Effects of Pregnancy on Digesta Kinetics and Ruminal Fermentation in Beef Cows[1]

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ABSTRACT: Four pregnant and four nonpregnant, ruminally cannulated beef cows were used to evaluate the effects of the last trimester of pregnancy on digesta kinetics and ruminal fermentation. Before breeding, cows were allotted randomly either to pregnant (PR) or to nonpregnant (NP) groups; PR cows were bred at the first estrus after synchronization. All cows were fed long-stem fescue hay at 15 g of DM/kg of BW based on BW 120 d before parturition of pregnant cows (average BW of 642 kg). Collection periods, each lasting 9 d, began at 96, 68, 41, and 10 ± 1.3 d before parturition. Particulate passage rate was greater ($P < .05$) and retention times and gastrointestinal fill were less ($P < .05$) for PR than for NP cows. Apparent total tract DM digestion, rate and extent (96-h in situ incubation) of NDF disappearance, and ruminal fluid kinetics were not affected ($P > .10$) by pregnancy. A physiological state × sampling day interaction was noted ($P < .05$) for ruminal NH_3 N, total VFA concentrations, and molar proportions of butyrate. Ruminal pH and individual VFA proportions, however, did not differ ($P > .10$) between PR and NP cows, except for valerate, which was less ($P < .05$) for PR than NP cows. Results suggest that in late pregnancy with restricted feeding, passage rate of particulates increases without substantial changes in fermentation or extent of digestion.

Key Words: Cattle, Rumen Digestion, Digesta Passage Rate

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Introduction

Female ruminants often decrease voluntary forage intake during the final weeks of pregnancy, which may result from compression of the rumen by the enlarging gravid uterus (Forbes, 1986). In sheep, particle and fluid passage increase, and proportions of ruminal branched-chain VFA decrease with advancing pregnancy (Coffey et al., 1989; Gunter et al., 1990). Conversely, in Angus × Hereford cows, Stanley et al. (1991) found that DMI increased from 65 to 24 d before calving, whereas ruminal fill decreased. These authors concluded that changes in ruminal fill do not account fully for changes in DMI during pregnancy. Our study was conducted to evaluate digesta kinetics and ruminal fermentation in limit-fed beef cows either nonpregnant or during the last trimester of pregnancy.

Experimental Procedures

Four trials were conducted with eight ruminally cannulated Angus or Red Angus crossbred, 3-yr-old beef cows. Cows were allotted randomly before breeding to either the pregnant (**PR**) or nonpregnant (**NP**) group. Estrus was synchronized in four cows in the pregnant group (SYNCHRO-MATE-B™, CEVA Laboratories, Overland Park, KS), after which these four cows were bred by natural service to a Hereford bull (72-h exposure after removal of the implant). Cows were determined to be pregnant by rectal palpation at 120 d before expected parturition. Based on actual calving dates, the gestation period was 271 ± 1.3 d. The remaining four cows served as nonpregnant controls. Except for the breeding period, all cows were housed together in a covered outdoor pen (27 m × 29 m) and were given ad libitum access to the same hay. Throughout the prebreeding, breeding, and gestational period, all cows had ad libitum access to water and trace mineral salt (guaranteed analysis

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[percentage of DM]: NaCl, 95; Fe, .20; Zn, .05; Cu, .04; Mn, .04; Mg, .04; and Co, .004). All surgical procedures and animal care were approved by the University Animal Care Committee and followed procedures outlined by the Consortium (1988).

At 120 d before parturition, all cows were weighed (average BW of 642 kg) and individually fed endophyte-free tall fescue hay daily at 15 g of DM/kg of BW until parturition by PR cows. The hay was fed in two equal amounts at 0700 and 1400 in separate Calan feeders (American Calan, Northwood, NH). To prevent orts and account for potential decreases in forage intake with advancing pregnancy, the quantity of hay fed during the collection period was 20% less than consumption during the previous 30 d. Calan gates were monitored twice daily to ensure exclusive access by the appropriate cow. Hay was grown under irrigation, harvested at boot-stage, and stored uncovered in small bales (approximately 30 kg/bale). Grab samples of fescue hay were obtained once weekly for analysis. Hay contained (percentage of DM): NDF, 51.0; ADF, 30.0; CP, 12.7; and ADIN, .22. Four collection periods, each lasting 9 d, were conducted at 96, 68, 41, and 10 ± 1.3 d before parturition by PR cows.

On d 1 of each trial at 0630, particulate passage rate was estimated using Yb as an external marker. Each cow was dosed with 140 g (DM) of Yb-labeled fescue hay (1.6 g of Yb) via the ruminal cannula. For all periods, hay that was labeled with Yb was obtained from weekly grab samples taken during the first adaptation period. Hay was labeled with $YbCl_3 \cdot 6H_2O$ according to procedures of Teeter et al. (1984). Rectal grab samples were taken before dosing and at 8, 12, 18, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96, 108, and 120 h after dosing.

On d 6 at 0630, 100-mL ruminal fluid samples were collected from the mid-ventral area of the rumen and cows were dosed with 200 mL of Co-EDTA (724.0 mg of Co; Uden et al., 1980). Mid-ventral ruminal samples were withdrawn by hand at 1, 3, 6, 9, 12, and 24 h after dosing. Immediately after withdrawal, ph was measured with a combination electrode, after which the sample was strained through four layers of cheesecloth, acidified with 1 mL of 7.2 *N* H_2SO_4/100 mL of strained fluid, and stored frozen (−40°C). Particulate matter retained on the cheesecloth was returned to the rumen after each sampling.

Also on d 6 at 0630, 12 polyester bags (10-cm × 20-cm; pore size, 53 ± 10 μm; Ankom, Spencerport, NY) that contained approximately 3 g of ground (2-mm screen) fescue hay obtained from grab samples during the initial adaptation period and six empty bags (one bag/incubation time) were suspended in the rumen for 0, 4, 8, 12, 24, 48, or 96 h. Upon removal, bags were washed under cold, running tap water until the rinse water was clear. Bags were then freeze-dried (Virtis Freeze Drier, Virtis, Gardner, NY), followed by drying for 24 h at 100°C in a forced-air oven. Contents of the bags were used to determine NDF in situ disappearance rates.

On d 9 of each collection period, immediately after removal of the last in situ bag, a blood sample (approximately 15 mL) was collected from each cow via jugular venipuncture. Blood samples were allowed to clot for 8 h at 0°C, centrifuged under refrigeration (4°C) at 2,300 × *g* for 15 min, and stored at −20°C.

Laboratory Analyses. Grab samples of fescue hay and fecal samples were dried in a forced-air oven at 60°C and ground to pass a 2-mm screen in a Wiley mill. Feed samples were analyzed for DM, ash, and Kjeldahl N (AOAC, 1984). Neutral detergent fiber and ADF were analyzed by nonsequential methods of Goering and Van Soest (1970). Acid detergent insoluble N was determined by Kjeldahl analysis of the ADF residue (Goering and Van Soest, 1970). Ytterbium was extracted from fecal samples by boiling in 20 mL of 3.1 *N* HCl with 1 mL of 10 *N* HNO_3. The acid extract of fecal samples was analyzed for Yb by atomic absorption spectroscopy with a nitrous oxide-plus-acetylene flame. To correct for background interference, standards and blanks were prepared using an extract from feces obtained before dosing. All samples contained 2,000 μg/mL of K as an ionization buffer.

All ruminal samples for fermentation analyses were thawed at room temperature and centrifuged at 10,000 × *g* under refrigeration (10°C) for 10 min. Cobalt concentration was analyzed by atomic absorption spectroscopy with an air-plus-acetylene flame, and ruminal NH_3 N concentrations were determined by the phenol-hypochlorite procedure of Broderick and Kang (1980). Volatile fatty acid concentrations in ruminal samples were quantified from the supernatant fractions as described by Goetsch and Galyean (1983), using 2-ethyl butyric acid as an internal standard.

Serum concentrations of progesterone and estradiol-17β ($\mathbf{E_2}$) were determined from blood samples obtained on d 9 of each collection period. Progesterone was determined using an ELISA technique (intraassay CV = 9.4%) outlined by Munro and Stabenfeldt (1984); E_2 was determined by a double-antibody, RIA technique (Diagnostic Products, Los Angeles, CA; intraassay CV = 9.2%). These two hormones were measured because they have been reported to influence gut motility (Forbes, 1986).

Calculations and Statistical Analyses. Fecal Yb extraction curves were fitted to a one-compartment model (Pond et al., 1988) using the nonlinear regression option of SAS (1987). Particulate passage rate, retention time, gastrointestinal fill, and fecal output were estimated using the parameter estimates from the one-compartment model (Krysl et al., 1988). Gastrointestinal fill was expressed as grams/kilogram of BW, based on BW at 120 d before parturition of PR cows. Measured DMI and estimated fecal output were used to calculate apparent DM digestion. Rate of NDF in situ disappearance was calculated from polyester

Table 1. Serum progesterone and estradiol-17β concentrations, particulate digesta kinetics, apparent total tract dry matter (DM) digestion, and in situ rate and extent of neutral detergent fiber (NDF) disappearance in pregnant and nonpregnant beef cows

Item	Nonpregnant	Pregnant	SE[a]
Progesterone, ng/mL*	1.7	3.2	.3
Estradiol-17β, pg/mL[b]	13.5	109.2	21.0
Particulate digesta kinetics			
Passage rate, %/h*	3.46	3.92	.07
Gastrointestinal mean retention time, h*	54.5	48.2	.5
Ruminal retention time, h*	35.0	30.6	.6
Intestinal transit time, h*	19.5	17.6	.6
Gastrointestinal fill, g/kg of BW[c]*	8.2	7.1	.3
Apparent DM digestion, %	52.1	52.9	2.0
In situ rate of NDF disappearance %/h	5.76	4.87	.74
In situ extent of NDF disappearance, %[d]	66.0	65.9	.9

[a]n = 16.
[b]Physiological state × sampling day interaction ($P < .05$).
[c]Based on BW at 120 d before parturition of pregnant cows.
[d]96-h in situ incubation.
*Row means differ ($P < .05$).

bags as described by Mertens and Loften (1980) using SAS (1987) nonlinear regression procedures. The model included a coefficient for lag time, but lag time was not included in the statistical analysis. Fluid passage and volume estimates were calculated by logarithmic regression of Co concentration against time after dosing (Uden et al., 1980). Particulate and fluid kinetic estimates, apparent DM digestion, rate and extent of NDF in situ disappearance, and serum progesterone and E_2 data were analyzed as a split-plot design with physiological state (PR vs NP) as the main-plot treatment and sampling day as the subplot treatment. Physiological state was tested against cow within treatment as an error term (Error A). The interaction of sampling day × physiological state and the main effect of sampling day were tested against residual error (Gill, 1986) using the GLM procedure of SAS (1987). Ruminal fermentation data were analyzed as a split-split-plot design. Sub-subplot effects included sampling time (main effect) and the two- and three-way interactions of sampling time with the other main effects, tested against residual error. No dependent variables analyzed were affected ($P > .10$) by interactions involving sampling time.

Results and Discussion

A physiological state × sampling day interaction was not detected ($P > .10$; Table 1) for serum progesterone concentration. Serum progesterone concentrations were greater ($P < .05$) in PR cows (3.2 ng/mL) than in NP cows (1.7 ng/mL). In contrast, serum E_2 concentration was affected ($P < .05$; Table 2) by a physiological state × sampling day interaction. At 10 d before parturition, pregnant cows (307.9 pg/mL) had greater ($P < .05$) serum E_2 concentrations than NP

Table 2. Effects of physiological state and day of pregnancy on serum estradiol-17β concentrations and ruminal volatile fatty acid (VFA) concentrations, molar proportions of butyrate, and ammonia concentrations in beef cows

Item	Nonpregnant	Pregnant	SE[a]
Serum estradiol-17β, pg/mL			
Day[b]			
96	15.8	42.3[d]	5.44
68	10.8	37.5[d]	5.47
41	13.6	49.0[d]	7.82
10*	13.8	307.9[e]	89.10
SE[d]	1.00	41.14	—
Total VFA, m*M*			
Day			
96	105.1[e]	110.4[d]	3.97
68*	114.7[d]	106.6[de]	3.37
41*	93.2[f]	100.0[e]	3.77
10	108.8[de]	105.6[de]	2.89
SE	2.49	2.60	—
Butyrate, mol/100 mol[c]			
Day			
96*	10.6[d]	11.2[d]	.25
68	10.2[de]	9.9[e]	.23
41	10.0[e]	9.9[e]	.24
10*	10.0[e]	9.6[e]	.17
SE	.16	.16	—
Ammonia N, mg/dL			
Day			
96	7.7[d]	7.9[d]	.47
68	5.6[e]	6.4[e]	.43
41	5.6[e]	6.1[e]	.48
10*	8.0[d]	6.9[e]	.55
SE	.35	.35	—

[a]n = 24 for all data, except for estradiol-17β, where n = 4.
[b]Day before average parturition of pregnant cows.
[c]Moles of butyrate/100 mol of total VFA.
[d,e,f]Column means within each item that do not have common superscripts differ ($P < .05$).
*Row means differ ($P < .05$).

cows (13.8 pg/mL). Within PR cows, serum E_2 concentrations did not differ ($P > .10$) among sampling days except at 10 d prepartum, when serum E_2 concentrations were greater ($P < .05$) than on previous sampling days. No differences in serum E_2 were noted ($P > .10$) among sampling days for NP cows. Both progesterone and E_2 concentrations in NP cows were indicative of the preovulatory concentrations associated with estrus in the cow (Pineda, 1989). The increased E_2 concentrations for NP cows can be attributed directly to high E_2 concentrations in one or two cows that exhibited estrous behavior during each collection period. Both progesterone and estrogens may have effects on voluntary intake by pregnant ruminants (Forbes, 1986). Estradiol 17-β infusions at amounts similar to daily secretion during pregnancy have caused a dose-dependent decrease in voluntary feed intake (Forbes, 1971) as a result of a direct effect on the ventro-medial hypothalamus (Baile and Forbes, 1974). Nonetheless, this decrease in DMI can be blocked if exogenous progesterone is administered (Muir et al., 1972).

Particulate passage rate (Table 1) was greater ($P < .05$) for PR than for NP cows, which agrees with results for pregnant sheep given ad libitum access to feed (Coffey et al., 1989) and for limit-fed, pregnant sheep (Faichney and White, 1988a; Gunter et al., 1990). Gastrointestinal mean retention time (**GMRT**), ruminal retention time (**RRT**), and intestinal transit time (**ITT**) were less ($P < .05$) in PR than in NP cows. Faichney and White (1988b) noted that with limit-fed ewes, total tract retention time was shorter as a result of decreased ruminal retention time, even though intestinal retention time increased as pregnancy progressed. Similarly, Coffey et al. (1989) and Gunter et al. (1990) reported decreased GMRT in pregnant vs nonpregnant ewes; however, the decrease was a result of a shorter RRT and unchanged ITT.

Pregnant cows had less ($P < .05$) gastrointestinal fill (Table 1) than NP cows. Stanley et al. (1991), using pregnant beef cows given ad libitum access to feed, reported a decrease in ruminal fill between 65 and 24 d prepartum. In our study, gastrointestinal fill (grams/kilogram of BW) for PR cows was 6.9, 7.8, 6.5, and 7.3, whereas gastrointestinal fill was 8.1, 8.0, 8.2, and 8.7 for NP cows (d 96, 68, 41, and 10 before parturition of PR cows, respectively). Different results for gastrointestinal fill with advancing pregnancy between our study and that of Stanley et al. (1991) may be the result of differences in feeding schemes (limit-fed vs ad libitum, respectively) and sampling methodology. Stanley et al. (1991) used ruminal evacuation to estimate ruminal fill, whereas we estimated total tract fill using the one-compartment model.

Greater particulate passage rate, and decreased ruminal retention times and gastrointestinal fill in PR than in NP cows, combined with no change in feed intake, suggest an increase in ruminal propulsitory activity. Displacement of the gastrointestinal tract by the enlarging gravid uterus may be a primary factor involved in decreasing ruminal capacity and, subsequently, ruminal particulate fill (Forbes, 1986; Stanley et al., 1991), but decreased ruminal capacity with no change in feed intake would require greater ruminal emptying, which could lead to the increased particulate passage rates. Pressure in the reticulum, reticuloruminal fold, rumen, omasum, and abomasum can stimulate motility; however, gross distention in these areas and in the duodenum can inhibit contractions (Baile and Forbes, 1974). Gastrointestinal fill has been one factor considered to control intake and to be limited by particulate passage, rather than controlling particulate passage. Therefore, other factors, such as circulating concentrations of estrogen and progesterone, may be responsible for altered particulate passage rate during late pregnancy. Forbes (1986) reviewed the literature regarding the effects of sex hormones on voluntary intake and noted that decreased ruminal retention time of particles during the last trimester of pregnancy probably resulted from high circulating concentrations of E_2. Furthermore, both E_2 and progesterone increase gut motility in nonpregnant cattle and sheep (Forbes, 1986), and, in our study, both these hormones exhibited greater concentrations in PR than in NP cows.

Apparent total tract DM digestion and rate and extent (96-h in situ incubation) of NDF disappearance were not influenced ($P > .10$ by physiological state. Gunter et al. (1990), with ewes limit-fed chopped alfalfa hay, also reported no change in apparent total tract DM digestion with advancing pregnancy. Faichney and White (1988b) and Coffey et al. (1989), studying ewes fed forage and concentrate diets, respectively, noted a small, but nonsignificant, decrease in DM digestibility as pregnancy progressed.

Ruminal fluid dilution rate and volume did not differ ($P > .10$; Table 3) between PR and NP cows; this finding agrees with the results of other research with pregnant beef cows (Stanley et al., 1991). In sheep, however, faster fluid passage and shorter retention times (Faichney and White, 1988a) have been associated with greater water consumption during pregnancy; water intake data were not collected for our study.

Ruminal NH_3 N concentration, total VFA concentration, and molar proportions of butyrate (Table 3) were affected ($P < .05$) by a physiological state × sampling day interaction. Ruminal NH_3 N did not differ ($P > .10$) between PR and NP cows until 10 d before parturition of PR cows, when PR cows (6.9 mg/dL) had lower ($P < .05$) concentrations than did NP cows (8.0 mg/dL). Total VFA concentration was less ($P < .05$; Table 3) in PR than in NP cows at 68 d; however, this relationship was reversed at 41 d. Molar proportions of butyrate were greater ($P < .05$) in PR cows at 96 and 10 d before parturition of PR cows. In

Table 3. Ruminal pH, ammonia N (NH_3 N) concentrations, volatile fatty acid (VFA) concentrations, and fluid kinetics in pregnant and nonpregnant beef cows

Item	Nonpregnant	Pregnant	SE[a]
Ruminal fluid			
Dilution rate, %/h	10.2	10.1	.5
Fluid volume, L	53.3	47.1	3.3
Ruminal pH	6.4	6.4	.04
Ruminal NH_3 N, mg/dL[b]	6.7	6.8	.29
Ruminal total VFA, m*M*[b]	108.5	102.6	1.6
	mol/100 mol		
Acetate	68.1	68.3	.3
Propionate	18.8	18.7	.1
Isobutyrate	.8	.8	.03
Butyrate[b]	10.2	10.2	.2
Isovalerate	1.0	1.0	.05
Valerate*	1.1	1.0	.03

[a]n = 24 for all data, except for fluid kinetics, where n = 16.
[b]Physiological state × sampling day interaction ($P < .05$).
*Row means differ ($P < .05$).

both PR and NP cows, these measures of ruminal fermentation were variable among collection days. Furthermore, differences noted between PR and NP cows and collection days, although significant, were small and perhaps not biologically important. Faichney and White (1988b) reported a decrease in these fermentation variables as pregnancy progressed, but only ruminal NH_3 N has been shown to differ between pregnant and nonpregnant ruminants (Gunter et al., 1990). Large variation among ewes may have precluded a sensitive test of the effects of pregnancy on total VFA concentrations in the study of Gunter et al. (1990). Decreased NH_3 N and VFA concentrations can result from increased fluid passage rate; both have been reported to be negatively correlated with fluid dilution rate (Prigge et al., 1984). However, fluid passage was not altered in our study. Alternatively, use of amino acids by fetal tissue and VFA by maternal tissue may decrease the supply of N for recycling and increase the osmotic gradient for VFA absorption, thereby accounting for decreases in ammonia and VFA concentrations (Faichney and White, 1988a).

Pregnancy did not alter ($P > .10$; Table 3) ruminal pH, and other than the interaction for butyrate, molar proportions of any VFA, except for valerate. Nonetheless, differences in valerate ($P < .05$) were small and probably of limited biological importance. Faichney and White (1988b) and Gunter et al. (1990) reported no differences in VFA proportions as a result of pregnancy in ewes.

Implications

During the third trimester of pregnancy in cows, even with feed intake held constant, digesta passage rate increased and ruminal retention time decreased without affecting dry mater digestion. These changes in digesta kinetics with advancing pregnancy in beef cows seem to be related to changes in circulating estradiol and progesterone concentrations.

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