Effects of Genetic Merit and Varying Dietary Protein Degradability on Lactating Dairy Cows

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ABSTRACT

Eighty two multiparous Holstein cows were blocked by genetic merit (high vs. low) and assigned to one of two treatments [high rumen-undegradable protein (RUP): rumen-degradable protein (RDP) vs. low RUP: RDP] from d 21 before to d 150 after calving to study the effects of these treatments on production and reproductive performance. Diets were isonitrogenous (dry cow 10.5% crude protein; lactating cow 19.3%), isoenergetic (dry cow 10.0 MJ of metabolizable energy (ME); lactating cow 10.9 MJ of ME) and fed as total mixed rations. Feeding more RUP significantly increased dry matter intake and milk yield, reduced body tissue mobilization, and lowered concentrations of serum nonesterified fatty acids (NEFA) and plasma urea. Expression of estrus at first ovulation was improved, first service conception rate was higher, and calving to conception interval was shorter for the high RUP group. Cows of high genetic merit produced more milk, mobilized more body tissue, and had higher concentrations of plasma growth hormone. The dry matter intake and concentrations of blood metabolites did not significantly differ with genetic merit. Expression of estrus at first ovulation was significantly lower for cows of high genetic merit. Serum NEFA concentrations were significantly higher, and estrus was not observed at first ovulation for cows of higher genetic merit fed the low RUP diet. The interaction between dietary RUP and genetic merit was not significant for other measures of performance or fertility. Feeding a low RUP: high RDP diet had negative effects on some aspects of production and reproductive performance. The effects of diet on NEFA concentrations and estrus display were greater in cows of high genetic merit, indicating that potential interactions should be evaluated in future reproductive studies involving protein and fertility.

(**Key words:** protein degradability, genetic merit, productivity, fertility)

Abbreviation key: ABV = Australian breeding value, **GGT** = glutaryl transferase, **GH** = growth hormone, **HD** = high degradable, **LD** = low degradability, **ME** = metabolizable energy, **MJME** = MJ of ME, **NEB** = negative energy balance, **NIRS** = near infrared spectrophotometry, **NSB** = nonspecific binding, **PEG** = polyethylene glycol, **Tris** = tris(hydroxymethyl)aminomethane.

INTRODUCTION

Continued genetic selection for milk production has increased the potential of dairy cows to produce milk. However, increased milk production is associated with more mobilization of body tissue (Veerkamp et al., 1995). The effects of dietary protein on the metabolic and physiological controls of nutrient partitioning require further definition in cows of high genetic merit. Rumen undegraded proteins increase the supply of AA provided from the diet to the small intestine. Sustained release of peptides and AA from slowly degraded dietary proteins may enhance rumen microbial protein synthesis, improve microbial protein yield, and increase milk yield (Ørskov et al., 1981, 1987). Feeding more RUP when dietary energy is limited may, however, lower energy balance and increase body tissue mobilization, and increase concentrations of NEFA and BHBA in blood (Ørskov et al., 1981, 1987). It is possible that selection of cows for higher milk production may accentuate the effects of feeding more dietary RUP to dairy cows and extend the period over which homeorhetic adaptations to lactation occur. This hypothesis has not been examined under controlled experimental conditions.

Detrimental effects of dietary CP intake on dairy cow fertility have been reported (Butler, 1998; Westwood et al., 1998); however, the effect of rumen protein degradability on the fertility of dairy cows has been examined infrequently.

Received March 15, 2000.
Accepted June 27, 2000.
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The objectives of this study were 1) to investigate the responses and reproductive performance of cows of high and low genetic merit fed isonitrogenous, isoenergetic diets that contained different ratios of RUP to RDP and 2) to evaluate the effects of the interaction between genetic merit and dietary protein degradability on the performance of lactating dairy cows.

MATERIALS AND METHODS

Cows and Feeding

Eighty-two multiparous, Holstein-Friesian dairy cows of high or low genetic merit were assigned to one of two dietary treatment groups in a 2 × 2 factorial arrangement. Parity ranged from 2nd to 10th parity, mean 4.4. Cows of high genetic merit were defined as those with an Australian Breeding Value (**ABV**) fat plus protein of greater than or equal to 31. Cows of low genetic merit were ABV of less than or equal to 15. Diets were formulated as low degradable (**LD**), 35%RUP: 65% RDP and high degradable (**HD**), 15% RUP and 85% RDP, and were isonitrogenous (19.3% CP) and isoenergetic [10.9 MJ of metabolizable energy (**MJME**)] (Table 1). Cows were adapted to the feeding system 6 wk before calving and fed a TMR containing a 50:50 blend of LD and HD dry cow diets. Cows were randomly allocated to dietary group and fed LD or HD dry cow TMR starting 3 wk before calving. Cows on the LD dry cow TMR were assigned the LD lactating TMR from calving until 150 DIM. Rations fed during the study were composed of chopped alfalfa and oat hay, and the rations differed only in the composition of the pelleted concentrate. Diets were fed for ad libitum intake as TMR in a Calan-Broadbent feeding facility (American Calan Inc., Northwood, NH), with cows maintained on an outdoor, shaded drylot adjacent to the feeding facility without access to other feeds. A maximum of 44 cows were present in the herd at any time, balanced for dietary and genetic merit group. The study was conducted over a 2-yr period. All work was carried out with the approval of the Animal Care and Ethics Committee of the University of Sydney, Australia.

Degradability of the diets for the lactating cows was verified by in sacco degradation studies performed for each ration with three cannulated, nonlactating beef heifers on three occasions during the study (Table 1) using the technique described by Orskov and McDonald (1979). Forages and TMR were sampled every 2 wk for DM, CP, CF, and ADF by scanning near infrared spectrophotometry (**NIRS**) (model 6250, Pacific Scientific, Silver Springs, MD). Subsamples of LD and HD dry cow and lactating TMR were submitted for wet chemistry analysis during the study. A comparison of NIRS and wet chemistry results showed internal consistency, indicating robustness of equations used for NIRS predictions.

Amino acid analyses were performed for the lactating TMR on three occasions during the study (Table 2).

Data and Sample Collection

Daily DMI was calculated for each cow as DM offered minus orts. All cows were weighed and scored for body condition every 7 d. The accuracy of the scales was verified by placing a known weight on the scales before and after weighing. Body condition was scored by the same two people during the study using a five-point scale of 0.25 increments where 1 = thin and 5 = obese (Edmondson et al., 1989).

Blood samples were taken once weekly ± 3.5 d from 3 wk before calving until wk 10 of lactation. Cows were bled while restrained in the self-locking stanchions of the Calan feeding facility at the same time of day relative to feeding. Blood was collected from the coccygeal vessels into heparinized and plain Vacutainers (Becton Dickinson Pty. Ltd., Lane Cove, NSW, Australia), and placed immediately on ice before centrifugation at 2000 × *g* for 20 min at 4°C. Serum and heparinized plasma were decanted into polypropylene tubes and stored at –20°C before analysis.

Serial blood samples were collected between d 14 and 28 of lactation from 13 cows selected randomly from the 82 study cows. There were three cows of high genetic merit fed the LD diet; three low genetic merit cows fed the LD diet, three high genetic merit cows fed the HD diet; and four low genetic merit cows fed the HD diet. These cows were removed from the Calan feeding facility 10 h before the sampling period. Indwelling jugular catheters were inserted and cows held in a confined area with access ad libitum to water and appropriate diets. Serial blood samples were collected at 20-min intervals from 0300 h until 0900 h and immediately centrifuged. Plasma was stored at –20°C until assayed for growth hormone and insulin content.

Plasma progesterone concentrations were determined using heparinized blood samples collected from the coccygeal vessels of each cow twice weekly for 24 d after mating. Plasma was harvested following centrifugation and frozen at –20°C before analysis.

Cows were milked twice daily. Daily milk yields were measured using Tru-test F meters (Tru-Test Distributors Ltd, Auckland, New Zealand) for the majority of study duration. For the latter stages of the study, Milk Master yield indicators (Alfa Laval Agri Pty Ltd, Reservoir 3073, Australia) were used to measure milk yields. Whole milk samples were collected at the afternoon milking every third day, beginning d 7 after calving. Samples were collected using Tru-test F meters (Tru-

Table 1. Ingredient and nutrient composition of the diets.

	TMR[1]				
Ingredient (% of TMR)	LD Dry	HD Dry	LD Lac 'A'	LD Lac 'B'	HD Lac
Forages (chopped)					
Alfalfa hay	. . .	. . .	35.0	35.0	35.0
Oaten hay	60.0	60.0	5.0	5.0	5.0
Pelleted concentrate	40.0	40.0	60.0	60.0	60.0
Components of pelleted concentrate (% of pellet) as formulated					
Sorghum, ground	10.6	5.3	24.8	24.5	27.8
Wheat middlings	22.4	23.9	7.3	10.5	4.3
Wheat, ground	. . .	8.0	. . .	. . .	12.0
Meatmeal	19.4	. . .	9.3	9.2	1.8
Cottonmeal	4.7	. . .	14.9	. . .	. . .
Soymeal	. . .	. . .	. . .	3.8	. . .
Protected canola	. . .	. . .	. . .	8.3	. . .
Tallow	. . .	7.0	3.3	3.4	4.5
Limestone	. . .	. . .	. . .	. . .	0.8
Dicalcium phosphate	1.0	0.9	. . .	. . .	1.6
Salt	. . .	. . .	. . .	0.01	0.1
Sodium bicarbonate	0.2	0.2	0.3	0.3	0.3
Urea	. . .	0.9	. . .	. . .	2.4
Dairy premix	0.1	0.1	0.1	0.1	0.1
Composition of TMR from NIRS analyses (% of DM)					
DM%	89.4	89.4	89.5	NT	89.8
CP	10.4	10.9	19.1	NT	19.5
RUP, % of CP, as formulated	44.7	14.2	34.8	NT	15.3
RUP, % of DM, as formulated	4.6	1.5	6.6	NT	3.0
RUP, % of CP, actual	NT	NT	40.1	NT	25.4
RUP, % of DM, actual	NT	NT	7.7	NT	5.0
Crude fiber	21.7	21.5	16.8	NT	16.6
ADF	27.1	26.8	21.0	NT	20.8
ME (MJME/kg DM)	9.9	10.0	10.9	NT	10.9

[1]LD dry = Low-degradable dry cow, HD dry = high-degradable dry cow, LD lac 'A' = low-degradable lactating cow (fed for the majority of the study), LD lac 'B' = low-degradable lactating cow (fed for 12 wk of the 2-yr study when a period of drought limited the availability of dietary ingredients).

NT = Not tested.

Test Distributors Ltd, Auckland, New Zealand), which provide a composite sample collected from the entire milking. Samples were preserved with potassium dichromate (434 samples) or sodium azide (2122 samples) and stored at 4°C before assay for milk progesterone. Collection of milk samples ceased 7 d after observation of the first standing estrus following calving. Milk progesterone profiles for each cow were examined to characterize luteal activity, in the absence of visual estrus, between calving and first standing estrus. Milk samples also were collected from each cow weekly ± 3.5 d, during the first 10 wk of lactation. Between wk 10 and 20, of lactation the sampling interval was monthly. Samples were preserved with 0.02% bronopol and refrigerated at 4°C before analysis for fat percent, protein percent, and lactose percent.

Calculation of Energy Balance

Weekly energy balance (MJ of ME) was calculated for each cow using equations described by AFRC (1993) using mean daily DMI per week, mean daily milk production per week, weekly change in BW, weekly milk fat percent, milk protein percent, and milk lactose percent. Missing data were estimated by averaging values for the week before and after the missing value. Less than 2.5% of data was missing from the variables examined using BMDP Dynamic AM (BMDP Statistical Software Inc., Los Angeles, CA). Daily ME intake was calculated with formulated values for ME for each ration.

$$\text{Milk energy} = (0.384*\text{Fat}\% + 0.223*\text{Protein}\% + 0.199*\text{Lactose}\% - 0.108)/0.63$$

$$\text{Maintenance} = (0.53(\text{BW}/1.08)0.67) + (0.0091\text{BW})/0.68$$

$$\text{Weight change} = (\text{BW change}*27.36)/0.6$$

$$\text{ME milk} = \text{uncorrected milk}*\text{Milk energy}$$

$$\text{Dietary ME} = 11.2*\text{Feed intake}*0.85$$

$$\text{ME balance} = \text{Dietary ME} - \text{ME maintenance} - \text{ME milk}$$

Analytical Procedures

Plasma urea, cholesterol, gamma glutaryl transferase (**GGT**), and calcium, were determined with commercially available kits using a Cobas Mira autoanalyzer (Roche Diagnostic Systems, Australia). Plasma urea concentration was determined using Urea MPR 2 and Precimat Urea reagents (Boehringer Mannheim GmbH Diagnostica, Australia). Gluco-quant glucose reagents (Boehringer Mannheim GmbH Diagnostica, Australia) were used to determine plasma glucose concentrations. Plasma cholesterol was assessed with Unimate reagents (Roche Products Pty. Ltd., Dee Why, NSW, Australia), and plasma activities of the enzyme GGT were determined with Trace gamma GT reagents (Trace Scientific Pty. Ltd., Melbourne, Victoria, Australia). Plasma calcium concentrations were measured with Trace calcium reagents (Trace Scientific Pty. Ltd., Melbourne, Victoria, Australia). Intraassay coefficients of variation for the urea, glucose, cholesterol, GGT, and calcium were 3.9, 11.2, 4.8, 7.0, and 1.4%, respectively. Interassay coefficients of variation for the urea, glucose, cholesterol, GGT, and calcium were 8.2, 9.7, 13.4, 20.6, and 15.3%, respectively.

Concentrations of plasma BHBA were determined by autoanalyzer according to the method of Zivin and Snarr (1973). The intraassay and interassay coefficients of variation for BHBA were 2.1 and 9.9%, respectively. Serum NEFA concentrations were determined using Wako NEFA C kits (Wako Pure Chemical Industries, Osaka, Japan), with modifications described by Rabiee (1995). The intraassay and interassay coefficients of variation for the NEFA assay were 2.2 and 5.8%, respectively.

Plasma growth hormone (**GH**) concentrations were quantified by a double antibody heterogenous radioimmunoassay. Bovine GH standard (ASP-11182B, A.F. Parlow, VCLA) in concentrations ranging from 1 to 64 ng/ml were added with rabbit anti-ovine GH antiserum [NIDDK-anti-oGH-2 (ASP - CO123080)] diluted to 1:10,000 (100 μl) to 700 μl of 0.05 phosphate buffer, (pH 7.4). Bovine GH standard was equipotent with the ovine GH standard (NIDDK-oGH-10-4) in this assay. Tubes were incubated at 4°C for 24 h and 10,000 cpm of ^{125}I-bGH were added, and tubes were vortexed and incubated for a further 24 h. Donkey anti-rabbit serum (diluted 1:4 in 0.05 *M* phosphate buffer containing 5% polyethylene glycol, **PEG**) was added and tubes were mixed and incubated for a further 24 h at 4°C. Antibody-bound ^{125}I-bGH was separated by centrifugation (1600 $\times$ *g* for 1 h), and the supernatant was aspirated. The pellets were counted in a gamma-counter (1470 Wizard Gamma Counter, Pharmacia, Wallac Oy, Finland) and bovine GH concentrations (ng/ml) calculated by Multicalc software (Pharmacia, Finland). The interassay coefficient of variation of low and high concentration quality controls for GH were 22.6% (n = 5) and 10.1% (n = 5), respectively. Intraassay coefficient of variation for the high quality control was 13.2% (n = 10).

Insulin concentrations were measured using the same protocol as that described for bovine GH, but using ovine insulin standard (lot no. 716-112B-108-1, Eli Lilly, Australia Pty Ltd, West Ryde NSW 2114, Australia), guinea pig anti-ovine insulin antiserum (100% cross reactive with bovine insulin; no. 016666-Novo, Denmark, CSIRO, Prospect, NSW, Australia), 10,000 cpm ^{125}I-insulin, and donkey anti-guinea pig serum (diluted 1:4). The interassay coefficient of variation of low and high concentration quality controls for insulin were 17.2% (1.33 ng/ml, n = 5) and 23.4% (ng/ml, n = 5), respectively. Intraassay coefficient of variation for the low quality control was 13.2% (n = 10).

Plasma progesterone concentrations were determined using a solid phase ^{125}I labeled progesterone assay (Spectria Veterinary Progesterone, Orion Diagnostica, Espoo, Finland). Standards, quality controls, and samples were assayed in duplicate. Fifty μmol of standards, quality controls, and samples were added to antibody-coated tubes. Phosphate buffered ^{125}I labeled progesterone (500 μmol) was added to each tube, and the tubes vortexed for 10 s. Supernatant was decanted from tubes following incubation at room temperature for 2 h. Radioactivity in tubes was counted using an automatic gamma counter (1470 Wizard Gamma Counter, Pharmacia, Wallac Oy, Finland). Intraassay

Table 2. Amino-acid analysis of low-degradable (LD) and high-degradable (HD) lactating cow TMR, mean (± SD) for three samples collected during study.

	AA (g/100 g protein)	
	LD TMR	HD TMR
Aspartic acid	1.74 (0.11)	1.36 (0.10)
Threonine	0.76 (0.04)	0.61 (0.05)
Serine	0.94 (0.03)	0.76 (0.09)
Glutamic acid	2.83 (0.13)	2.30 (0.29)
Proline	1.30 (0.09)	1.07 (0.13)
Glycine	1.27 (0.09)	0.82 (0.17)
Alanine	1.19 (0.03)	0.90 (0.08)
Valine	0.98 (0.06)	0.79 (0.07)
Methionine	0.27 (0.03)	0.23 (0.01)
Isoleucine	0.74 (0.05)	0.62 (0.04)
Leucine	1.46 (0.04)	1.23 (0.09)
Tyrosine	0.58 (0.07)	0.48 (0.06)
Phenylalanine	0.90 (0.04)	0.71 (0.06)
Lysine	0.85 (0.06)	0.62 (0.06)
Histidine	0.49 (0.03)	0.35 (0.01)
Arginine	1.28 (0.12)	0.79 (0.12)
Cystine and cysteine	0.23 (0.02)	0.20 (0.01)
Protein recovery %	90.47 (2.50)	79.23 (5.86)

coefficient of variation was 5.4%. Interassay coefficient of variation was 13.1%.

Milk progesterone concentration was determined using one of two commercially available radioimmunoassay kits. The concentration of progesterone in 1654 of 2556 samples was determined utilizing a commercially available liquid phase progesterone kit (Orion Diagnostica, Espoo, Finland). Lyophilized progesterone antiserum was reconstituted 24 h prior to sample analysis by adding 12 ml of tris(hydroxymethyl)aminomethane (**Tris**) buffer, pH 7.4, gently mixing the vial until dissolved; antiserum was stored at 4°C prior to use. Lyophilized milk standards (0 to 50 nmol/L) were reconstituted 24 h before use with the addition of 1.00 ml of distilled water, mixed, and refrigerated at 4°C until required. Milk samples and kits, except PEG, were removed from storage at 4°C and allowed to equilibrate to room temperature. One vial of assay buffer 1 (0.01 mol/L Tris buffer, containing 0.1% sodium azide, pH 7.4) was diluted with distilled water to a total volume of 100 ml.

Samples were homogenized by vortex mixer and diluted 1:2 with buffer 1 to give a total sample volume of 1.0 ml. Polystyrene radioimmunoassay tubes were used for the assay. Twenty-five microliters of zero standard was pipetted into the nonspecific binding (**NSB**) tubes, and 25 μl of standards (0 to 50 nmol/L) and diluted quality control milks and milk samples were pipetted into the appropriate tubes. Labeled ^{125}I progesterone (100 μl) was pipetted into all tubes, followed by 100 μl of reconstituted antiserum into all tubes, with the exception of total count and NSB tubes. Assay buffer 2 (100 μl) was pipeted into NSB tubes, all assay tubes were mixed for 10 s on a vortex mixer, and the tubes were incubated at 4°C for 30 min. Cold (4°C) PEG was decanted into each tube (500 μl), tubes were vortexed for 10 s, and the tubes were incubated at 4°C for a further 30 min. All tubes, except total tubes were centrifuged at 4°C for 60 min at 2000 rpm. The supernatant was aspirated and discarded, and the tubes counted on an automatic gamma counter (1470 Wizard Gamma Counter, Pharmacia, Wallac Oy, Finland).

Liquid-phase kits were unavailable for the processing of samples during later stages of the study, therefore, solid-phase, ^{125}I-labeled progesterone assay kits (Orion Diagnostica, Espoo, Finland) were used for the last 900 samples processed. Kits were identical to those described for plasma progesterone, with laboratory procedures analogous to those described for plasma progesterone. Interassay and intraassay coefficients of variation for the liquid phase kit were 11.7 and 3.6%, respectively. Interassay and intraassay coefficients of variation for the solid-phase kit were 15.5 and 6.7%, respectively. Comparison of samples assayed in duplicate by both liquid and solid kit types demonstrated that milk progesterone concentrations obtained from each kit type were significantly correlated ($r^2 = 0.89$, $P < 0.0001$) and showed no evidence of bias.

Reproductive Management

The reproductive health of all cows that had not been inseminated during the previous 42 d was monitored every 3 wk ± 3.5 d, beginning 3 wk ± 3.5 d after calving, by palpation of the reproductive tract per rectum. The uterine tract and ovaries were palpated per rectum by the same two veterinarians during the study and assessed for normal structure and function. Although cases of reproductive pathology (retained fetal membranes, metritis, endometritis, pyometra, and cystic ovarian disease) were recorded and treated according to good veterinary practices, treatment was not administered for nonpathological cases of anestrum, nor for cows recorded as 'no visual heat,' but detected with palpable corpora lutea.

Milk progesterone concentrations were used to indicate resumption of ovarian activity after calving. Cattle were observed for estrus for at least 14 h per d between 0400 and 1800 h by study staff trained in the signs of estrus in the dairy cow. Sensitivity of estrus detection was increased by placing KaMaR heatmount detectors (Steamboat Springs, Colorado) on the tailhead of each cow at d 7 after calving. All estrus signs were recorded as primary (standing to be ridden) or secondary (activated or missing KaMaR riding other cows, bellowing, pacing, or other behavioral changes). Cows were bred on the observation of primary heat signs and on the observation of secondary heat signs in the absence of primary signs. All cows were artificially inseminated 6 to 18 h after the detection of estrus. Cows were not bred before d 45 of lactation. For the purpose of semen allocation, cows were paired, one HD and one LD cow per pair, and allocated randomly to one of 15 bulls (Semex Australia Pty. Ltd., Bacchus Marsh, Victoria 3340, Australia). Cows were inseminated with one straw of semen, previously frozen at –18°C and thawed to 37°C before intrauterine placement. Insemination was performed by the same technician during the study. Semen quality was assessed on three occasions during the study. Two semen straws were selected at random from the semen storage tank and thawed to 37°C. Semen was microscopically examined and scored as good to excellent.

Calving to first ovulation interval was defined by the first increase in milk progesterone >6 nmol/L after calving (Spectria Veterinary Progesterone radioimmunoassay literature, Orion Diagnostica, Finland). Ovulation was assumed to have occurred 5 d prior to elevation of

progesterone concentration. Some estrus events detected by an activated KaMaR detector in the absence of other signs of estrus were, retrospectively, identified as being accompanied by 'ovulation.' Calving to first estrus interval was, therefore, defined as interval from calving to estrus for events accompanied by ovulation (progesterone concentration <6 nmol/L at the time of estrus). Similarly, interval from calving to first service interval was defined as interval from calving to first service accompanied by ovulation, as indicated by low milk or plasma progesterone concentration. Conception or pregnancy rate to first or to all services was calculated only for services accompanied by low plasma progesterone concentrations.

Plasma progesterone concentrations were used to define early establishment of pregnancy (initial conception), defined by a persistently elevated milk or plasma progesterone concentration for more than 24 d after insemination. In contrast, evidence of pregnancy palpable per rectum at d 42 after mating was used to define successful pregnancy to service (successful pregnancy). Interval from calving to initial conception was calculated using plasma progesterone concentrations. The interval from calving to successful pregnancy was calculated using palpation records. Early embryonic death, for the purpose of this study, was defined as loss of conceptus between initial conception diagnosis and confirmation of pregnancy by rectal palpation.

Statistical Analyses

Initially, data were assessed for outliers and normality using BMDP Dynamic 2D (BMDP Statistical Software Inc., Los Angeles, CA). Missing variables were estimated by regressing that variable on all variables that had acceptable values in the case with the missing value, using BMDP Dynamic AM. Repeatedly measured variables (DMI, milk yield, energy balance, NEFA, BHBA, glucose, urea, cholesterol, BW, and BCS) were assessed by repeated measures analysis of variance using BMDP 2V, initially using four-group evaluation and later a 2×2 factorial arrangement. We used genetic merit as a covariate in repeated measure analyses to investigate interactions between dietary group and outcomes. Conversely, repeated measure analyses used to investigate the influence of genetic merit on each variable were calculated with diet as a covariate. Differences between precalving BW for cows of high and low genetic merit indicated that BW 1wk before calving should be used as a covariate in BW analyses.

Effects of dietary protein degradability and genetic merit on time from calving to first ovulation, first estrus, first service, conception and pregnancy were examined using survival analysis (BMDP Dynamic 1L). Survival analysis is used to analyze the length of time to a response, an event occurring at a specific point in time, often called a death. Survivor function was estimated for each dietary and genetic merit group, and a test of equality of the survivor functions across dietary groups was performed using the Mantel-Cox test statistic (BMDP Dynamic 1L). When an event was not recorded for a cow, that cow was treated as censored. The effects of genetic merit on dietary group survival function, and the effects of dietary group on genetic merit survival function were examined using Cox's proportional hazards model (BMDP Dynamic 2L). Cox's proportional hazards regression model assumes that time-failure rates may be modeled as log-linear functions of the covariates.

The effects of diet and genetic merit on expression of estrus at first ovulation and all ovulations, conception, and pregnancy to first service, and conception and pregnancy to all services were investigated by creating two-way frequency tables (BMDP 4F). The Mantel-Haenszel χ^2 test of independence was used to test the null hypothesis that there was no significant relationship between diet and estrus expression, between diet and conception rate, between genetic merit and estrus expression, or between genetic merit and conception rate to first or all services.

One cow was culled at d 77 of lactation as a result of chronic vagal indigestion. Milk progesterone concentrations were not determined, in error, for that cow. Reproductive data for that cow was, therefore, excluded from analysis.

RESULTS

Daily DMI

Mean DMI was significantly higher for the LD dietary group than the HD group ($P = 0.003$; Table 3). Pattern of change of DMI with time differed significantly between dietary groups ($P = 0.02$), because DMI was higher for the LD group until wk 12 to 13 of lactation, when DMI for each dietary group converged. Genetic merit did not significantly increase mean daily DMI, or change in DMI through time ($P > 0.2$; Table 3).

Milk Yield

Mean milk yield differed significantly between dietary groups ($P < 0.01$; Table 3; Figure 1). Mean uncorrected milk production by the LD group was 4.0 L/d greater than for the HD group. The pattern of change in milk yield over time differed significantly between dietary groups ($P = 0.03$). Peak milk yield and persistency of milk production was greater for the LD group relative to the HD group.

Table 3. Mean (± SE) values for repeated measures analysis of covariance for blood metabolites (wk –3 to 10 of lactation), blood hormones (24 samples collected during intensive sampling period), and DMI, uncorrected milk yield, energy balance, BCS, and BW (wk –3 to wk 17 of lactation). Diet, controlling for genetic merit, and genetic merit controlling for diet.

	Low Degradable		High Degradable		Low genetic merit		High genetic merit	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Mean daily DMI (kg), 3 wk precalving to wk 17	21.7[ax]	1.9	20.0[ax]	1.9	20.6	1.9	21.0	1.9
Mean daily DMI (kg), 3 wk precalving	14.6	3.3	14.3	3.1	14.6	2.8	14.2	3.6
Mean daily DMI (kg), calving to wk 17	22.8[ay]	4.6	20.9	4.0	21.7	4.1	22.1	4.8
Mean daily uncorrected milk yield, L/d	39.0[ax]	4.3	35.0[ax]	4.2	35.6[c]	4.2	38.3[c]	4.3
Mean daily energy balance, MJME/d	28.7	16.2	26.8	15.9	33.5[bx]	16.0	21.9[bx]	16.0
Weekly BCS, 1–5 scale	3.20	0.17	3.13	0.17	3.23[ax]	0.17	3.13[ax]	0.17
Weekly BW, kg	607.8	40.9	593.2	39.9	596.4[bx]	39.9	604.5[bx]	40.9
Plasma								
BHBA	1.76[x]	0.38	1.65[x]	0.37	1.62	0.37	1.79	0.38
Cholesterol, mmol/L	4.01[y]	0.63	3.83[y]	0.62	3.86[y]	0.62	3.98[y]	0.63
Glucose, mmol/L	3.42	0.18	3.47	0.17	3.47[y]	0.17	3.42[y]	0.18
Gamma glutaryl transferase, mmol/L	19.9	2.8	20.2	2.7	20.2	2.7	19.9	2.8
Calcium, mmol/L	2.11	0.12	2.11	0.11	2.13	0.11	2.09	0.12
Urea, mmol/L	8.53[b]	0.54	8.99[b]	0.53	8.77	0.53	8.75	0.54
Growth hormone, ng/ml	1.94	3.08	2.41	3.37	1.25[c]	3.08	3.24[c]	3.37
Insulin, ng/ml	0.82	0.63	0.93	0.69	0.83	0.63	0.92	0.69
Serum								
NEFA, μmol/L	321.6[by]	74.5	387.9[by]	72.7	336.2	72.7	375.9	74.5

[a]Repeated measures analysis, $P < 0.01$; [b]Repeated measures analysis, $P < 0.05$; [c]Repeated measures analysis, $P < 0.1$.
[x]Variable*sampling time, $P < 0.01$; [y]Variable*sampling time, $P < 0.05$; [z]Variable*sampling time, $P < 0.1$.

Cows of high genetic merit produced significantly more milk across the first 17 wk of lactation compared with low genetic merit cows ($P = 0.05$; Table 3). A mean uncorrected milk yield of 38.3 L per cow daily was recorded for the high genetic potential group, compared with 35.6 L for the low genetic merit group. Genetic merit did not significantly influence ($P > 0.2$) the pattern of milk production over the first 17 wk of lactation.

Energy Balance, BCS, and BW

Differences between dietary groups in mean energy balance, BCS, and BW were not significant ($P > 0.15$; Table 3). The HD dietary group, however, tended to mobilize more BCS during early lactation than the LD group ($P = 0.17$). Energy balance during early lactation was significantly lower for high genetic merit cows compared with cows of low genetic merit ($P = 0.04$, Figure 2; Table 3). Further, the interaction between genetic merit and stage of lactation was highly significant for energy balance ($P = 0.009$). Cows of high genetic merit had a lower energy balance nadir, and were slower to return to positive energy balance after calving than cows of lesser genetic merit. Mean BCS differed significantly between genetic merit groups ($P = 0.005$), and pattern of change of BCS was significantly different (P

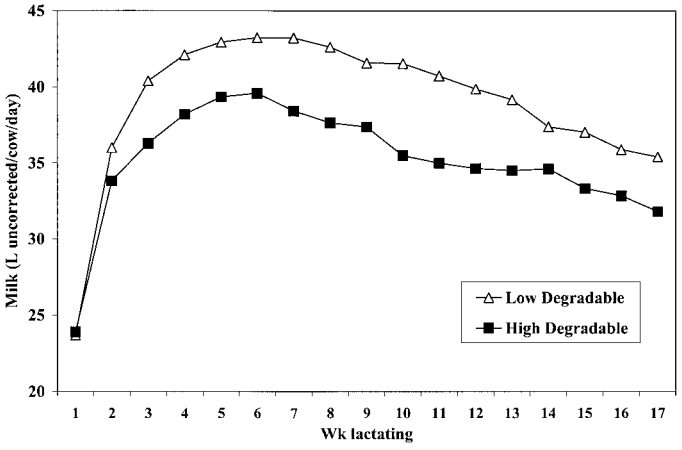

Figure 1. Effect of degradability of dietary protein on uncorrected milk production.

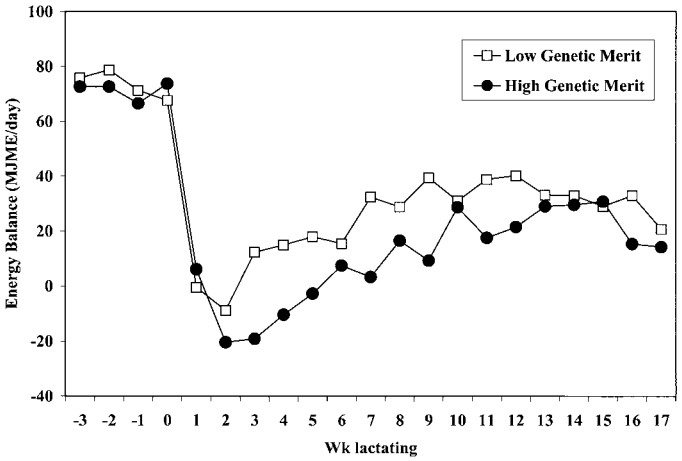

Figure 2. Effect of genetic merit of cows on energy balance.

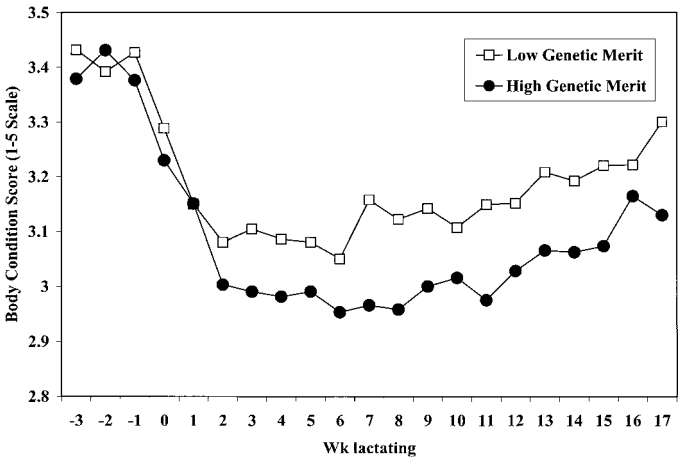

Figure 3. Effect of genetic merit of cows on body condition score [Scale 1–5 where 1 = thin and 5 = obese (19)].

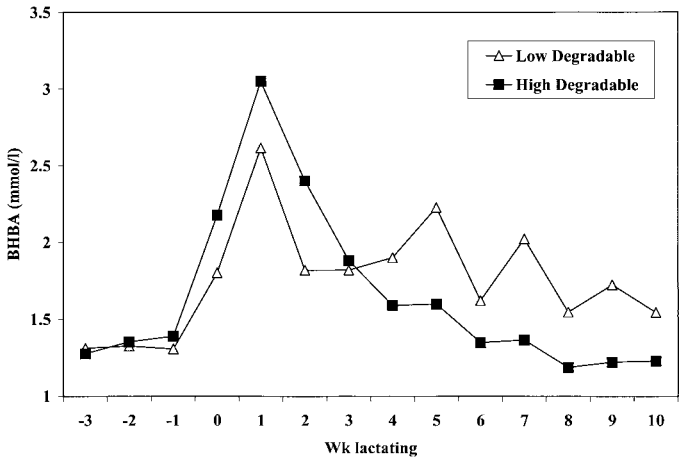

Figure 5. Effect of degradability of dietary protein on plasma concentration of BHBA.

< 0.0001; Figure 3) because high genetic merit cows mobilized more body tissue during early lactation than cows of low production potential. The BW was significantly lower ($P = 0.04$; Figure 4) for cows of high genetic merit, when BW 1 wk before calving was used as a covariate. The rate of BW change over the first 17 wk of lactation significantly differed ($P < 0.0001$) between genetic groups, because cows of high genetic merit lost more and were slower to regain weight during early lactation.

Diet, Blood Metabolites, and Blood Hormones

Mean plasma BHBA did not differ between dietary groups ($P > 0.2$; Table 3); however, the pattern of change in BHBA concentrations over time differed significantly between groups ($P = 0.008$; Figure 5). Plasma BHBA concentrations peaked approximately 0.4 mmol/L higher for the HD dietary group compared with the LD group; however, BHBA concentrations remained higher for longer in LD cows compared with cows fed the diet with more degradable protein.

Serum NEFA concentrations were significantly higher in the HD group than cows fed the less degradable protein source ($P < 0.02$). The pattern of change in serum NEFA concentrations differed significantly between dietary groups ($P < 0.05$; Figure 6), because NEFA concentrations in the LD group decreased more rapidly following the initial peak than those of the HD group.

Mean plasma cholesterol, glucose, GGT, and calcium concentrations did not differ significantly between di-

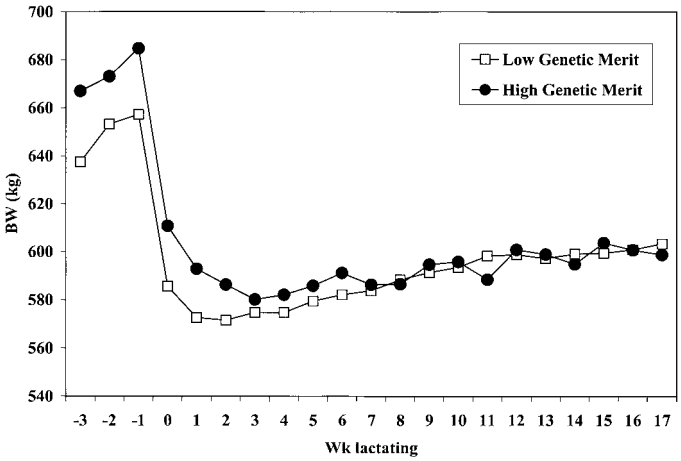

Figure 4. Effect of genetic merit of cows on BW.

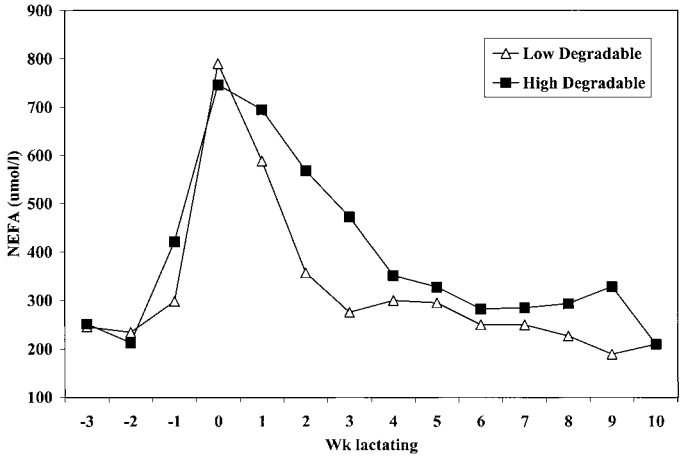

Figure 6. Effect of degradability of dietary protein on serum concentration of NEFA.

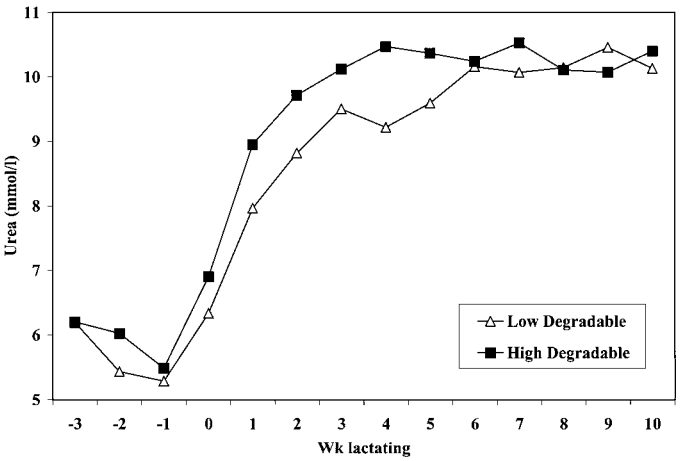

Figure 7. Effect of degradability of dietary protein on plasma concentrations of urea.

etary groups ($P > 0.2$; Table 3) and the interaction between stage of lactation and dietary group did not significantly affect plasma glucose, GGT, or calcium concentrations ($P > 0.2$). The pattern of change in plasma cholesterol concentrations differed significantly between dietary groups ($P < 0.02$) cholesterol concentrations increased more rapidly between wk 5 and wk 10 of lactation for the LD dietary group.

Mean plasma urea was significantly higher for the HD dietary group compared with the LD group ($P < 0.03$), and the pattern of change in plasma urea differed between dietary groups ($P < 0.06$). During the first 6 wk of lactation, plasma urea concentrations were higher for the HD group; however, between wk 6 and 10 of lactation, plasma urea concentrations were similar for cows fed either diet (Figure 7).

Concentrations of plasma growth hormone and plasma insulin from serially collected samples were not significantly different for subsets of cows fed HD or LD diets between wk 2 and 4 after calving ($P > 0.2$; Table 3). Change in concentrations of growth hormone and insulin with time during the 8-h collection period did not differ significantly between dietary groups ($P > 0.2$).

Genetic Merit, Blood Metabolites, and Blood Hormones

Neither mean concentrations of serum NEFA, plasma BHBA, GGT, calcium, glucose, cholesterol ($P > 0.2$; Table 3), nor the pattern of change in serum NEFA, plasma BHBA, GGT, calcium, and urea differed significantly between genetic merit groups ($P > 0.2$). However, the pattern of change of glucose concentration was significantly different between genetic groups ($P = 0.04$), because the nadir of plasma glucose concentration was reached later, and low concentrations of plasma glucose persisted for a longer period for cows of high genetic merit. The pattern of change of plasma cholesterol concentrations differed significantly between genetic groups because concentrations were not significantly different for the first 4 wk of lactation, however, between wk 5 and 10 of lactation, concentrations were higher for cows of high genetic merit.

Genetic merit and plasma growth hormone concentrations were associated positively (Table 3); the relationship approached significance ($P < 0.07$). Insulin concentrations did not significantly differ between genetic merit groups, and patterns of change of growth hormone and insulin across the 8-h sampling period were not affected by genetic merit ($P > 0.2$).

Dietary Group × Genetic Merit by Sampling Week Interactions

No significant interactions were found ($P > 0.2$) for DMI, milk yield, BW, BCS, plasma cholesterol, calcium, glucose, GGT, urea, or calculated energy balance (Table 3). Similarly, the interaction between diet, genetic merit, and sampling time across an 8-h period did not significantly influence plasma growth hormone or insulin concentrations ($P > 0.2$). Cows of high genetic merit fed the HD diet had persistently elevated mean serum NEFA concentrations and a higher peak plasma BHBA concentration compared with other cows ($P = 0.0001$ and $P = 0.13$; Figures 8 and 9, respectively).

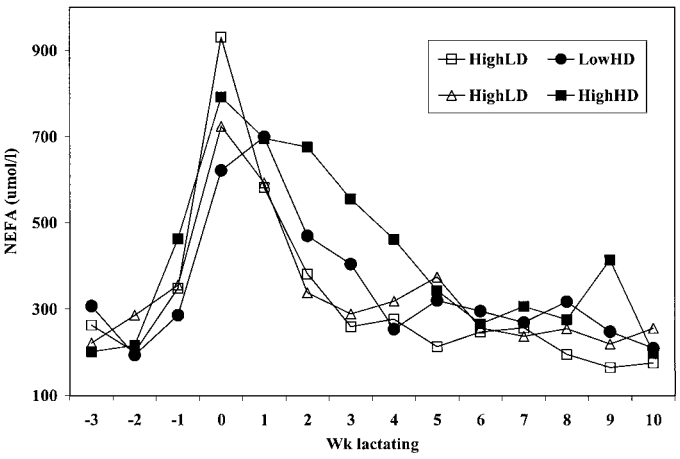

Figure 8. Effect of interaction between genetic merit of cows and degradability of dietary protein on concentrations of serum NEFA (Low LD = cows of low genetic merit fed protein of low degradability; Low HD = cows of low genetic merit fed protein of high degradability; High LD = cows of high genetic merit fed protein of low degradability; High HD = cows of high genetic merit fed protein of high degradability).

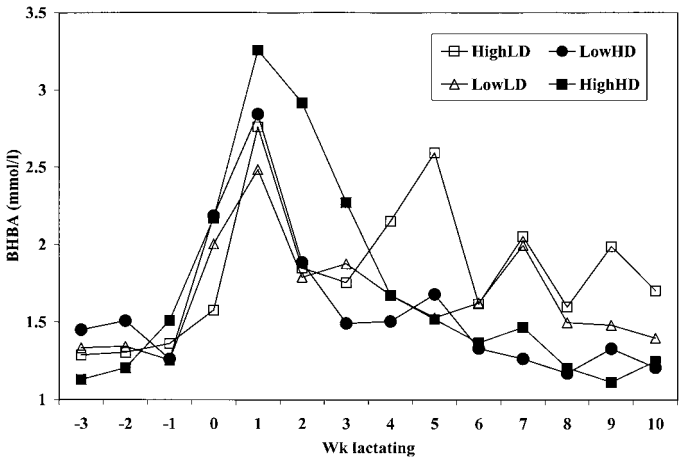

Figure 9. Effect of interaction between genetic merit of cows and degradability of dietary protein on concentrations of plasma BHBA (Low LD = cows of low genetic merit fed protein of low degradability; Low HD = cows of low genetic merit fed protein of high degradability; High LD = cows of high genetic merit fed protein of low degradability; High HD = cows of high genetic merit fed protein of high degradability).

Resumption of Ovarian Activity

Calving to first ovulation interval did not differ significantly between diet and genetic groups ($P > 0.1$; Table 4). Interval from calving to first estrus was not significantly different for dietary and genetic groups ($P > 0.1$; Table 4).

Expression of Estrus

Cows fed the HD ration tended have a greater incidence of silent estrus at first ovulation (Table 5). When the effects of diet were controlled for, cows of high genetic merit were at significantly greater risk of silent estrus at first ovulation ($P < 0.02$, Table 5) and a significant interaction between diet and genetic merit was found ($P = 0.006$). No cows of high genetic merit fed the HD ration showed estrus at first ovulation.

Forty-eight percent of ovulations recorded in this study were not accompanied by detected estrus activity. Neither diet nor genetic merit significantly influenced expression of estrus at all ovulations ($P > 0.99$; Table 5), and the interaction between diet and genetic merit was not significant for display of estrus at all ovulations ($P > 0.15$).

Calving to First Service, Conception, and Pregnancy Interval

Neither diet, nor genetic merit significantly affected days to first service ($P > 0.2$, Table 4). Calving to initial conception interval tended to be shorter for the LD

Table 4. The effect of dietary protein degradability and cow genetic merit on days to first ovulation, first estrus, first service and conception.

	Low degradable		High degradable		Low genetic merit		High genetic merit	
Median days to:	Days (Q1–Q3)[a]	% Censored	Days (Q1–Q3)[a]	% Censored	Days (Q1–Q3)[a]	% Censored	Days (Q1–Q3)[a]	% Censored
First ovulation	31.0 (22.0–54.0)	0	31.0 (19.3–46.0)	2.4	32.0 (23.0–54.8)	0	30.0 (20.0–45.0)	2.5
First estrus	67.0 (48.0–80.0)	2.5	60.5 (47.3–86.5)	2.4	66.5 (48.3–78.3)	0	59.0 (47.0–93.0)	5.0
First service	76.0 (57.0–84.0)	2.5	63.5 (55.0–91.8)	2.5	70.5 (56.0–83.5)	0	73.0 (55.0–96.0)	5.0
Initial conception	84.0 (70.0–112.0)	15.0[c]	101.5 (72.5)[b]	29.3[c]	96.5 (74.2–139.8)	22.0	93.0 (63.0–127.0)	22.5
Successful pregnancy	103.0 (73.0)[b]	30.0	109.0 (74.3)[b]	34.2	101.5 (74.3–148.8)	24.4	109.0 (72.0)[b]	40.0

[a]Range from quartile 1 to quartile 3.
[b]Could not be estimated accurately.
[c]$P = 0.11$.

Table 5. The effect of dietary protein degradability and cow genetic merit on the observation of estrus at first and all ovulations after calving.

	Low Degradable		High Degradable		Low genetic merit		High genetic merit	
	No. of cows	(%)	No. of cows	(%)	No. of cows	(%)	No. of cows	(%)
Estrus expression at first ovulation								
Expressed estrus	11	27.5	5	12.5	13	31.7	3[a]	7.7[a]
Silent	29	72.5	35	87.5	28	68.3	36	92.3
Estrus expression at all ovulations								
Expressed estrus	68	53.5	74	50.3	75	55.1	67	48.6
Silent	59	46.5	73	49.7	61	44.9	71	51.4

[a]Significantly different from low genetic merit (Mantel-Haenszel statistic $P < 0.02$).

dietary group after controlling for the influence of genetic merit ($P = 0.10$; Table 4; Figure 10). Almost 30% of HD cows failed to conceive by d 150 of lactation. In comparison, only 15% of LD cows failed to conceive by d 150 of lactation. After controlling for the influence of diet, calving to initial conception interval did not significantly differ between genetic groups ($P = 0.64$; Table 4). Interval from calving to successful pregnancy was not significantly different between dietary or genetic groups ($P > 0.3$; Table 4).

Conception and Pregnancy Rates

Feeding the HD diet resulted in poorer initial conception at first service ($P < 0.02$) because 72.0% of the LD group cows conceived to first service, compared with 45.0% of the HD group (Table 6). The number of successful pregnancies to first service, in contrast, did not differ significantly between dietary groups ($P > 0.2$; Table 6). Genetic merit did not significantly affect initial conception or pregnancy to first service ($P > 0.2$; Table 6).

A nonsignificant trend towards higher conception rate to all services was found for the LD dietary group ($P = 0.17$; Table 6); however, conception rate to all services was not significantly different between genetic groups ($P > 0.7$). The number of successful pregnancies to all services did not differ significantly for diet or genetic groups ($P > 0.5$).

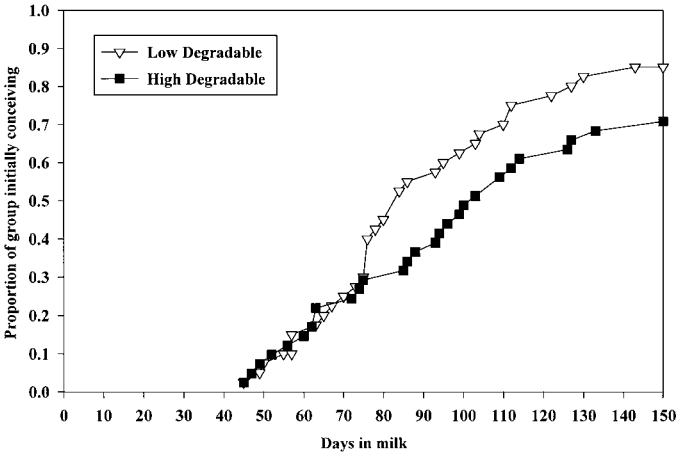

Figure 10. Effect of degradability of dietary protein on proportion of cows conceiving during the first 150 d of lactation.

Reproductive Pathology

The incidence of cystic ovarian disease and metritis was low and not influenced by genetic merit of cows or dietary treatment.

DISCUSSION

Production and Physiological Responses

Feeding more RUP as part of an isonitrogenous ration significantly increased DMI, an observation consistent with some (Orskov et al., 1981; Kaim et al., 1987), but not all studies (Bruckental et al., 1986; Garnsworthy and Jones, 1987; Carroll et al., 1994). Feeding more RUP can increase DMI by increasing intestinal delivery of AA, while supplementary dietary RDP may improve DM digestibility and increase DMI when rumen N is limiting. Feeding excessive RDP may depress DMI (Erb et al., 1976). Peak DMI for both dietary groups exceeded 23 kg of DM per cow daily. The HD ration contained 2.4% urea as formulated; therefore, at peak DMI urea intake exceeded published recommendations (Huber and Kung, 1981). The relatively high feed intakes and milk yields of the HD group may reflect adaptation to feeding of high concentrations of urea. A gradual introduction of NPN to a ration may facilitate adaptation to high concentrations in the diet (Erb et al., 1976; Huber and Kung, 1981). The significantly greater milk yield of LD cows is consistent with many other studies and provides further support for systems of nutritional evaluation that use protein degradability in predictions of response to feed.

The increased yield of milk stimulated by additional RUP was accounted for by increased feed intake because energy balance, BW, and BCS were not signifi-

cantly different between dietary groups. The HD cows, however, tended to mobilize more body tissue early in lactation, a finding consistent with others (Bruckental et al., 1986; Kaim et al., 1987). Greater requirements for AA precursors of milk protein synthesis, for gluconeogenesis, for the provision of endogenous AAN for urea synthesis, or increased energetic costs associated with the metabolism and excretion of excess dietary N may have induced slightly more mobilization of body tissue by the HD group. Others have found that feeding more RUP in isonitrogenous rations can lower energy balance (Carroll et al., 1994; Son et al., 1996) or increase BW loss (Orskov et al., 1981), or not significantly change the partitioning of nutrients (Blauweikel and Kincaid, 1986; Foldager and Huber, 1979; Garnsworthy and Jones, 1987; Jones and Garnsworthy, 1988). Variation in the response to RUP can be ascribed to different concentrations of dietary protein and carbohydrates, AA profiles of RUP, carbohydrate components of diets, stage of lactation and genetic potential of cows used in studies.

Plasma glucose concentrations were not significantly affected by diet, in agreement with other studies (Blauweikel and Kincaid, 1986; Foldager and Huber, 1979). Jones and Garnsworthy (1988), however, found a negative relationship between dietary RUP content and plasma glucose concentrations. Although irreversible loss of glucose in lactose was greater for the high RUP group, compensatory mechanisms may have acted either through increasing DMI and gluconeogenesis to increase glucose flux. Alternatively, plasma levels were maintained by decreased glucose uptake by tissues.

The tendency to greater adipose tissue mobilization by the HD dietary group was reflected in significantly higher serum NEFA concentrations. This finding differs from other studies (Blauweikel and Kincaid, 1986), in which significant differences in NEFA concentrations were not found between cows fed isonitrogenous diets containing high or low RUP. Garnsworthy and Jones (1987) and Jones and Garnsworthy (1988) reported higher NEFA concentrations for dairy cows fed more RUP. While diet did not influence mean plasma BHBA, concentrations were persistently higher for the LD group during early lactation. Greater lactogenic drive and requirement for glucose in the LD group may have stimulated ketone production in the higher producing group. The significant week × diet interaction found for plasma cholesterol provides further evidence of a lower energy balance in the HD group, because plasma cholesterol concentrations and energy balance are positively correlated after calving (Lean et al., 1992).

The lack of significant difference in plasma growth hormone and insulin concentrations between groups offered rations varying in protein degradability in serially collected blood samples taken from a subset of cows during early lactation, is similar to the report of Blauweikel and Kincaid (1986). However, the low numbers of cows used in this study diminished the power with which differences between groups could be detected.

Greater plasma urea concentrations for the HD group may have reflected excess dietary N intake relative to milk N demand, or increased catabolism of lean tissue in response to deficiencies in the diet of AA limiting for milk production. This finding is consistent with some (Foldager and Huber, 1979; Kaim et al. 1987), but not all studies (Carroll et al., 1994).

There was no significant effect of genetic merit on DMI, a finding consistent with other studies (Diab et al., 1996; Gibson et al., 1987; Lukes et al., 1989). Studies in which diets high in forages were fed (Gibson, 1986; Grainger et al., 1985; Holmes, 1988) found that genetic merit and feed intake were positively related. Veerkamp et al. (1995) found that feed intake was not significantly different for dairy cows of high and low genetic merit fed low forage: high concentrate diets, however, high genetic merit cattle ate significantly more when the forage: concentrate ratio was increased. Despite similar DMI, cows of high genetic merit produced significantly more milk over the first 17 wk of lactation, a finding consistent with most other studies (Grainger et al., 1985; Holmes, 1988; Veerkamp et al., 1995).

Table 6. The effect of dietary protein degradability and cow genetic merit on initial conception and pregnancy rates to first and all artificial inseminations.

	Low degradable		High degradable		Low genetic merit		High genetic merit	
	No. of cows	%	No. of cows	%	No. of cows	(%)	No. of cows	(%)
Cows conceived to first service								
Initial conception	28	71.8	18[a]	45.0[a]	23	56.1	23	60.5
Successful pregnancy	22	56.4	17	42.5	20	48.8	19	50.0
Cows conceived to all services								
Initial conception	36	52.2	31	40.8	35	44.9	32	47.8
Successful pregnancy	29	42.0	28	36.8	32	41.0	25	37.3
Embryonic death	8	9.5	3	3.2	4	4.2	7	8.9

[a]Significantly different from Low Degradable (Mantel-Haenszel statistic $P < 0.05$).

The greater efficiency of milk synthesis of high genetic merit cows resulted mainly from mobilization of body tissue. Other workers (Lukes et al., 1989; Veerkamp et al., 1995) also report that cows of higher genetic merit partition more energy and protein reserves in body tissues to milk. Greater demand for precursors to support higher milk yields of the high genetic merit group was not adequately compensated by increased DMI or greater entry of precursors to gluconeogenic pathways, because plasma glucose was significantly lower for those cows.

Surprisingly, serum NEFA nor plasma BHBA concentrations did not differ significantly between genetic merit groups. It was hypothesized that lower energy balance and greater body tissue loss in cows of high genetic merit would be reflected in higher serum NEFA concentrations. Despite a lower energy balance for high genetic merit cows, Lukes et al. (1989) also found no significant relationship between genetic merit and plasma NEFA. The similar NEFA concentrations between genetic groups in this study may reflect greater utilisation of NEFA for de novo synthesis of milk fat, because milk fat % was significantly higher for cows of high genetic merit (J. K. Garvin, unpublished).

Growth hormone is a key metabolic regulator (Bauman and Currie, 1980) responsible for partitioning of nutrients in favor of milk production in early lactation. Higher concentrations of growth hormone in cows of higher genetic merit group have also been observed by others (Barnes et al., 1985; Lukes et al. 1989; Kazmer et al., 1986). Genetic merit did not significantly affect plasma urea concentrations. In contrast, Barnes et al. (1985) reported a tendency towards higher blood urea concentrations for cows of superior genetic merit, an observation that was attributed to increased efficiency of catabolism of ingested protein for the high genetic merit group. Greater catabolism of body tissue by cows of high genetic merit may have also increased plasma urea concentrations in this group (Barnes et al., 1985).

It was hypothesized that feeding more RUP would promote greater milk yield and DMI, and that this response would be accentuated for cows of high genetic merit. However, neither DMI nor milk yield was significantly affected by the interaction between genetic merit and diet. Further, the interaction between genetic merit and diet was not significant for energy balance, BW change or body condition loss. Evidence for an interaction between diet and genetic merit was, however, provided in serum NEFA and plasma BHBA concentrations. Cows of superior genetic merit fed the HD diet had higher peak BHBA concentrations in early lactation, and higher NEFA concentrations persisted later in lactation, reflecting greater body tissue mobilization compared with other groups. If the AA supply from the HD diet was inadequate to support milk production, cows of superior genetic merit may have mobilized additional body tissue in response to greater demand for milk precursors.

Reproductive Performance

Resumption of ovarian activity, as indicated by time to first increase in milk progesterone after calving, was not significantly affected by diet, a finding in agreement with Son et al. (1996). Greater energy deficits during early lactation have been related to delayed calving to first ovulation interval (Butler and Smith, 1989). While the LD group produced significantly more milk, energy balance did not differ significantly between diets and the lack of significance in time to first ovulation between dietary groups was expected. Calving to first estrus interval did not differ significantly between dietary groups, despite a trend towards less expression of estrus at first ovulation for the HD group.

First-service conception rate was significantly lower for the HD group. There was also a nonsignificant tendency towards reduced conception rate to all services, and delayed calving to conception interval was found for HD cows. Conception rates were also lower in studies of cows fed diets containing more rapidly rumen degradable proteins, compared with those fed isonitrogenous rations containing less degradable proteins (Bruckental et al., 1989; Folman et al., 1981; Son et al., 1996).

The mechanisms by which a greater RDP:RUP ratio may have reduced conception rates were not determined, however, lower energy balance during early lactation, and greater concentrations of byproducts of RDP metabolism may have contributed to lower conception rates in the HD group. Feeding more RDP tended to reduce energy balance and increase loss of body condition in this study. Greater magnitude and duration of NEB after calving has been associated with lower mean LH concentrations and LH pulse frequency (Butler and Smith, 1989), impaired follicular development (Lucy et al., 1991) and lower progesterone production (Villa-Godoy et al., 1990).

Plasma urea concentrations were greater for the HD group and high concentrations of urea in blood or plasma are associated with decreased plasma progesterone concentrations (Butler, 1998). Plasma progesterone concentrations during the estrus cycle before insemination have been positively associated with conception rates (Folman et al., 1973). Other mechanisms by which RDP can adversely influence conception have been proposed and include increased concentrations of urea in the uterus, changed uterine pH, and altered mineral flux across the uterine wall (Butler, 1998). Higher plasma urea concentrations may, therefore,

have altered the uterine environment for the HD dietary group and lowered gamete or embryo viability.

Pregnancy to first and all services, and time to successful pregnancy was not significantly different for HD and LD groups, despite significantly lower initial conception rates at first true service for HD cows. Pregnancy did not differ between dietary groups probably because embryonic loss was greater for the LD group (8, compared with 3 for HD). Loss of conceptus was ascribed to incorrect breeding of early pregnant cows (two cows), surgery for left displaced abomasum (2), severe systemic coliform mastitis (1), or was of unknown etiology (3). In contrast, loss of conceptus in the HD group was ascribed to incorrect breeding in early pregnancy (1) and of unknown etiology (2).

Cows of superior genetic merit had a significantly lower energy balance, which was hypothesized to delay onset of ovarian activity after calving. While the magnitude of negative energy balance (**NEB**) was significantly greater for superior genetic cows, calving to NEB nadir interval was not different between genetic groups and the interval to progesterone rise did not significantly differ. Time from calving to NEB nadir was a more significant determinant of onset of ovarian activity after calving than the magnitude of NEB (Canfield and Butler, 1990). A lower EB may, however, have contributed to less estrus expression at first ovulation for cows of high genetic merit, because cows in NEB were less likely to show estrus at first ovulation compared with those in positive energy balance (Spicer et al., 1990; Villa-Godoy et al., 1990).

It was hypothesized that a genetic merit by diet interaction may exist for reproductive outcomes because cows of higher genetic merit fed the HD diet showed evidence of greater mobilization of body tissue. While estrus expression at first ovulation was significantly lower for cows of superior genetic merit fed the HD diet, conception and pregnancy did not differ significantly. Low numbers of cows used limited the power with which differences between the four groups could be detected, and ad libitum feeding is likely to have prevented excessive mobilization of body tissue by cows of high genetic merit. Further studies to investigate the interaction between high concentrations of rapidly degradable protein in diets of modest energy status and higher genetic merit are warranted. Higher cow numbers, and restricted feeding protocols that emphasize the physiological response by cows of superior genetic merit to early lactation would permit a more sensitive assessment of the effects of a cow genetic merit by dietary protein interaction on reproductive performance.

CONCLUSIONS

Feeding more RDP was associated with lower DMI and milk yield, a tendency towards increased mobilization of body tissue, and significantly lower conception rates at first service. While cows of high genetic merit produced significantly more milk, DMI was unaffected by genetic merit, and mobilization of body tissue supplied additional nutrients to support greater milk production. This finding suggests that we may be selecting cows that are less well adapted to survival in the herd, as reproductive failure is associated with greater tissue loss. The significant interaction between genetic merit and diet on concentrations of serum NEFA, and the lower expression of estrus at first ovulation for cows of high genetic merit fed more RDP indicates that interactions between diet and genotype are important. We also found evidence that cows can compensate for, or to adapt to high concentrations of rapidly degradable dietary protein. This study indicates that feeding more protected protein when dietary energy is not limiting will benefit the productivity and fertility of high producing lactating dairy cows.

ACKNOWLEDGMENTS

The authors thank the industry and university partners of the Collaborative Research Grant for support of this study; Millmaster Feeds (Sydney, N.S.W., Australia), Dairy Farmers Co-Operative (Wetherill Park, N.S.W. Australia), Elanco Animal Health (North Ryde, N.S.W. Australia), Wrightson Seeds Australia (Laverton, Victoria, Australia), Department of Employment, Education and Training, New South Wales Dairy Farmers Association (Canberra, A.C.T., Australia), New South Wales Dairy Corporation (Chippendale, N.S.W. Australia), Alfa Laval Agri Pty (Hamilton, New Zealand), Dairy Research and Development Corporation (Melbourne, Victoria, Australia), Milk Marketing, Semex Australia Pty Ltd (Bacchus Marsh, Victoria, Australia), University of Sydney, University of Western Sydney and the Foundation for Research Science and Technology (New Zealand). Appreciation is also extended to Jim Della Vedova, study manager, Steve Allingham, farms manager, and laboratory staff at the Dairy Research Unit and the Rural Veterinary Centre, University of Sydney.

REFERENCES

Agricultural and Food Research Council. 1993. Pages 21–32 *in* Energy and Protein Requirements of Ruminants. CAB International, Wallingford, U.K.

Barnes, M. A., G. W. Kazmer, R. M. Akers, and R. E. Pearson. 1985. Influence of selection for milk yield on endogenous hormones and metabolites in Holstein heifers and cows. J. Anim. Sci. 60:271–284.

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.

Blauwiekel, R., and R. L. Kincaid. 1986. Effect of crude protein and solubility on performance and blood constituents of dairy cows. J. Dairy Sci. 69:2091–2098.

Bruckental, I., H. Tagari, S. Amir, H. Kennit, and S. Zamwell. 1986. The effect on the performance of dairy cattle of plant protein concentration and of urea or urea-phosphate supplementation in the diet. Anim. Prod. 43:73–82.

Bruckental, I., D. Drori, M. Kaim, H. Lehrer, and Y. Folman. 1989. Effects of source and level of protein on milk yield and reproductive performance of high-producing primiparous and multiparous dairy cows. Anim. Prod. 48:319–329.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and post partum reproductive function in dairy cattle. J. Dairy. Sci. 72:767–783.

Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle J. Dairy Sci. 81:2533–2539.

Canfield, R. W., and W. R. Butler. 1990. Energy balance and pulsatile LH secretion in early post partum dairy cows. Domest. Anim. Endocrinol. 7:323–330.

Carroll, D. J., F. R. Hossain, and M. R. Keller. 1994. Effect of supplemental fish meal on the lactation and reproductive performance of dairy cows. J. Dairy Sci. 77:3058–3072.

Diab, I. A., and J. K. Hillers. 1996. Effect of selection for milk yield and dietary energy on yield traits, bovine somatotropin, and plasma urea nitrogen in dairy cows. J. Dairy Sci. 79:682–688.

Edmondson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78.

Erb, R. E., H. A. Garverick, C. J. Callahan, R. S. Patton, and E. L. Monk. 1976. Dietary urea for dairy cattle III. Effect on ovarian activity. Theriogenology 5:203–211.

Foldager, J., and J. T. Huber. 1979. Influence of protein percent and source on cows in early lactation. J. Dairy Sci. 62:954–964.

Folman, Y., M. Rosenberg, Z. Herz, and M. Davidson. 1973. The relationship between plasma progesterone concentration and conception in post partum dairy cows maintained on two levels of nutrition. J. Reprod. Fertil. 34:267–278.

Folman, Y., H. Neumark, M. Kaim, and W. Kaufmann. 1981. Performance, rumen and blood metabolites in high yielding cows fed varying protein percents and protected soybean. J. Dairy Sci. 64:759–768.

Garnsworthy, P. C., and G. P. Jones. 1987. The influence of body condition at calving and dietary protein supply on voluntary food intake and performance in dairy cows. Anim. Prod. 44:347–353.

Gibson, J. P. 1986. Efficiency and performance of genetically high and low milk-producing British Friesian and Jersey cattle. Anim. Prod. 42:161–182.

Gibson, J. P., A. C. Field, and G. Weiner. 1987. Concentrations of blood constituents in genetically high and low milk-production lines of British Friesian and Jersey cattle around calving and in early lactation. Anim. Prod. 44:183–199.

Grainger, C., W. F. Davey, and C. W. Holmes. 1985. Performance of Friesian cows with high and low breeding indexes. I. Stall feeding and grazing experiments and performance during the whole lactation. Anim. Prod. 40:379–388.

Holmes, C. W. 1988. Genetic merit and efficiency of milk production by the dairy cow. Pages 195–215 *in* Nutrition and Lactation in the Dairy Cow, P. C. Garnsworthy, ed. Butterworths, London.

Huber, J. T., and L. Kung. 1981. Protein and non protein nitrogen utilisation in dairy cattle. J. Dairy Sci. 64:1170–1195.

Jones, G. P., and P. C. Garnsworthy. 1988. The effects of body condition at calving and dietary protein content on dry matter intake and performance in lactating dairy cows given diets of low energy content. Anim. Prod. 47:321–333.

Kaim, M., H. Neumark, Y. Folman, and W. Kaufmann. 1987. The effect of two concentrations of dietary protein and of formaldehyde treated soya-bean meal on the performance of high yielding dairy cows. Anim. Prod. 44:333–345.

Kazmer, G. W., M. A. Barnes, R. M. Akers, and R. E. Pearson. 1986. Effect of genetic selection for milk yield and increased milking frequency on plasma growth hormone and prolactin concentration in Holstein cows. J. Anim. Sci. 63:1220–1227.

Lean, I. J., T. B. Farver, H. F. Troutt, M. L. Bruss, J. C. Galland, R. L. Baldwin, C. A. Holmberg, and L. D. Weaver. 1992. Time series cross correlation analysis of postparturient relationships among serum metabolites and yield variables in Holstein cows. J. Dairy Sci. 75:1891–1900.

Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early post partum dairy cows. J. Dairy Sci. 74:473–482.

Lukes, A. J., M. A. Barnes, and R. E. Pearson. 1989. Response to selection for milk yield and metabolic challenges in primiparous dairy cows. Domest. Anim. Endocrinol. 6:287–298.

Orskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499–503.

Orskov, E. R., G. W. Reid, and I. McDonald. 1981. The effects of protein degradability and food intake on milk yield and composition in cows in early lactation. Br. J. Nutr. 45:547–555.

Orskov, E. R., G. W. Reid, and C. A. G. Tait. 1987. Effect of fish meal on the mobilisation of body energy in dairy cows. Anim. Prod. 45:345–348.

Rabiee, A. R. 1995. Transovarian uptake of metabolites in sheep and cattle. Ph.D. thesis, University of Sydney, Australia.

Son, J., R. J. Grant, and L. L. Larson. 1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 79:822–830.

Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-1 in dairy cows: Relationships among energy balance, body condition, ovarian activity and estrus behavior. J. Dairy Sci. 73:929–937.

Veerkamp, R. F., G. Simm, and J. D. Oldham. 1995. Genotype by environment interactions: Experience from Langhill. Pages 59–65 *in* Breeding and Feeding the High Genetic Merit Dairy Cow. Occasional Publ. No. 19, Br. Soc. Anim. Sci.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, E. P. Stanisiewski, and R. L. Fogwell. 1990. Influence of energy balance and body condition on estrus and estrous cycles in Holstein heifers. J. Dairy Sci. 73:2759–2765.

Westwood, C. T., I. J. Lean, and R. C. Kellaway. 1998. Indications and implications for testing of milk urea in dairy cattle: A quantitative review. Part 2. Effect of dietary protein on reproductive performance. N.Z. Vet. J. 46:123–130.

Zivin, J. A., and J. F. Snarr. 1973. An automated colorimetric method for the measurement of 3-Hydroxybutyrate concentration. Anal. Biochem. 52:456–461.