Effect of dietary protein content on the fertility of dairy cows during early and mid lactation

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ABSTRACT

Ninety autumn-calving Holstein dairy cows (45 primiparous and 45 multiparous; mean parity, 3.1) were allocated to 1 of 3 treatments; 173, 144, or 114 g of crude protein (CP)/kg of dry matter (DM) from calving until d 150 of lactation. On d 151 of lactation, half the animals receiving 114 g of CP/kg of DM went onto 144 g of CP/kg of DM, half of the animals receiving 144 g of CP/kg of DM went onto 173 g of CP/kg of DM, and half of the animals receiving 173 g of CP/kg of DM went onto 144 g of CP/kg of DM, with the remaining animals staying on their original treatments. This resulted in 6 treatments in mid to late lactation: 114/114; 144/144; 173/173; 114/144; 144/173; and 173/144 g of CP/kg of DM. Overall, 95.3% of cows intended for breeding conceived during a 6-mo breeding period. The average pregnancy rates to first service and first plus second service were 30.9% [standard error of the difference (SED), 0.05] and 56.7% (SED, 0.05) respectively. The average 100 d in-calf rate from the start of the breeding period was 70.5%, and at least one abnormal progesterone profile was observed in 62% of animals. An increase in dietary protein content decreased the requirement for treatment of metritis. There was no effect of dietary protein content on any of the reproductive or progesterone measures; for example, days to conception, calving interval, 100 d in-calf rate (from commencement of breeding), days to onset of luteal activity, average luteal phase, average interovulatory interval, or average interluteal interval. An increase in dietary protein content decreased the average daily energy balance. A more positive energy balance was associated with an increased requirement for the treatment of metritis in the current study. Cumulative energy balance was positively associated with conception. There was no effect of the concentration of plasma urea on any of the reproductive variables; however, the concentration of serum leptin was favorably

associated with the time to progesterone increase above 3 ng/mL, which has been deemed essential for embryo survival. Additionally, the average peak concentration of progesterone and the duration of the average luteal phase were favorably associated with the interval from calving to conception. The latter relationships emphasize the importance of progesterone in achieving and maintaining pregnancy.

Key words: reproduction, energy balance, progesterone, conception

INTRODUCTION

Postpartum nutrition plays a significant role in the onset of ovarian cyclicity, the expression of normal estrous cycles, and conception rates (Robinson et al., 2006). However, the importance of protein nutrition on the reproductive performance of the modern high-yielding dairy cow is ambiguous (Laven and Drew, 1999). There are numerous studies reporting direct negative effects (Larson et al., 1997), indirect or associative effects (Barton et al., 1996), or no effects (Kenny et al., 2001) of elevated dietary protein content on reproductive performance. For example, work by Jordan and Swanson (1979) showed that an increase in dietary CP content increased the interval from calving to first ovulation and reduced the pregnancy rate. However, other studies have failed to support this observation (Laven and Drew, 1999). Westwood et al. (1998) also reported confounding evidence on the effect of dietary protein concentration on conception rates. However, Westwood et al. (1998) concluded that a stronger relationship existed between dietary CP concentration and conception rates in older animals compared with primiparous animals. Work by Butler (2001) illustrated that elevated blood urea concentration coupled with suboptimal early luteal progesterone concentrations are detrimental to embryo survival. Interestingly, Orihuela (2000) indicated that a severe protein deficiency will interfere with reproductive processes, as will an excessively high protein concentration in the diet. The detrimental effects of protein deficiency were previously emphasized by Westwood et al. (1998) who stated that low-CP diets

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result in suboptimal microbial protein synthesis in the rumen and are associated with reproductive failure.

The current study was undertaken to investigate possible reasons for the inconsistencies in the effects of dietary protein concentration on reproductive performance presented in the literature. The specific aim of the present study was to examine the response of high-yielding Holstein cows to low-protein diets, in terms of effects on energy balance and fertility. The effects of 3 levels of total dietary CP on a range of reproductive variables are reported.

MATERIALS AND METHODS

Animals and Housing

Ninety Holstein Friesian dairy animals (45 primiparous and 45 multiparous; mean parity 3.1) were used in the study. Calving commenced on August 29 and ended on December 23. Following calving, animals were housed as a group in free stalls (cubicles) with concrete flooring. The cubicle to cow ratio was $\geq 1:1$ at all times, meeting the recommendations set by FAWC (1997). Cubicles had a bed measurement of 2.20 m long and 1.25 m wide, were fitted with rubber mats, and bedded with sawdust thrice weekly. Concrete passageway floors were scraped at least 4 times daily using an automated system. Cows were milked twice daily through a 50-bail rotary parlor at 0530 and 1630 h, with cows traveling about 35 m to the milking parlor. Lights were left on at all times. Animals were inseminated from a minimum of 42 d postcalving, on the first observed standing heat following the commencement of the breeding period (November 23).

Experimental Design, Diets, and Feeding

The experiment involved allocating 90 freshly calved Holstein Friesian dairy animals to 3 dietary treatments that differed in overall CP levels, as formulated, in the complete diet (DM basis); 180, 150, and 120 g/kg of DM. At d 151 of lactation, half of the animals in each treatment were allocated (balanced for parity, milk yield, calving date, and BW) to an alternative dietary CP concentration, and the remaining animals were maintained on their original diets. Half of the animals receiving 120 g of CP/kg of DM went onto 150 g of CP/kg of DM, half of the animals receiving 150 g of CP/kg of DM went onto 180 g of CP/kg of DM, and half of the animals receiving 180 g of CP/kg of DM went onto 150 g of CP/kg of DM. This resulted in 6 treatments (1 to 151/151 to 305 DIM): 120/120; 150/150; 180/180; 120/150; 150/180; and 180/150. Primiparous animals were assigned in a balanced manner to treat-

ments based on heifer rearing regimen, calving date, and BW. Multiparous animals were assigned to treatments according to parity, previous lactation milk yield, calving date, and BW. The diet was presented as a TMR; animals had free access to water at all times and were fed between 1000 and 1100 h daily using a diet feeder. The concentrate to forage ratio (DM basis) was 0.55:0.45 for all diets. The forage component of the diet consisted of 0.60 grass silage and 0.40 maize silage (DM basis). Samples of grass and maize silage were taken weekly and analyzed using near infrared reflectance spectroscopy (Park et al., 1998) and twice weekly for measurement of nitrogen and ammonia nitrogen using methods outlined by Steen (1989). Two concentrates were formulated to contain 229 and 117 g of CP/kg of DM to achieve target protein levels in the overall diets of 180 and 120 g of CP/kg of DM respectively. Diets containing 150 g of CP/kg of DM were produced by complementing the forage component with equal amounts of concentrates containing 117 and 229 g of CP/kg of DM. The energy and protein concentrations of individual ingredients were based on published values (AFRC, 1993), which were used in the initial formulation of the concentrate proportion of the diet as presented in Table 1. Concentrate samples were taken weekly during the experiment and analyzed for DM, ash, ADF, NDF, and nitrogen as described by Cushnahan and Gordon (1995). The TMR diets were offered ad libitum using feed boxes that were placed on a computer-recorded load cell system, with controlled access to the boxes using an electronic identification system. This enabled the DMI of individual animals to be recorded continuously via automatic feeding gates (Calan gate feeder, American Calan, Northwood, NH), from which a daily intake was calculated and then averaged on a weekly basis.

Following approval from an ethical review committee, the experiment was conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and sampling techniques.

Measurements

Milk yields were recorded daily at each milking throughout the experiment. Milk composition (fat, protein, lactose, and SCC) was determined weekly from one consecutive a.m. and p.m. milking. Separate analysis was completed for a.m. and p.m. samples and milk composition was calculated on the basis of recorded a.m. and p.m. milk yields. Milk composition was determined using an infrared milk analyzer. Body weight and BCS (scale: 0–5; Edmonson et al., 1989) were recorded weekly throughout lactation. Locomotion scores were carried out every 2 wk using the method

Table 1. Effect of dietary protein concentration in early and mid lactation on animal performance and energy balance (1 to 140d)

Item	Dietary CP concentration (g/kg of DM)			Significance	
	114	144	173	SED ¹	P-value
Milk yield (kg/d)	25.4 ^c	31.8 ^b	35.4 ^a	1.14	***
DMI (kg/d)	16.5 ^b	18.0 ^a	18.6 ^a	0.35	***
ME requirement ² (MJ/d)	191.3 ^c	222.3 ^b	242.3 ^a	6.34	***
ME intake (MJ/d)	204.2 ^b	222.8 ^a	231.0 ^a	4.54	***
Daily energy status ² (MJ/d)	12.78 ^a	0.539 ^{ab}	-11.05 ^b	6.20	**
Cumulative energy status ² (MJ)	414 ^a	-537 ^a	-1,801 ^b	520	***

^{a-c}Within rows, means not sharing a common superscript differ significantly.

¹SED = standard error of the difference.

²ME requirement, daily energy balance, and cumulative energy balance were calculated using the equations described by Thomas (2004).

** $P < 0.01$; *** $P < 0.001$.

described by Manson and Leaver (1988). Blood samples were collected weekly between 0930 and 1130 h from the coccygeal vein using uncoated, heparin-coated, and fluoride oxalate-coated tubes (Becton Dickinson, Oxford, UK) from calving until d 100 of lactation and then every 2 wk thereafter. Plasma was recovered by centrifugation for analysis of glucose, (fluoride oxalate tubes) total protein, albumin, globulin, BHBA, NEFA, and urea (heparinized tubes), respectively, by clinical analyzer (Olympus UK Ltd, Middlesex, UK). Concentrations of NEFA were determined using a standard kit (Wako Chemicals GmbH, Neuss, Germany). Uncoated tubes provided serum for leptin RIA. Plasma and serum samples were stored at -20°C until analyzed. The RIA were balanced for dietary treatments and parity, and control samples were included in each assay.

Milk samples were collected twice weekly for use in progesterone analyses (Tuesday and Friday; a.m. milk samples) until pregnancy confirmation at d 30 postinsemination. Pregnancy was reconfirmed at d 60 postinsemination and in the event of embryo loss, milk sampling for progesterone recommenced. Each sample had preservative added (Lactab Mark III, Thompson and Cooper Ltd., Runcorn, UK) and was stored at 4°C until analysis (within 4 wk). Milk progesterone concentrations were determined using an ELISA kit (Ridge-way Science Ltd., Lydney, UK) based on the method of Sauer et al. (1986).

Reproductive Performance

All fertility events were recorded until 6 mo after the commencement of breeding. Insemination details included date, hour, sire, and inseminator. On average, cows were inseminated 12 h after visual observation of estrus. Pregnancy was diagnosed via an ultrasound scan carried out by a veterinary surgeon at d 30 and d

60 postinsemination. Late embryo loss (>60 d postinsemination) was defined by the return of an animal to estrus that had previously been diagnosed as pregnant at d 60 postinsemination. Conception was defined by parturition, marking the commencement of the subsequent lactation.

Stockpersons examined animals for metritis weekly. Metritis was classified as the presence of uterine discharge from the vagina 21 d or more after calving. Animals displaying uterine discharge were presented for veterinary inspection. Following confirmation of metritis, animals were treated with Pevidine (Novartis Animal Health UK Ltd., Surrey, UK; iodine-based solution, 60 mL, 1:5 dilution, administered through a stainless steel catheter into the uterine body), pessaries (Utocyl, combination of antibiotics; 6 pessaries administered into the uterus for the prevention of postpartum metritis, leucorrhoea, endometritis, and pyometra; Novartis Animal Health UK Ltd., Surrey, UK), or both. Additional intervention products included controlled internal drug-releasing insert (CIDR) and progesterone-releasing intravaginal device (PRID; used for the treatment of anestrus or follicular ovarian cysts); Estrumate (prostaglandin analog cloprostenol, which causes luteolysis followed by return to estrus; Schering-Plough Animal Health, Middlesex, UK); and Fertagyl (synthetic equivalent of GnRH; gonadorelin, which optimizes time of ovulation and improves conception rate; Janssen Animal Health, Saunderton, UK).

Definitions of Progesterone Measures

Royal et al. (2000) and McCoy et al. (2006) provide full descriptions defining ovarian activity and abnormal progesterone profiles, respectively. Ovarian activity measures were defined as follows. Onset of luteal activity (OLA) was defined by the first of at least 2 consecutive

progesterone concentrations ≥ 3 ng/mL in composite milk. The luteal phase of an individual estrous cycle was defined as the time between the first elevated progesterone concentration measuring ≥ 3 ng/mL and the final consecutive milk progesterone concentration measuring ≥ 3 ng/mL. The interovulatory interval (**IOI**) was defined as the time between the first progesterone increase of one cycle to the first progesterone increase of the next cycle. The interluteal interval (**ILI**) was defined as the time between the demise of one corpus luteum and the rise of the next. It is the interval from the first progesterone concentration < 3 ng/mL at the end of one cycle until the last consecutive progesterone concentration < 3 ng/mL in composite milk at the start of the next cycle.

Abnormal progesterone patterns were defined as follows. Delayed ovulation type I (**DOV I**) was defined as progesterone concentrations < 3 ng/mL in composite milk for ≥ 45 d (prolonged anovulation). Delayed ovulation type II (**DOV II**) was defined as progesterone concentrations < 3 ng/mL in composite milk for ≥ 12 d after OLA (prolonged ILI). Persistent corpus luteum type I (**PCL I**) was defined as progesterone concentrations ≥ 3 ng/mL for ≥ 19 d on the first luteal phase (delayed luteolysis of the corpus luteum during the first estrous cycle). Persistent corpus luteum type II (**PCL II**) was defined as progesterone concentrations ≥ 3 ng/mL for ≥ 19 d on subsequent luteal phases (delayed luteolysis of the corpus luteum during subsequent estrous cycles).

Calculation of Energy Balance

The average daily energy balance for each animal was calculated each week of lactation using the equations described by Thomas (2004): [Energy balance = ME intake - ME requirement $\{-10 + (ME_{\text{preg}} + ME_{\text{maintmilk}} \times Lwt^{0.75}) + [(0.0013 \times Lwt)/K_m]\}$, where Lwt = liveweight and K_m = efficiency of energy use for maintenance $[0.35 \times (ME/\text{gross energy}) + 0.503]$. Missing values were estimated from the week before and the week after the week with missing data. Less than 2% of the data were missing.

Statistical Analyses

A repeated-measures approach using the REML procedure available in GenStat (Payne et al. 1993) was used to analyze the data set. The model fitted fixed effects for parity, dietary protein concentration, and stage of lactation (weeks from calving) for each dependent variable. The model included all 2-level interactions between those independent variables. There was no significant effect of dietary protein concentration on

BCS change over the first 3 wk of lactation and the deviation from the mean BCS of multi- and primiparous animals during this period was used as a covariate in this experiment.

Multiple linear regression analysis was performed for several independent variables on all the reproductive variables. Independent variables used in the multiple linear regression analysis were milk yield, milk energy, daily energy balance, cumulative energy balance, average daily energy balance in the first 3 wk of lactation, average daily energy balance in the first 6 wk of lactation, and weeks to energy nadir. A Spearman's correlation matrix was also performed on all continuous variables.

A logistic regression model was used to analyze the following data; pregnancy rate to first service, pregnancy rate to first and second service, 100 d in calf rate, intervention, delayed ovulation types I and II, persistent corpus luteum types I and II, and whether or not there was more than one abnormal progesterone profile observed.

RESULTS

For the purpose of the analysis, data after d 150 of lactation are not presented because only 16 cycles, from a total of 412 cycles across all animals, occurred on a changed diet (half the animals changed diets at 150 d postcalving). The composition (as-fed) of diets targeted to contain 120, 150, and 180 g of CP/kg of DM were 114, 144, and 173 g of CP/kg of DM, respectively. All diets were isoenergetic.

Effect of Protein Concentration on Energy Measures (0–150 d)

Dietary protein concentration effects on production measures (milk yield, DMI, ME requirement, ME intake, daily energy balance, and cumulative energy balance) are presented in Table 1. A full description of the effects of dietary protein on production measures is given in Law et al. (2009). Increased dietary protein concentration significantly ($P < 0.001$) increased milk yield, DMI, and ME requirement. Increasing dietary protein concentration from 114 to 144 or 173 g/kg of DM significantly ($P < 0.001$) increased ME intake. Increasing dietary CP concentration from 114 or 144 to 173 g/kg of DM significantly decreased daily energy balance and cumulative energy balance.

Effect of Protein Treatment on Reproductive Variables

An increase in the dietary protein concentration significantly ($P < 0.05$) decreased the proportion of

Table 2. Effect of dietary protein concentration on the incidence of metritis and the proportion of cows requiring intervention with a reproductive treatment, within each protein treatment

Reproductive ailment/ treatment ¹	Protein treatment (g of CP/kg of DM)			Significance
	114	144	173	
Metritis	0.45 ^b	0.25 ^a	0.13 ^a	*
Pevidine	0.31 ^b	0.19 ^{ab}	0.06 ^a	*
Pessaries	0.34 ^b	0.11 ^{ab}	0.04 ^a	*
CIDR/PRID	0.29	0.22	0.40	NS
Estrumate	0.31	0.31	0.42	NS
Fertagyl	0.58	0.41	0.55	NS

^{a,b}Within rows, means not sharing a common superscript differ significantly.

¹Pevidine = 1% iodine-based solution for treating metritis (Novartis Animal Health UK Ltd., Surrey, UK); pessaries (used for treating metritis) = Utocyl, containing a combination of antibiotics (Novartis Animal Health UK Ltd.); CIDR = controlled internal drug-releasing insert (Animal Reproductive Technologies Ltd., Leominster, UK); PRID = progesterone-releasing intravaginal device (CEVA Animal Health Ltd., Chesham, UK); Estrumate = prostaglandin analog, cloprostenol (Schering-Plough Animal Health, Middlesex, UK); and Fertagyl = synthetic GnRH (Janssen Animal Health, Saunderton, UK).

NS = $P > 0.05$; * $P < 0.05$.

animals diagnosed as having metritis (Table 2). Following veterinary inspection, all animals with metritis were treated with Pevidine (iodine-based solution), pessaries, or both. An increase in dietary protein concentration significantly ($P < 0.05$) decreased the requirement to administer Pevidine or pessaries for the treatment of metritis. There were no significant ($P > 0.05$) effects of dietary protein content on the incidence of any other fertility intervention treatments.

The effects of protein treatment on selected reproductive variables are presented in Table 3. No statistically significant ($P > 0.05$) effects of dietary protein concentration on any of the parameters listed in Table 3 were found. However, there was a tendency for animals on the 114 g of CP/kg of DM diet to have a higher 100 d in-calf rate (82.7%) compared with those on 144 (66.7%) and 173 g of CP/kg of DM (62.1%). The 100 d in-calf rate was defined as an animal becoming preg-

nant during the 100-d period after the commencement of breeding (November 23). This variable indicates the proportion of animals returning to the autumn calving regimen in the subsequent year. There also appears to be some evidence of a beneficial effect of 114 g of CP/kg of DM on overall conception rate, with 100% of cows becoming pregnant (144 g of CP/kg of DM, 92.9%; 173 g of CP/kg of DM, 86.7%). The overall interval to OLA and the pregnancy rate to first service rate in the current study were 32.2 d and 30.6%, respectively.

Effect of Energy Status on Reproductive Variables

Several individual energy measures were significantly associated with the incidence of metritis [average daily energy balance in first 21 d ($P < 0.01$); average daily energy balance over 40 wk ($P < 0.05$); average cumulative energy balance over 40 wk ($P < 0.05$)]. In all cases,

Table 3. Predicted means for effects of protein concentration on fertility measures

Fertility measure ¹	CP concentration (g/kg of DM)			Significance ¹		
	173	144	114	SED	P-value	Overall
Onset of luteal activity (d)	30.9	33.2	32.4	4.6	NS	32.2
Pregnancy to first service (%)	27.6	29.7	34.5	8.6	NS	30.6
Pregnancy to first and second service (%)	51.7	63	55.2	9.2	NS	56.6
Services per conception	2.69	2.32	2.44	0.38	NS	2.48
100 d in-calf rate ² (%)	62.1	66.7	82.7	8.2	NS	70.5
Cumulative pregnancy rate ³ (%)	86.7	92.9	100.0	5.4	NS	93.2
Calving interval (d)	398	399	398	13.7	NS	398.3

¹There was no significant effect of protein concentration on any of the above fertility measures; SED = standard error of the difference.

²100 d in-calf rate was taken from the commencement of breeding (November 23, 2005).

³Cumulative pregnancy rate was calculated over a 6-mo breeding period.

NS = $P > 0.05$.

Table 4. Effect of blood constituents on reproductive variables

Blood constituent	Reproductive variable	r-value ²	Significance
Leptin (ng/mL)	Time to progesterone rise ¹	-0.207	*
Glucose (mmol/L)	Peak progesterone concentration	0.144	*
Globulin (g/L)	Peak progesterone concentration	-0.163	**
Glucose (mmol/L)	Duration of interluteal interval	-0.174	**
Total protein (g/L)	Peak progesterone concentration	-0.211	***

¹Days to progesterone increase above 3 ng/mL.²Correlation coefficient.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

a more-positive energy balance elicited an increased requirement for treatment. Following Spearman's correlation matrix analysis it was found that the average cumulative energy balance was positively associated with conception ($P < 0.01$; $r = 0.351$); that is, a more-positive average cumulative energy balance was associated with a higher conception rate. Additionally, it was found that a more-negative daily ($P < 0.01$; $r = -0.294$) and cumulative ($P < 0.05$; $r = -0.261$) energy balance was associated with a longer interval to progesterone increase (>3 ng/mL) after ovulation.

Effect of Parity on Reproductive Variables

Parity had a significant influence on the average IOI ($P < 0.01$), the average ILI ($P < 0.01$), the average peak progesterone concentration ($P < 0.05$), the time to progesterone increase ($P < 0.01$), and occurrence of more than one abnormal progesterone profile ($P < 0.05$). Primiparous animals had a shorter average IOI (23.5 vs. 26.9 d; SED, 1.19), average ILI (8.2 vs. 11.2 d; SED, 0.97), and time to progesterone increase (5.8 vs. 6.6 d; SED, 0.27) compared with multiparous animals. Primiparous animals had a higher average peak progesterone concentration (34.1 vs. 30.3 ng/mL; SED, 1.70) than multiparous animals, and a lower percentage of primiparous animals displayed more than one abnormal profile than multiparous animals (19.2 vs. 39.9%; SED, 6.64).

Effect of Blood Measures on Reproductive Variables

There was no statistically significant ($P > 0.05$) relationship between plasma urea and any of the fertility measures. Using a Spearman's correlation matrix and scatter graphs to verify relationships (Table 4), it was observed that leptin concentration was negatively associated with time to progesterone increase ($P < 0.05$); globulin concentration was negatively associated with peak progesterone concentration ($P < 0.01$); glucose concentration was positively associated with peak progesterone concentration ($P < 0.05$); glucose

concentration was negatively associated with duration of the ILI ($P < 0.01$); and total protein concentration was negatively correlated with peak concentration of progesterone ($P < 0.001$).

Correlations Between Reproductive Variables

Correlations between reproductive variables are presented in Table 5. In the present study, a shorter IOI was associated with a longer interval from calving to conception ($P < 0.01$). The occurrence of a persistent corpus luteum was associated with a longer calving interval ($P < 0.001$) and with a lower 100 d in-calf rate ($P < 0.01$). Progesterone concentration during the first 7 d post-breeding was positively related to conception ($P < 0.001$). An increase in the average peak concentration of progesterone significantly decreased the calving interval ($P < 0.001$). The interval to OLA was unfavorably associated with peak progesterone concentration in the first cycle ($P < 0.01$). The requirement for intervention was associated with a decrease in the 100 d in-calf rate ($P < 0.001$), and an increased calving interval ($P < 0.001$).

Progesterone Measures and Abnormal Hormonal Patterns

There was no significant effect of dietary protein concentration on any of the progesterone measures. The average interval to OLA was 32.2 d [interquartile range (IQR), 19.2 to 40.0], the average luteal phase was 19.1 d (IQR, 10.9 to 24.6), the average ILI was 9.2 d (IQR, 6.6 to 10.6), the average IOI was 24.8 d (IQR, 21.0 to 28.0), and the average number of cycles per cow was 4.5 (IQR, 3.0 to 6.0). There was no effect of dietary protein concentration on the incidence of any abnormal hormonal patterns following logistic regression analysis. In the current study, 62% of the animals displayed one or more abnormal progesterone profiles. A total of 81% of these animals received an intervention treatment during the breeding period. Delayed ovulation occurred in 20.7% of animals on their first cycle (DOV I) and

Table 5. Correlations between reproductive variables

Reproductive variable ¹	Reproductive variable	r-value ¹	Significance
Persistent corpus luteum type II	100 d in-calf rate	-0.317	**
Intervention required	100 d in-calf rate	-0.583	***
Average interovulatory interval	Days to conception from calving	0.354	**
Peak progesterone of first cycle	Days to onset of luteal activity	-0.342	**
Intervention required	Calving interval	0.732	***
Persistent corpus luteum type II	Calving interval	0.442	***
Average peak concentration of progesterone	Calving interval	-0.402	***
Progesterone concentration during first 7 d post-breeding	Conception	0.332	***

¹Correlation coefficient.** $P < 0.01$; *** $P < 0.001$.

at least one prolonged ILI (delayed ovulation in subsequent cycles) occurred in 27.6% of animals (DOV II). Delayed luteolysis during the first cycle was observed in 19.5% of animals (PCL I) and at least one delayed luteolysis in subsequent cycles occurred in 20.7% of animals (PCL II). Overall, 41% of animals displayed at least one delayed ovulation (DOV I and DOV II) and 40% displayed at least one persistent corpus luteum (PCL I and PCL II). When assessing the incidence of abnormal progesterone profiles in terms of cycles, it was found that 30.3% of all cycles had prolonged luteal phases (either PCL I or PCL II), 11.7% of all cycles had a prolonged ILI (DOV II) and 44.9% of all cycles had an IOI greater than or equal to 24 d.

DISCUSSION

Effects of Dietary Protein Concentration on Reproductive Variables

Altering dietary protein concentration had no direct influence on conventional reproductive variables (Table 3). The present study had a relatively low pregnancy to first service rate (30.6%) compared with values reported by Mayne et al. (2002) and Royal et al. (2000); 37.1 and 39.7%, respectively. Interestingly, Canfield et al. (1990) stated that animals on a high protein diet, with excess RDP, had a significantly lower (31%) conception to first service rates than animals on a low protein diet (48%) that met the requirements of RDP. The current study failed to illustrate a significant difference in pregnancy to first-service rates between dietary protein concentrations but indicated a tendency for animals on a low protein diet to have a higher pregnancy to first service rate. Despite obtaining a low conception rate to first service, the calving interval in the current experiment was shorter than that reported by Mayne et al. (2002; 398 vs. 407 d). No significant difference in the 100 d in-calf rate was apparent between dietary protein concentrations; however, the results indicate a tendency for animals receiving 114 g of CP/kg of DM

to have a substantially higher 100 d in-calf rate than animals receiving 144 or 173 g of CP/kg of DM. Clark et al. (1985) indicated that an increase in dietary CP concentration had a tendency to increase the number of services per conception. Results from the current study partially agree with this. Animals receiving 173 g of CP/kg of DM had the highest number of services per conception; however, animals receiving 144 g of CP/kg of DM had fewer services per conception than animals receiving 114 g of CP/kg of DM.

An increase in dietary protein concentration reduced the requirement to administer treatments for metritis. An increase in the requirement for treatment of metritis would suggest a suppression of the animal's ability to deal with an immune challenge. Houdijk et al. (2001) stated that competition for metabolizable energy does not produce a breakdown of immunity, but only competition for metabolizable protein. These conclusions are based on the proteinaceous nature of the immune system. In agreement, the results of the present study indicate that a higher dietary protein concentration was associated with a more competent immune defense in terms of reduced incidence of metritis in early lactation. The immune defense mechanisms against bacterial contamination include the attraction of neutrophils to the uterus endometrium and lumen, the subsequent phagocytosis of bacteria, and the release of antimicrobial proteins (highly proteinaceous). Contrary to these findings, a secondary study carried out by Young et al. [F. J. Young, J. McNair, M. Welsh (Agri-Food and Biosciences Institute, Veterinary Science Division, Belfast, BT4 3ND, Northern Ireland, UK), D. C. Patterson, and C. S. Mayne; unpublished data] on the *in vitro* immune competence of dairy cows receiving 114, 144, and 173 g of CP/kg of DM indicated that cows receiving 114 g of CP/kg of DM had a higher immune competence than those on 144 or 173 g of CP/kg of DM (quantifying IFN- γ production). However, this study was carried out in mid lactation (150 d) at which point the risk of metritis is low and factors affecting immune competence may be different from those in early lactation.

Abnormal Hormonal Patterns and Correlations Between Reproductive Variables

In the current experiment, 62% of animals displayed at least one abnormal progesterone profile, which is higher than reported in a previous study (McCoy et al., 2006; 41%). Overall, 30.3% of estrous cycles were prolonged because of delayed luteolysis. An extended luteal phase in the absence of pregnancy will increase the IOI. The average IOI in the present study was 24.8 d (44.9% of all cycles >24). Royal et al. (2000) suggested that an IOI longer than or equal to 24 d would be associated with reduced fertility (lower pregnancy rates). This was observed in the present study, as the duration of the IOI was negatively associated with the interval from calving to conception.

Post-ovulation, the luteal phase is extremely important in maintaining regular reproductive cycles and high reproductive performance. In the present study, the occurrence of a persistent corpus luteum was associated with a longer calving interval. An extended luteal phase can occur in 2 situations: 1) when there is early embryo loss following maternal recognition of pregnancy, or 2) when there is delayed luteolysis in the absence of an embryo (pregnancy). A luteal phase >16 d would suggest that maternal recognition of the embryo has occurred. Larson et al. (1997) illustrated that nonpregnant animals, with maternal recognition of the embryo (progesterone concentrations >3 ng/mL after d 19 post-breeding), had lower progesterone concentrations at d 4.5 postinsemination than pregnant animals. In support of this, Wathes et al. (2003) reported that a delay in progesterone increase in the early luteal phase (the progesterone concentrations on d 4 and d 5) in high-yielding cows will reduce the ability of cows to become pregnant. In such cases the dam recognizes the presence of the embryo but because of a delay in embryo maturity (as a result of a reduced progesterone concentration in the first 7 d postinsemination), the embryo is incapable of maintaining pregnancy. In the present study there was a positive correlation between the average progesterone concentration in the first 7 d post-breeding and conception, which further strengthens this hypothesis.

Delayed luteolysis in the absence of pregnancy can also be caused by a suboptimal uterine environment that can disrupt normal hormonal and luteolytic mechanisms (Kindahl et al., 1999; Sheldon et al., 2006). In a more favorable uterine environment, prostaglandin $F_{2\alpha}$ acts on the corpus luteum and causes it to regress and cease progesterone production, removing the inhibitory effect on LH release and allowing the next ovulation to occur. Sheldon et al. (2006b) stated that in a suboptimal uterine environment (presence of bacterial pathogens) there

is an increase in prostaglandin E_2 production because of the stimulatory effect of lipopolysaccharides found on the uterine pathogen *Escherichia coli*. Elevated prostaglandin E_2 has a luteotrophic effect, which increases progesterone secretion and subsequently prolongs the luteal phase. Prostaglandin E_2 is important in the growth, development, and maintenance of a normal corpus luteum (Arosh et al., 2004). Interestingly, Lamming and Darwash (1998) reported that cows that have suboptimal uterine environments and experience persistent corpora lutea are more at risk of late embryonic loss after becoming pregnant.

The average interval to OLA in the current experiment was 32.2 d, which was an improvement on the previous figures of 36.1 and 37.0 d cited by McCoy et al. (2006) and Opsomer et al. (2000), respectively. An extended time to OLA is caused by the inability of the ovulatory follicle to produce adequate amounts of estradiol to allow ovulation. In the present study an increase in the interval to OLA was associated with a decrease in the peak progesterone concentration of the first cycle. This would suggest that factors causing a delay in OLA have subsequent negative effects on the functionality of the corpus luteum, which will reduce the probability of embryo survival and pregnancy. Progesterone is essential for embryo development, and a delay in luteinization caused by a reduction in the competence of the corpus luteum will result in a delayed increase in circulating progesterone levels after ovulation, significantly reducing the probability of embryo survival (Wathes et al., 2003). Butler (2000) concluded that during a period of negative energy balance the ability of follicles to produce sufficient estradiol for ovulation appears to be dependent on the availability of insulin and IGF-1. Detrimental effects of negative energy balance on IGF-1 and luteal progesterone concentrations have been shown by Pushpakumara et al. (2001) and Spicer et al. (1990), respectively. In the present study a more-negative energy balance was associated with an increased interval from ovulation to a progesterone increase above 3 ng/mL. Starbuck et al. (2001) stated that animals with progesterone concentrations <1 ng/mL on d 5 after mating had pregnancy rates <10%.

Effect of Blood Constituents on Reproductive Variables

In the current study, blood urea concentrations did not significantly affect any of the reproductive variables. Interestingly, Butler et al. (1996) stated that blood urea concentrations >19 or 20 mg/dL would result in a 20% decrease in pregnancy rate after insemination. An increase in blood urea nitrogen concentration will decrease the uterine pH, making the uterine

environment more hostile and unsuitable for the early developing embryo (Elrod and Butler, 1993). However, in the current experiment, only 0.54% of all blood samples had urea concentrations above this threshold (using the conversion mmol/L divided by 0.357 = mg/dL), which may explain the lack of any effect of urea on fertility measures. Plasma ammonia concentrations, which were not recorded in this experiment, may have better illustrated the toxic effects of elevated protein on reproductive performance. An increase in plasma ammonia concentration occurs when the detoxification capacity of the liver is breached. Ureagenesis (conversion of absorbed ammonia into urea in the liver) has been reported to be less efficient in animals with fatty liver (Strang et al., 1998), subsequently reducing the capacity to detoxify. This would suggest that negative energy balance can amplify the negative effects of elevated dietary protein on reproductive performance.

In the current study, an increase in leptin concentration significantly reduced the interval to progesterone rise >3 ng/mL after ovulation. Adipose tissue is the biggest contributor to plasma leptin in ruminants (Chilliard et al., 2001) and is positively correlated with body condition (Kokkonen et al., 2005). Leptin concentration in blood is highly sensitive to alterations in energy status, with a more severe negative energy balance being associated with a greater loss of adipose tissue and subsequently large reductions in leptin production (Houseknecht et al., 1998; Wathes et al., 2003). In previous studies conducted by Woods (2005) and Reist et al. (2003), a positive correlation between leptin and IGF-1 was observed that could explain the observed relationship between leptin and time to progesterone increase in the current experiment. Unfortunately, there was no analysis of IGF-1 against interval to progesterone rise after ovulation in the current study because of limitations in the number of IGF-1 samples analyzed. Wathes et al. (2003) suggested that IGF-1 was important in dictating the progesterone output of the corpus luteum. Those authors also stated that increased progesterone secretion from the corpus luteum was related to a large dominant follicle (high estradiol production) that took less time to mature. In support of this, Starbuck et al. (2000) reported that a longer interval to progesterone increase after ovulation is associated with poor estradiol secretion during the follicular phase leading up to ovulation.

CONCLUSIONS

Much of the previous work on protein nutrition of dairy cattle has focused on increasing the protein concentration of the diet to increase milk yield, whereas the present study focused on the effects of reducing

the protein concentration of the diet as a means of improving the energetic balance and fertility of the high-yielding Holstein dairy cow. The results from the current study suggest that there is little or no effect of altering dietary protein concentration on the reproductive performance of the contemporary high-yielding dairy cow. This view is shared by Laven et al. (2007), who stated that the evidence for negative effects of elevated concentrations of dietary nitrogen on fertility of dairy cows was not conclusive, as many studies have shown little or no effect. Additionally, the high level of abnormal estrous cycles observed in the current study would suggest that there are more serious underlying physiological problems that may inhibit any potential benefit of altering the dietary CP concentration.

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