Effect of Protein Level in Prepartum Diets on Metabolism and Performance of Dairy Cows¹

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ABSTRACT

Multigravid Holstein cows ($n = 75$) were used in a randomized block design to evaluate the effect of prepartum diets formulated to supply surplus energy and incremental concentrations of protein on the nutritional status of dairy cows at parturition. Cows were blocked according to expected calving date and assigned to one of five diets: 9.7, 11.7, 13.7, 14.7, and 16.2% crude protein (CP). Dietary treatments were initiated 28 d before expected calving date and fed until parturition. A common diet was fed postpartum. Dry matter intake and milk yield were recorded daily through 90 d postpartum. Increasing the protein concentration from 9.7 to 14.7% of dry matter during the last 28 d of gestation improved responses of cows during lactation. Increasing dietary protein up to 14.7% also increased milk yield response to recombinant bovine somatotropin (rbST) during the ninth week of lactation and yields of 305-d 2 $\times$ mature equivalent milk, milk protein, and milk fat. Plasma aspartate aminotransferase tended to be highest in cows fed 13.7 and 14.7% CP prepartum, but decreased linearly postpartum in response to dietary protein levels. There were no treatment differences for plasma insulin-like growth factor-1 (IGF-1) at d 60 postpartum (before rbST provision), but IGF-1 on d 90 (after rbST provision) was higher in plasma of cows fed 14.7% CP than the other diets except 13.7% CP. Close-up diets containing 13.7% CP and surplus energy produced the most beneficial outcomes during the subsequent lactation.

(**Key words:** periparturient, protein, body condition, prepartum)

Abbreviation key: AST = plasma aspartate aminotransferase, EB = energy balance, ECM = energy-cor-

rected milk yield, MUN = milk urea nitrogen, PME = previous 305-d 2 $\times$ mature equivalent milk yield, PUN = plasma urea nitrogen.

INTRODUCTION

The periparturient period (21 d prepartum to 28 d postpartum) may be the most critical time in a dairy cow's production cycle. This period is characterized by rapid fetal growth (Eley et al., 1978; Bell et al., 1995; McNeill et al., 1997), metabolic transitions to support the ensuing lactation (Bauman and Currie, 1980), lactogenesis (Capuco et al., 1997), and ruminal adaptations to a change in diet. Improving the nutrition of the cow during this period may reduce prepartum tissue mobilization and enhance health, DMI, and milk production.

An increase in tissue reserves of the cow during the periparturient period is difficult to achieve because DMI is typically depressed during the last week before calving (Bertics et al., 1992). However, the critical issue relative to enhancing the tissue reserves at parturition is actual nutrient delivery, which is a function of DMI, nutrient density of the diet, and efficiency of nutrient utilization. Nutrient delivery becomes complicated in the transition cow because of the intake depression, which may be accompanied by a decrease in rumen volume (Forbes, 1968) and an increase in rate of passage, as observed in the beef cow (Stanley et al., 1993). The depression in DMI may not be a critical issue if nutrient delivery to the cow can be achieved by increasing the nutrient density of the diet and total digestive tract efficiency.

NRC (1989) recommendations for the dry cow do not suggest increasing the concentration of dietary nutrients as the cow approaches parturition, but it is common practice to introduce feedstuffs common to the lactation diet into the dry cow diet 14 to 21 d before parturition. This practice increases the energy and protein densities of the diet in an effort to improve the tissue reserves of the cow and acclimate the rumen microbial population as currently recommended by the NRC

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(2001). Grummer (1995) suggested that the energy content, particularly rumen soluble carbohydrates, of prepartum diets is more important than the protein content. Recent research (Wu et al., 1997; Putnam and Varga, 1998; Greenfield et al., 2000) has not demonstrated benefits postpartum from increasing protein in prepartum diets above NRC (1989) recommendations. All of these studies utilized energy levels above NRC (1989) recommendations; however, Putnam and Varga (1998) used energy levels considerably higher than the other two (1.72 vs. 1.5 Mcal/kg of NE_L). Putnam and Varga (1998) reported an increase in nitrogen retention in late-gestation dairy cows in response to higher levels of dietary protein, but milk production was unaffected; however, prepartum intake was held constant. It is possible that postpartum benefits occur when protein is increased in prepartum diets that contain surplus energy (above 1.7 Mcal/kg of NE_L) and are provided ad libitum.

The purpose of our study was to determine whether increasing dietary protein in prepartum diets high in energy would enhance the nutritional status or milk production of dairy cows.

MATERIALS AND METHODS

Cows and Treatments

Seventy-five multiparous Holstein cows were blocked by projected calving date and assigned to treatment groups that were balanced for BW, body condition, and previous lactation milk yield. The Cornell Net Carbohydrate and Protein System model 3.0 (Fox et al., 1992) was used to evaluate all diets on the basis of protein and to provide energy (1.54 Mcal/kg of NE_L) above NRC (1989) recommendations for nonlactating, pregnant dairy cows. The experimental diets (Table 1) were: 9.7% CP (negative control that supplied less RDP and RUP than the cow's needs); 11.7% CP [NRC (1989) recommendations]; 13.7% CP (supplied sufficient RDP without additional RUP); 14.7% CP (same RDP as 13.7% but additional RUP); and 16.2% CP (same RDP as 13.7% but higher RUP than 14.7% CP). The experimental diets consisted of alfalfa hay, prairie hay, corn silage, common grain mix, and a top dress respective to dietary treatment. Corn grain was replaced by soybean meal to increase dietary CP. Once ruminal protein needs had been met (13.7% CP diets), expeller soybean meal (Soyplus, West Central Soy, Ralston, IA) was used to replace corn and (or) solvent soybean meal. Treatments were initiated 28 d before projected calving date and terminated at parturition. Cows not on treatment for at least 14 d before parturition were excluded from the dataset and replaced as soon as possible. Cows were fed a common diet postpartum (Table 1).

This study was conducted from late June of 1998 until early June of 1999. Cows were housed in a tie-stall facility at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) during the prepartum treatment period and the first 90 d postpartum, then moved to a free-stall facility for the remainder of their lactation. Environmental conditions in the tie-stall barn were controlled through the use of an air conditioner and heater. From dry-off until initiation of treatments, all cows were fed a common diet (DM basis) consisting of 9 kg/d of corn silage (same corn silage as used in study, harvested with a John Deere silage chopper equipped with a processor), 4.5 kg/d of a 12% CP grain mix fortified with minerals and vitamins, and free choice prairie grass hay. Cows were housed prior to treatment in either a freestall facility or on a limited brome/fescue hay pasture, depending on the season. All cows received the common diet referred to previously, regardless of housing.

Experimental diets were fed as TMR and available ad libitum by cows and allowed for 10%orts. Forages were processed in a Rota-Mix feed wagon with a particle length of 1.9 to 4.0 cm. Feed was mixed and offered once daily prepartum and twice daily during lactation. Feeding times were 0500 and 1700 h. Daily feed intakes for individual cows were recorded until d 90 of lactation. Normal herd management practices were followed during the experiment. Recombinant bST (Posilac, Monsanto, St. Louis, MO) was administered to all cows on d 63 ± 6 of lactation and every 2 wk thereafter. The Kansas State University Institutional Animal Care and Use Committee approved all practices.

Sampling and Routine Analysis

Forage samples were collected weekly and composited monthly for analysis. Grain mixes were sampled by batch and composited monthly for analysis. Samples were frozen until analyzed by Dairy One (Ithaca, NY). Nonfiber carbohydrate fraction was calculated as $(100\% - CP\% - NDF\% - fat\% - ash\%)$. Values for TDN were calculated according to Weiss et al. (1992). For concentrates, TMR, and byproducts, the NE_L was calculated using the NRC (1989) dairy equations, and forage NE_L was calculated using the approach of Van Soest and Fox (1992). Each respective NE_L was calculated at 3 $\times$ maintenance. Silage samples were analyzed weekly for DM content, and the amount fed was adjusted accordingly.

The same individual scored condition of cows weekly throughout the study (1 to 5 scale in increments of 0.25; Wildman et al., 1982). Consecutive 2-d BW measurements were obtained weekly in the a.m. during the prepartum phase and immediately after the a.m. milk-

Table 1. Experimental diets.

Item	Prepartum diet, % CP					Postpartum
	9.7	11.7	13.7	14.7	16.2	
	% of DM					
Alfalfa hay	15.00	15.00	15.00	15.00	15.00	28.35
Prairie grass hay	20.00	20.00	20.00	20.00	20.00	...
Corn silage, processed	30.00	30.00	30.00	30.00	30.00	20.49
Corn grain, ground ¹	32.42	27.74	23.06	18.70	12.69	28.15
Whole cottonseed ²	...	...	...	...	...	9.56
Soybean meal, 48% CP	...	4.68	9.36	4.36	...	2.94
Expeller soybean meal ³	...	...	...	9.36	19.73	...
Expeller soybean meal ⁴	...	...	...	...	...	7.85
Limestone	0.60	0.60	0.60	0.60	0.60	1.00
Dicalcium phosphate	0.74	0.74	0.74	0.74	0.74	0.67
Sodium bicarbonate	...	...	...	...	...	0.48
Trace mineral salt ⁵	0.50	0.50	0.50	0.50	0.50	0.24
Magnesium oxide	0.50	0.50	0.50	0.50	0.50	0.17
Vitamin A, D, E ⁶	0.20	0.20	0.20	0.20	0.20	0.09
Sodium selenite premix ⁷	0.04	0.04	0.04	0.04	0.04	0.01

¹Particle size approximately 700 microns.²Linted.³Soyplus (West Central Soya, Ralston, IA).⁴Soybest (Grain States Soya, West Point, NE).⁵Composition: not less than 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, and 0.004% Co.⁶Contributed 4912 IU of vitamin A, 2358 IU of vitamin D, and 24 IU of vitamin E per kg of diet DM.⁷Contributed 0.06 mg of Se per kg of diet DM.

ing during the first 90 d postpartum. Cows and calves were weighed approximately 6 h after parturition, and BW loss due to calving was calculated as the average of the most recent 2-d weights prepartum minus post-calving weight. Calving difficulty was scored on a five-point scale (1 = no problem; 2 = slight problem, 3 = needed assistance, 4 = considerable force, 5 = caesarian). Health observations were recorded daily.

Energy balance (EB) during the 28-d prepartum period (Table 2) was estimated using the equation for lactating cows (Smith et al., 1997). By substituting gravid uterus accretion energy requirements (Bell et al., 1995) for lactational energy requirements and including an estimate (VandeHaar et al., 1999) of energy required for mammary development during the last 21-d prepartum [$EB = NE_L \text{ intake} - (0.08 * BW^{0.75} + 40$

kcal/kg of $BW^{0.75} + 2 \text{ Mcal}]$ an equation for calculating EB for dry cows was obtained.

Blood samples were taken from the coccygeal vein on d 28, 21, 10, 5, 3, and 1 before projected calving date and d 3, 7, 15, 20, 25, 60, and 90 postpartum at 0500 h. Five cows per treatment were used to determine plasma aspartate aminotransferase activity in samples obtained on d 10, 5, 3, and 1 prepartum and d 3, 7, 15, 20, 25, 60, and 90 postpartum. Blood was collected into EDTA-containing Vacutainer tubes, which were placed in an ice bath until blood was processed. Tubes were centrifuged at $500 \times g$ for 20 min. Plasma was removed, and 3-ml aliquots were stored frozen at -20°C . Urine samples were obtained on the same days and time as blood into a 40-ml plastic container and frozen. Ketones were determined daily in fresh urine from d 10 prepartum.

Table 2. Incidence of health disorders.

Health disorders	Prepartum diet, % CP				
	9.7	11.7	13.7	14.7	16.2
Subclinical ketosis	1	3	1	0	4
Displaced abomasum	0	0	0	0	0
Retained placenta	1	0	0	1	0
Lameness	13	8	10	8	13
Mastitis	2	1	2	0	5
Fatty liver	0	0	0	0	1
Milk fever	0	1	2	0	0
Subclinical milk fever	4	3	0	1	4

tum to d 28 postpartum using Keto-stix reagent strips sensitive to acetoacetic acid (Bayer Corporation Diagnostics Division, Elkart, IN).

Udder edema was scored daily from d 28 prepartum to d 21 postpartum at the same time of day as blood and urine collections. A four-point scale was used to assess edema (1 = no edema; 2 = edema seen but not sensed through palpation, 3 = edema seen and sensed through palpation; 4 = edema present on the caudal ventral abdominal wall, udder swollen, and hard).

Cows were milked twice daily at 0530 and 1630 h. Daily milk production was measured during the first 90 d postpartum, and milk samples (a.m. and p.m. composites) were obtained weekly. Milk samples were analyzed for protein, fat, lactose, SNF, urea nitrogen (MUN), and SCC by the DHI laboratory, Manhattan, Kansas. Milk fat, protein, and lactose were determined by near-infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska MN). MUN was determined by chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN) and SCC by flow cytometry (Somacount 500, Bentley Industries). Routine DHI monthly milk weights and composition (a.m., p.m. samples) were used to assess performance from 90 d postpartum until the end of lactation. Lactations were standardized to 305-2X-ME.

Laboratory Analyses

Plasma was analyzed for aspartate aminotransferase by a colorimetric assay described by Babson et al. (1962). Triacylglycerols were determined with a colorimetric assay (Infinity Triglycerides Reagent procedure number 343; Sigma Diagnostics, St. Louis, MO). A Technicon auto analyzer II (Technicon Industrial Systems, Tarrytown, NY) was used to measure plasma glucose, plasma urea nitrogen (PUN), total α -amino N in plasma, and urinary creatinine. Plasma glucose was determined by a peroxidase indicator reaction with glucose oxidase (Technicon industrial method number SE-4-0036FJ4). Plasma urea nitrogen was measured by a diacetyl-monozime assay (Technicon industrial method 339-01). Total plasma α -amino N was determined by a trinitrobenzenesulfonic acid assay (Technicon industrial method 512-77T). Urinary creatinine was measured by a picric acid assay (Technicon industrial method 339-11). A microcentrifugal analyzer was used for an enzymatic determination of D(-)- β -hydroxybutyric acid and acetoacetic acid in plasma (Williamson et al., 1962). Before plasma IGF-1 analysis, IGF-1 was extracted from insulin-like growth factor binding protein by formic acid-ethanol extraction (Bruce et al., 1991). Concentration of the extracted IGF-1 was mea-

sured by radioimmunoassay using anti-human IGF-1 polyclonal antiserum (rabbit) and recombinant human IGF-1 as the standard (GroPeP, North Adelaide, Australia). Recombinant human IGF-1 was iodinated with ^{125}I (Amersham Pharmacia Biotech Inc., Piscataway, NJ) using pre-coated IODO-GEN tubes (Pierce, Rockford, IL). Briefly, for the radioimmunoassay, standard or samples were incubated overnight at 4°C with IGF-1 polyclonal antiserum at a dilution of 1 to 150,000 and iodinated IGF-1. During the next day, killed *Staphylococcus aureus* protein A-positive cells (Roche, Indianapolis, IN) were added to the assay tubes (0.5 mg/tube) and incubated for 4 h at room temperature to precipitate the antibody-IGF-1 complex. Each tube was given 2 ml of assay buffer and centrifuged for 30 min at $2230 \times g$ and 4°C. Assay buffer contained 30 mM sodium phosphate, 10 mM EDTA, 0.02% protamine sulfate, 0.02% Na azide, and 0.05% Tween-20 at pH 7.5. After centrifugation, the supernatant was decanted, and the pellets were counted in a gamma counter (TM Analytic, Inc., Tampa, FL). The intraassay and interassay coefficients of variation for the IGF-1 radioimmunoassay were 8 and 18%, respectively. Incubation blocks were nested within assay.

Statistical Analyses

The trial was a randomized block design with incomplete blocks based on expected calving date. Nineteen blocks with an average size of four cows were utilized due to a limited availability of cows with close expected calving dates. Fifteen cows were assigned to each treatment; treatments were balanced for BCS, BW, and previous 305-d $2\times$ mature equivalent milk yield (PME). Dry matter intake and milk production data collected the day of calving were not included in the dataset because of the inherent problems associated with data collected on the day of calving. Data for statistical analyses started at 28 d before actual calving date. Analysis of variance was conducted on weekly data using SAS (1990); however, data presented in tabular form are averages based on either the late-gestation or early-lactation period. All data were analyzed as a randomized incomplete block design using the mixed procedure of SAS (Littell et al., 1996) with a split-plot analysis for variables measured over time. For variables not measured over time, the model included covariates (see below) and diet, with block being included as a random effect. For models with measures over time, the model statement included covariates (see below), diet, time, and diet $\times$ time. Block and diet $\times$ block were included as random effects. The method of Satterthwaite was used for calculation of denominator degrees of freedom for *F*-tests. Initial cow characteristics (BCS, BW, and

Table 3. Chemical characteristics of experimental diets.

Item	Prepartum diet, % CP					Postpartum
	9.7	11.7	13.7	14.7	16.2	
CP, %	9.73	11.68	13.73	14.68	16.20	18.17
RDP, % of DM ¹	5.36	6.49	7.92	8.05	8.94	10.27
RUP, % of DM ¹	4.37	5.20	5.82	6.62	7.26	7.90
ADF, %	23.3	23.6	23.4	24.2	24.7	19.9
NDF, %	36.6	37.3	37.2	38.1	39.5	30.9
Nonfiber carbohydrate, %	42.0	40.7	37.6	35.0	31.4	37.8
NE _L , Mcal/kg	1.57	1.59	1.54	1.54	1.54	1.72
Crude fat, %	3.1	3.5	3.3	3.6	3.9	5.8
Ash, %	6.2	6.5	6.6	6.8	7.0	7.9
Calcium, %	0.42	0.43	0.45	0.46	0.46	0.66
Phosphorus, %	0.34	0.38	0.40	0.41	0.41	0.53
Magnesium, %	0.32	0.33	0.34	0.35	0.35	0.31
Potassium, %	1.18	1.28	1.37	1.42	1.48	1.57
Sodium, %	0.10	0.10	0.11	0.12	0.12	0.31
Chlorine, %	0.32	0.32	0.32	0.32	0.32	0.53
Sulfur, %	0.13	0.14	0.16	0.16	0.17	0.20
DCAD, meq/kg ²	173	195	208	225	234	261

¹Based on feed analysis from DHI Forage Testing Laboratory (Ithaca, NY) using a *Streptomyces griseus* protease technique.

²Dietary cation anion difference: (Na + K) – (Cl + S).

PME) and interactions between initial cow characteristics and diet were included as covariates for all datasets except initial characteristics. Covariates were dropped from the model one at a time, starting with the least significant and continuing until all remaining covariates were significant ($P < 0.05$). Data are presented as least square means. Contrast statements for linear, quadratic, cubic, and quartic effects (based on unequal spacing of dietary protein level) were used to delineate responses to dietary protein. Prepartum and postpartum data were analyzed separately. Statistical significance was set at $P < 0.05$, and trends were noted at $P < 0.15$.

RESULTS

General Observations

Experimental diets (Table 1) were formulated to contain 1.50 Mcal/kg of NE_L DM (Table 3). Chemical characteristics of individual feed components and top-dress components are listed in Tables 4 and 5. Forage quality was higher than expected, thus diets averaged 1.56 Mcal/kg DM (range 1.54 to 1.59). The higher NE_L content of the diets satisfied our original intent because they provided more energy than recommended by NRC (1989). The diets varied in NFC content [100 – (NDF + fat + protein + ash)] from 42% for the 9.7% CP diet to 31% for the 16.2% CP diet because soybean meal was substituted for corn to achieve incremental amounts of dietary protein (Table 1). The amount of fat increased as the amount of expeller soybean meal increased in

the diet and ranged from 3.1% in the 9.7% CP diet to 3.9% in the 16.2% CP diet.

Cows used in the study are characterized in Table 6. Characteristics were similar among treatments, except those fed diets 13.7 and 14.7% CP tended to be younger than cows fed 9.7, 11.7, and 16.2% CP diets. Also, cows fed 13.7% CP had a shorter calving interval and fewer DIM during the previous lactation than those fed the other diets. The intent of this study was to initiate the experimental diets to cows 28 d before projected calving date, but variation in actual calving date resulted in treatments being fed an average of 31.7 d prepartum (Table 6).

Parturitional Events

A significant cubic effect ($P < 0.05$) was observed for calving difficulty score (Table 7). Cows fed 9.7 and 11.7% CP had higher calving difficulty scores than cows fed the other diets. Cows fed the low protein diets may have been limited in nutrients available to support calving. Calf birth weights were similar among treatments (mean = 41 kg) and were not considered a deciding factor in calving difficulty. Placental weights were not measured.

Health Observations

Health disorders were recorded daily and reported as the number of incidents observed (Table 2). In all cases except lameness, the reported number of incidents represents the number of cows observed with

Table 4. Individual chemical characteristics of experimental diets.

Item	Prairie hay	Alfalfa hay	Corn silage	Cottonseed	Dry grain mix ¹	Lact grain mix ²
DM, %	92.8	90.5	34.7	91.7	89.0	88.7
CP, %	5.58	21.26	9.21	26.85	8.62	18.5
Soluble protein, %	21.0	33.5	55.1	32.5	25.0	16.6
RDP, % of DM ³	2.7	14.0	6.8	12.8	2.8	8.0
RUP, % of DM ³	2.8	7.2	2.3	13.9	5.8	10.5
ADF, %	47.5	30.2	28.4	32.7	3.1	5.7
NDF, %	70.5	39.8	46.8	45.4	9.0	13.7
Nonfiber carbohydrate, %	13.5	26.1	34.9	5.2	72.0	54.6
NE _L , Mcal/kg	1.21	1.34	1.54	2.29	2.02	1.92
Crude fat, %	2.75	2.21	3.09	24.95	6.0	5.16
Ash, %	4.69	10.48	5.96	4.6	9.0	7.9
Calcium, %	0.55	1.64	0.38	0.17	1.54	1.3
Phosphorus, %	0.01	0.28	0.30	0.71	0.99	0.78
Magnesium, %	0.16	0.22	0.16	0.39	1.22	0.43
Potassium, %	1.03	2.61	1.57	1.1	0.36	0.97
Sodium, %	0.011	0.023	0.008	0.0045	0.61	0.72
Chlorine, %	0.22	0.21	0.05	0.07	1.41	0.54
Sulfur, %	0.12	0.25	0.11	0.26	0.12	0.21

¹Grain mix in prepartum diets.²Grain mix in postpartum diets.³Based on feed analysis from DHI Forage Testing Laboratory (Ithaca, NY) using a *Streptomyces griseus* protease technique.

a particular disorder. In the case of lameness, a new incidence was recorded for the same cow if she appeared normal for 7 d between incidents. It is difficult to associate the incidence of lameness with the experimental diets because the affected cows experienced mild clinical laminitis during the previous lactation. Regardless,

no difference was observed among treatments, and it is unlikely that lameness affected the impact of the experimental diets on performance traits.

The incidence of subclinical ketosis (Keto-stix value > 80 mg/dl) was 7, 20, 7, 0, and 27% for diets 9.7, 11.7, 13.7, 14.7, and 16.2% CP, respectively. No clinical cases

Table 5. Individual chemical characteristics of dietary treatments.

Item	Topdress ¹				
	9.7% CP	11.7% CP	13.7% CP	14.7% CP	16.2% CP
DM, %	87.0	88.1	88.5	88.9	89.8
CP, %	7.7	17.6	30	35.9	44.6
Soluble protein, %	18.0	20	18	12	8
RDP, % of DM ²	3.39	7.57	17.1	15.08	19.6
RUP, % of DM ²	4.31	10.03	12.9	20.8	25
ADF, %	2.7	4.1	3.1	7.7	10.5
NDF, %	6.6	9.7	9.5	14.7	23.1
Nonfiber carbohydrate, %	80.1	67.3	53.4	39.4	18.9
NE _L , Mcal/kg	2.09	2.07	1.92	1.94	1.92
Crude fat, %	2.0	3.9	3.0	4.8	6.8
Ash, %	2.0	3.2	4.11	5.19	6.59
Calcium, %	0.02	0.11	0.31	0.34	0.37
Phosphorus, %	0.22	0.42	0.55	0.58	0.63
Magnesium, %	0.09	0.16	0.23	0.26	0.28
Potassium, %	0.34	0.83	1.37	1.67	2.02
Sodium, %	0.006	0.011	0.061	0.105	0.074
Chlorine, %	0.05	0.06	0.05	0.07	0.04
Sulfur, %	0.1	0.16	0.24	0.28	0.3

¹Topdresses were 100% corn; 76.6% corn and 23.4% soybean meal (48%); 53.3% corn and 46.6% soybean meal (48%); 26.6% corn, 23.4% soybean meal (48%), and 50% expeller soybean meal (Soyplus; West Central Soya, West Point, NE); 100% expeller soybean meal (Soyplus) for the diets 9.7, 11.7, 13.7, 14.7, and 16.2, respectively.

²Based on feed analysis from DHI Forage Testing Laboratory (Ithaca, NY) using a *Streptomyces griseus* protease technique.

Table 6. Initial characteristics of cows.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7	11.7	13.7	14.7	16.2		L	Q	C	Qu
BCS	2.88	2.92	2.90	2.92	2.95	0.13	0.75	0.93	0.85	0.94
BW, kg ²	668	671	676	672	664	20	0.94	0.68	0.87	0.94
305-2X-ME milk, kg ³	10,858	10,805	10,807	10,850	10,897	360	0.93	0.84	0.98	0.97
Fat yield, kg	366	369	383	389	359	14	0.78	0.16	0.21	0.78
Protein yield, kg	327	329	344	332	330	12	0.74	0.48	0.73	0.47
SCC ⁴	2.43	2.49	2.47	2.06	2.86	0.38	0.74	0.44	0.32	0.39
Gestation period, d	281	280	280	280	280	1.2	0.52	0.52	0.79	0.75
Previous lactation, d	338	324	308	352	333	15	0.88	0.36	0.45	0.06
Days on treatment	32.0	30.3	30.8	32.0	33.4	1.7	0.48	0.22	0.76	0.87
Age at calving, mo	53.7	55.4	47.7	45.9	55.5	4.4	0.57	0.24	0.09	0.89
Calving interval, d	396	387	369	413	400	15	0.63	0.32	0.60	0.06

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²Body weight of cows at the beginning of study.

³Previous 305-d 2× mature equivalent lactation performance in kg.

⁴Previous 305-d 2× mature equivalent lactation performance, *100,000/ml.

of ketosis were observed. Three cows were treated for clinical milk fever (one on the 11.7% CP diet and two on the 13.7% CP diet), one cow was diagnosed with fatty liver (16.2% CP diet), none with displaced abomasal disorder, and two experienced a retained placenta (one each on diets 9.7% and 14.7% CP). Subclinical milk fever (staggered gait, droopy, cold ears) was observed in 27, 20, 0, 7, and 27% of the cows fed 9.7, 11.7, 13.7, 14.7, and 16.2% CP diets, respectively. These cows returned to normal state following one treatment with Cal-Gel (Jorgensen Laboratories, Inc., Loveland, CO). A quartic ($P < 0.01$) relationship was observed between diet and prepartum udder edema scores (Table 8), but postpartum udder edema scores were unaffected by dietary treatment (Table 9). Cows fed 14.7% CP exhibited less edema prepartum than other treatments.

Production Responses

A quadratic ($P < 0.13$) tendency best described the relationship between dietary CP and DMI prepartum (Table 8). Cows fed 14.7% CP consumed slightly more, whereas cows fed 16.2% CP consumed less than cows fed the other diets. This trend was similar, but of slightly smaller magnitude, when DMI was expressed as a percentage of BW. Cows fed 16.2% CP experienced a 29% drop in intake during the last 3 wk before parturition, whereas DMI for cows fed the other diets only decreased by an average of 20%. Prepartum treatment influenced ($P < 0.05$) intake and intake as a percentage of BW in the first 90 d postpartum (Table 9). Postpartum DMI was best described by a quartic response ($P = 0.02$) with cows fed 9.7 and 16.2% CP consuming the least DM and cows fed 11.7% CP consuming the most.

Table 7. Parturitional characteristics.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7	11.7	13.7	14.7	16.2		L	Q	C	Qu
Var. from exp. cal. date ² , d	3.38	3.09	4.22	3.28	4.75	0.67	0.12	0.39	0.89	0.14
CDS ³	1.33	1.53	1.00	1.07	1.07	0.13	0.02	0.90	0.05	0.27
Calf birth wt, kg	39.7	43.8	39.2	41.6	40.2	2.3	0.85	0.52	0.33	0.26
BW before calving, kg ⁴	709	708	704	713	710	8	0.82	0.73	0.86	0.46
BW after calving, kg ⁵	651	643	649	648	655	9	0.72	0.44	0.81	0.77
BW loss at calving, kg ⁶	58	65	56	65	55	6	0.80	0.48	0.89	0.20

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²Variation from expected calving date, absolute value basis.

³Calving difficulty score—five point scale: 1 = no problem, 2 = slight problem, 3 = needed assistance, 4 = considerable force, 5 = caesarian).

⁴Most recent consecutive 2-d BW taken weekly.

⁵Body weight taken within 6 h after parturition.

⁶The difference between the BW before calving and after calving.

Table 8. Responses to dietary protein during the prepartum period.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7%	11.7%	13.7%	14.7%	16.2%		L	Q	C	Qu
DMI, kg/d	15.6	15.9	15.5	16.3	14.6	0.6	0.31	0.13	0.38	0.20
DMI, % of BW	2.24	2.26	2.20	2.31	2.10	0.08	0.30	0.23	0.46	0.24
Intake drop, % ²	20.4	19.0	19.1	22.9	29.2	6.5	0.33	0.37	0.89	0.87
Intake drop, kg ³	3.5	3.4	3.4	4.0	4.9	1.1	0.36	0.48	0.87	0.82
Average BW, kg	693	689	685	688	694	7	0.94	0.32	0.79	0.82
BW change, kg/28 d	37.5	36.0	33.1	35.9	37.0	7.5	0.92	0.71	0.93	0.82
Average BCS ⁴	3.01	3.02	3.07	3.21	3.01	0.07	0.37	0.23	0.10	0.18
BCS change per 28 d	0.22	0.18	0.28	0.43	0.25	0.08	0.24	0.58	0.10	0.35
Energy balance, Mcal/d ⁵	4.82	5.22	4.05	4.89	2.51	0.90	0.07	0.18	0.70	0.25
Urinary ketones, mg/dl	0.55	0.05	0.00	0.00	0.15	0.21	0.01	0.002	0.20	0.69
Edema score ⁶	1.47	1.48	1.43	1.33	1.47	0.12	0.99	0.31	0.05	0.01
AP, kg/d ⁷	1.55	1.70	1.70	1.89	1.75	0.07	0.01	0.16	0.51	0.05

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²Defined as the intake of week three prepartum minus the intake on week one prepartum divided by the intake on week three prepartum.

³Defined as the daily intake of week three prepartum minus the daily intake on week one prepartum.

⁴BCS scale = 1- to 5-point system (Wildman et al., 1982).

⁵Energy balance equals NE_L intake minus fetal energy (FE) minus NE_M. NE_M calculated as 0.08 * BW^{0.75} (Smith et al., 1997). Equation was modified with FE, 40 kcal/kg of BW^{0.75}, in place of lactational energy by use of a FE.

⁶Udder edema score: 1 = no edema, 2 = edema seen but not sensed through palpation, 3 = edema seen and sensed through palpation, 4 = edema present on the ventral abdominal wall, udder swollen and hard.

⁷Absorbable protein calculated according to NRC (1989) as $[0.64 \times (0.0716 \times (\text{NE}_L) - 0.19)] + [0.8 \times (\text{RUP})(\text{CP})]$; where NE_L is in Mcal/d, CP is in kg/d, and RUP is a percentage of CP (NRC, 1989).

Cows gained an average of 36 kg of BW during the treatment period, with no differences among treatments (Table 8). Body condition score change was not in agreement with BW change, suggesting that frame size differed among groups. No attempt was made to measure frame size. Cows fed 14.7% CP exhibited the

highest BCS at calving and had the largest gain in BCS (0.43) during the prepartum period.

Cows fed 16.2% CP were in negative EB during the last 14 d prepartum, whereas cows fed the other diets only reached negative EB during the last 7 d prepartum (Figure 1). Omitting the estimate for mammary devel-

Table 9. Postpartum period (d 1 to 90) responses to dietary protein during the prepartum period.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7	11.7	13.7	14.7	16.2		L	Q	C	Qu
DMI, kg/d	24.4	25.9	24.6	24.9	24.4	0.9	0.49	0.09	0.82	0.02
DMI, % of BW	4.03	4.26	4.00	4.05	4.07	0.12	0.49	0.07	0.60	0.01
Average BW, kg	610	616	613	618	611	9	0.57	0.05	0.72	0.01
BW change, kg/90 d	-39	-19	-21	-23	-38	10	0.90	0.08	0.86	0.83
Average BCS ²	2.31	2.35	2.43	2.55	2.31	0.09	0.40	0.18	0.15	0.37
BCS change per 90 d	-0.60	-0.68	-0.66	-0.73	-0.72	0.13	0.47	0.87	0.89	0.69
Energy balance, Mcal/d ³	3.31	5.82	4.05	2.38	4.23	0.94	0.63	0.50	0.01	0.50
Urinary ketone, mg/dl	0.6	5.9	0.9	3.1	5.2	3.0	0.48	0.96	0.15	0.44
Edema score ⁴	2.25	2.26	2.20	2.24	2.39	0.13	0.57	0.46	0.55	0.92
AP, kg/d ⁵	3.34	3.56	3.37	3.42	3.34	0.13	0.49	0.09	0.82	0.02

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²BCS scale = 1 to 5 point system (Wildman et al., 1982).

³Energy balance equals NE_L intake minus lactational energy minus NE_M. NE_M calculated as 0.08 * BW^{0.75}. LE calculated as milk yield (kg) + milk energy content (Smith et al., 1997). Milk energy content was defined as $(226.09 + 89.5 \times \% \text{ fat} + 49.83 \times \% \text{ protein})/1000$ (Tyrrell and Reed, 1965).

⁴Udder edema score: 1 = no edema, 2 = edema seen but not sensed through palpation, 3 = edema seen and sensed through palpation, 4 = edema present on the ventral abdominal wall, udder swollen and hard.

⁵Absorbable protein calculated according to NRC (1989) as $[0.64 \times (0.0716 \times (\text{NE}_L) - 0.19)] + [0.8 \times (\text{RUP})(\text{CP})]$; where NE_L is in Mcal/d, CP is in kg/d, and RUP is a percentage of CP (NRC, 1989).

Figure 1. Effect of prepartum protein on energy balance in late gestation with cows fed 9.7% CP ($\diamond$), 11.7% CP ($\blacksquare$), 13.7% CP ($\blacktriangle$), 14.7% CP ($\bullet$), and 16.2% CP ($\circ$). Effects in model: linear contrast ($P < 0.07$) prepartum and cubic contrast ($P < 0.01$) postpartum. Energy balance prepartum equals NE_L intake minus fetal energy (FE) minus net energy for maintenance (NE_M). NE_M calculated as $0.08 \times BW^{0.75}$. FE calculated as $40 \text{ kcal/kg of } BW^{0.75}$ (Smith et al., 1997). Equation was modified with FE in place of lactation energy (LE) by use of a fetal energy equation (Bell et al., 1995). Energy balance postpartum LE instead of FE, LE calculated as milk yield (kg) + milk energy content (Smith et al., 1997). Milk energy content was defined as $(226.09 + 89.5 \times \% \text{ fat} + \% \text{ protein})/1000$ (Tyrrell and Reid, 1965).

opment resulted in only cows fed the 16.2% CP in a negative EB during the last 7 d of gestation. Either our energy balance estimates were flawed or body condition scoring was not sufficiently sensitive to detect differences among treatments in EB.

Prepartum treatment influenced (cubic effect, $P = 0.01$) energy balance during the first 90 d postpartum (Table 9). Cows fed 14.7% CP had the lowest average postpartum EB (2.38 Mcal/d) and were in negative EB longer than cows fed the other diets (Figure 1). Prepartum treatment did not influence change in BW or BCS for the first 90 d postpartum.

Prepartum treatment influenced milk yield (Table 10) during the first 90 d (quartic, $P < 0.05$). Milk yield was lowest for cows fed 13.7% CP. Peak milk yield and days to peak did not differ among treatments. However, response to rbST injection during the ninth week postpartum was significantly influenced by prepartum protein level (quadratic, $P < 0.05$). Cows fed 13.7 and 14.7% CP exhibited the greatest response ($>2 \text{ kg/d}$) to rbST, whereas cows fed 9.7% CP exhibited only a 0.6 kg of milk/d response during the second week after rbST injection, and cows fed 16.2% CP did not respond. The lack of response to rbST accounts for much of the difference in 305-d 2 $\times$ -mature energy milk yield among treatments (quadratic response, $P = 0.02$). Cows fed 16.2% CP produced 1473 kg less milk during the 305-d lactation than cows fed 13.7% CP, and those fed 9.7% CP

produced 1028 kg less milk than those fed 13.7% CP. Prepartum treatment had a significant quadratic affect ($P < 0.01$) on 305-d 2 $\times$ mature equivalent milk protein yield and elicited cubic responses ($P < 0.02$) in 305-d 2 $\times$ mature equivalent milk fat yield. Cows fed the 13.7 and 14.7% CP diets during the prepartum period had the highest yields of milk protein and fat, whereas those fed the 9.7 and 16.2% CP diets had the least. Milk protein percentage tended to be lower in milk from cows fed 9.7 and 16.2% CP during the first 90-d postpartum (quadratic, $P = 0.06$). Milk fat percentage was similar among treatments but, during the first 90 d of lactation, cows fed 14.7% CP produced the most fat, cows fed 13.7% CP produced the least, and cows fed the other diets were intermediate (cubic, $P < 0.03$). Energy-corrected milk yield (ECM) during the first 90 d postpartum was lowest for cows fed 13.7% CP and highest for cows fed 14.7% CP (cubic response, $P < 0.05$). Cows fed 9.7 and 14.7% CP produced the most ECM and were the most efficient, whereas those fed 11.7% CP were least efficient. Somatic cell count declined (linear, $P < 0.05$) as dietary CP increased from 9.7 to 14.7%, which is consistent with the recorded incidences of mastitis.

Metabolic Responses

Plasma acetoacetate was similar among diets prepartum but exhibited a cubic response ($P < 0.01$) postpartum (Table 11). This prepartum response of plasma acetoacetate did not agree with the quadratic response ($P < 0.01$) from the urinary ketones, which were high in cows fed the 9.7 and 16.2% CP diets. Cows fed 16.2% CP had the highest plasma acetoacetate concentration postpartum, which agrees with the observed incidence of subclinical ketosis. However, the plasma acetoacetate response does not agree with the urinary ketones that were high in the cows fed the 11.7 and 16.2% CP diets. Although not significantly different from cows fed the other diets, those receiving 9.7 and 16.2% CP had higher numerical values for plasma BHBA (quadratic, $P = 0.18$) prepartum. Unlike the plasma acetoacetate concentrations, BHBA concentrations demonstrated responses similar to urinary ketones for both the prepartum and postpartum periods. Postpartum BHBA was best described by a cubic contrast ($P = 0.10$), and cows fed 11.7% CP had the highest plasma BHBA concentration postpartum and the highest DMI. However, cows fed 16.2% CP had the second highest plasma BHBA concentration postpartum. Total plasma triglycerides were similar among treatments both prepartum and postpartum. Plasma glucose was not affected by treatment prepartum but was lowest in cows fed the 16.2% CP diet and highest in those fed the 14.7% CP diet postpartum (cubic, $P < 0.05$). Prepartum plasma total

Table 10. Milk yield responses to dietary protein during the prepartum period.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7	11.7	13.7	14.7	16.2		L	Q	C	Qu
Day 0 to 90										
Milk yield, kg/d	39.0	39.5	37.7	39.5	38.9	1.9	0.88	0.72	0.85	0.02
Milk fat, %	3.70	3.62	3.60	3.74	3.69	0.08	0.87	0.46	0.47	0.37
Milk protein, %	3.04	3.11	3.10	3.09	2.99	0.05	0.53	0.06	0.88	0.80
SNF, %	8.72	8.75	8.67	8.79	8.59	0.08	0.33	0.25	0.54	0.15
Lactose, %	4.89	4.97	4.83	4.95	4.84	0.06	0.16	0.46	0.11	0.36
Fat yield, kg/d	1.44	1.41	1.35	1.47	1.41	0.06	0.78	0.89	0.03	0.19
Milk protein, kg/d	1.19	1.21	1.15	1.22	1.16	0.05	0.73	0.72	0.92	0.28
SNF, kg/d	3.42	3.42	3.25	3.50	3.36	0.17	0.55	0.95	0.09	0.93
Lactose, kg/d	1.95	1.93	1.83	1.97	1.91	0.11	0.92	0.26	0.55	0.01
SCC, 100,000/ml	4.19	2.79	3.59	1.34	4.00	1.20	0.02	0.35	0.08	0.92
MUN ² , mg/dl	17.04	15.72	16.04	15.35	15.21	0.43	0.18	0.04	0.18	0.74
ECM ³ , kg/d	40.1	40.0	38.0	40.9	39.6	1.7	0.71	0.94	0.05	0.50
Efficiency of ECM yield ⁴	1.74	1.58	1.67	1.70	1.64	0.05	0.51	0.43	0.04	0.92
Peak milk yield, kg/d	47.2	45.9	44.1	45.8	45.9	1.8	0.55	0.40	0.96	0.53
Peak milk yield, d	37.7	38.4	37.7	36.1	35.8	3.6	0.62	0.78	0.84	0.87
rbST response, kg/d ⁵	0.81	0.93	0.78	1.43	0.31	0.54	0.77	0.40	0.47	0.35
rbST response, kg/d ⁶	0.62	1.43	2.13	2.41	-0.08	0.81	0.98	0.02	0.22	0.63
305-d ME yield ⁷										
Milk, kg	10,170	10,396	11,198	10,924	9725	422	0.98	0.02	0.15	0.75
Fat, kg	359	356	389	392	336	14	0.99	0.02	0.02	0.87
Protein, kg	308	322	340	340	297	14	0.90	0.01	0.16	0.85

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²Milk urea nitrogen.

³ECM = Energy-corrected milk calculated as $0.327 \times \text{milk yield} + 12.95 \times \text{fat yield} + 7.2 \times \text{protein yield}$.

⁴ECM/DMI, kg/kg.

⁵Difference in milk production between the first injection time on rbST and week prior to injection.

⁶Difference in milk production between the second injection time and week prior to injection.

⁷305-d 2× mature equivalent milk yield.

α -amino N increased as dietary protein increased and was best described as a quadratic effect ($P < 0.05$) because plasma total α -amino N plateaued at the higher levels of dietary protein. PUN increased linearly ($P < 0.01$) with dietary protein prepartum. Conversely, postpartum PUN concentrations were negatively related to prepartum protein (linear, $P < 0.01$). Plasma urea N reflects the status of the cow at one point in time, whereas MUN reflects the average PUN over the extended interval between milkings. Milk urea nitrogen (Table 10) response to treatment was best described as quadratic ($P < 0.05$) and did not correlate well with DMI. Prepartum urinary creatinine concentrations were not significantly different among diets but tended to be lower in cows fed 16.2% CP. The creatinine concentration in urine decreased by approximately 46, 46, 39, 37, and 16% across parturition for diets 9.7, 11.7, 13.7, 14.7, and 16.2% CP, respectively. The urine creatinine concentration among treatment groups during the postpartum period exhibited a linear response ($P < 0.01$) with the lowest values in urine from cows fed 11.7% CP.

Plasma aspartate amino transferase (AST) showed a tendency for a cubic ($P = 0.11$) response prepartum; cows fed 13.7 and 14.7% CP had the highest AST activ-

ity during this period (Figure 2). Activity of AST during the postpartum period tended to decrease linearly ($P = 0.15$) with dietary protein level fed during the prepartum period. Plasma AST increased an average of 38% from d 1 prepartum to d 3 postpartum. Peak AST activity occurred on d 3 postpartum from cows fed 13.7 and 14.7% CP, on d 7 postpartum in those fed 11.7 and 16.2% CP, and on d 15 in cows fed 9.7% CP.

Dietary protein level had no effect on IGF-1 prepartum. Plasma IGF-1 declined approximately 50% in all cows from d 21 prepartum through d 3 postpartum. Postpartum IGF-1 response to treatment was best described as quartic ($P < 0.01$). The significant treatment effect on IGF-1 during the first 90 d postpartum was primarily due to a differential response by treatment groups to rbST injection (Figure 3). No difference was observed in IGF-1 among treatment groups on d 60 postpartum (before rbST provision). However, IGF-1 was higher ($P < 0.05$) in plasma from cows fed 14.7% CP on d 90 (after rbST provision) compared with the other diets. Cows fed 13.7% CP exhibited a lesser IGF-1 response to rbST than those fed 14.7% CP ($P < 0.05$) but a response greater than those fed 9.7, 11.7, and 16.2% CP ($P < 0.05$).

Table 11. Metabolic responses to dietary protein.

Item	Prepartum diet, % CP					SEM	Contrast ¹			
	9.7	11.7	13.7	14.7	16.2		L	Q	C	Qu
Prepartum										
Plasma glucose, mg/dl	66.1	68.7	67.4	68.0	68.4	1.3	0.28	0.52	0.26	0.60
Plasma IGF-1, ng/ml	167	166	162	168	145	14	0.28	0.39	0.56	0.56
Plasma TAA ² , mM	2.06	2.17	2.23	2.31	2.30	0.05	0.09	0.05	0.09	0.94
PUN ³ , mg/dl	5.44	7.36	10.69	11.81	13.28	0.43	0.01	0.81	0.15	0.70
Plasma TAG ⁴ , mg/dl	18.9	18.9	18.7	18.1	18.9	1.47	0.81	0.84	0.70	0.80
Plasma AST ⁵ , mmol/h	2.16	2.15	2.42	2.49	2.06	0.16	0.67	0.12	0.11	0.84
Urinary creatinine, mg/dl	47.1	46.4	49.7	47.6	40.1	3.7	0.34	0.17	0.32	0.82
Plasma Acac ⁶ , mg/dl	0.75	0.77	0.80	0.78	0.81	0.04	0.25	0.86	0.97	0.56
Plasma BHBA ⁷ , mg/dl	4.84	4.42	4.51	4.44	4.82	0.29	0.99	0.18	0.93	0.68
Postpartum										
Plasma glucose, mg/dl	62.4	61.4	62.5	64.6	59.9	1.6	0.63	0.18	0.02	0.23
Plasma IGF-1, ng/ml	106	114	124	133	106	11	0.35	0.55	0.62	0.004
Plasma TAA ² , mM	2.58	2.63	2.73	2.66	2.65	0.06	0.29	0.20	0.69	0.45
PUN, mg/dl ³	16.76	15.12	15.37	14.68	14.75	0.50	0.01	0.25	0.40	0.33
Plasma TAG ⁴ , mg/dl	18.0	17.4	17.3	18.4	17.8	1.1	0.96	0.67	0.54	0.467
Plasma AST ⁵ , mmol/h	3.06	2.96	2.68	2.81	2.72	0.20	0.15	0.68	0.87	0.51
Urinary creatinine, mg/dl	25.4	24.9	30.3	29.8	33.9	2.3	0.01	0.43	0.65	0.43
Plasma Acac ⁶ , mg/dl	0.69	0.68	0.69	0.68	0.72	0.03	0.31	0.35	0.01	0.22
Plasma BHBA ⁷ , mg/dl	5.67	7.15	5.27	5.98	6.23	0.72	0.93	0.86	0.10	0.27

¹Contrasts: L = Linear, Q = quadratic, C = cubic, Qu = quartic.

²Total α -amino nitrogen.

³Plasma urea nitrogen.

⁴Triacylglycerol.

⁵Aspartate amino transferase.

⁶Acetoacetate.

⁷Beta hydroxybutyrate.

DISCUSSION

The utilization of labile tissue protein by the dairy cow to support lactation during the early postpartum period has led some (Botts et al., 1979; Van Saun, 1991; Grummer, 1995) to theorize that improving protein reserves prepartum will reduce the incidence and (or) severity of metabolic disorders associated with parturition and, thus improve lactational performance. The

Figure 2. Effect of prepartum protein on plasma aspartate amino transferase in late gestation with cows fed 9.7% CP ($\diamond$), 11.7% CP ($\blacksquare$), 13.7% CP ($\blacktriangle$), 14.7% CP ($\bullet$), and 16.2% CP ($\circ$). Effects in model: cubic tendency ($P < 0.11$) prepartum and linear tendency ($P < 0.15$) postpartum.

most efficacious way to improve a cow's protein status during the prepartum period is unclear. Some researchers have investigated the influence of prepartum dietary protein levels on postpartum performance (Moorby et al., 1996; Putnam and Varga, 1998; Huyler et al., 1999). Others have fed rumen-protected amino acids (Wu et al., 1997; Carson et al., 1998), varied the energy density of the diet (Flipot et al., 1988; Grum et al., 1996; Minor et al., 1998), evaluated various dietary protein-to-energy ratios (VandeHaar et al., 1999), or increased RUP (Huyler et al., 1999; Putnam and Varga, 1998; Van Saun et al., 1993) in prepartum diets. These studies have been inconclusive with regard to effects of prepartum protein on postpartum performance. Contrary to dietary influence, an injection of bST prepartum has been shown to improve protein status of dairy cows (Simmons et al., 1994).

Studies with dairy cows during the periparturient period are complicated by factors that can mask effects from the dietary treatments being evaluated in the study. We attempted to control the most obvious factors by balancing treatment groups for BCS, BW, PME, and projected calving date. Initial cow characteristics (BCS, BW, and PME) and their interactions with diet were used as covariates in the statistical model. The intent of our study was to determine whether varying the

Figure 3. Effect of prepartum protein on plasma IGF-1 in late gestation with cows fed 9.7% CP (grid lines), 11.7% CP (dots), 13.7% CP (horizontal lines), 14.7% CP (wavy lines), and 16.2% CP (diagonal lines). Bars with unlike letters differ ($P < 0.05$).

metabolizable protein supply in prepartum diets influenced body protein reserves when surplus energy [above NRC (1989) recommendations] was included in the diet. However, the source of energy shifted because the increase in dietary protein was accomplished by replacing corn grain with soybean meal that contained 4.5% fat. Diets were similar in NE_L , but fat and protein replaced carbohydrate, and the diets ranged in NFC content from 42% in the lowest to 31% in the highest protein diet. Several studies (Coppock et al., 1972; Johnson and Otterby, 1981; Minor et al., 1998) have indicated that increasing dietary NFC stimulates DMI during the late prepartum period. Cows fed the 16.2% CP, 31% NFC diet consumed numerically the least DM and experienced the largest drop in intake (4.9 kg/d, 29.2%) during the last 3 wk before parturition. Because prepartum DMI and intake depression during the last week before calving was similar among cows fed the other diets, which contained higher NFC to protein ratios, the depression in intake may have been due in part to limited NFC in the diet. These results support data of VandeHaar et al. (1999) and of Minor et al. (1998) who showed an increase in DMI prepartum when NFC was increased (43.8% of DM) by adding corn grain and starch to diets containing higher CP (13.2 and 14.4%) than recommended for the dry cow (NRC, 1989).

Increasing maternal tissue reserves during the dry period has been suggested to be essential to support metabolic transitions associated with the periparturient period (Bereskin and Touchberry, 1967; Ferrell et al., 1976). Maternal weight gains of at least 0.6 to 0.7 kg/d have been recommended for the dry period to provide energy reserves necessary for lactation (Cowan et al., 1981; Broster and Broster, 1984). A gain in BW of 1.1 to 1.2 kg/d would correspond to maintenance of the cow

because the gravid uterus gains about 0.7 kg/d (Bell et al., 1995) and mammary gain is around 0.4 to 0.5 kg/d (VandeHaar et al., 1999). In our study, the change in BW from wk 4 to 1 prepartum averaged 36 kg or approximately 1.7 kg/d for all cows, with no significant treatment effects. Cows appeared to gain 0.6 kg/d in BW, which is in the suggested range to support lactation requirements. The allocation of weight gain between maternal and fetal tissues appeared to be similar among treatments, as calf birth weights did not differ. Prepartum BCS tended to be influenced by prepartum treatment; with cows fed 14.7% CP experiencing the largest increase (0.43) between wk 4 and 1 prepartum.

Aldrich et al. (1993) reported that rumen microbial efficiency is maximal when the NSC (sugar and starch) to RDP ratio in lactating diets falls between 6.0 and 3.4. Our ratios of NFC (starch, sugars, pectins, and organic acids) to RDP were 7.8, 6.3, 4.8, 4.4, and 3.5 for diets containing 9.7, 11.7, 13.7, 14.7, and 16.2% CP, respectively. We purposefully utilized the 9.7 and 11.7% CP levels knowing that they were deficient in RDP to have a negative control (9.7% CP) and a treatment that met NRC (1989) recommendations (11.7% CP) to compare to diets that were sufficient in RDP (13.7, 14.7, and 16.2% CP). Increasing protein in diets sufficient in RDP was accomplished by increasing the amount of RUP in the diet. Since peak response appeared to occur at 13.7% CP, additional increases in RUP did not improve performance. This suggests that the primary consideration with respect to meeting protein requirements in prepartum diets is to meet the needs of the rumen microbial population.

The nutritional status of a dairy cow is influenced by DMI, nutrient density of the diet, and nutrient digestibility. Some (Clark and Davis, 1980; Curtis et al., 1985; Grummer, 1995) have suggested that the nutrient density of the dry cow diet during the immediate prepartum period should be increased to offset the generally observed depression in DMI. Diets for our study were formulated using the Cornell Net Carbohydrate and Protein System model 3.0 (Fox et al., 1992) and were designed to deliver sufficient ruminally available protein to support rumen function, with the exception of the 9.7 and 11.7% CP diets. The expected range in metabolizable protein was 1222 to 1628 g/d, based on an assumed DMI of 10.5 kg/d. Because one of our objectives was to determine the effect of prepartum dietary protein on DMI, intake was not regulated. The calculated metabolizable protein ranged from 1547 to 1888 g/d, based on observed intakes. Cows fed 16.2% CP consumed less and consequently had less metabolizable protein than those fed 14.7% CP. Calculated EB data (Figure 1) indicates that cows fed 16.2% CP were in negative EB during the last 2 wk before calving,

whereas cows on the other diets experienced a negative EB only during the last week prepartum. Thus, cows fed the 16.2% CP diet probably mobilized more tissue reserves prepartum. This, in turn, may have reduced nutrient reserves available to support lactation and contributed to the lack of a milk response to an injection of exogenous rbST and, therefore, the lowest complete lactation milk yield. Aspartate amino transferase has been used to evaluate protein status in dairy cows (Zurek et al., 1995; Xu et al., 1998) and responds to both an increase in protein intake and tissue protein catabolism. Plasma AST activity followed a pattern similar to plasma total α -amino N and protein intake in all cows except those fed the 16.2% CP diet.

Prepartum DMI for all cows was near peak on d 21 and lowest on d 1 prepartum, and PUN decreased 20, 17, 8, 7, and 2% between d 21 and d 1 prepartum for diets 9.7, 11.7, 13.7, 14.7, and 16.2% CP, respectively. The concentration of PUN is primarily a function of ruminal ammonia levels (Butler, 1998) but can be influenced by the metabolic use of amino acids for gluconeogenesis in fasting situations (Finco, 1980; Butler, 1998). The relative influence of these two factors on PUN concentration cannot be separated in our study, but observed PUN concentrations appear to reflect intake on d 1 prepartum except for cows fed the 16.2% CP diet. Their PUN remained essentially the same as it was on d 21 (14.30 and 14.03, respectively), which suggests, in the face of a decrease in DMI, that these cows were actively using amino acids to support energy needs.

A comparison of plasma BHBA concentrations between d 21 and 1 prepartum sheds some light on the influence of diet on the metabolic status of cows in our study. Plasma BHBA levels were similar among diets on d 21 but responded quadratically ($P < 0.05$) to protein level in the diet on d 1 prepartum (4.07, 4.06, 3.91, 4.22, and 4.00 for d 21 vs. 7.16, 5.01, 5.37, 5.78, and 6.74 for d 1 respective to diets 9.7, 11.7, 13.7, 14.7, and 16.2% CP). On d 1, cows fed diets 9.7 and 16.2% CP had the highest plasma BHBA. The increase in plasma BHBA by cows fed 9.7 and 16.2% CP between d 21 and 1 prepartum was greater than for cows fed the other diets, which agrees with the observed urinary ketones. The higher concentration of plasma BHBA in conjunction with the higher values of urinary ketones suggests that these cows were mobilizing more tissue reserves than cows fed the other diets. However, no clinical and only nine subclinical cases of ketosis were recorded for the 75 cows in this study, and four of the subclinical cases consumed the 16.2% CP diet.

Our working hypothesis was that cows adequately prepared during the dry period to withstand the demands of early lactation would have more body reserves

to support sustained milk production with higher persistency than cows entering lactation with fewer nutrient reserves. As a test of this, milk and IGF-1 responses to exogenous rbST were evaluated. Cows consuming the 11.7, 13.7, and 14.7% CP treatments showed increases in milk yield in response to rbST and increases in IGF-1 concentrations between d 60 (before rbST) and 90 (after rbST). Cows fed 9.7 and 16.2% CP diets did not show significant increases in milk yield in response to the rbST injection, but all cows experienced an increase in plasma IGF-1 concentration although the increase was less for cows fed 9.7 or 16.2% CP. The greater IGF-1 concentrations of cows fed 11.7, 13.7, and 14.7% CP was assumed to be due to a greater responsiveness of IGF-1 production to rbST.

CONCLUSIONS

Prepartum diets were formulated to provide incremental increases in protein with the lowest levels beginning below NRC (1989) recommendations; however, all cows consumed more protein than recommended because DMI was higher than predicted. Full lactation 305-d 2 $\times$ -mature equivalent milk, milk protein, and milk fat yields improved with increased prepartum protein up to the 13.7% level. Our results suggest that lactation responses of cows is improved when fed prepartum diets containing 1.54 Mcal/kg of NE_L and 13.7% CP.

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REFERENCES

- Aldrich, J. M., L. D. Muller, G. A. Varga, and L. C. Griel, Jr. 1993. Nonstructural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance of dairy cows. *J. Dairy Sci.* 76:1091–1105.
- Babson, A. L., P. O. Shapiro, P. A. R. Williams, and G. E. Phillips. 1962. The use of diazonium salt for the determination of glutamic-oxaloacetic transaminase in serum. *Clin. Chim. Acta* 7:199–205.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529.
- Bell, A. W., R. Slepetis, and R. A. Ehrhardt. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. *J. Dairy Sci.* 78:1954–1961.
- Bereskin, B., and R. W. Touchberry. 1967. Some effects of pregnancy on body weight and paunch girth. *J. Dairy Sci.* 50:220–224.

- Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration in early lactation. *J. Dairy Sci.* 75:1914–1922.
- Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cow. *J. Dairy Sci.* 62:433–440.
- Broster, W. H., and V. S. Broster. 1984. Reviews of the progress of dairy science: Long term effects of plane of nutrition on the performance of the dairy cow. *J. Dairy Sci.* 52:149–196.
- Bruce, L. A., T. Atkinson, J. S. M. Hutchinson, R. A. Shakespear, and J. C. MacRae. 1991. The measurement of insulin-like growth factor 1 in sheep plasma. *J. Endocrinol.* 128:R1–R4.
- Butler, W. R. 1998. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J. Dairy Sci.* 81:2533–2539.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477–487.
- Carson, V. M., N. L. Whitehouse, K. Kolinsky, B. D. Garthwaite, M. S. Piepenbrink, and C. G. Schwab. 1998. Interactions of prepartum and postpartum feeding of rumen inert amino acids on lactational performance of Holstein cows. *J. Dairy Sci.* 81(Suppl. 1):1152. (Abstr.)
- Clark, J. H., and C. L. Davis. 1980. Some aspects of feeding high producing dairy cows. *J. Dairy Sci.* 63:873–885.
- Coppock, C. E., C. H. Noller, S. A. Wolfe, C. J. Callahan, and J. S. Baker. 1972. Effect of forage-concentrate ratio in complete feeds fed ad libitum on feed intake prepartum and the occurrence of abomasal displacement in dairy cows. *J. Dairy Sci.* 55:783–789.
- Cowan, R. T., G. W. Reid, J. F. D. Greenhalgh, and C. A. G. Tait. 1981. Effects of feeding in late pregnancy and dietary protein concentrations during early lactation on food intake, milk yield, live weight change and nitrogen balance of cows. *J. Dairy Res.* 48:201–205.
- Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfield. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. *J. Dairy Sci.* 68:2347–2360.
- Eley, R. M., W. W. Thatcher, F. W. Bazer, C. J. Wilcox, R. B. Becker, H. H. Head, and R. W. Adkinson. 1978. Development of the conceptus in bovine. *J. Dairy Sci.* 61:467–473.
- Ferrell, C. L., W. N. Garnett, and N. Hinman. 1976. Growth, development and composition of udder and gravid uterus of beef heifers during pregnancy. *J. Anim. Sci.* 42:1477–1489.
- Flipot, P. M., G. L. Roy, and J. J. Dufour. 1988. Effect of peripartum energy concentrations on production performance of Holstein cows. *J. Dairy Sci.* 71:1840–1850.
- Finco, D. R. 1980. Kidney function. Pages 338–400 in *Clinical Biochemistry of Domestic Animals*. 3rd ed. J. J. Kaneko, ed. Academic Press, Washington, DC.
- Forbes, J. M. 1968. The physical relationships of the abdominal organs in the pregnant ewe. *J. Agric. Sci. (Camb.)* 70:171–177.
- Fox, D. G., C. J. Sniffen, J. D. O'Connor, J. B. Russell, and P. J. Van Soest. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *J. Anim. Sci.* 70:3578–3596.
- Greenfield, R. B., M. J. Cecava, T. R. Johnson, and S. S. Donkin. 2000. Impact of dietary protein amount and rumen undegradability on intake, peripartum liver triglyceride, plasma metabolites, and milk production in transition dairy cattle. *J. Dairy Sci.* 83:703–710.
- Grum, D. E., J. K. Drackley, L. R. Hansen, and J. D. Cremin, Jr. 1996. Production, digestion, and hepatic lipid metabolism of dairy cows fed increased energy from fat or concentrate. *J. Dairy Sci.* 79:1836–1849.
- Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.* 73:2820–2833.
- Huyler, M. T., R. L. Kincaid, and D. F. Dostal. 1999. Metabolic and yield responses of multiparous Holstein cows to prepartum rumen-undegradable protein. *J. Dairy Sci.* 82:527–536.
- Johnson, D. G., and D. E. Otterby. 1981. Influence of dry period diet on early postpartum health, feed intake, milk production, and reproductive efficiency of Holstein cows. *J. Dairy Sci.* 64:290–295.
- Littell, R. C., G. A. Milliken, S. W. Walter, and R. D. Wolfinger. 1996. SAS systems for Mixed Models. SAS Institute Inc., Cary, NC.
- McNeill, D. M., R. Slepatis, R. A. Ehrhardt, D. M. Smith, and A. W. Bell. 1997. Protein requirements of sheep in late pregnancy: partitioning of nitrogen between gravid uterus and maternal tissues. *J. Anim. Sci.* 75:809–816.
- Minor, D. J., S. L. Trower, B. D. Strang, R. D. Shaver, and R. R. Grummer. 1998. Effects of nonfiber carbohydrate and niacin on periparturient metabolic status and lactation of dairy cows. *J. Dairy Sci.* 81:189–200.
- Moorby, J. M., R. J. Dewhurst, and S. Marsden. 1996. Effect of increasing digestible undegraded protein supply to dairy cows in late gestation on the yield and composition of milk during the subsequent lactation. *Anim. Sci.* 63:203–213.
- National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. National Academy Press, Washington, DC.
- National Research Council. 2001. Nutrient Requirements of Dairy Cattle 7th rev. ed. National Academy Press, Washington DC.
- Putnam, D. E., and G. A. Varga. 1998. Protein density and its influence on metabolite concentration and nitrogen retention by Holstein cows in late gestation. *J. Dairy Sci.* 81:1608–1618.
- SAS User's Guide: Statistics, Version 6 Third edition. 1990. SAS Inst., Inc., Cary, NC.
- Simmons, C. R., W. G. Bergen, M. J. VandeHaar, D. J. Sprecher, C. J. Sniffen, E. P. Stanisiewski, and H. A. Tucker. 1994. Protein and fat metabolism in cows given somavuboe before parturition. *J. Dairy Sci.* 77:1835–1847.
- Smith, T. R., A. R. Hippen, D. C. Beitz, and J. W. Young. 1997. Metabolic characteristics of induced ketosis in normal and obese dairy cows. *J. Dairy Sci.* 80:1569–1581.
- Stanley, T. A., R. C. Cochran, E. S. Vanzant, D. L. Harmon, and L. R. Corah. 1993. Periparturient changes in intake, ruminal capacity, and digestive characteristics in beef cows consuming alfalfa hay. *J. Anim. Sci.* 71:788–795.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. *J. Dairy Sci.* 48:1215–1223.
- Van Saun, R. J. 1991. Dry cow nutrition: The key to improving fresh cow performance. *Vet. Clin. North Am. Food Anim. Pract.* 7:599–620.
- Van Saun, R. J., S. C. Idleman, and C. J. Sniffen. 1993. Effect of undegradable protein amount fed prepartum on postpartum production in first lactation Holstein cows. *J. Dairy Sci.* 76:236–244.
- VandeHaar, M. J., G. Yousif, B. K. Sharma, T. H. Herdt, R. S. Emery, M. S. Allen, and J. S. Liesman. 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. *J. Dairy Sci.* 82:1282–1295.
- Van Soest, P. J., and D. G. Fox. 1992. Discounts for net energy and protein. Pages 40–68 in *Proc. Cornell Nutr. Conf. Feed Manuf.*, Rochester, NY. Cornell Univ., Ithaca, NY.
- Weiss, W. P., H. R. Conrad, and N. R. St. Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values for forages and concentrates. *Anim. Feed Sci. Technol.* 39:95–110.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Trout, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production variables in high producing Holstein dairy cattle. *J. Dairy Sci.* 65:495–501.
- Williamson, D. H., J. Mellanby, and H. A. Krebs. 1962. Enzymatic determination of D(-)- β -hydroxybutyric acid and acetoacetic acid in blood. *Biochem. J.* 82:90–98.
- Wu, Z., R. J. Fisher, C. E. Polan, and C. G. Schwab. 1997. Lactational performance of cows fed low or high ruminally undegradable protein prepartum and supplemental methionine and lysine postpartum. *J. Dairy Sci.* 80:722–729.
- Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *J. Dairy Sci.* 81:1062–1077.
- Zurek, E., G. R. Foxcraft, and J. J. Kennelly. 1995. Metabolic status and interval to first ovulation in postpartum dairy cows. *J. Dairy Sci.* 78:1909–1920.