prairie hay and addition of sufficient CSM to meet 125% of the protein requirements of a 50-kg, nonlactating ewe (NRC, 1975).

Hay and supplement grab samples were obtained daily and composited within each period. Hay refusal subsamples were composited by ewe during each period to examine the possibility of sorting. Dry matter (100 C) and ash content (550 C) were determined by standard procedures (AOAC, 1984). Dietary fiber constituents (neutral detergent fiber, acid

[4]Morton IOFIXT Trace Mineralized salt. Morton Salt Division of Morton Thiokol, Inc. Chicago, IL.

detergent fiber, acid detergent lignin) were determined according to Goering and Van Soest (1970). The Kjeldahl procedure was used for all N analyses (AOAC, 1984). Nitrogen content of hay and supplement was subdivided into soluble, insoluble, insoluble-available and insoluble-nonavailable N. Soluble and insoluble N fractions were determined by the .15 M NaCl procedure of Waldo and Goering (1979). Insoluble-nonavailable N was represented by acid detergent insoluble N (ADIN), and was determined as described by Goering and Van Soest (1970). Insoluble-available N was calculated by subtraction of soluble N and ADIN fractions from total N.

At 0700 on d 14 of each collection period, 50 ml of ruminal contents were withdrawn from each ewe (time 0). Ewes were dosed via ruminal cannula with 50 ml of cobalt-ethylenediaminetetraacetate (Co-EDTA; Uden et al., 1980) containing 176 mg of Co, after which CSM was fed to supplemented ewes. Additional ruminal samples were collected from each ewe at 1, 3, 6, 9, 12, 15, 18, 21 and 24 h after supplementation. Ruminal pH was determined immediately with a combination electrode. Samples were strained through four layers of cheesecloth, acidified with 1 ml of 7.2 N H_2SO_4/100 ml of strained fluid and stored frozen. In addition, on d 14 at 0700, 30 to 80 ml of cecal contents were removed at time 0. Ewes were dosed via cecal cannula with 20 ml of chromium-EDTA (Binnerts et al., 1968) containing 53.6 mg of Cr. Additional cecal samples were collected at 3, 6, 9, 12, 15, 18, 21 and 24 h after supplementation. The pH of cecal contents was determined as described previously for ruminal samples, and samples were acidified with .2 ml of 7.2 N H_2SO_4/20 ml of contents and frozen.

Ruminal samples were thawed at room temperature and centrifuged at 10,000 × g for 10 min; cecal samples were centrifuged twice at 30,000 × g for 20 min. Supernatant fractions were analyzed for ammonia by the phenol-hypochlorite procedure of Broderick and Kang (1980), and for either Co or Cr by atomic absorption spectroscopy with air/acetylene or nitrous oxide/acetylene flames, respectively. Volatile fatty acid concentrations (VFA; 2-ethylbutyric acid as an internal standard) were determined on supernatant fractions as described by Goetsch and Galyean (1983). Ruminal and cecal fluid dilution rates were calculated by regressing the natural logarithm of Co or Cr concentration on time post-dosing (0-h sample not included). Fluid volume was estimated by dividing the marker dose by extrapolated concentration at 0 h.

On d 14 of each period, blood samples (approximately 10 ml) were collected via jugular venipuncture at 0, 1, 3, 6, 9, 12, 15, 18, 21 and 24 h after supplementation. Blood samples were allowed to clot at room temperature for 30 min, centrifuged at 2,300 × g for 15 min at 4 C and stored at −20 C for subsequent analyses. Serum insulin (Sanson and Hallford, 1984) and growth hormone (GH; Hoefler and Hallford, 1987) were measured using validated double antibody radioimmunoassay procedures. Serum free fatty acids were determined colorimetrically using procedures outlined by Smith (1975). Serum glucose and urea N (BUN) were determined colorimetrically using ortho-toluidine[5] and diacetyl-monoxime[6] assay procedures, respectively.

Immediately after the 24-h ruminal sample was obtained, 14.1 g (dry matter) of ytterbium (Yb)-labeled prairie hay containing 139.3 mg Yb were dosed into each ewe via ruminal cannulae. Chopped hay was labeled with $YbCl_3 \cdot H_2O$[7] by the immersion labeling procedure of Teeter et al. (1984). Rectal grab samples were collected at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96 and 108 h post-dosing. Fecal samples were dried at 50 C; Yb was extracted using the method described by Hart and Polan (1984). Ytterbium content was determined by atomic absorption spectroscopy with a nitrous oxide/acetylene flame. Fecal Yb excretion curves were fitted to a one-compartment model (Pond et al., 1982; McCollum and Galyean, 1985a) that provided estimates of particulate passage rate, retention time, fecal output and gastrointestinal dry matter fill.

Immediately following labeled forage dosing, nylon bags (9 × 16 cm; pore width 26.5 ± 5.1 μm, pore length 47.3 ± 6.4 μm) containing approximately 3 g of prairie hay and CSM in separate bags (CSM only in supplemented ewes) were placed into the rumen of each ewe for a

[5] Direct (ortho toluidine) Glucose Test Set, Stan Bio Lab., Inc., San Antonio, TX 78202.

[6] Direct (diacetyl-monoxime) Urea Nitrogen Test Set, StanBio Lab., Inc., San Antonio, TX 78202.

[7] Research Chemicals Inc., P. O. Box 14588, Phoenix, AZ 85063.

48-h incubation period. Two bags of forage and one blank bag were incubated in each control ewe and two forage, two supplement and one blank bag in each supplemented ewe. Bags were removed, rinsed under running water until clean, dried at 50 C, reweighed and a .5-g subsample was subjected to a 48-h acid-pepsin digestion (Tilley and Terry, 1963). In addition, in vitro dry matter disappearance (48-h ruminal inoculum and 48-h acid-pepsin digestion) was determined on prairie hay and CSM using ruminal fluid from each ewe (Tilley and Terry, 1963). The in situ + acid pepsin method was adopted because ewes were supplemented daily and conditions could not be reproduced with standard in vitro procedures without reinoculation of tubes. The measurement of digestibility is critical for calculation of forage intake when marker techniques are used to estimate fecal output. Therefore, estimated intake from fecal output data was computed with both estimates of digestibility for comparison with actual intake values.

Data were subjected to analysis of variance for crossover designs (Cochran and Cox, 1957) with effects for ewe, period and treatment considered in the model. Blood and fermentation measurements at various sampling times were analyzed by analysis of variance as a split-plot design with effects for ewe, period, treatment, time, time × treatment and ewe within treatment included in the model. No time × treatment interaction was noted for blood or fermentation measurements; thus, values were pooled over time. Significant differences among treatment means were determined by the F-test associated with the analysis of variance. Actual observed significance levels are reported in tables for variables with the F-test significant at $P<.15$ or less.

Results and Discussion

Daily voluntary intake of prairie hay was increased ($P<.04$) from 23.7 to 28.3 g/kg of body weight by CSM supplementation (table 2). Analysis of hay refusals revealed no evidence of sorting by the ewes. Intensive sampling of the rumen and cecum during intake measurement periods did not appear to affect voluntary intake, because values did not differ from those observed during adaptation periods. Similar responses to protein supplementation of low-quality forages have been reported in cattle (Redman et al., 1980; Church and Santos, 1981; McCollum and Galyean, 1985b) and sheep (Egan, 1965; Cook and Harris, 1968). However, Branine et al. (1985) noted no effect of protein supplementation on voluntary intake of cattle consuming prairie hay containing 7.5% crude protein.

Estimates of forage digestibility using the in vitro procedure (Tilley and Terry, 1963) were 8.7 and 6.8% lower than those achieved using the in situ + acid pepsin modification for control and supplemented ewes, respectively (table 1). Forage intake estimates from fecal output derived with a pulse dose of Yb-labeled hay, coupled with indigestibility determined by using in situ + acid pepsin, were within 1%

TABLE 2. VOLUNTARY INTAKE, PARTICULATE PASSAGE RATE, RETENTION TIMES AND GASTROINTESTINAL FILL OF EWES FED PRAIRIE HAY WITH AND WITHOUT COTTONSEED MEAL

Item	Treatment		SE[a]	Significance level[b]
	Control	Cottonseed meal		
Hay intake, g dry matter/kg body wt	23.7	28.3	2.0	P<.04
Particulate passage rate, %/h	3.72	3.76	.22	NS[c]
Total mean retention time, h	55.6	50.3	2.6	NS
Ruminal retention time, h	33.0	32.2	2.3	NS
Intestinal transit time, h	22.6	18.1	1.0	P<.03
Estimated gastrointestinal fill, g/kg body wt	12.9	14.3	.3	P<.05

[a]Standard error of treatment means based on six observations/mean.

[b]Observed significance level of row means.

[c]NS = nonsignificant (P>.15).

of measured forage intake for both control (23.5 g/kg body weight) and supplemented (28.0 g/kg body weight) ewes. However, when fecal output was coupled with values from standard in vitro procedures, forage intake estimates were only within 15 and 20% of actual intake for control (20.1 g/kg body weight) and supplemented (22.6 g/kg body weight) ewes, respectively. Because actual digestibility values were not determined, intake estimates from in vitro and in situ procedures were not statistically compared.

Cottonseed meal supplementation had no effect (P>.15) on particulate passage rate (PPR), with supplemented and control ewes averaging 3.76 and 3.72%/h, respectively (table 2). In contrast, McCollum and Galyean (1985b) found that CSM supplementation increased PPR in steers that were fed a hay diet comparable (6.1% crude protein) to that consumed by ewes in the present study. Ruminal retention time was not affected (P>.15) by supplementation; however, intestinal transit time was less (P<.03) for ewes receiving supplement (table 2); thus, total mean retention time tended (P>.15) to be lower in supplemented than control ewes. Thornton and Minson (1973) found that ruminal organic matter retention time was highly correlated with daily intake of digestible organic matter in sheep fed grasses and legumes. Increased voluntary intake of low-quality forages with protein supplementation is usually attributed to increased rate of digestion and(or) rate of passage (Ellis, 1978). Further evidence suggests that gut fill is the primary factor regulating forage intake (Freer, 1981). Estimated gastrointestinal fill was greater (P<.05) in supplemented than in control ewes (table 2). If fill was not limiting intake of this hay, or if protein supplementation allowed expansion of fill, voluntary intake would be increased by supplementation because a greater amount of digesta fill would be processed at the same rate as in control ewes. Elevated gastrointestinal fill was not observed by McCollum and Galyean (1985b) in steers receiving a CSM supplement with low-quality prairie hay. As previously stated, these investigators reported an increase in PPR and decreased total mean retention time in CSM-supplemented compared with control steers. These findings emphasize that various mechanisms can be involved in the control of voluntary intake by ruminants fed low-quality forages, and suggest the need for further research on the nature of these mechanisms.

Ruminal fluid volume (table 3) was not influenced by supplementation (P>.15). Ruminal fluid dilution rate, however, tended to be increased (P<.14) from 9.4%/h in control ewes to 11.3%/h in supplemented ewes. Ruminal outflow rate (liters/h) also tended to be increased (P<.11) by supplementation. Estell et al. (1985) found that protein supplementation of a mixed-hay diet did not influence fluid dilution rate measurements, but protein concentration of hay in that study was double the level used in the present study.

TABLE 3. RUMINAL AND CECAL FLUID VOLUME AND DILUTION RATE OF EWES FED PRAIRIE HAY WITH AND WITHOUT COTTONSEED MEAL

Item	Treatment		SE[a]	Significance level[b]
	Control	Cottonseed meal		
Ruminal fluid				
Volume, liters	4.5	4.1	.3	NS[c]
Dilution rate, %/h	9.4	11.3	.7	P<.14
Outflow rate, liters/h	.17	.18	.01	P<.11
Cecal fluid				
Volume, liters	.5	.6	.1	NS
Dilution rate, %/h	18.1	16.3	2.2	NS
Outflow rate, liters/h	.09	.10	.02	NS

[a]Standard error of treatment means based on six observations/mean.

[b]Observed significance level of row means.

[c]NS = nonsignificant (P>.15).

Cecal fluid volume (table 3) was not influenced by supplementation (P>.15), with control and supplemented ewes averaging .5 and .6 liters, respectively. These values are similar to those previously reported for sheep (Hecker, 1971b; Grovum and Hecker, 1973; Hoover, 1978) and represented 11 and 15% of the rumen volume for control and supplemented ewes, respectively. Cecal dilution rate did not differ (P>.15) between control (18.1%/h) and supplemented ewes (16.3%/h). In contrast, Caton et al. (1985) reported an increased cecal dilution rate in wethers receiving CSM in addition to prairie hay (control: 8.6%/h; supplemented: 11.0%/h). Cecal fluid outflow rate was not influenced by supplementation (P>.15).

Ruminal pH did not differ (P>.10) between supplemented and control ewes (6.1 and 6.2 averaged over all sampling times, respectively; table 4). No differences in ruminal pH have been observed in cattle receiving protein supplements on low-quality forages (Topps et al., 1965; Wagner et al., 1983; McCollum and Galyean, 1985b). Ruminal pH below 6.7 may provide a less favorable environment for cellulolytic bacteria, resulting in a potential reduction in fiber digestion (Mertens, 1977). Cecal pH was not influenced (P>.15) by supplementation (table 4). Similarly, Caton et al. (1985) found that CSM supplementation of poor-quality prairie hay had no influence on cecal pH. Hoover (1978) stated cecal contents commonly have a pH of 7.0 or greater.

Mean ruminal ammonia concentrations (table 4) did not differ (P>.15), but were numerically higher in supplemented (3.8 mg/100 ml) vs control (2.9 mg/100 ml) ewes. Highest ruminal ammonia concentrations were observed 1 h after supplementation (0700) in both treatment groups, and remained fairly stable thereafter (figure 1). Increased ruminal ammonia concentration has been reported with protein supplementation of low-quality forages in cattle (Wagner et al., 1983; McCollum and Galyean, 1985b). In contrast, Judkins et al. (1987) observed no consistent ruminal ammonia response in cattle to protein supplementation on dormant blue grama rangeland. Ammonia concentrations in both control and supplemented ewes were low (<5 mg/100 ml), and may have limited efficiency of microbial protein synthesis (Satter and Slyter, 1974). Based on the low available N (total N − ADIN) level of this hay (5.0%), however, low ruminal ammonia concentrations would be expected.

As with ruminal ammonia, cecal ammonia concentration (table 4) was not influenced (P>.15) by supplementation. Highest cecal ammonia concentrations were noted at 12 and 18 h after supplementation in supplemented and control ewes, respectively (figure 1). Caton et al. (1985) reported that CSM supplementation (106 $g \cdot head^{-1} \cdot d^{-1}$) increased cecal ammonia concentrations in wethers fed prairie hay (7.4% crude protein). These researchers also reported higher ammonia concentrations for control (22.0 mg/100 ml) and supplemented (23.7 mg/100 ml) animals than observed in the present study. Studies compiled by Hoover (1978) clearly implicated the large intestine as a major source of recycled N in ruminants. Cecal proteolytic activity is high, and consequently, ammonia concentrations are frequently higher than in the rumen (Hecker, 1971a), as was the case in the present study. There is a lack of agreement concerning the source of N entering the cecal ammonia pool, but it is generally

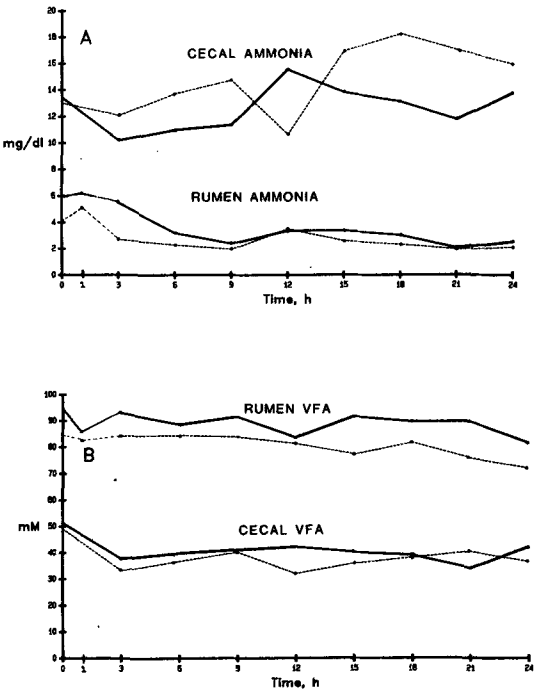

Figure 1. Time course changes after supplementation in A) cecal (upper two lines) and ruminal (lower two lines) ammonia concentrations and B) ruminal (upper two lines) and cecal (lower two lines) total VFA concentration in control (−−·−−·−−) and cottonseed meal-supplemented (−·−·−) ewes fed prairie hay.

TABLE 4. RUMINAL AND CECAL pH, AMMONIA AND VOLATILE FATTY ACIDS IN EWES FED PRAIRIE HAY WITH AND WITHOUT COTTONSEED MEAL

	Rumen				Cecum			
Item	Control	Cottonseed meal	SE[a]	Significance level[b]	Control	Cottonseed meal	SE	Significance level
pH	6.2	6.1	.06	P<.15	7.0	7.1	.03	NS[d]
Ammonia, mg/100 ml	2.9	3.8	.7	NS	14.7	12.7	1.4	NS
TVFA[c], mM	80.4	88.6	3.0	P<.13	38.0	40.9	1.3	NS
	mol/100 mol				mol/100 mol			
Acetate	76.2	75.6	.4	NS	73.0	74.1	.8	NS
Propionate	14.7	15.0	.3	NS	19.0	18.9	.3	NS
Butyrate	7.9	8.1	.4	NS	4.0	3.8	.2	NS
Isobutyrate	.5	.4	.04	NS	1.4	1.1	.1	NS
Valerate	.3	.4	.03	P<.12	1.2	1.1	.1	NS
Isovalerate	.5	.5	.1	NS	1.3	1.0	.2	NS

[a]Standard error of treatment means based on 60 observations/mean for rumen and 54 observations/mean for cecal data.

[b]Observed significance level of row means.

[c]TVFA = total volatile fatty acids.

[d]NS = nonsignificant (P>.15).

agreed that ammonia is the primary nitrogenous compound absorbed from the hindgut (Hoover, 1978). Ammonia is passively absorbed into the bloodstream and such absorption is facilitated by pH >7.0 (Chalmers et al., 1976). Estimates of cecal N contribution to total BUN range from 13 to 40% (Hecker, 1971b; Nolan et al., 1976).

Total VFA concentration in the rumen averaged over all sampling times tended (P<.13) to be increased from 80.4 to 88.6 mM with CSM supplementation. In contrast, McCollum and Galyean (1985b) and Judkins et al. (1987) found no difference in total VFA concentration when steers consuming poor-quality roughage diets were given protein supplements. Time course changes in total VFA concentration after supplementation are depicted in figure 1.

Cottonseed meal supplementation did not (P>.15) alter the molar proportions of any of the individual VFA. Wagner et al. (1983), working with cattle grazing dormant rangeland, reported no differences in molar proportions of VFA as a result of protein supplementation. Supplemented ewes had similar molar proportions of acetate, propionate and butyrate to control ewes. In contrast, McCollum and Galyean (1985b), working with CSM-supplemented steers fed prairie hay, reported a shift in molar proportion of ruminal VFA from acetate to propionate. Similar shifts have been noted by Judkins et al. (1987) with protein-supplemented steers grazing dormant blue grama rangeland.

Total cecal VFA concentration did not differ (P>.15) in supplemented (40.9 mM) compared with control ewes (38.0 mM). Concentrations of cecal total VFA throughout the 24-h sampling period (figure 1) remained relatively constant over time, which is consistent with patterns observed in sheep (Williams, 1964; Faichney, 1968; Caton et al., 1985). Caton et al. (1985) reported CSM supplementation increased total cecal VFA concentration at 4 h after supplementation in supplemented wethers (67.7 mM) when compared with control wethers (63.1 mM) fed only prairie hay.

As in the rumen, supplementation had no influence (P>.15) on molar proportions of individual cecal VFA. Supplemented ewes had similar molar proportions of acetate, propionate and butyrate to control ewes (table 4). Molar proportions of isobutyrate, valerate and isovalerate also were similar between control and supplemented ewes (table 4).

Ewes receiving CSM had higher (P<.05) serum insulin concentrations (table 5) than control ewes. These results agree with those of Johnson et al. (1981), who reported increased serum insulin as a result of abomasal casein infusion in steers fed prairie hay. Insulin concentration remained relatively constant over time (figure 2). The higher insulin concentration corresponded with a nonsignificant decrease (P>.15) in blood glucose concentrations for supplemented ewes (75.3 mg/dl) compared with control ewes (80.0 mg/dl; table 5). Decreased blood glucose concentrations in response to elevated insulin concentrations have been previously observed in sheep receiving exoge-

TABLE 5. SERUM INSULIN, GROWTH HORMONE, GLUCOSE, UREA NITROGEN AND FREE FATTY ACID LEVELS IN EWES FED PRAIRIE HAY WITH AND WITHOUT COTTONSEED MEAL

Item	Treatment		SE[a]	Significance level[b]
	Control	Cottonseed meal		
Insulin, ng/ml	.3	.4	.02	P<.05
Growth hormone, ng/ml	3.0	2.5	.15	P<.07
Insulin:growth hormone ratio	.13	.21	.03	P<.08
Serum glucose, mg/dl	80.0	75.3	2.5	NS[c]
Serum urea N, mg/dl	10.3	11.1	.8	NS
Free fatty acids, mM	.34	.19	.04	P<.06

[a]Standard error of treatment means based on 60 observations/mean.

[b]Observed significance level of row means.

[c]NS = nonsignificant (P>.15).

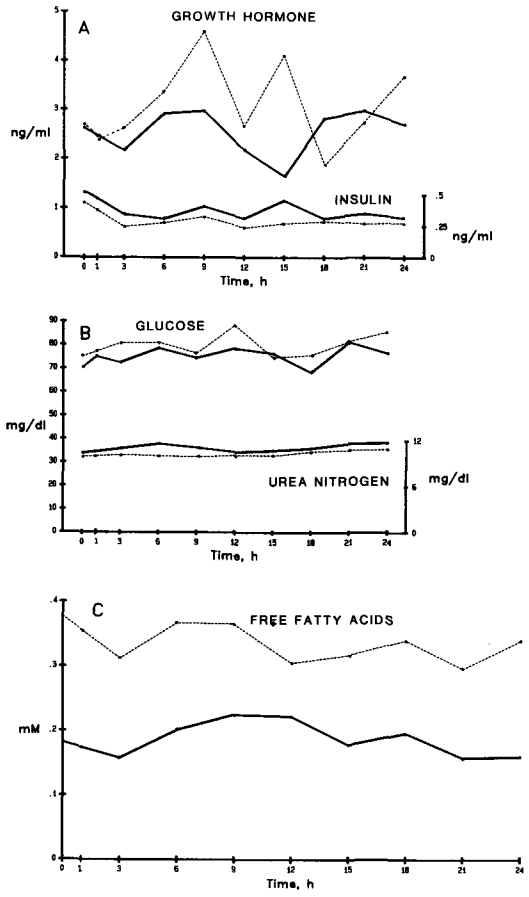

Figure 2. Time course changes after supplementation in A) serum growth hormone (upper two lines) and serum insulin (lower two lines); B) serum glucose (upper two lines) and serum urea N (lower two lines) and C) serum free fatty acids in control (– – • – – • – –) and cottonseed meal-supplemented (– • – • –) ewes fed prairie hay.

nous injections of insulin (Baile and Martin, 1971). Blood glucose concentration fluctuated, but followed a pattern similar to the reverse of insulin concentration (figure 2).

Control ewes exhibited higher ($P<.07$) GH concentrations and lower ($P<.08$) insulin:GH ratios than supplemented ewes. Bassett (1974) reported average GH concentrations of 2.0 to 2.5 ng/ml in Border Leicester and Merino wethers receiving 800 g of leucence chaff:oat grain (1:1), which agrees with the GH concentrations observed in the present study. The higher concentration of GH in control ewes may indicate an attempt to mobilize body energy stores and liberate free fatty acids into the plasma. A high concentration of serum GH in animals on a low-quality diet is in line with the findings of de Boer et al. (1985) that GH concentration increased in feed-restricted (34% of ad libitum intake) dairy cows compared with ad libitum-fed cows at various times pre- and postpartum. Another possible explanation for differences in GH concentrations is that supplemented ewes were able to incorporate GH into the cells more rapidly. Supplemented ewes may have had an increased number of GH receptors, allowing for increased incorporation of GH into the cell and lower serum concentrations. Serum free fatty acids were higher ($P<.06$) in control ewes when compared with supplemented ewes (table 5, figure 2), as would be expected with higher GH concentrations in control vs supplemented ewes. Blood urea N concentration did not differ ($P>.15$) between treatments (table 5) and remained relatively constant over time (figure 2), which agrees with ruminal and cecal ammonia data.

In conclusion, these data are interpreted to show that CSM supplementation increased prairie hay intake in mature ewes by expanding gastrointestinal fill rather than increasing particulate passage rate. Supplementation altered ruminal fluid dynamics but had minimal effects on cecal fluid dynamics. Ruminal and cecal fermentation measurements were generally not altered by CSM supplementation. Cottonseed meal supplementation altered serum insulin and GH concentrations. The extent to which the changes observed in response to supplemental CSM in this study, especially altered insulin and GH concentrations, result from consumption of protein alone or to the associated increase in dry matter intake is not clear. Further study is needed to separate the effects of protein per se from increased voluntary intake that occurs as a result of protein supplementation.

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