Bypass supplementation of grazing pregnant beef cows

by Jess Lee Miner

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
The objective was to determine if supplementing additional rumen-bypass protein vs only an oil-seed meal could reduce prepart urn weight loss. Other objectives were to determine the effects of supplement (S) on forage digestibility, ad libitum intake and blood metabolite concentrations. During two winters (trials 1 and 2) approximately 60 prepartum beef cows were grazed on native foothills range. Cows were randomly allotted to five S groups and supplemented on alternate days in early afternoon with either (g/d): none (control) 570 soybean meal (SOY); 450 soybean meal and 230 blood meal (SOY+BM); 140 soybean meal, 16 urea and 450 corn gluten meal (SOY+COM) or 570 soybean meal and 210 animal fat (SOY+FAT). Palpable condition scores and body weights were determined at trial initiation (mid-December) and ending (early March). Each month (sampling period) neutral detergent fiber (NDF) fermentation rate of grazed forage was measured via nylon bags. Cobalt EDTA and Cr mordant were used to measure ruminal fluid and particulate mass and dilution rate. At the intervals nylon bags were removed, blood samples were obtained and ruminal ammonia and pH were measured with a meter. Serum was analyzed for concentration of glucose, albumin, total protein, urea nitrogen, total bilirubin, creatinine, cholesterol and amino acids. Cows in control gained the least (P<.01) body weight in both trials 1 (-1.9 kg) and 2 (-46.4 kg). Additional bypass protein increased (P=.06) weight gain of SOY+BM (-1.8 kg) and SOY+CGM (-15.0 kg) compared to SOY (-20.1 kg) in trial 2. Except for cholesterol blood metabolites were not affected by S but the interaction with period was often significant. For example, during cold temperatures and snow cover bilirubin was elevated most in control and least in SOY+BM. Fermentation rate was increased (P<.01) by supplementation in trial 1. It was higher (P=.07) for SOY+BM (2.6%/h) and S0Y+.CGM (2.8%/h) than for SOY (2.3%/h). Fermentation rate was not influenced by S in trial 2 but the same trends were observed. Ruminal pH was lower (P=.03) for SOY than control in trial 1 and lower for SOY+BM and SOY+OGM than for SOY in trials 1 (P=.01) and 2 (P=.09). Ruminal ammonia was lowest (P=.03) in control but not different between other S groups. Fluid dilution rate was lower and volume higher for SOY+BM and SOY+CGM than SOY in both trials 1 (P<.06) and 2 (P<.14). Particulate dilution rate followed the same trend (P=.03). Bypass protein additions to rumert-degradable protein supplement can reduce prepartum weight loss of grazing cows. In addition, bypass protein can enhance NDF fermentation and increase ruminal retention time. Cows supplemented with SOY plus bypass protein were least affected by changes in environment as indicated by blood metabolites.

BYPASS SUPPLEMENTATION OF GRAZING PREGNANT BEEF COWS

by

Jess Lee Miner

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The objective was to determine if supplementing additional rumen-bypass protein vs only an oil-seed meal could reduce prepartum weight loss. Other objectives were to determine the effects of supplement (S) on forage digestibility, ad libitum intake and blood metabolite concentrations. During two winters (trials 1 and 2) approximately 60 prepartum beef cows were grazed on native foothills range. Cows were randomly allotted to five S groups and supplemented on alternate days in early afternoon with either (g/d): none (control) 570 soybean meal (SOY); 450 soybean meal and 230 blood meal (SOY+BM); 140 soybean meal, 16 urea and 450 corn gluten meal (SOY+CGM) or 570 soybean meal and 210 animal fat (SOY+FAT). Palpable condition scores and body weights were determined at trial initiation (mid-December) and ending (early March). Each month (sampling period) neutral detergent fiber (NDF) fermentation rate of grazed forage was measured via nylon bags. Cobalt EDTA and Cr mordant were used to measure ruminal fluid and particulate mass and dilution rate. At the intervals nylon bags were removed, blood samples were obtained and ruminal ammonia and pH were measured with a meter. Serum was analyzed for concentration of glucose, albumin, total protein, urea nitrogen, total bilirubin, creatinine, cholesterol and amino acids. Cows in control gained the least ($P<.01$) body weight in both trials 1 (-1.9 kg) and 2 (-46.4 kg). Additional bypass protein increased ($P=.06$) weight gain of SOY+BM (-1.8 kg) and SOY+CGM (-15.0 kg) compared to SOY (-20.1 kg) in trial 2. Except for cholesterol blood metabolites were not affected by S but the interaction with period was often significant. For example, during cold temperatures and snow cover bilirubin was elevated most in control and least in SOY+BM. Fermentation rate was increased ($P<.01$) by supplementation in trial 1. It was higher ($P=.07$) for SOY+BM (2.6%/h) and SOY+CGM (2.8%/h) than for SOY (2.3%/h). Fermentation rate was not influenced by S in trial 2 but the same trends were observed. Ruminal pH was lower ($P=.03$) for SOY than control in trial 1 and lower for SOY+BM and SOY+CGM than for SOY in trials 1 ($P=.01$) and 2 ($P=.09$). Ruminal ammonia was lowest ($P=.03$) in control but not different between other S groups. Fluid dilution rate was lower and volume higher for SOY+BM and SOY+CGM than SOY in both trials 1 ($P<.06$) and 2 ($P<.14$). Particulate dilution rate followed the same trend ($P=.03$). Bypass protein additions to rumen-degradable protein supplement can reduce prepartum weight loss of grazing cows. In addition, bypass protein can enhance NDF fermentation and increase ruminal retention time. Cows supplemented with SOY plus bypass protein were least affected by changes in environment as indicated by blood metabolites.

INTRODUCTION

The nutrient concentration (digestible energy, crude protein, vitamin and mineral content) of forage normally declines with maturity. In Montana during the winter months dormant forage alone usually does not provide adequate nutrition to maintain body weight in gestating beef cows. Forage can be harvested during times of high quality and stored for winter feed. However, at times standing forage is physically or economically prohibitive to harvest. Since standing forage is often exposed for grazing, it is usually less expensive to harvest by grazing and supplement those nutrients deficient in the forage.

To date, the primary objective of supplementing winter grazing has been to feed nutrients deficient in the forage. However, supplement has also been shown to affect forage intake (Siebert and Hunter, 1981) and grazing behavior (Adams, 1985).

Responses to protein supplementation are common. However, it is not known whether the effect occurs due to enhanced microbial nutrition in the rumen or due to increased supply of amino acids presented to the small intestine. Providing additional protein which is primarily digested in the small intestine (rumen-bypass) has increased growth rate of calves (Klopfenstein et al., 1978). The value of bypass protein for gestating beef cows has not been

established. The objective of this study was to determine if supplementing additional rumen-bypass protein vs only an oil-seed meal could reduce prepartum weight and condition loss and thus have a probable impact on postpartum interval. Other objectives were to determine the effect of rumen-degradable protein with or without additional bypass protein on factors related to digestibility, ad libitum intake and blood metabolite concentrations.

LITERATURE REVIEW

Effect of Prepartum Nutrition on Reproduction

The most important factor reducing net calf crop is failure of cows to become pregnant (Dziuk and Bellows, 1983). The time between parturition and first estrus, (postpartum interval) is highly correlated with preparatum energy intake (Wiltbank et al., 1962; Dunn et al., 1969; Clanton and Zimmerman, 1970; Bellows and Short, 1978; Bellows et al., 1982). In contrast, Phillips and Vavra (1981) did not find an effect of prepartum energy consumption on postpartum interval. However, they fed gestating cows at 120, 100 and 80% of NRC (1976) energy recommendations, whereas the aforementioned investigators limited energy intake to 50-60% of NRC (1976) recommendations. Thus, it seems that once the energy requirement is met, increasing energy consumption does not shorten postpartum interval. Postpartum interval has also been shortened by increasing postpartum energy consumption, but this effect seems less important than the prepartum energy effect (Wiltbank et al., 1962; Dunn et al., 1969). In fact, Bellows and Short (1978) found lengthened postpartum intervals in cows fed at a high energy level vs low energy level postpartum when prepartum energy consumption was deficient. They hypothesized that the high

postpartum energy consumption stimulated milk production which in turn increased postpartum interval.

Bellows and Short (1978) found that shortened postpartum interval was related to decreases in precalving condition score loss and to a lesser extent related to decreases in precalving body weight loss. Clanton and Zimmerman (1970) found that in situations where protein intake was low, a higher energy intake did not increase weight gain in prepartum heifers. They also found no effect from increased protein intake when energy consumption was low and concluded that the first limiting nutrient should be supplemented first.

Need for Supplement

Cordova et al. (1978) and Allison (1985) state that consumption of available protein and energy by prepartum cows grazing winter range is usually below NRC (1976) recommendations by such magnitude that subsequent reproduction is impaired. Although energy- and(or) protein-dense supplements are readily consumed and can preclude lengthened postpartum interval, their cost compared to that of the grazed forage reduces the attractiveness of supplementation.

Supplement Effect on Intake

In addition to increasing specific nutrient consumption, supplements have been shown to affect intake of forage as reviewed in Table 1 and by Allison (1985) and Siebert and Hunter (1981) and to affect grazing activity (Adams, 1985; Adams et al., 1986). It is generally accepted that high energy supplements derived from grain sources depress voluntary forage intake while supplements rich in natural protein enhance forage intake and digestibility. Causes for the effect of supplemental energy on intake and digestibility are not clear and since these are not directly involved with my objectives will not be discussed. The effect of protein supplements on intake and digestibility probably involves increased rumen microbial activity (Allison, 1985) or decreased retention time (Siebert and Hunter, 1981). Exceptions to this trend can be expected when forage crude protein content is above 8 to 10% (Mison, 1985).

Although Table 1 can be summarized in a variety of ways, it appears that energy supplements depressed forage intake in over half the cases, natural protein supplements of high ruminal degradability increased intake in 11 of 17 cases, urea enhanced intake in 4 of 4 cases, and bypass protein increased intake in all 10 cases reviewed.

Table 1. Forage Intake as Influenced by Protein or Energy Supplementation.

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Branine and Galyean, 1985/ New Mexico	307 kg steers	YB/IVOMD	1000 daily	summer blue grama OM basis: 13.0% CP 38.5% ADIN 80.6% NDF 53.7% ADF 13.8% ADL 14.6% Ash	None	1.63% BW (OM)
					.5 kg corn	2.53% BW (OM)
					1.0 kg corn	1.35% BW (OM)
Branine et al., 1985/ New Mexico	332 kg steers	direct		prairie hay 7.5% CP 12.9% ADIN 64.3% NDF 40.9% ADF 5.3% ADL 9.3% Ash	None	2.33% BW
					.72 kg cottonseed meal	2.27% BW
Kartchner, 1981/ Montana	mature beef cows	Cr_2O_3/ ADL	alternate days	winter range 6.0% CP 57.2% IVDMD 47.9% ADF 5.3% ADL	None	84.1 g DM/ kg BW·75
					.75 kg cottonseed meal	80.0 g DM/ kg BW·75
					1.70 kg barley	78.5 g DM/ kg BW·75
	mature beef cows		three times/wk	winter range 8.1% CP 49.3% IVDMD 51.1% ADF 9.4% ADL	None	66.2 g DM/ kg BW·75
					.71 kg soybean meal	76.8 g DM/ kg BW·75
					.66 kg barley	63.6 g DM/ kg BW·75

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Adams et al., 1986/ Montana	3-and 6-year-old beef cows	Cr_2O_3 ADL		winter range 52.3% DMD (ADL) 48.7% DMD	None	2.0% BW 1.8% BW
Adams, 1985/ Montana	291 kg steers	IVDMD		Russian wild ryegrass	None	3.1% BW
			0730 daily	6.6% CP 62.5% IVDMD	.3 kg corn	2.6% BW
			1330 daily	47.8% ADF 5.1% ADL	.3 kg corn	2.9% BW
Turner et al., 1983/ Montana	3- and 4-year-old beef cows	Cr_2O_3/ IVOMD	three times/wk	winter range	None	1.1% BW
					.91 kg (15% CP)	1.2% BW
					.91 kg (30% CP)	1.4% BW
					1.81 kg (15% CP)	1.7% BW
					None	1.2% BW
					.91 kg (15% CP)	1.3% BW
					.91 kg (30% CP)	1.2% BW
					1.81 kg (15% CP)	1.3% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Gill and England, 1984/ England	119 kg Friesian steers	direct	supplement mixed with forage	ryegrass silage 10.3% CP 39.0% Cellulose 6.0% ash	None	1.9% BW (DM)
					50 g fishmeal/ kg silage DM	2.0% BW (DM)
					63 g ground-nut meal/kg DM	2.0% BW (DM)
Hovell et al., 1986/ England	40 kg wether lambs	direct		chopped hay 5.2% CP 59.0% IVOMD 66.0% NDF 40.0% ADF 5.9% ADL 7.0% ash	all hays equalized to 11.6% CP with urea additions	71 g DM/ kg $BW^{\cdot 75}$
				chopped hay 9.6% CP 49.0% IVOMD 74.0% NDF 41.0% ADF 7.6% ADL 6.0% ash		62 g DM/ kg $BW^{\cdot 75}$
				chopped hay 9.3% CP 39.0% IVOMD 76.0% NDF 45.0% ADF 8.0% ADL 7.0% ash		52 g DM/ kg $BW^{\cdot 75}$

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
				chopped hay 8.6% CP 28.0% IVOMD 75.0% NDF 46.0% ADF 9.0% ADL 10.0% ash		45 g DM/ kg $BW^{.75}$
Rittenhouse et al., 1970/ Nebraska	295-620 kg cattle	Cr_2O_3/ ADL	daily	sandhills winter range 5.3% CP 42.0% DMD	soybean meal/ corn/corn starch mixes to provide daily:	

MCal DE/ kg $BW^{.75}$	g CP/ kg $BW^{.75}$	g/kg $BW^{.75}$
None	None	51
.020	1.16	46
.041	1.16	49
.061	1.16	44
.081	1.16	37
.020	2.07	47
.041	2.07	48
.061	2.07	40
.081	2.07	50
.020	3.00	50
.041	3.00	52
.061	3.00	44
.081	3.00	45

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day		Daily Forage Intake
Mullins et al., 1983/ Australia	215 kg steers	direct	daily	prairie hay 2.5% CP	None		1.0% BW
					188 g CP from urea		1.6% BW
					188 g CP from urea/231 g CP from HCHO-cotton-seed meal		1.9% BW
					431 g CP from urea & 231 g CP from HCHO-cotton-seed meal & 400 g maize		1.7% BW
Hennessy et al., 1983/ Australia	142 kg Hereford steers	direct	twice daily	pasture hay 3.9% CP 41.0% IVOMD 8.0% Ash	cottonseed, meat & fish meal; pelleted kg/d	sorghum grain kg/d	
					None	None	2.0% BW
					.6	None	2.6% BW
					1.2	None	2.9% BW
					None	.56	1.4% BW
					None	1.12	1.6% BW
					.6	.56	2.6% BW
					.6	1.12	2.5% BW
					1.2	.56	2.2% BW
					1.2	1.12	2.2% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Sriskandarajah and Kellaway, 1982/ Australia	280 kg Friesian heifers	direct	daily	ground wheat straw & 15 g urea-N/kg straw 45.0% DOM	None	1.6% BW
					.5 kg cotton-seed meal	1.7% BW
				ground alkali treated wheat straw & 15 g urea-N/kg straw 63.0% DOM	None	2.4% BW
					.5 kg cotton-seed meal	2.4% BW
Lee et al., 1985/ Australia	mature 397 kg beef cows	direct	twice daily	chopped native hay 17.5% CP 8.1 MJ ME/kg	cottonseed, meat & fish meal, 43% CP ($g/kg\ BW^{.75}$):	
					None	1.2% BW (OM)
					5.25	1.4% BW (OM)
					10.50	1.8% BW (OM)
					15.75	2.1% BW (OM)
					21.00	2.0% BW (OM)
Smith and Warren, 1986a/ Australia	32 kg lambs	direct	morning daily	annual pasture hay 7% CP 43% IVDMD 40% CF	None	1.2% BW
					27 g CP from:	
					rolled lupins	1.7% BW
					pelleted lupins	1.9% BW
					soybean meal	1.8% BW
					pelleted cotton-seed meal	2.1% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Smith and Warren, 1986b/ Australia	32 kg lambs	direct	morning daily	oat stubble hay 4.1% CP 40.0% IVDMD 36.0% CF	None	1.2% BW
					2.0 MJ ME/ lamb from:	
					oats	1.5% BW
					oats & urea	1.5% BW
					oats & IBDU	1.6% BW
					pelleted cotton-seed meal	1.7% BW
					whole lupins	1.7% BW
Fishwick et al., 1978/ Scotland	425 kg beef cows	direct	0730 daily	oat straw 2.2% CP 45.3% CF 4.7% ash	2.0 kg barley & 60 g urea	1.5% BW
					2.0 kg barley & 192 g IBDU	1.5% BW
					2.0 kg barley	1.5% BW
			four times daily		60 g urea added to straw	1.6% BW
Hennessy and Murison, 1982/ Australia	350 kg steers	direct	0900 daily and 1600 daily	pasture hay 4.6% CP 43.5% IVOMD	None	-
					cottonseed meal & molasses	11-19% increase

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Forero et al., 1980/ Oklahoma	lactating 428 kg beef cows	CR_2O_3/ acid insoluble ash	morning daily	warm season pasture hay 34.8% DMD	1.22 kg of 15% CP (natural)	1.6% BW
					1.22 kg of 40% CP (natural)	2.2% BW
					1.22 kg of 40% CP (coated urea)	2.0% BW
					1.22 kg of 40% CP (urea)	2.0% BW
					2.44 kg of 20% CP (urea)	1.9% BW
Kempton and Leng, 1979/ Australia	26.3 kg lambs	direct		70% oat hulls, 30% solka floc & 25 g urea/ kg DM 3.1% CP	None	1.9% BW
					g/kg DM:	
					25 urea	2.4% BW
					75 HCHO-casein	2.5% BW
					75 casein	2.5% BW
					25 urea & 75 HCHO-casein	3.1% BW
					25 urea & 75 casein	2.4% BW
	24.5 kg lambs	direct		70% oat hulls, 30% solka floc, & 25 g urea/ kg DM 3.1% CP	None	2.6% BW
					g/kg DM:	
					150 casein	2.8% BW
					100 casein & 50 HCHO-casein	3.0% BW
					50 casein & 100 HCHO-casein	3.4% BW
					150 HCHO-casein	3.2% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
Kempton and Leng, 1979/ Australia	35 kg lambs	direct		70% oat hulls & 30% solka floc 3.1% CP 59.0% OMD	None	1.7% BW (OM)
					g/kg DM:	
					25 urea	2.7% BW (OM)
					25 urea & 150 casein	2.7% BW (OM)
					25 urea & 150 HCHO-casein	3.1% BW (OM)
Orskov et al., 1973/ Great Britain	25 kg lambs	direct	0800, 1200, 1600 & 2000 daily	pelleted barley & urea 13% CP	None	3.4% BW
					by bottle:	
					17 g fish protein	4.0% BW
					34 g fish protein	3.7% BW
					51 g fish protein	4.0% BW
					10 g urea	3.4% BW
	35 kg lambs	direct	0800, 1200, 1600 & 2000 daily	pelleted barley & urea 13% CP	None	3.1% BW
					by bottle:	
					17 g fish protein	3.4% BW
					34 g fish protein	3.4% BW
					51 g fish protein	3.5% BW
					10 g urea	3.0% BW
	45 kg lambs	direct	0800, 1200, 1600 & 2000 daily	pelleted barley & urea 13% CP	None	2.8% BW
					by bottle:	
					17 g fish protein	3.1% BW
					34 g fish protein	3.5% BW
					51 g fish protein	3.1% BW
					10 g urea	2.8% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
	20 kg lambs	direct		barley & fish meal 19.8% CP	None	3.6% BW
					by bottle:	
					lactose @ 10% of intake	3.4% BW
					lactose @ 20% of intake	3.2% BW
					added to diet:	
					lactose @ 10% of intake	3.9% BW
					lactose @ 20% of intake	3.7% BW
	30 kg lambs	direct		barley & fish meal 19.8% CP	None	3.6% BW
					by bottle:	
					lactose @ 10% of intake	3.2% BW
					lactose @ 20% of intake	3.1% BW
					added to diet:	
					lactose @ 10% of intake	3.1% BW
					lactose @ 20% of intake	3.5% BW

Table 1. continued

Investigator	Animals	Methods	Time Supplement Fed	Basal Diet	Supplement Per Day	Daily Forage Intake
	40 kg lambs	direct		barley & fish meal 19.8% CP	None	3.3% BW
					by bottle:	
					lactose @ 10% of intake	2.7% BW
					lactose @ 20% of intake	2.4% BW
					added to diet:	
					lactose @ 10% of intake	3.0% BW
					lactose @ 20% of intake	2.8% BW
Stakelum, 1986/ Ireland	520 kg dairy cows	compared pasture clippings pre- & post-grazing	daily	16 kg standing forage/cow 33.5% CP 75.1% IVOMD 26.8% MADF	None	2.5% BW
					3.3 kg barley	2.3% BW
				24 kg standing forage/cow 37.7% CP 73.6% IVOMD 24.3% MADF	None	3.4% BW
					3.3 kg barley	2.8% BW

[a]Acronyms:

ADF = acid detergent fiber
ADIN = acid detergent fiber
ADL = acid detergent lignin
BW = body weight
CF = crude fiber
CP = crude protein
Cr_2O_3 = chromic oxide
DM = dry matter
DMD = dry matter digestibility
DOM = digestible organic matter
HCHO = formaldehyde treated
IBDU = di-ureido isobutane
IVDMD = in vitro dry matter digestibility
IVOMD = in vitro organic matter digestibility
MADF = modified acid detergent fiber
ME = metabolizable energy
MJ = megajoules
NDF = neutral detergent fiber
OM = organic matter
OMD = organic matter digestibility.
YB = ytterbium

Forages of higher quality (low fiber, high crude protein and digestibility) were consumed in higher amounts than those of lower quality (Table 1). Thus, it appears and is generally accepted (Allison, 1985; Siebert and Hunter, 1981) that forage digestibility has a major affect on voluntary intake.

Grazing behavior manipulation may also affect forage intake. Adams (1985) showed that a grain supplement fed to grazing steers depressed intake more when administered in the morning than when fed in the early afternoon. He attributed the response to an interference with time periods of high grazing activity.

Rumen kinetics, other than digestion, are also potentially involved with ad libitum intake regulation. Egan and Moir (1965) increased intake of oat chaff by sheep with duodenal infusions of casein more than with infusions of urea. They could not attribute the response to increased forage digestibility but did note increased rumen volume when casein was infused. A larger rumen volume coupled with a similar dilution rate would result in increased outflow rate (liters/h) and would be compatible with increased intake. Kempton and Leng (1979) also increased the voluntary intake of forage of sheep by feeding formaldehyde-treated casein. Orskov et al. (1973) obtained similar results with a high starch diet and presentation of fish-protein concentrate to the abomasum in sheep. Johnson et

al. (1982) however, did not find intake of a concentrate by steers to increase with abomasal infusions of casein. They suggested that no response occurred due to the already high level of intake and digestibility.

Assessing Efficacy of a Supplement

Conventional measures

The end objective of beef cow supplementation is to increase the amount of beef produced per unit of input. However, the evaluation of reproductive performance requires many animals (Bellows and Short, 1978) yet evaluation of the biological mechanisms of a supplement's effect requires techniques not easily applied to the number of animals studied by Bellows and Short (1978). For this reason, and the difficulty of obtaining large numbers of animals, alternative measurements are desirable which are correlated with reproductive performance. Two such measures are condition score and body weight changes of cows (Dziuk and Bellows, 1983). A major limitation of using weight or condition score is that by the time measurable changes are noted it may be too late to alter management. These measurements are also limited in explaining the mechanisms by which nutritional status is affected and in allowing for further exploration. Intake of forage is a factor affected by supplementation and can be determined along with the

associated effects of digestibility and digesta passage rates.

Blood metabolites

Amino acids. Plasma amino acid concentrations have been used to determine individual amino acid requirements (Ahmed, 1982) in steers and the status of protein metabolism in gestating ewes (Lynch, 1984; Lynch and Jackson, 1986). Young et al. (1981) and Ahmed (1982) showed with steers that infusion of a limiting essential amino acid caused no change in its plasma concentration whereas infusion at a rate assumed to be above requirement elevated the concentration of that amino acid. Ahmed (1982) also stated that increasing the supply of a limiting amino acid could lower plasma concentration of other essential amino acids since those could then be utilized to a greater extent in protein synthesis. Lynch and Jackson (1986) found increased serum concentration of the amino carriers alanine and glutamine with decreased protein content of isocaloric diets fed to gestating ewes. They also found that with low protein intake concentration of branched chain, ketogenic and essential amino acids decreased. They concluded that catabolism of muscle and subsequent gluconeogenesis occurred to a greater extent in the ewes fed low protein diets. Hennessy et al. (1981) found that the ratios of methionine:valine, glycine:valine, glycine:leucine, glycine:

branched chain amino acids and essential:nonessential amino acids were lower in protein supplemented than in control steers.

Glucose. Plasma glucose concentration in ewes was not affected by variation in energy intake (Lynch and Jackson, 1983ab) nor was it correlated with degree of body fatness in ewes (McNiven, 1984). However, plasma glucose concentration has been positively correlated with short postpartum interval in dairy cows (Rowlands et al., 1977; Thompson et al., 1982; Wilson et al., 1985), with high energy intake in ewes (McNiven, 1984), with energy balance in beef cows (Russel and Wright, 1983) and with protein and energy content of forage eaten by elk (Weber and Wolfe, 1984). Thus it seems that plasma glucose may aid in evaluating nutritional status.

Albumin, globulin and total protein. Serum albumin concentration has been positively correlated with level of protein intake of gestating ewes (Lynch and Jackson, 1983ab) and with postpartum interval, number of services per conception and conception rate in dairy cows (Rowlands et al., 1977 Wilson et al., 1985). Lynch and Jackson (1983b) speculated that albumin is lower in protein restricted situations due to decreased synthesis by the liver. Thus, albumin concentration may reflect liver function. In addition, Bull et al. (1984) found that beef heifers restricted in intake of crude protein had lower serum

albumin concentrations than animals fed a diet of higher protein content. Weber and Wolfe (1984) reported a similar effect in elk. Globulin, on the other hand, tended to become more concentrated in the situations where albumin concentration was lowered. Thus, serum total protein concentration is often influenced by the opposing variations of albumin and globulin. Thus total protein concentration and can be difficult to interpret without knowledge of either globulin or albumin levels.

Urea nitrogen. Serum urea nitrogen concentration increased with increased dietary crude protein (Preston et al., 1965). Preston et al. (1965) also state that isonitrogenous diets lower in digestible energy (DE) result in higher urea nitrogen concentration. The contribution of ammonia released from rumen microbial degradation of dietary protein to urea nitrogen would be reduced with increased DE intake since microbial protein synthesis is dependent on DE intake (Kropp et al., 1976; Petersen et al., 1985). In addition to ruminal ammonia absorption, catabolism of amino acids derived from body tissue is a major contributor to urea nitrogen. Amino acids cycled through gluconeogenesis may supply 12 to 30% (or more in pregnant ruminants) of the glucose requirement (Macrae and Lobley, 1986). Thus urea nitrogen concentration may indicate protein or energy status and more specifically, may be an indication of the adequacy of glucogenic substrates.

Bilirubin. Total bilirubin, a product of erythrocyte degradation, has been shown to increase in serum concentration in pregnant beef heifers fed either protein or energy restricted diets (Thompson et al., 1982; Bull et al., 1984) and in the urine of pregnant ewes fed protein deficient diets (Lynch and Jackson, 1983a). Urinary bilirubin content can be elevated due to hepatocellular damage (Kaneko, 1980 as cited by Lynch and Jackson, 1983a). If serum and urinary bilirubin concentrations are correlated, then serum bilirubin may indicate liver function. However, Lynch and Jackson (1983a) did not produce associated changes in serum bilirubin concentration. Lynch and Jackson (1983b), in contrast, did increase serum total bilirubin concentration by restricting protein and energy consumption and noted an increase associated with advancing gestation of ewes as did Bull et al. (1984) with gestating heifers.

Creatinine and cholesterol. Creatinine is the dephosphorylated form of phosphocreatine, a cellular form of short term energy storage. Serum creatinine concentration has been elevated by dietary restriction of protein in gestating beef heifers (Bull et al., 1984), protein and energy of gestating ewes (Lynch and Jackson, 1983b) but not with only protein restriction of gestating ewes (Lynch and Jackson, 1983a). The investigators above also noted increases associated with advancing gestation. These

studies produced similar fluctuations in serum cholesterol concentration. Weber and Wolfe (1984) also noted increased serum cholesterol with low vs high diet quality as indicated by the amount of dry vs green forage eaten.

The blood metabolites discussed above have been used clinically for diagnosis of metabolic dysfunction (Benjamin, 1978). The fluctuations in concentration caused by nutritional manipulation, although statistically significant, were within the normal range of healthy animals as reported by Benjamin (1978). The individual animal variation in comparison to mean fluctuation will probably preclude their use as single animal interpretations. While not perfectly consistent indicators of nutritional status, measurement of several different metabolites do indicate relative nutritional status between groups of animals on different nutritional regimens. Blood metabolites also provide more specific indication of metabolic inadequacy than do measures such as weight change or postpartum interval. For example, high urea nitrogen and low glucose concentrations may indicate a deficiency in glucogenic substrate.

The objective of this study was to determine if supplementing additional rumen-bypass protein vs only an oil-seed meal could reduce prepartum weight and condition loss and thus have a probable impact on postpartum interval. Other objectives were to determine the effect of rumen-

degradable protein with or without additional bypass protein on factors related to digestibility, ad libitum intake and blood metabolite concentrations.

MATERIALS AND METHODS

Animals

Two trials were conducted at the Red Bluff Research Ranch, Norris, Montana. Trial 1 was initiated December 15, 1984 and concluded March 1, 1985. Trial 2 was initiated December 15, 1985 and concluded March 6, 1986. For each trial pregnant 3- to 6- yr-old crossbred cows, (trial 1, n=55; trial 2, n=61) were randomly selected within age from the ranch herd of 150 cows, within the restriction that breeding date be in the first half of herd breeding dates. Cows used in trial 1 were bred to Angus bulls. Cows used in trial 2 were bred to the same Angus bull except that 3-yr-old cows were bred to a different Angus bull. Expected average calving dates were approximately March 15 each year. A random fraction (trial 1, n=20; trial 2, n=23) were fitted with rumen cannulas one month prior to use. Genotypes were various combinations of Angus, Hereford and(or) Tarentaise. Initial mean body weight and condition score (1=thinnest to 10=fattest; Bellows et al., 1971), for trial 1 were 491 kg and 5.4, and for trial 2 were 520 kg and 4.5 respectively. The cause of variation between trials in body weight and condition score was not apparent and was attributed to a random year effect. For 6 mo preceeding initiation of either trial cows grazed native pasture and had no access to supplement.

Pasture

During each trial, cows freely grazed a 324 ha native range pasture with long, north-facing, slight to moderate slopes and areas of steep slopes and rock outcrops. Elevation at the study site ranged from 1,400 to 1,900 m and annual precipitation was from 350 to 406 mm. A 1980 Soil Conservation Service survey described the pasture as a silty range site in good condition with vegetation composed of 65% grasses and 35% forbs and woody species. Dominant grasses included bluebunch wheatgrass (Agropyron spicatum), needleandthread (Stipa comata), Idaho fescue (Festuca idahoensis) and basin wildrye (Elymus cinereus). Estimated carrying capacity was 1.2 ha per animal unit month (USDA-SCS, 1976).

Treatments

Prior to each trial cows were randomly allotted within age to five supplement treatments (S) designated as control, SOY, SOY+BM, SOY+CGM and SOY+FAT (table 2). Control received range forage only. The SOY group and all other S groups received the equivalent of .2 kg/d of rumen degradable protein. Previous studies (Petersen and Clanton, 1986) have suggested that ruminal protein needs must be satisfied before an animal response to the protein presented to the small intestine can be anticipated. Therefore, these

supplements were formulated to meet a specific experimental criteria. The SOY supplement was expected to meet ruminal protein needs. Blood meal and corn gluten meal were added to SOY+BM and SOY+CGM to determine the response to additional protein reaching the small intestine. In order to determine if a response to the bypass protein supplements was due to a protein or energy effect, animal fat was added to the fifth supplement. Miller and Cramer (1969) showed that degradation of animal fat in the rumen is incomplete. Therefore, the SOY+FAT supplement was designed to differentiate between a bypass protein or energy effect.

Table 2. Supplement Composition[a] (kg/d).

			Crude Protein			
S	Ingredient	Amount fed	Total	Bypass	Rumen degradable	TDN
Control						
SOY	soybean meal	.57	.24	.05	.20	.43
SOY+BM	soybean meal	.45	.20	.04	.16	.34
	blood meal	.23	.20	.16	.04	.14
	total	.68	.40	.20	.20	.48
SOY+CGM	soybean meal	.14	.06	.01	.05	.10
	corn gluten meal	.45	.29	.19	.10	.37
	urea	.016	.05		.05	
	total	.61	.40	.20	.20	.47
SOY+FAT	soybean meal	.57	.24	.05	.20	.43
	animal fat	.21				.41
	total	.78	.24	.05	.20	.84

[a]Supplements in trial 1 were also balanced to provide daily 9.5 g phosphorus, 23.6 g potassium and 18,000 international units vitamin A. Supplements in both trials contained 50 g dried molasses and 4.5 g Molasses Booster (Feed Flavours, inc.).

Supplements in trial 1 also contained added sources of phosphorus, potassium and vitamin A (Table 2) whereas trial 2 supplements did not. At the initiation of trial 2 all cows were injected im with 20,000,000 IU of vitamin A and during the trial had free access to a loose iodized salt mixture containing 30% dicalcium phosphate and 30% potassium chloride. In both trials all supplements additionally provided 35 g/d of a chromic oxide premix (25% Cr_2O_3 and 75% finely ground corn) for determination of fecal output (Dunn, 1986).

With the exception of control, treatments were designed to have an equal effect on rumen function. Any response, either in rumen kinetics or otherwise, was expected to be due to an action of supplement via some postruminal avenue. It was not possible to formulate SOY isonitrogenous with SOY+BM and SOY+CGM while maintaining equivalant amounts of rumen degradable natural protein. It was decided that supplements being nonisonitrogenous would not confound interpretation of results if ruminal ammonia concentrations were similar. Thus SOY was not isonitrogenous with SOY+BM or SOY+CGM.

On alternate days all cows were gathered with the aid of horses and supplemented individually at approximately 1300. The control cows were handled in the same manner as others except were not offered supplement. Some animals were reluctant to consume their prescribed supplement.

Thus, if after six daily attempts to entice consumption by top dressing the supplement with other ingredients a cow still refused supplement, she was moved to another treatment and replaced by a cow orginally allotted to another treatment. Approximately four such switches were made in each trial. In addition, one cow in SOY+CGM received her supplement via rumen cannula. It was assumed that these problems would have no effect on the results of either trial. Within 14 d of the onset of each trial, all cows were consuming supplements.

Measurements

Ruminal flow kinetics

During three sampling periods (1=9; 2=4; and 3=1 wk precalving) in each trial, ruminal samples were obtained from the reticulum for ruminal pH, fluid and particulate volume and dilution rate measurements.

Cobalt Ethylenediaminetetraacetic acid (CoEDTA) and chromium modant were used as markers for estimating dilution rate and volume in the rumen of fluid and particulate matter, respectively. These markers were synthesized as described by Uden et al. (1980). Resulting Co and Cr concentrations were 8.3 and 3.0% in each marker, respectively. The range forage used for the chromium mordant marker was obtained from the rumen of at least six cows 2 wk before each period. At approximately 1100, a time

which coincided with grazing activity (Dunn, 1986), freshly consumed forage was removed via rumen cannula from the uppermost portion of the dorsal sac.

Doses of 100 g of chromium mordant and 20 g of CoEDTA were administered via rumen cannula. Cows were dosed at 0800 in trial 1 and 1200 in trial 2. Digesta samples (100 ml) were obtained near the reticulo-omasal orifice and mixed with 1 ml of 5% HgCl to cease microbial fermentation at 5, 10, 15, 25, 35, 48, 72, and 96 h post dosing in trial 1 and 1, 5, 10, 20, 30, 48 and 75 h post dosing in trial 2. The 96 h sampling interval was eliminated in trial 2 because markers were not detectable after 72 h in trial 1. The 15 h interval was eliminated in trial 2 due to logistical problems associated with accessing cows in the pasture. Supplementation time was the same in both trials.

Digesta samples were immediatedly frozen. After thawing, ruminal fluid was separated from particulate matter by centrifugation at 1000 x g for 15 min and filtration through paper with particle retention >10 um. Analysis for Co was via atomic absorption spectrophotometry (AA) as described by Perkin-Elmer (1971). Particulate matter was dried at 65 C and ground in a Wiley mill through a 1.0 mm screen. Duplicate 1 g particulate samples were prepared by the procedure of Williams et al. (1962) and analyzed for chromium (Perkin-Elmer, 1971).

Digesta samples obtained before initial dosing were composited and used to make Co and Cr standards. Eight samples of unknown high and low concentration were aspirated during each 3 h period of AA operation and duplicated within a range of 3%. Ruminal dilution rates and volumes were calculated with the equations in table 3. Marker concentrations in the first post dosing sample of both trials were below the concentration of later sampling times and varied considerably. Thus, inadequate mixing was assumed and these values were excluded from the analyses.

Fermentation rate

Fermentation rate of neutral detergent fiber in situ was measured via incubation in nylon bags as described by Orskov (1982). Incubated samples were composites obtained prior to each period from three esophageally fistulated cows on at least four separate days. After an overnight fast, plugs were removed from and collection harnesses fitted to fistulated cows which were allowed to graze for .5 h. Collected samples were frozen, freeze dried and ground in a Wiley mill through a 2.0 mm screen, mixed, and 3.0 g placed in each nylon bag. A sample was retained for NDF analysis (Goerning and Van Soest, 1970). Bags were constructed from nylon mesh fabric* of 44 um pore size. Finished bags were

*Nitex 44 Fabric (#HD3-44), H. R. Williams Mill Supply, Kansas City, MO 64108.

double zig-zag stitched with polyester thread and had 533 cm^2 of surface area. Bags containing no sample (blanks) were also incubated. All bags were suspended in the rumen via attachment to a 75 cm circular stringer composed of tygon tubing, bath plug chain, and fishing swivels.

In trial 1 eight bags containing sample were suspended in the rumen at 0800 and one bag was removed at each of the time intervals described for dilution rate markers. In trial 2 six bags were incubated, each being removed at the sampling times described for dilution markers in trial 2. Upon removal all bags were immediately frozen and later washed by hand in groups by time removed until rinse water in a tub was clear. All bags were then subjected to NDF analysis in groups of 12, dried and weighed. Calculations of NDF disappearance rate (Robinson, et al., 1986; Table 3) involved subtraction of incubation and analysis effect on blanks.

Ruminal pH and ammonia

In both trials ruminal ammonia concentration and pH were measured with a portable meter and ion-specific electrode (Anonymous, 1984). Ruminal fluid samples (50 ml) were obtained from near the reticulo-omasal orifice at -5, 0, 5, 10, 20, 30, 43, 67 and 91 h after supplementation on the first day of each sampling period in trial 1. In trial 2 samples were obtained at 0, 5, 10, 20, 30, 48 and 75 h

Table 3. Equations Used to Calcuclate Ruminal Neutral Detergent Fiber (NDF) Fermentation Rate, Fluid and Particulate Volume and Dilution Rate and NDF Digestibility (Trials 1 and 2).

A	initial nylon bag weight
B	forage sample weight
C	percent NDF of sample
D	nylon bag + sample weight after incubation and NDF analysis
E	Correction for loss of weight in blank bags
F	1-[(A+BC-D-E)/BC] = residual NDF
F=G-HI+J	
G	fermentable NDF
H	rate of loss of fermentable NDF
I	duration of incubation
J	unfermentable NDF
K=-LM+N	
K	nlog of marker concentration
L	dilution rate
M	time span between dosing and sampling
N	nlog of marker concentration at time of dosing
O	rumen volume
$O = P/e^N$	
P	quantity of marker in initial dose

post supplementation. Supplementation time was the same in both trials. Ammonia concentration was not determined in period 1 of trial 1 since the meter had not yet been acquired and was not determined in trial 2 due to instrument malfunction. Ruminal pH was not measured in period 1 of either trial for the same reasons.

Cow weights, condition scores and calf birthweights

Palpable condition score (1 = thinnest to 10 = fattest; Bellows et al., 1971) by two technicians and two consecutive day body weights after 12 h of feed and water deprivation were recorded at the start and finish of each trial. Calf sex, birth dates and weights were also recorded.

Blood metabolites

During three periods (1=9, 2=4 and 3=1 wk precalving) in each trial blood samples were obtained from either the jugular vein (trial 1) or from an artery or vein near the base of the tail (trial 2). Although most animals were sampled easily from the jugular vein in trial 1, it was thought that any effect of stress would be minimized with the tail bleeding technique since animals would not be restrained in a squeeze chute. In trial 1 nine blood samples were obtained from each of four rumen cannulated cows per treatment during each period. In trial 1 samples were obtained -5, 0, 5, 10, 20, 30, 43, 67 and 91 h post

supplementation. In trial 2 all cows (N=61) were sampled 0 and 48 h post supplementation. In addition, four rumen cannulated cows per treatment were bled at 5, 10, 20, 30, and 75 h after supplementation. In both trials two 10 ml samples were obtained; one in a non treated plastic syringe (trial 1) or vacutainer (trial 2; Becton-Dickerson, Rutherford, NJ) and one in a syringe (trial 1) or vacutainer (trial 2) containing 2 mg/ml of sodium fluoride and an anticoagulant. Samples in containers with anticoagulant were immediately centrifuged at 500 x g for 30 minutes. Plasma was decanted into two 12 x 75 mm polystyrene (trial 1) or polypropylene test tubes and immediately frozen. Other samples were allowed to coagulate for 4 h then centrifuged and serum decanted and frozen. Plasma was analyzed for glucose (GL) concentration while serum was analyzed for concentration of urea nitrogen (UN), total bilirubin (BIL), creatinine (CRE), albumin (AL), cholesterol (CHO) and total protein (TP) (Technicon Instruments Corp., Tarrytown, NY).* No serum was obtained during period 1 of trial 1.

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*Analyses conducted by Marsh Laboratory, Montana State University, Bozeman.

Statistical Analyses

Ruminal ammonia concentration and pH data were pooled to represent a 48 h supplementation cycle. In trial 1 the 43 h sampling interval represents -5, 43 and 91 h intervals while the 20 h interval represents 20 and 67 h intervals. In trial 2 the 0 h intervals represents 0 and 48 h intervals while the 30 h interval represents 30 and 75 h intervals.

All measures were analyzed within trial via analysis of variance using the general linear models procedure of SAS (1984) as a split plot design as described by Gill and Hafs (1971). For ammonia concentration, pH and blood metabolites sampling period, sampling interval, S X period interaction and S X interval interaction were tested with the error mean square. Supplement was tested with the mean square for cow nested within S (Tables 4, 5, and 13). Measurements involving markers and NDF fermentation rate were analyzed similarly (Tables 16 and 17). Cow body weight and condition score change and calf birthweight were also analyzed via analysis of variance (Tables 10 and 11). Main effects included supplement and cow age. Regressions included initial cow body weight and condition score. Age classes included 3-, 4-, and 5- to 6-yr of age. Supplement means were separated with single degree of freedom orthogonal contrasts. Standard errors were pooled by averaging the

standard error of each subclass mean using the appropriate error term.

RESULTS

Climatic

The mean median daily temperature was lower during period 2 of trial 1 (-6.5 C) than during either period 1 (-4.2 C) or period 3 (1.1 C; NOAA, 1985). The ambient temperature was also declining with each day during period 2 whereas climatic conditions remained relatively constant in the other two periods. Snow cover in the pasture was greatest during period 2.

In trial 2 the mean median daily temperature was warmer than in trial 1 during sampling periods (period 1 = -2.3 C; period 2 = 2.1 C; period 3 = 9.2 C; NOAA, 1986). Snow cover was greater in trial 2 except during the sampling periods.

Blood Metabolites

Trial 1

Plasma glucose concentration (Table 6) was not affected (Table 4) by S but the S X period interaction was important ($P<.01$). All S groups had lower ($P<.01$) GL in period 2 than 1. However, glucose for SOY+BM in period 3 was the same as in period 1 whereas in all other S groups it remained lower.

Neither AL nor TP concentration (Table 6) were affected by S, but both were affected by the S X period interaction (Table 4). Albumin was .62 mg/dl lower for control in period 3 than 2. However, AL was similar between periods

for all other S groups. Total protein concentration for control and SOY+CGM was lower in period 3 but not lower for other S groups. Serum TP was higher in period 3 for SOY.

Urea nitrogen concentration (Table 6) was not affected by S (Table 4) but was influenced (P<.01) by the S X period interaction. Serum UN was higher in period 3 than 2 for control (10.88 vs 4.43 mg/dl), SOY+BM (9.99 vs 8.02 mg/dl) and SOY+CGM (10.99 vs 8.55 mg/dl) but only slightly higher for SOY and SOY+FAT.

Total bilirubin concentration (Table 6) was not affected (Table 4) by S but the S X period interaction was significant. In period 3 BIL was lower than in period 2 for all S groups except SOY+CGM. Total bilirubin concentration was lower (P<.03) 5 and 10 h after supplementation than immediately before supplementation (Table 8).

Creatinine concentration (Table 6) when was higher (P=.05; Table 4) for control than other S groups. No other significant main effects were detected. The S X period interaction was significant. In period 3 CRE was higher than in period 2 for all S groups except SOY+CGM.

Cholesterol concentration (Table 6) was affected (P<.01; Table 4) by S. Cholesterol concentration for SOY+BM (95.3 mg/dl) and SOY+CGM (90.2 mg/dl) was lower (P<.01) than for SOY (113.6 mg/dl) or SOY+FAT (142.3 mg/dl). The S X period interaction (P<.01) resulted because CHO was lower in period 3 for all S groups except SOY+BM.

Table 4. Least-Squares Analysis of Variance for Precalving Concentrations of Glucose (GL), Albumin (AL), Total Protein (TP), Urea Nitrogen, Total Bilirubin (BIL), Creatinine (CRE) and Cholesterol (CHO; Trial 1).

		GL		AL		TP		UN		BIL		CRE		CHO	
Item	df[a]	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Supplement (S)[b]	4	593.0	.34	1.933	.66	1.279	.96	120.30	.54	.0352	.67	1.0959	.15	24,207.6	<.01
Control vs others	1	829.4	.21	6.930	.16	.847	.76	1.26	.93	.0457	.39	2.6210	.05	7,938.6	.03
SOY vs SOY+BM, SOY+CGM	1	26.0	.46	.132	.84	.095	.92	277.19	.19	.0583	.34	.5408	.33	12,658.1	<.01
SOY+BM vs SOY+CGM	1	703.7	.25	.380	.73	.351	.84	14.06	.76	.0177	.59	.0018	.95	621.0	.51
SOY+FAT vs SOY+BM, SOY+CGM	1	816.3	.22	.000	.99	3.739	.52	358.47	.14	.0283	.50	.4916	.35	87,033.5	<.01
Cow(S)	15	487.2	<.01	3.176	<.01	8.447	<.01	147.60	<.01	.0587	<.01	.7643	<.01	1356.9	<.01
Sampling period (PER)	2	9,528.7	<.01	.745	<.01	.145	.40	496.77	<.01	.1516	<.01	1.3068	<.01	373.3	.01
Sampling interval (I)	5	161.7	<.01	.025	.95	.083	.85	18.35	.13	.0438	<.01	.0410	.08	47.8	.56
S X PER	8	145.1	<.01	1.297	<.01	2.468	<.01	66.58	<.01	.0332	<.01	.0918	<.01	961.0	<.01
S X I	20	30.1	.85	.015	1.00	.199	.50	6.05	.93	.0040	.92	.0178	.64	45.8	.77
Error	399	44.8		.109		.205		10.68		.0067		.0206		60.9	

[a]Degrees of freedom of cow(S) for AL, TP, UN, BIL, CRE and CHO was 13; degrees of freedom of error for AL was 237 and for TP, UN, BIL, CRE and CHO was 234; degrees of freedom for PER was 1 for all metabolites except GL.

[b]Mean square for cow(S) used as error term for supplement and least-squares means separated by orthogonal contrasts.

Table 5. Least-Squares Analysis of Variance for Precalving Concentrations of Glucose (GL), Albumin (AL), Total Protein (TP), Urea Nitrogen (UN), Total Bilirubin (BIL), Creatinine (CRE) and Cholesterol (CHO; Trial 2).

		GL		AL		TP		UN		BIL		CRE		CHO	
Item	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Supplement (S)[a]	4	49.5	46	.442	.88	.547	.79	162.61	<.01	.0295	.32	.0703	.93	5,850.3	<.01
Control vs others	1	169.4	.08	.603	.53	0.003	.96	491.81	<.01	.0009	.85	.0017	.94	139.2	.72
SOY vs SOY+BM, SOY+CGM	1	1.1	.89	.059	.84	1.907	.41	121.85	.02	.0490	.16	.1947	.44	638.7	.45
SOY+BM vs SOY+CGM	1	4.3	.78	.219	.70	.034	.87	.42	.89	.0803	.08	.0848	.61	1,753.5	.21
SOY+FAT vs SOY+BM, SOY+CGM	1	33.4	.43	1.016	.41	.524	.53	83.48	.05	.0100	.53	.0940	.59	21,711.8	<.01
Cow(S)	60	53.5	<.01	1.496	<.01	1.307	<.01	21.65	<.01	.0247	<.01	.3234	<.01	1,084.5	<.01
Sampling period (PER)	2	1,036.0	<.01	20.819	<.01	28.032	<.01	125.34	<.01	.5693	<.01	1.0848	<.01	6,450.9	<.01
Sampling interval (I)	4	129.4	<.01	.170	<.01	.137	.32	146.60	<.01	.0061	.79	.1260	<.01	76.8	.20
S X PER	8	21.5	.04	.019	.89	.129	.35	11.46	<.01	.0407	<.01	.0236	.12	1,569.7	<.01
S X I	16	20.6	.01	.014	.99	.066	.91	10.48	<.01	.0142	.44	.0091	.86	39.2	.72
Error	568	10.5		.042		.116		3.61		.0140		.2914		51.1	

[a]Mean square for cow(S) used as error term for supplement and least-squares means separated by orthogonal contrasts.

Table 6. Least-Squares Means for Precalving Concentrations of Glucose, Albumin, Total Protein, Urea Nitrogen, Total Bilirubin, Creatinine and Cholesterol by Sampling Period (Trial 1).

Metabolite	Sampling period	Supplement					SE[a]	SE[b]
		Control	SOY	SOY+BM	SOY+CGM	SOY+FAT		
Glucose,	1	60.9	66.8	62.4	62.2	66.3		
mg/dl	2	45.6	49.0	48.6	41.5	52.6		
	3	54.1	60.0	63.0	57.1	60.3	1.32	4.31
Albumin,	2	2.70	2.76	2.88	2.72	2.75		
g/dl	3	2.08	2.72	2.86	2.76	2.86	.065	.349
Total Protein,	2	8.09	7.53	7.69	8.16	7.40		
g/dl	3	7.64	7.99	7.83	7.60	7.59	.089	.572
Urea Nitrogen,	2	4.43	5.53	8.02	8.55	5.61		
mg/dl	3	10.88	7.09	9.99	10.99	6.81	.644	2.393
Total Bilirubin,	2	.177	.147	.081	.072	.142		
mg/dl	3	.076	.092	.042	.106	.065	.0161	.048
Creatinine,	2	1.819	1.624	1.569	1.608	1.445		
mg/dl	3	1.945	1.905	1.696	1.640	1.576	.0283	1.683
Cholesterol,	2	101.6	116.2	96.7	92.9	136.7		
mg/dl	3	91.8	111.0	94.0	87.6	147.9	1.54	7.25

[a]Pooled standard error of the least-squares means calculated using error mean square as error term.

[b]Pooled standard error of the least-squares means calcuclated using mean square for cow(S) as error term.

Table 7. Least-Squares Means for Precalving Concentrations of Glucose, Albumin, Total Protein, Urea Nitrogen, Total Bilirubin, Creatinine and Cholesterol by Sampling Period (Trial 2).

Metabolite	Sampling period	Supplement					SE[a]	SE[b]
		Control	SOY	SOY+BM	SOY+CGM	SOY+FAT		
Glucose, mg/dl	1	57.5	58.7	58.3	58.8	59.6		
	2	52.4	55.0	56.4	55.7	57.6		
	3	52.0	54.5	54.9	53.5	55.9	.71	1.60
Albumin, g/dl	1	4.20	4.30	4.31	4.20	4.55		
	2	3.61	3.78	3.79	3.66	3.94		
	3	3.67	3.78	3.79	3.69	3.97	.045	.266
Total Protein, g/dl	1	7.21	7.36	7.09	7.14	7.00		
	2	6.60	6.79	6.73	6.67	6.46		
	3	6.46	6.63	6.49	6.37	6.25	.071	.248
Urea nitrogen, mg/dl	1	6.28	9.97	11.89	11.03	10.20		
	2	6.66	10.59	13.02	12.58	9.83		
	3	8.22	11.02	13.09	13.91	10.98	.425	1.01
Total Bilirubin, mg/dl	1	.335	.384	.319	.391	.344		
	2	.353	.334	.308	.323	.304		
	3	.434	.460	.323	.446	.480	.0257	.0341
Creatinine, mg/dl	1	1.769	1.818	1.693	1.743	1.791		
	2	1.868	1.912	1.769	1.856	1.856		
	3	1.906	1.916	1.829	1.907	1.978	.0262	.1236
Cholesterol, mg/dl	1	103.2	97.0	87.9	92.8	114.2		
	2	96.6	93.0	81.6	94.2	127.3		
	3	81.0	81.3	72.1	85.6	124.5	1.55	7.16

[a]Pooled standard error of the least-squares means calculated using error mean square as error term.

[b]Pooled standard error of the least-squares means calculated using mean square for cow(S) as error term.

Table 8. Least-Squares Means for Precalving Concentrations of Glucose (GL), Albumin (AL), Total Protein (TP), Urea Nitrogen (UN), Total Bilirubin (BIL), Creatinine (CRE) and Cholesterol (CHO) by Sampling Interval (Trial 1).

Sampling Interval, hours post supplementation	GL, mg/dl	AL, g/dl	TP, g/dl	UN, mg/dl	BIL, mg/dl	CRE, mg/dl	CHO, mg/dl
0	55.2	2.72	7.82	7.1	.1169	1.649	108.7
5	54.6	2.75	7.78	7.7	.0625	1.718	108.7
10	59.0	2.72	7.74	6.9	.0725	1.698	106.8
20	57.5	2.68	7.69	8.4	.0886	1.672	108.2
30	56.1	2.69	7.71	8.5	.1219	1.707	106.1
44	57.9	2.70	7.75	8.2	.1381	1.651	107.3
SE[a]	.87	.051	.068	.50	.0127	.0222	1.21

[a]Pooled standard error of the least-squares means.

Table 9. Least-Squares Means for Precalving Concentrations of Glucose, Albumin, Total Protein, Urea Nitrogen, Total Bilirubin, Creatinine and Cholesterol by Sampling Interval (Trial 2).

Metabolite	Sampling Interval hours post-supplementation	Supplement					SE[a]	SE[b]
		Control	SOY	SOY+BM	SOY+CGM	SOY+FAT		
Glucose, mg/dl	0	53.4	53.3	56.4	53.6	54.6		
	5	54.0	55.9	56.1	57.1	57.3		
	10	55.8	60.2	57.7	56.9	60.1		
	20	53.6	54.9	55.7	56.9	59.4		
	30	53.0	56.1	56.7	55.6	57.2	.927	2.095
Albumin, g/dl	0	3.75	3.93	3.92	3.80	4.07		
	5	3.88	3.95	3.93	3.85	4.14		
	10	3.83	4.00	3.96	3.88	4.17		
	20	3.79	3.90	3.99	3.81	4.17		
	30	3.87	3.98	4.02	3.92	4.22	.059	.349
Total Protein, g/dl	0	6.65	6.90	6.73	6.74	6.46		
	5	6.92	7.05	6.82	6.66	6.53		
	10	6.78	6.89	6.83	6.84	6.61		
	20	6.71	6.89	6.71	6.65	6.64		
	30	6.74	6.90	6.77	6.74	6.61	.097	.326
Urea nitrogen, mg/dl	0	6.79	9.08	10.84	10.48	8.50		
	5	7.55	9.60	11.70	12.39	9.39		
	10	6.71	10.44	11.19	11.93	10.21		
	20	7.01	11.55	14.52	13.27	12.17		
	30	7.19	11.98	15.09	14.45	11.42	.543	1.329
Total Bilirubin, mg/dl	0	.400	.381	.334	.350	.369		
	5	.358	.442	.325	.404	.386		
	10	.392	.355	.336	.421	.362		
	20	.376	.378	.243	.376	.403		
	30	.344	.407	.343	.380	.361	.0338	.0449
Creatinine, mg/dl	0	1.894	1.913	1.761	1.851	1.893		
	5	1.934	1.958	1.800	1.891	1.927		
	10	1.796	1.834	1.722	1.823	1.890		
	20	1.798	1.877	1.788	1.813	1.860		
	30	1.816	1.827	1.752	1.801	1.806	.0344	.1624
Cholesterol, mg/dl	0	91.8	89.8	81.2	91.6	121.8		
	5	94.3	90.5	80.8	90.0	117.9		
	10	93.5	91.0	80.3	92.4	119.4		
	20	95.3	91.9	82.0	90.4	127.2		
	30	93.1	89.0	78.4	89.9	123.5	2.04	9.403

[a]Pooled standard error of the least-squares means calculated using error mean square as error term.

[b]Pooled standard error of the least-squares means calculated using mean square for cow(S) as error term.

Trial 2

Plasma glucose concentration (Table 7) was lower (P=.08; Table 5) for control than other S groups. Glucose concentration was lower (P<.01) in period 2 than 1 for all S groups. In period 3 GL was lower than in period 2 for SOY+BM, SOY+CGM and SOY+FAT only.

Neither AL nor TP (Table 7) was affected (Table 5) by S nor by the S X period interaction. Both AL and TP were lower (P<.01) in period 2 and 3 than in period 1. Total protein concentration was also lower (P<.01) in period 3 than 2.

Urea nitrogen concentration (Table 7) for control was lower (P<.01; Table 5) than other S groups. Concentration of UN was higher (P<.01) for SOY+BM and SOY+CGM than for SOY and higher (P=.05) than for SOY+FAT. Each S group had higher (P<.09) UN in period 3 than 1.

Total bilirubin concentration (Table 7) was lower (P=.08; Table 5) for SOY+BM than for SOY+CGM. Total bilirubin concentration was affected (P<.01) by the S X period interaction. In period 2 BIL was higher than in period 1 for SOY and SOY+CGM. In period 3 BIL was higher than in period 1 for all S groups except SOY+BM and SOY+CGM. In period 3 BIL was lower for SOY+BM than any other S group.

Creatinine concentration (Table 7) was not affected (Table 2) by S or the S X period interaction. Creatinine

concentration was higher (P<.01) in period 2 than 1 and higher (P<.01) in period 3 than 2.

Supplement affected (P<.01; Table 5) cholesterol concentration (Table 7). Cholesterol concentration was higher (P<.01) for SOY+FAT than for SOY+BM and SOY+CGM. The S X period interaction also affected (P<.01) CHO. Period 2 CHO was lower than period 1 for control, SOY and SOY+BM but higher for SOY+FAT. In period 3 CHO was lower than in either period 1 or 2 for all S groups except SOY+FAT.

Cow Weights, Condition Scores and Calf Birthweights

Trial 1

Cow body weight change (Table 12) was affected (P<.01; Table 10) by S. Control gained -1.9 kg which was less (P<.01) than other S groups. No significant differences were detected among the other S groups. Neither cow age group, initial cow weight nor initial condition score affected body weight change.

Cow condition score change (Table 12) was affected (P=.02; Table 10) by S. Control lost 1.46 units, which was more (P<.01) than other S groups. Cows in SOY+BM and SOY+CGM lost less (P=.03) condition than SOY. Cow age group was not significant but initial weight (P=.03) and initial condition score (P=.05) were. The regression coefficients (P<.05) of condition score change on initial body weight and condition score were .0026 and -.35 respectively.

Table 10. Least-Squares Analysis of Variance for Calf Birthweight and Precalving Cow Body Weight and Condition Score Change (Trial 1).

Item	Calf birthweight			Body weight change			Condition score change		
	df	Mean square	P	df	Mean square	P	df	Mean square	P
Supplement[a]	4	42.85	.80	4	15,394	<.01	4	1.4193	.02
Control vs others	1	1.18	.88	1	56,212	<.01	1	3.6081	<.01
SOY vs SOY+BM SOY+CGM	1	5.18	.75	1	2,793	.29	1	1.0929	.03
SOY+BM vs SOY + CGM	1	54.50	.31	1	1,013	.52	1	0.0000	.99
SOY+FAT vs SOY+BM SOY+CGM	1	14.96	.59	1	4,095	.20	1	.2887	.43
Cow age	2	59.57	.32	2	2,828	.32	2	.0151	.97
Initial cow weight	1	712.32	<.01	1	777	.57	1	2.2512	.03
Initial condition score	1	45.82	.35	1	4,320	.19	1	1.8070	.05
Error	45	51.33		45	2,390		47	.4583	

[a]Least-squares means separated by orthogonal contrasts.

Table 11. Least-Squares Analysis of Variance for Calf Birthweight and Precalving Cow Body Weight and Condition Score Change (Trial 2).

Item	Calf birthweight			Body weight change			Condition score change		
	df	Mean square	P	df	Mean square	P	df	Mean square	P
Supplement[a]	4	19.29	.88	4	14,486	<.01	4	.4123	.03
Control vs others	1	29.27	.51	1	50,086	<.01	1	.6129	.04
SOY vs SOY+BM SOY+CGM	1	.44	.94	1	5,445	.06	1	.9150	.01
SOY+BM vs SOY+CGM	1	15.28	.63	1	4,595	.08	1	.2403	.19
SOY+FAT vs SOY+BM SOY+CGM	1	24.43	.55	1	99	.80	1	.0172	.73
Cow age	2	273.83	.02	2	1,387	.40	2	.0202	.86
Initial cow weight	1	14.10	.65	1	1,226	.37	1	.9128	.01
Initial condition score	1	1.01	.90	1	1,084	.40	1	6.7967	<.01
Error	49	66.49		50	1,477		44	.1375	

[a]Least-squares means separated by orthogonal contrasts.

Table 12. Least-Squares Means for Calf Birthweight and Precalving Cow Body Weight and Condition Score Change (Trials 1 and 2).

Trial	Supplement	Calf birthweight, kg	Body weight change, kg	Condition score change
1[a]	Control	38.4	-1.9	-1.46
	SOY	38.1	31.7	-1.18
	SOY+BM	39.2	38.2	-.76
	SOY+CGM	37.7	44.4	-.76
	SOY+FAT	39.1	30.7	-.56
	SE[c]	1.03	7.04	.212
2[b]	Control	37.8	-46.4	-.95
	SOY	38.7	-20.1	-.93
	SOY+BM	38.4	-1.8	-.46
	SOY+CGM	39.2	-15.0	-.69
	SOY+FAT	38.0	-10.1	-.63
	SE	1.14	5.37	.122

[a]January 15 to March 1, 1985.
[b]December 15, 1985 to March 6, 1986.
[c]Pooled standard error of the least-squares means.

Calf birthweight (Table 12) was not affected (Table 10) by S, cow age group or initial condition score. The regression coefficient ($P<.01$) of birthweight on initial cow body weight was .0464.

Trial 2

Cow body weight change (Table 12) was affected ($P<.01$; Table 11) by S. Control gained -46.4 kg which was less ($P<.01$) than other S groups. Cows in SOY gained -20.1 kg which was less ($P=.06$) than cows in SOY+BM (-1.8 kg) and SOY+CGM (-15.0 kg). Cows in SOY+BM tended to lose less ($P=.08$) weight than cows in SOY+CGM. Neither cow age group,

initial cow weight nor initial condition score significantly influenced weight change.

Cow condition score change (Table 12) was affected (P=.03) by S. Control lost .95 units which was more (P=.04) than for other S groups. Cows in SOY+BM and SOY+CGM lost less (P=.01) than cows in SOY. Cow age group was not significant. The regression coefficients (P=.01) of condition score change on initial weight and condition score were .00219 and -.73 respectively.

Calf birthweight (Table 12) was not affected (Table 11) by S group.

Ruminal pH and Ammonia

Trial 1

Ruminal ammonia concentration (Table 14) for control (6.7 mg/dl) was 1.7 to 4.9 mg/dl lower (P=.03) than for other S groups. Ammonia concentration was higher in period 3 than 2 for control, SOY+BM and SOY+FAT.

Ruminal pH (Table 14) was affected (P<.01; Table 13) by S. Control pH (6.859) was higher (P=.03) than for other S groups. Mean pH for SOY+BM (6.442) and SOY+CGM (6.443) was lower (P=.01) than for SOY (6.753) or SOY+FAT (6.778). Ruminal pH was higher (P<.01) during period 3 (6.845) than 2 (6.626).

Table 13. Least-Squares Analysis of Variance for Precalving Ruminal Ammonia Concentration and pH (Trials 1 and 2).

	Trial 1				Trial 2		
		Ammonia		pH		pH	
Item	df	Mean square	P	Mean square	P	df	Mean square	P
Supplement(S)[a]	4	113.81	.09	1.6029	<.01	4	.4343	.19
Control vs others	1	278.71	.03	1.9715	.03	1	.1753	.41
SOY vs SOY+BM SOY+CGM	1	104.30	.15	2.4507	.01	1	.7939	.09
SOY+BM vs SOY+CGM	1	102.18	.16	0.0000	.99	1	.2421	.34
SOY+FAT vs SOY+BM SOY+CGM	1	43.00	.35	3.4514	<.01	1	1.2611	.04
Cow(S)	13	45.30	<.01	.2953	<.01	16	.2479	<.01
Sampling period (PER)	1	104.72	.02	.1580	<.01	1	.3410	<.01
Sampling interval (I)	5	151.04	<.01	.4596	<.01	4	.7586	<.01
S X PER	4	63.07	.01	.0779	<.01	4	.1717	<.01
S X I	20	19.00	.52	.0291	.31	16	.0118	.77
Error	199	19.83		.0210		227	.0316	

[a]Mean square for cow(S) used as error term for supplement and least-squares means separated by orthogonal contrasts.

Table 14. Least-Squares Means for Precalving Ruminal Ammonia Concentration and pH by Sampling Period (PER; Trials 1 and 2).

Item	PER	Supplement					SE[a]	SE[b]
		Control	SOY	SOY+BM	SOY+CGM	SOY+FAT		
Trial 1								
Ammonia,	2	4.5	8.7	7.9	12.3	8.1		
mg/dl	3	8.9	8.1	10.6	10.7	10.3	.96	1.45
pH	2	6.859	6.677	6.384	6.482	6.727		
	3	6.862	6.830	6.499	6.403	6.829	.0328	.1229
Trial 2								
pH	2	6.703	6.646	6.586	6.613	6.731		
	3	6.661	6.667	6.322	6.514	6.738	.0376	.1051

[a]Pooled standard error of the least-squares means calculated using error mean square as error term.
[b]Pooled standard error of the least-squares means calculated using mean square for cow(S) as error term.

Table 15. Least-Squares Means for Precalving Ruminal Ammonia Concentration and pH by Sampling Interval (Trials 1 and 2).

Sampling Interval (hours post-supplementation)	Trial 1		Trial 2
	Ammonia, mg/dl	pH	pH
0	7.5	6.825	6.765
5	10.5	6.519	6.429
10	6.1	6.566	6.566
20	12.1	6.681	6.679
30	8.5	6.612	6.652
43	9.4	6.728	
SE[a]	.73	.0242	.0270

[a]Pooled standard error of the least-squares means.

Trial 2

Ruminal pH (Table 14) tended to be lower (P=.09; Table 13) for SOY+BM (6.454) and SOY+CGM (6.564) than for SOY (6.657). Ruminal pH was lower (P=.04) for SOY+BM and SOY+CGM than for SOY+FAT (6.735). A significant S X period interaction resulted because pH was lower in period 3 than 2 only for SOY+BM and SOY+CGM.

Fermentation Rate

Trail 1

Ruminal NDF fermentation rate was slower (P<.01; Table 16,18) for control (1.85%/h) than for other S groups. Fermentation rate for SOY+BM (2.62%/h) and SOY+CGM (2.84%/h) was faster (P=.07) than for SOY (2.25%/h). During period 3 fermentation rate (Table 19) was faster than during either period 1 (P=.04) or period 2 (P=.07).

Trial 2

Ruminal NDF fermentation rate (Table 18) was not affected (P=.20; Table 17) by S but tended to be faster (P=.08) for SOY+CGM (4.9%/hr) than for SOY+BM (4.3%/h). All three periods had different (P<.01) rates of NDF fermentation.

Ruminal Flow Kinetics

Trial 1

Ruminal fluid dilution rate (Table 18) was slower (Table 16) for SOY+BM (8.4%/h) and SOY+CGM (6.7%/h) than for either SOY (11.4%/h; P=.06) or SOY+FAT (10.9%/h; P=.07). Fluid dilution rate was also slower (P<.01) during period 1 than either period 2 or 3 (Table 19). The S X period interaction was not significant.

Ruminal fluid volume (Table 18) tended to be greater (P=.09; Table 16) for control (38.9 liters) than for other S groups. Fluid volume was greater for SOY+BM and SOY+CGM than for SOY (P=.05) Fluid volume (Table 19) was higher (P<.01) in period 1 than in either period 2 or 3.

Trial 2

Ruminal fluid dilution rate (Table 18) was slower (P<.01) for SOY+BM (6.9%/h) and SOY+CGM (7.2%/h) than for SOY+FAT (11.8%/h). Fluid dilution rate (Table 19) was faster (P<.01) in period 1 than in either period 2 or 3.

Dilution rate of ruminal particles (Table 18) was slower (P=.03; Table 16) for SOY+BM (2.33%/h) and SOY+CGM (2.75%/h) than for SOY (3.48%/h). Particulate dilution rate (Table 19) was faster (P<.03) for period 2 than for either period 1 or 3.

Fluid volume (Table 18) was greater ($P<.01$) for SOY+BM (41.3 liters) and SOY+CGM (42.1 liters) than for SOY+FAT (20.7 liters).

Table 16. Least-Squares Analysis of Variance for Precalving Ruminal Fluid Dilution Rate, Fluid Volume and Neutral Detergent Fiber (NDF) Fermentation Rate (Trial 1).

Item	Fluid dilution rate			Fluid volume			NDF fermentation rate		
	df	Mean square	P	df	Mean square	P	df	Mean square	P
Supplement(S)[a]	4	.4145	.19	4	1,229.1	.04	4	.01310	.04
Control vs others	1	.2039	.35	1	1,423.4	.09	1	.03606	<.01
SOY vs SOY+BM SOY+CGM	1	.9536	.06	1	1,986.3	.05	1	.01207	.07
SOY+BM vs SOY+CGM	1	.1422	.43	1	15.5	.85	1	.00264	.37
SOY+FAT vs SOY+BM SOY+CGM	1	.8815	.07	1	2,043.9	.05	1	.00350	.30
Cow(S)	12	.2162	.04	12	431.6	.05	15	.00308	.60
Sampling period (PER)	2	.7180	<.01	2	2,878.7	<.01	2	.00944	.09
S X PER	8	.0834	.53	8	372.9	.11	8	.00407	.37
Error	24	.0920		24	196.5		25	.00353	

[a]Mean square for cow(S) used as error term for supplement and least-squares means separated by orthogonal contrasts.

Table 17. Least-Squares Analysis of Variance for Precalving Ruminal Fluid and Particulate Dilution Rate, Fluid Volume and Neutral Detergent Fiber (NDF) Fermentation Rate (Trial 2).

Item	Fluid dilution rate			Particulate dilution rate			Fluid volume			NDF fermentation rate		
	df	Mean square	P	df	Mean square	P	df	Mean square	P	df	Mean square	P
Supplement(S)[a]	4	.382	.03	4	.02535	.25	4	834.6	.04	4	.01539	.20
Control vs others	1	.006	.82	1	.00235	.71	1	270.5	.33	1	.00608	.39
SOY vs SOY+BM SOY+CGM	1	.286	.13	1	.09243	.03	1	648.6	.14	1	.02138	.11
SOY+BM vs SOY+CGM	1	.006	.82	1	.01067	.43	1	3.9	.90	1	.02739	.08
SOY+FAT vs SOY+BM SOY+CGM	1	1.503	<.01	1	.02575	.23	1	2,900.1	<.01	1	.00109	.71
Cow(S)	18	.109	.14	18	.01660	.25	18	265.8	.48	18	.00774	.08
Sampling period (PER)	2	.412	<.01	2	.05800	.02	2	577.6	.13	2	.27561	<.01
S X PER	8	.109	.18	8	.00818	.74	8	188.8	.68	8	.00583	.26
Error	31	.070		31	.01270		31	265.6		31	.00434	

[a]Mean square for cow(S) used as error term for supplement and least-squares means separated by orthogonal contrasts.

Table 18. Least-Squares Means for Precalving Ruminal Fluid and Particulate Dilution Rates, Fluid Volume and Neutral Detergent Fiber (NDF) Fermentation Rate (Trials 1 and 2).

Item	Supplement					SE[a]
	Control	SOY	SOY+BM	SOY+CGM	SOY+FAT	
Trial 1						
Fluid dilution rate, %/h	7.7	11.4	8.4	6.7	10.9	1.47
Fluid volume, liters	38.9	15.7	34.4	32.7	17.1	6.55
NDF fermentation rate, %/h	1.85	2.25	2.62	2.84	2.51	.180
Trial 2						
Fluid dilution rate, %/h	9.0	8.7	6.9	7.2	11.8	1.01
Particulate dilution rate, %/h	3.14	3.48	2.33	2.75	3.17	.395
Fluid volume, liters	27.6	33.8	41.3	42.1	20.7	5.00
NDF fermentation rate, %/h	4.14	4.16	4.28	4.95	4.48	.270

[a]Pooled standard error of the least-squares means.

Table 19. Least-Squares Means for Precalving Ruminal Fluid and Particulate Dilution Rates, Fluid Volume and Neutral Detergent Fiber (NDF) Fermentation Rate by Sampling Period (Trials 1 and 2).

Item	Period			SE[a]
	1	2	3	
Trial 1				
Fluid dilution rate, %/h	6.65	10.49	9.94	.743
Fluid volume, liters	42.9	18.9	21.5	3.43
NDF fermentation rate, %/h	2.25	2.30	2.69	.146
Trial 2				
Fluid dilution rate, %/h	10.40	7.99	7.74	.618
Particulate dilution rate, %/h	2.75	3.60	2.56	.263
Fluid volume, liters	27.0	37.5	34.8	3.81
NDF fermentation rate, %/h	4.42	3.17	5.61	.154

[a]Pooled standard error of the least-squares means.

DISCUSSION

During trial 1 fecal output was estimated by Dunn (1986). He used the same animals described for this study. His results have been used to interpret my data. However, it should be recognized that his measurements were made during days between my sampling periods. The technique involved measurement of chromic oxide dilution (Raleigh et al., 1980) of all cows (n=55) adjusted by concurrent total fecal collection of 20 cows (four per S group) replicated once. Daily organic matter fecal ouput as a percent of body weight was .54, .68, .86, .59 and .79 for control, SOY, SOY+BM, SOY+CGM, and SOY+FAT respectively. The pooled standard error was .04. Dunn (1986) found that forage intake in trial 1 was increased ($P<.05$) in SOY, SOY+BM, and SOY+FAT compared to control. It was assumed that the contribution of supplement to fecal output was relatively insignificant and that forage digestibility was similar across S groups. However, digestibility probably was not similar across S groups. Fermentation rate was higher for SOY than control and higher for SOY+BM and SOY+CGM than for SOY (Table 18). Although we lack forage ruminal retention time data for trial 1, the dilution rates of fluid and particles in trial 2 had proportional differences between treatments. If we assume the same was true in trial 1 then forage ruminal retention times for SOY+BM and SOY+CGM were equal to that of

control. The residence time for SOY and SOY+FAT by the same rationale may have been less than for control. Consequently, forage digestibility for SOY+BM and SOY+CGM was most probably higher than for SOY.

Fecal output numerically was 27% greater for SOY+BM than for control. Forage intake probably was significantly higher if we assume different digestibility. This result is consistent with the rumen bypass protein effect on intake of a low protein forage diet reported by Egan and Moir (1965) and of a barley diet (Orskov et al., 1973). Egan and Moir (1965) attributed the intake response to improved protein status brought forth by increased protein presented to the small intestine and not to enhanced forage digestibility. It appears that supplements of the present study had both effects.

The soybean meal fraction of each supplement was expected to enhance fermentation rate compared to control. Indeed, this was the effect observed in trial 1. However, SOY+BM and SOY+CGM groups had higher fermentation rates than SOY in both trials 1 (P=.07) and 2 (P=.11). Fermentation rate was also numerically higher for SOY+FAT than for SOY and was different than for SOY+BM and SOY+CGM. Ruminal degradation rate of blood meal and corn gluten meal is less than that of soybean meal. Lipid coating has been used to slow ruminal degradation of protein (Van Soest, 1982). Thus

ruminal ammonia concentration might have been higher at later sampling intervals post supplementation for SOY+BM, SOY+CGM and SOY+FAT than for SOY. However, the S X sampling interval interaction was not significant. Neither ruminal ammonia concentration nor serum UN were significantly higher in SOY+BM and SOY+CGM than in SOY but both were numerically higher. It could be hypothesized that nitrogen recycling to the rumen may have enhanced NDF fermentation rate. Orskov (1982) states that in situ fermentation rate of whole barley can be enhanced by urea addition until ruminal ammonia concentration exceeds 23.5 mg/dl. Our values ranged from 4 to 11 mg/dl. However, he also states that the optimum concentration of ruminal ammonia is different for different substrates and that optimum concentration was only 2 mg/dl for caustic soda-treated barley straw. Considering that the forage diet in this study was more similar to the barley straw than the barley described above, it may be that even if ruminal ammonia concentration was higher for SOY+BM and SOY+CGM than for SOY, it was not the factor which enhanced fermentation rate.

An alternative, and more likely, explanation of the effect of SOY+BM, SOY+CGM and SOY+FAT on fiber fermentation rate could involve increased availability of other nutrients limiting microbial activity. Mir et al. (1986) increased the digestibility of barley straw from 34.8 to 63.1% digestibillty with additions of valine, leucine and

isoleucine. Clark and Petersen (1985) observed a similar effect with additions of methionine, arginine or cysteine. Blood meal and corn gluten meal, although degraded slowly in the rumen, when fed in combination with soybean meal may have supplied certain carbon skeletons in greater quantity than soybean meal alone. Or even more likely, SOY+BM, SOY+CGM and SOY+FAT may have supplied these nutrients over a longer period of time than SOY, thus improving fermentation rate and digestibility.

That fermentation rate in trial 1 was as high for SOY+FAT as for SOY+BM and SOY+CGM, is not consistent with numerous experiments cited by Palmquist and Conrad (1978). These have shown dietary fat to depress fermentation of fiber in the rumen. However, when added at the rate of 10% of the diet or less, fat usually did not depress digestibility of other dietary components (Palmquist and Conrad, 1978). In fact, these authors found nonsignificant increases in acid detergent fiber digestibility in dairy cows when fat was included at 6 and 20% of a high grain ration. The depressing effect of fat on digestibility is probably due to free, long-chain fatty acids inhibiting rumen bacteria and can be minimized by increasing the calcium content of the diet (Palmquist et al., 1986). Calcium is involved in the formation of soaps which are presumed nontoxic to rumen bacteria (Palmquist et al., 1986). One can thus hypothesize that the relatively higher

calcium content of forages compared to grains would help to prevent a depressing effect of dietary fat. Most studies involving fat feeding have been with grain rations. Hungate (1966) stated that when fat was added to a complete ration, digestibility was depressed more than when one component of the diet contained the added fat. He further stated that lipid coating of feed reduced enzymatic attack by bacteria. Since animal fat was only a part of the ration in SOY+FAT, this is probably why fermentation rate was not depressed.

Increased fermentation rate in SOY+BM and SOY+CGM may be the cause of lower ruminal pH of these groups. Ruminal pH decreases during periods of elevated volatile fatty acid production (Van Soest, 1982).

In trial 2 NDF fermentation rates appeared to be nearly twice that of trial 1. One explanation of the difference may involve differences in forage quality, yet NDF content of esophageal extrusa samples used in the in situ technique were not different between trials and ranged from 62-65% NDF. More likely the difference was due to differences in incubation times between the two trials. Nylon bags were incubated for up to 96 h in trial 1, but only up to 72 h in trial 2. The equation used to describe rate of NDF disappearance attempts to account for an undigestible fraction of the substrate. Although this equation fit the data of trail 1 better than others evaluated, the data of trial 2 was simply loglinear. In other words, incubation

times were not long enough to allow for description of an undigestible residue. However, theoretically, if no undigestibile fraction existed then the equation used should predict the same fermentation rate as a simple loglinear model. Thus, there is no apparent explanation for the numerically higher fermentation rates in trial 2.

The higher fluid dilution rate in SOY compared to control may have been caused by the effect of soybean meal on fermentation rate. Fluid dilution rate increases in response to increased fluid osmolality (Van Soest, 1982). However, this fails to explain the increased dilution rate of SOY over control in trial 2 since fermentation rates were equal. It also does not explain the lower fluid dilution rates in either trial for SOY+BM and SOY+CGM. Due to these inconsistencies and the probability that particulate dilution rate by S group ranked similarly to fluid dilution rate, another mechanism must be involved. Many endogenous compounds have been shown to affect voluntary food intake and consequently rumen kinetics (Morley, 1980; Baile et al., 1986; Forbes, 1986). Some of these may be involved in the responses of enhanced voluntary intake observed by Orskov et al. (1973) and Egan and Moir (1965) and by the same reasoning may have influenced intake and dilution rates in this study. For example, Barry et al. (1982) showed that serum somatostatin concentration could be reduced, and herbage intake increased, by infusing casein into the

abomasum of growing lambs. The interactive effects of endocrine regulation of feed intake and digestive kinetics are not completely understood but it is probable that they affected the dilution rates in this study.

In the SOY group, increased fluid dilution rate was associated with decreased fluid volume. This association seems inconsistent with the hypothesis that dilution rate was elevated due to increased osmolality in SOY. By that hypothesis an influx of water to the rumen in an effort to reduce osmolality would be the cause of increased dilution rate (Van Soest, 1982). This water influx should not reduce fluid volume. Thus, the mechanism of increased fluid dilution rate in this instance cannot be explained by the common theory that osmolality governs dilution rate.

The actual fluid volumes in all treatments and periods are lower than values reported by Branine and Galyean (1985) and may not be accurate. Error may have been encountered in extrapolation of cobalt concentration to the time of initial dosing. The relative differences across treatments, however, are probably valid. One should realize that fluid volume in this context describes the content of aqueous solution in the rumen and not physical volume by the general definition.

The SOY supplement had a positive influence on cow body weight change compared to control (Table 12) in both trials. On the other hand, SOY+BM and SOY+CGM had an additive effect

on weight change only in trial 2. Cows lost weight in trial 2 whereas most gained weight in trial 1. Differential response to similar supplemental regimens in different years have been reported previously (Kartchner, 1981; Phillips and Vavra, 1981; Stanton et al., 1983).

The year effect in this study was probably not a function of ambient temperature. S. K. Beverlin (unpublished data) found mean daily temperature not different between the two years. Observation did indicate that more forage was covered by snow in trial 2 for longer periods of time than in trial 1. The quantity of forage available also appeared to be less in trial 2, possibly due to lower precipitation during the preceeding growing season (NOAA, 1985; NOAA, 1986). In both trials at least some forage was always available for grazing. However, initial grazing and selection may have shifted the quality of available forage in the latter parts of trial 2 more than the effect in trial 1. No differences betwen years were noted in NDF content of esophageal extrusa. The possibility of finding differences may have been precluded by the procedure used in obtaining extrusa. Fistulated cows were fasted overnight and during collections were not allowed to stray beyond a limited area. This area included the facility used for supplementation, was near the salt box and was thus one of the most often used areas in the pasture. Thus, forage quality might be expected to be constantly low

and not necessarily representative of the diets of the experimental animals. In addition, fasting may influence diet selectivity of grazing beef cattle (R. Cochran, personel communication). Selectivity influences quality of the sample collected (Lesperence et al., 1974). Fermentation rate in situ can not be used to indicate changes in forage quality either since the extrusa incubated in nylon bags was obtained early in each trial and used in each of the three sampling periods. Thus, the interaction of forage quantity and quality remains a possible cause for the year effect on cow body weight change. At any rate, the nutrient deficiency which potentiated a response to supplementing bypass protein in trial 2 was absent in trial 1.

Given the digestible organic matter intake described above, microbial protein synthesis can be estimated by using protein synthesis rates measured by Kropp et al (1976) and Petersen et al. (1985). In turn, equations of NRC (1984) and Orskov (1982) can be used to calculate a metabolic protein requirement of gestating beef cows. These estimations indicate that cows in the SOY group may have been deficient in metabolically available protein in trial 1. The same would be true if forage intake between trials was similar. Since, in trial 1 ruminal ammonia concentration was above 5 mg/dl, addition of nitrogen to the rumen would not be expected to enhance microbial yield

(Satter and Slyter, 1974). Thus, as was our hypothesis, rumen bypass protein should alleviate weight loss compared to rumen degradable protein. Consumption of bypass protein may increase the quantity of metabolically available protein in two ways. Obviously, the bypassed protein contributes to the pool of available protein. Microbial yield may also be enhanced, since washout of bacteria is related to passage of unfermented substrate out of the rumen (Van Soest, 1982).

Alleviation of body weight loss would be expected if the amino acids derived from bypass protein substituted for amino acids of tissue origin in meeting amino acid requirements described by NRC (1984). Amino acids may also increase the efficiency of acetate utilization (Macrae and Lobley, 1986). Several amino acids are known precursors of oxaloacetate, a required intermediate for oxidation of acetate in the tricarboxylic acid cycle (Stryer, 1981).

Another possible effect of bypass protein involves the animal requirement for essential amino acids. In trial 1, serum amino acid concentrations were measured on samples composited across sampling interval within sampling period 2 (M. K. Petersen and J. L. Miner, unpublished data). Branched chain amino acid concentration was highest (P=.07) in SOY+BM and SOY+CGM which, according to Lynch and Jackson (1986), may indicate reduced muscle catabolism in these S groups. Compared to control, SOY and SOY+FAT, the concentration of lysine in SOY+BM was nearly doubled while

it was reduced in SOY+CGM. According to the reasoning of Ahmed (1982) SOY+CGM may have supplied a limiting essential amino acid which increased the usage of lysine. For example, methionine may have been limiting since its concentration was not affected by feeding corn gluten meal. Corn gluten meal protein is relatively rich in methionine but low in lysine (Anonymous, 1982). Lysine probably was not limiting since lysine concentraton was elevated when lysine-rich blood meal was fed.

Weight change in SOY+FAT was intermediate between SOY+BM and SOY+CGM. The animal fat in SOY+FAT may have increased the amount of soybean meal that escaped rumen degradation. Lipids have been used to protect protein sources from fermentation although the lipid coating used has been thicker than it would have been in this study (Van Soest, 1982).

The probability that forage intake was higher in SOY+BM and SOY+FAT than SOY probably accounts for a significant portion of the observed differences in weight change in trial 2. That forage intake of SOY+CGM was numerically lower than SOY yet body weight change was numerically higher lends support to the hypothesis that the supply of a limiting amino acid was increased.

Condition score loss was greater for control than other S groups but appeared similar to SOY in trial 1 and was the same as SOY in trial 2. Condition score is probably a

measure of external fatness and thus indicated that although SOY had a more positive weight change, loss of external fat in SOY was similar to control. Therefore since weight loss was different for SOY than control and SOY lost less condition then the loss of weight from body tissues other than subcutaneous fat must have been greater for control than SOY.

The demand for glucose or glucogenic substrates may have been the cause of protein tissue mobilization in control. The regulation of lipolysis may not be strictly related to the regulation of body protein catabolism. Certainly, increased fat mobilization and lipolysis would not significantly contribute to the pool of glucogenic substrates. This reasoning would explain why SOY could lose less body weight and a similar amount of body fat compared to control. Soybean meal supplementation probably increased the quantity of microbial protein presented to the small intestine. An increased plasma glucose pool would not necessarily accompany increased dietary contribution to the pool since glucose of dietary origin may merely replace that derived from body protein catabolism. As described above in situations where the supply of oxaloacetate is rate limiting compared to the concentration of acetate available for oxidation, efficiency of acetate oxidation can be increased by increasing the supply of amino acids (Macrae and Lobley, 1986). It is conceivable that the additional amino acids

supplied by SOY+BM and SOY+CGM could increase the efficiency of metabolism (turnover and oxidation) of body fat and thus reduce the quantity of fat mobilized. This theory assumes that the priority of reducing protein catabolism is greater than the priority for increased efficiency of acetate metabolism. The endocrine regulation of lipolysis and lipogenesis has not been as well defined for ruminants as for nonruminants (Van Soest, 1982). The low condition score loss in SOY+FAT may be attributed to the high supply of fatty acids in the SOY+FAT supplement.

Forage intake differences may also explain some of the differences in condition score change. The problem with this explanation is that, at least in trial 1, SOY+CGM did not exhibit higher forage intake than SOY but did lose less condition score. On the other hand SOY had higher intake than control but did not have a different condition score change compared to control.

During trial 1 the lower glucose concentrations in period 2 compared to period 1 may have been a result of the environmental conditions. Snow covered much of the forage during period 2 but was nearly absent during periods 1 and 3. Snow cover has been associated with reduced forage availability and reduced digestibility, the effect being greater for unsupplemented heifers than for heifers fed a soybean meal supplement as shown by Rittenhouse et al., 1970). In addition, ambient temperature was less during

period 2 (-6.5 C) than either period 1 (-4.2 C) or period 3 (1.1 C). Glucose turnover and oxidation are increased dramatically in sheep during acute cold exposure; the use being oxidation by skeletal muscles for thermogenesis and the increased supply probably due to increased glycogenolysis and gluconeogenesis, (Sasaki and Weekes, 1986). However, plasma glucose concentration has been shown to increase during cold exposure in most but not all cases (Sasaki and Weekes, 1986). Thus, our results may reflect the combined effect of snow cover and cold.

The failure of all S groups except SOY+BM to restore plasma glucose concentration by period 3 to a level consistent with period 1 may involve the stress of advancing gestation. The gravid uterus in late gestation accounts for a major part of the cows utilization of glucose and glucogenic substrates (Prior and Scott, 1977; Ferrel and Ford, 1980). During trial 2 glucose concentration was lower in control than other S groups; probably indicating an insufficiency of glucogenic substrate. Glucose concentration in all S groups declined from period 1 to period 2. This may reflect the increasing fetal requirement for glucose and glucogenic substrate and is consistent with the linear decline in GL with gestation observed by Bull et al. (1984). Glucose concentration did not decline from period 2 to 3 for control or SOY but did for SOY+BM, SOY+CGM and SOY+FAT. However, concentrations in the latter were

still not lower than for control or SOY. This could indicate that a minimum concentration was reached earlier in control and SOY. Regulation of glucose concentration in the beef cow may be such that only in extreme situations does it fall below 50 mg/dl.

Serum urea nitrogen concentration was higher in supplemented groups and higher in SOY+BM and SOY+CGM than SOY in both trials although the response was not significant in trial 1. This probably indicates that the additional amino acids absorbed were deaminated by the liver and(or) fetus. It also lends support to the hypothesis that the demand for glucogenic metabolites has a major effect on other aspects of metabolism.

Albumin concentrations in trial 1 were below, and in trial 2 were above the normal range reported by Benjamin (1978). The decline with advancing gestation in trial 2 is consistent with results of Bull et al. (1984). It is not clear why no differences between S groups were found since higher protein intake did increase albumin concentration as reported by Lynch and Jackson (1983ab). Variation in protein intake between S groups was similar to the variation imposed by Lynch and Jackson (1983ab). However, our results may indicate that protein supplied by SOY+BM and SOY+CGM was not excessive and was utilized for a need of higher priority than albumin production.

Total bilirubin concentration was much higher in trial 2 when most cows lost body weight than in trial 1 when most cows gained weight. In both trials the concentration was within the normal range reported by Benjamin (1978). Concentration increased with advancing gestation in both trials which is consistent with results of Bull et al. (1984). Bull et al. (1984) also found BIL to be higher in prepartum heifers restricted in protein intake. In trial 2 SOY+BM had lower BIL than other S groups during period 3. This may indicate that cows in SOY+BM had a greater ability to cope with the demands of advancing gestation. Elevations in BIL may indicate impaired liver function (Lynch and Jackson, 1983ab). Since BIL was higher during period 2 than period 3 of trial 1 the hypothesis concerning environmental stress in period 2 is supported.

The higher serum creatinine concentration in control compared to other S groups in trial 1 is consistent with the response reported by Bull et al. (1984) to protein restriction. Creatinine concentration increased with advancing gestation which is also consistent with results reported by Bull et al. (1984). This response in trial 1 seems to indicate that CRE is more sensitive to factors associated with advancing gestation than to the environmental stress of period 2.

Serum cholesterol concentration was elevated by the high lipid diet of SOY+FAT. Increased lipid intake has

previously been shown to elevate CHO (Talavera et al., 1985). With advancing gestation CHO has been shown to decline in dairy cows (Blum et al., 1983) and to rise in beef heifers (Bull et al., 1984). The reason for this discrepency is unclear. In general, CHO declined with advancing gestation in this study and the significance is unclear.

In general the blood metabolites showed that even during trial 1 when most cows gained body weight there were signs of stress in unsupplemented animals. It should be emphasized that body weight change was determined precalving. In both trials the subtraction of fetal and placental weight would reveal that most cows were actually losing body weight.

SUMMARY

In conclusion, supplementation of pregnant, winter grazing beef cows with SOY significantly improved NDF fermentation rate, forage intake and body weight change. Addition of blood meal to soybean meal further enhanced forage intake but caused unexpected increases in fermentation rate and rumen volume and declines in ruminal fluid dilution rate. When all experimental animals were in a weight loss state blood meal reduced weight loss by 18.3 kg compared to SOY. When cows were in a weight gain situation there was no benefit in body weight change to blood meal. The addition of corn gluten meal did not stimulate forage intake but did improve fermentation rate in trial 1. Both bypass protein sources increased ruminal fluid volume and decreased fluid dilution rate. Animal fat also stimulated forage intake. It did not reduce NDF fermentation rate and did reduce condition score loss. Blood metabolites indicated improved nutritional status in SOY and further improvement in SOY+BM and SOY+CGM. Glucose and urea nitrogen data supported the hypothesis that glucogenic substrates are often more in demand than supply. None of the supplements affected calf birthweight.

Under these circumstances .5 kg of soybean meal per day in some years will be more than enough supplement to prevent

body weight loss. The addition of bypass protein to soybean meal in some years will improve body weight change.

RECOMMENDATIONS

In hindsight, it is obvious that this thesis research could have been better. Several techniques could have yielded results with more accuracy and precision. Additional measurements could have provided information which would aid in interpretation of the treatment effects.

In order to evaluate the contribution of nutrients from supplement it is vital that the contribution of nutrients from forage is known. Directly measuring intake of free roaming cows is impossible. Thus we are forced to estimate intake by measuring fecal output and digestibility. However, there appears to be ample room for improvement in intake estimation.

Fecal output is usually assumed to be overestimated when measured by the chromic oxide dilution. This is apparently due to incomplete fecal recovery of dosed chromic oxide. Total collection in fecal bags is considered the standard for adjusting fecal output estimated by chromic oxide dilution. The problem is that one cannot be certain that total collection accurately measures fecal output in a natural situation. Field observations indicated that cows fitted with collection bags often became bruised and walked at a much slower pace than other cows. Some cows fitted with bags spent considerable effort trying to get rid of their bags. This, in turn, often resulted in feces being

lost from the bag. It is also probable that intake was depressed to some degree in cows fitted with bags. Measurement of this depressed intake could also be biased downward. Behavior of cows fitted with collection bags should be measured (vibration recorders and pedometers) and compared to that of the other cows in the herd.

More complete recovery of chromic oxide may be possible if total collection in bags was in fact total collection. Vogel et al. (1985) recovered 99 to 106% of dosed chromium in the feces of Hereford steers. Overestimation of actual supplement intake could also account for incomplete marker recovery.

We had no estimate of diurnal variation in chromium excretion. The time span between obtaining the first and last fecal grab sample for a given collection period was often at least three hours. Since grab samples were obtained first from cows fitted with bags the correction factor applied the other cows could be biased. If chromium excretion during this period declined and was below the two day average then fecal output would be biased upward even with the correction factor.

Thus my recommendation is to determine the fecal chromium excretion curve in the field. This would enable one to estimate the bias in fecal output estimation due to the time span of grab sampling. It would of course be desirable to sample all cows at precisely the same time. It

would also be desirable to compare estimates of fecal output betweeen the two techniques in a situation where spillage from bags could be completely eliminated. For this trial the experimental diet should be similar to that in the field.

Since we have shown that both fermentation rate and digesta retention time can be influenced by supplementation, the assumption of equal forage digestibility across treatment groups cannot be valid. The accuracy with which in vitro measurements reflect digestibility in the field is also questionable. One alternativee method of estimating digestibility would be to directly measure digestibility of harvested range forage. This value could be compared to in vitro digestibility and an adjustment factor obtained. The adjustment could then be applied to in vitro measurements such as those in this study. This method is still unable to determine differences in digestibility between supplement groups. It would be difficult to apply the effect of supplement in vitro although adjustment factors could be derived as above. A more accurate method of measuring digestibility may involve use of situ digestion. If mean ruminal forage retention time was known then nylon bags could be incubated in the rumen for mean retention time. Extent of ruminal digestion would thus be measured. If the bags used were sufficiently small they could be passed through the reticulo-omasal orifice and subsequently

collected in the feces by use of a total collection bag. Loss of weight would represent total tract digestibility. This method assumes that weight loss from the bags represents digestion outside of the bags. This assumption has been accepted at least regarding ruminal fermentation. Another assumption is that postruminal passage of bags would be equal to that of digesta. This could be determined by marking digesta and measuring the passage rate for each. This technique, like the in vitro technique should be validated by comparison to direct digestibility measurement. Either alternative promises to yield a more accurate estimate of digestibility than face value in vitro estimates.

Supplementation with bypass protein enhanced fermentation rate compared to soybean meal only supplementation at least in trial 1. Knowledge of the mechanism involved could lead to a more economical way of increasing forage digestibility. It seems likely that either an increased or more even supply of nitrogen for rumen microorganisms through either urea recycling or a slow rate of degradation could be involved. On the other hand, the effect may be due to an increased or more consistent supply of other microbial nutrients. I would suggest a metabolism trial in which these factors could be applied independently. This type of trial could provide insight as to the mechanism(s) involved.

Although we measured ruminal digestion rate of our supplements we did not determine total tract digestibility. Interpretation of performance depends partly on whether bypass protein is ever digested. Again I suggest a metabolism trial utilizing duodenal or abomasal and ileal cannulas to determine site of digestion. This would necessitate assessment of bacterial and nonprotein nitrogen as well.

A major criticism of this research is the lack of data pertaining to reproductive performance. Nutritional treatments were terminated in early March and all cows were then provided luxurious nutritional treatment. The possibility exists that days to postcalving conception could have been affected. This may not have been highly correlated to body weight or condition as we have measured condition. It appears that cows in SOY lost weight from a different tissue than those in control. Suppose a form of body condition more specific than external fatness is the true determinant of first postcalving estrus. This factor would be correlated with condition score but suppose it could be affected independently of condition score by supplying a specific limiting nutrient. We did preferentially supply specific nutrients compared to experiments where protein or energy intake were varied and postpartum interval measured. However, it is distinctly

possible that excessive feeding during the postpartum period could mask any such effect.

Even without the above argument I believe nutritional treatment should be maintained until supplemental feeding is no longer required due to spring growth of forage. The cost of research would probably be elevated due to lengthened postpartum interval or lack of some cows to conceive. However, the philosophy for ag experiment station operation involves experimentation. We can't tell producers for example that .5 kg/d of soybean meal is most economical prepartum without saying that much more supplement may also be necessary until conception. It is fine to study mechanisms through a window but how can we justify ignoring the big picture when it comes to the bottom line. We can say a supplement should be economical but cannot expect producers to do the product testing.

The most intriguing result of this research in my opinion was the increased fecal output of SOY+BM compared to control. Since this response to postruminal protein addition has been previously reported it seems to offer realistic potential. Supplementation may not be necessary if forage intake could be sufficiently increased. It appears that grass, crop residues etc. will always be a less expensive source of nutrients than any presently known form of supplement. It also appears likely that the intake response is due to an endocrine mechanism.

In situ fermentation rates in trial 2 were higher than in trial 1. Significant fermentation occurred at least up to 72 h. I suggest that at least two forage samples in bags be incubated past 100 h. The time of day that these bags are removed from the rumen would not be important. It would not be practical to remove bags more frequently during the first 10 h of incubation though this would be necessary to calculate a lag time. Since rate of fermentation appeared to be constant the first 40 to 50 h of incubation the timing of bag removal probably is not important. Emphasis should be placed on preventing interruption of fermentation during bag removal. For example expose only the bag to be removed. It would also be easier to determine a period effect if all bags for all three periods contained the same forage.

Esophageal fistual collections are intended to provide a forage sample representative of that consumed by the herd. Thus it would be preferred to have fistulated animals differ only by the fact that they are fistulated. The biggest problem with our collections was that location of sampling was not always the same as the pasture area where the herd grazed.

Measurement of particulate dilution rate was limited in precision since Cr concentration of ruminal digesta became undetectable 48 h after dosing and sometimes earlier. We dosed with 100 g of mordant. I suggest a larger dose.

Precision of fluid dilution rate measurement was limited by the apparent slow equilibration of CoEDTA. If this was the problem, then dosing with an aqueous solution of CoEDTA should help to alleviate the problem.

Marker analysis by AA was made more difficult due to unstable air line pressure. The line pressure regulator on the tank should be replaced or frequent adjustments are necessary. Samples of fluid and fiber should be obtained prior to marker administration for construction of standards. This would apply also to fecal Cr analysis. For sample preparation a more convenient method of obtaining purified water would decrease lab time.

Measurement of ruminal ammonia using the specific ion electrode obviously has serious limitations since we discarded all such data for trial 2. I suggest verification of the technique by comparison to a proven one such as distillation as with macro-kjeldahl. Ruminal ammonia is one measurement that should be made frequently between times of supplementation. It could reveal that a supplement is or is not causing a response due to nitrogen availability for rumen microorganisms.

If all the experimental cows were bled two or three times per period it probably would be unneccessary to sample individual cows several times per day. It appears that diurnal variation in metabolite concentrations could be ignored if all animals were sampled at the same time

(Lindsay, 1978). Group differences can be maximized by sampling immediately pre-feeding (Coggins and Field, 1978). I also suggest that future studies are designed such that environmental stress in ensured. Cows should be sampled during warm and cold periods and in the absence and presence of snow cover. The ability of cows to maintain blood metabolite concentrations during stress may be more related to subsequent fertility than mean concentrations.

Concentrations of metabolites at calving could be better indicators of adequacy of prepartum nutrition than concentrations precalving. This appears to be true for albumin as an indicator of precalving protein adequacy (Rowlands, 1978).

Beta-hydroxybutyrate may be a better indication of the adequacy of glucogenic substrate than glucose (Lindsay, 1978; Russel, 1978). With hesitation, I suggest the use of plasma free fatty acids as an indicator of general energy status. These two metabolites, when interpreted together, may help delineate the type of any energy deficiency. Great care in preventing a sampling effect on free fatty acids would be necessary. This metabolite is readily influenced by animal excitement (Lindsay, 1978; Russel, 1978).

Fluid volume was not similar between treatments in this study. Therefore the significance of body weight change data could be questioned. Cows in SOY+BM gained more weight than those in SOY. However, Fluid volume in SOY+BM was

significantly greater. It may be important to fast cows more than one day prior to recording body weights. Another option would be to reweigh the cows one or two weeks after the the last time that supplements are fed.

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