An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior of high-yielding Holstein-Friesian dairy cows

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ABSTRACT

Reproductive performance in the high-yielding dairy cow has severely decreased in the last 40 yr. The aim of this study was to compare the effectiveness of 4 nutritional strategies in improving the reproductive performance of high-yielding dairy cows. It was hypothesized that offering cows a high-starch ration in early lactation would enhance the onset of luteal activity, and that decreasing the severity of negative energy balance in the early postcalving period would improve reproductive parameters. Nutritional regimens aimed at improving fertility were applied to 96 Holstein-Friesian dairy animals. Upon calving, animals were allocated in a balanced manner to one of 4 dietary treatments. Primiparous animals were balanced according to live weight, body condition score and calving date. Multiparous animals were balanced according to parity, previous lactation milk yield, liveweight, body condition score and calving date. Treatment 1 was based on an industry best practice diet (control) to contain 170 g of crude protein/kg of dry matter. Treatment 2 was an individual cow feeding strategy, whereby the energy balance (EB) of individual animals was managed so as to achieve a predetermined target daily EB profile (± 10 MJ/d). Treatment 3 was a high-starch/high-fat combination treatment, whereby an insulino-genic (high-starch) diet was offered in early lactation to encourage cyclicity and followed by a lipogenic (low-starch, high-fat) diet to promote embryo development. Treatment 4 was a low-protein diet, containing 140 g of crude protein/kg of dry matter, supplemented with protected methionine at an inclusion level of 40 g per animal per day. The nutritional strategies implemented in this study had no statistically significant effects on cow fertility measures, which included the onset of

luteal activity, conception rate, in-calf rate, and the incidence of atypical cycles. The individual cow feeding strategy improved EB in early lactation but had no benefit on conception rate to first insemination. However, conception rate to second insemination, 100-d pregnancy rate (from the commencement of breeding), and overall pregnancy rate tended to be higher in this group. The high-starch/high-fat treatment tended to decrease the proportion of delayed ovulations and increase the proportion of animals cycling by d 50 postcalving. Animals that failed to conceive to first insemination had a significantly longer luteal phase in the first cycle postpartum and a longer inter-ovulatory interval in the second cycle postpartum. With regards to estrous behavior, results indicate that as the size of the sexually active group increased, the intensity of estrus and the expression of mounting or attempting to mount another cow also increased. Furthermore, cows that became pregnant displayed more intense estrous behavior than cows that failed to become pregnant.

Key words: nutrition, fertility, reproductive performance, high-yielding dairy cow

INTRODUCTION

Reproductive performance in the modern high-yielding Holstein-Friesian dairy cow has decreased at such a rate, and to such an extent, over the last 40 yr that it is predicted that this high-performance production system will become unsustainable by 2020 if the current rate of decrease continues (Maas et al., 2009).

It is considered unlikely that this decrease in reproductive performance has a direct genetic origin, as conception rates in nonlactating Holstein-Friesian heifers have remained high (at 70–80%) during a period when milk production has increased by 218% (Beam and Butler, 1999). However, in general, the increase in milk energy output has not been matched by a proportionate increase in energy intake, resulting in a negative energy balance (NEB), which forces, and is

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counterbalanced by, the mobilization of body reserves. Negative energy balance has been determined as an underlying causal factor of poor reproductive performance in high-yielding dairy cows (Jorritsma et al., 2003) and has been associated with a delay in the onset of luteal activity (OLA; Jolly et al., 1995), an extended interval to first service (Butler et al., 1981) and decreased conception rates (Domecq et al., 1997). Specifically, NEB impairs ovarian function through a decrease in the maximum diameter of ovarian dominant follicles (Lucy et al., 1991; Mackey et al., 1999). Smaller dominant follicles produce less estradiol, suppressing the pulsatile secretion of LH (Butler, 2001), and decreasing ovarian responsiveness to LH (Butler, 2001). Collectively, these events increase the proportion of follicles that fail to ovulate (Mackey et al., 1999).

Several diet components have the potential to influence the extent of the postpartum NEB as well as the circulating concentrations of specific blood metabolites and hormones. For example, dietary CP content has been shown to affect milk output and thereby influence NEB (Law et al., 2009a). The latter authors reported decreased milk production and improved EB in cows offered a diet of 114 g of CP/kg of DM (low CP) compared with those offered 173 g of CP/kg of DM (high CP). Diets high in CP also increase blood urea concentrations (Law et al., 2009a), and Butler et al. (1996) argued that blood urea concentrations above 19 or 20 mg/dL would result in a 20% decrease in pregnancy rate post-AI. The inclusion of protected fat in dairy cow rations increases the energy density of the diet and also has the potential to decrease the extent of the NEB (van Knegsel et al., 2007a). However, supplemental fat in excess of 50 to 60 g/kg of DM may decrease feed intake (Schauff and Clark, 1992) and offset any potential beneficial effect on NEB. Nonetheless, the inclusion of supplemental fat is often preferred to starch inclusion, as a means of increasing the energy density of the diet, as high dietary starch levels can be detrimental to digestion, milk composition, and cow health (Staples et al., 1998). Importantly, however, diets high in starch content increase the supply of glucogenic precursors and can increase circulating insulin concentrations (van Knegsel et al., 2007b) which, in turn, are associated with enhanced cyclicity early postpartum (Gong et al., 2002). Supplementation of the diet with fat can increase cholesterol concentration (Grummer and Carroll, 1988), which serves as a precursor for progesterone synthesis (Staples et al., 1998). The inclusion of dietary fat has also proved beneficial to blastocyst growth rate in lactating dairy cows (Fouladi-Nashta et al., 2007). More recently, it has been suggested that the type of fat offered to dairy cows is more important than the

quantity of fat, with particular emphasis on the effects of n-3 and n-6 fats on reproductive performance (Childs et al., 2008). However, much of the published literature has focused on the effects of including calcium salts of palm fatty acids on dairy cow fertility (Garnsworthy et al., 2008a,b; Staples et al., 1998).

Despite the key importance of nutrition, poor reproductive performance remains multifactorial in origin with management factors also making a significant contribution. For example, a decline has occurred in the observed expression, intensity, and detection of animals in estrus (Van Eerdenburg et al., 1996; Kerbrat and Disenhaus, 2004) and poor heat detection is a major contributor to decreased reproductive performance in modern high-yielding dairy cows (Reimers et al. 1985) due to shorter and less intense estrous expression (Lopez et al., 2004). Lyimo et al. (2000) found that maximum estradiol concentrations, which are influenced by NEB, were related to total estrous expression.

The aim of the current study was to evaluate the effect of 4 distinctive nutritional regimens or strategies on the reproductive performance of contemporary high-yielding Holstein-Friesian cows.

The hypotheses attached to the use of the nutritional regimens applied in the current study were as follows: (1) adjusting the protein content of individual cow diets upwards or downwards can be used to more closely align cows to an optimal and more uniform energy balance trajectory; (2) offering a high-starch diet post-calving decreases the interval from calving to the onset of luteal activity; (3) switching cows to a low-starch/high-fat diet at 50 d postcalving improves reproductive parameters; and (4) offering a decreased protein ration supplemented with protected methionine decreases plasma urea concentrations in dairy cows while sustaining milk production and improving reproductive parameters.

MATERIALS AND METHODS

Animals and Housing

The experiment involved 108 Holstein-Friesian animals (40 primiparous and 68 multiparous; mean parity 2.1), calving between August 24 and December 19, 2007. Following calving, animals were housed as a single group in freestalls with concrete flooring. The cubicle-to-cow ratio was >1:1 at all times, meeting the recommendations set by FAWC (1997). All cubicles had a rubber mat measuring 2.20 m long and 1.25 m wide and sawdust bedding was renewed 3 times weekly. Concrete passageway floors were scraped at least 4 times daily by an automated system. Lights were left

on at all times. Cows were milked twice daily through a 50-point rotary parlor, commencing at 0530 and 1530 h, with cows walking approximately 35 m to the parlor.

Experimental Design, Diets, and Feeding

Immediately after calving, animals were allocated to 1 of 4 dietary treatments in a balanced manner. Primiparous animals were assigned to treatments based on their live weight (LW), BCS, and calving date. Multiparous animals were assigned to treatments according to parity, previous lactation milk yield, LW, BCS, and calving date. Treatment 1 (control) reflected an industry best practice diet. It was formulated using the UK feed rationing program Feed into Milk (Thomas, 2004) and contained 170 g of CP/kg of DM and 12.5 MJ of ME/kg of DM. Treatment 2 was an individual cow feeding strategy, whereby the calculated EB of individual cows was manipulated to maintain a target daily energy balance (DEB) profile (± 10 MJ/d) based on that observed in cows offered 150 g of CP/kg of DM (Law et al., 2009a; Figure 1). To achieve this, cows were offered 5.22 kg of DM of 1 of 3 concentrates differing in CP (via the parlor feeder), so as to achieve total dietary CP concentrations of 200 g of CP/kg of DM (high), 170 g of CP/kg of DM (medium), or 140 g of CP/kg of DM (low). After calving, all animals were initially offered 170 g of CP/kg of DM (medium) for 3 wk, after which the CP content of the ration was altered (if required) to dictate milk yield and subsequent DEB, until d 210 of lactation. Following any dietary adjustment, a monitoring period of 3 wk followed before any further dietary adjustments were made. For example, if after 3 wk, the energy balance was below the target energy balance for that specific week of lactation, the CP content of the diet was changed from high to medium or from medium to low, depending on which diet was currently being offered. Similarly, if energy balance for the previous 3 wk was above the target, the CP content of the diet was increased from low to medium or medium to high, depending on which diet was currently being offered. Similarly, if energy balance for the previous 3 wk was above the target, the CP content of the diet was increased from low to medium or medium to high, depending on which diet was currently being offered. Treatment 3 (high-starch/high-fat), a fertility improver ration, was based on previous work by Gong et al. (2002) that had shown improved reproductive performance by feeding an insulinogenic or lipogenic diet at different stages of the reproductive cycle. In the current study, an insulinogenic (high-starch) diet was offered for 50 d after parturition to encourage renewed luteal activity. Then, to prevent detrimental effects of high insulin levels on oocyte quality (Fouladi-Nashta et

Figure 1. Example of the energy balance (EB) profile used to determine the dietary protein level offered to animals on the individual cow treatment (based on Law et al., 2009a). The solid line (◆) illustrates the target average daily EB for each week of lactation; the dashed line (---) illustrates the upper and lower thresholds for each week of lactation. The diagonally striped zone illustrates EB below target, whereas the solid gray zone illustrates EB above target. Color version available in the online PDF.

al., 2005), a lipogenic (low-starch/high-fat) diet, supplemented with 750 g of protected fat per day (as-fed basis; Megalac, calcium salts of palm fatty acids; Volac International, Royston, UK), was offered between d 51 and 120 of lactation. Treatment 4 was a low-protein diet, containing only 140 g of CP/kg of DM but supplemented with protected methionine (MetaSmart; Kemin UK, Ltd., North Lincolnshire, UK) at an inclusion level of 40 g per animal per day. This treatment was designed to assess the effects of offering a decreased CP ration supplemented with protected methionine (the first limiting AA for milk production; Newbold, 2006) on the nitrogen efficiency of milk production and reproductive performance.

The concentrate-to-forage ratio of the TMR was 60:40 (DM basis) in the control, high-starch/high-fat, and low-protein treatments. Animals on the above treatments were also offered 1 kg of concentrate in the milking parlor each day. The individual cow TMR had a concentrate-to-forage ratio of 45:55 (DM basis) as an additional 6 kg (as fed) of concentrate was offered in the milking parlor daily (to facilitate the alteration of dietary CP content). The formulations of each of the concentrates used are presented in Table 1.

The forage component of all diets consisted of 60% grass silage and 40% maize silage (DM basis). The TMR diets were offered ad libitum and were freshly mixed each day between 1000 and 1100h. Individual DMI were recorded continuously by using feed boxes placed on computer-linked load cells with access to the boxes controlled by a gated system with electronic identifiers (Calan Inc., Northwood, NH). Feed allocation

Table 1. Formulations (g/kg) of the concentrates used in preparing the treatments

Item	Treatment						
	Control	Individual cow			High-starch/high-fat		
		Base	High-protein	Low-protein	High	Low	Low-protein
Barley	186	185	25	280	314	50	198
Wheat	186	185	25	278	314	50	197
Citrus pulp	116	163	30	120	60	245	170
Soyhulls	115	160	25	120	60	230	170
Soybean meal	160	110	420	75	100	150	125
Rape meal	160	110	418	70	95	155	60
Megalac ¹	20	30	—	—	—	63	20
Trace minerals and vitamins	5	5	5	5	5	5	5
Salt	4	4	4	4	4	4	4
Limestone	13	13	13	13	13	13	13
Calcined magnesium	5	5	5	5	5	5	5
Molasses	30	30	30	30	30	30	30
Protected methionine	—	—	—	—	—	—	4
ME (MJ/kg of DM)	13.0	13.1	12.5	12.7	12.8	13.5	13.0
CP (g/kg of DM)	221.0	180.0	419.0	157.0	178.0	205.0	176.0
S starch (g/kg of DM)	252.0	249.0	58.0	370.0	416.0	78.0	264.0

¹Volac Ltd., Orwell, Hertfordshire, UK.

was calculated on a daily basis and included an excess of 10% of the total feed consumed on the previous day. An average daily intake was calculated for each animal for each week of lactation.

Measurements

Estrous behavior was observed during one 30-min period every 12 h at 0900 and 2100 h for the first 150 d of the breeding period (commencing November 30, 2007). Individual behavioral activities were defined according to a scoring system developed by Van Eerdenburg et al. (1996; Table 2) involving 9 key activities previously illustrated as the main behaviors expressed during estrus, each allocated an associated number of points (score). After each 30-min observational period, the total number of points scored was calculated. A total score ≥ 50 points during a single period or consecutive observational periods (an aggregate score for a particular cycle) was taken to indicate an animal in estrus (Van Eerdenburg et al., 1996).

Milk samples for progesterone analysis were collected on Mondays, Wednesdays, and Fridays (all a.m.) from parturition until 100 d into the breeding period, or until confirmation of pregnancy (whichever was sooner). Milk samples were taken aseptically and to each was added a preservative tablet (Lactab Mark III; Thompson and Cooper Ltd., Runcorn, UK). Samples were stored at 4°C until analysis. Milk progesterone concentration was determined using a competitive ELISA kit (Ridgeway Science Ltd., Gloucestershire, UK). The assay is based on the method of Sauer et al. (1986). Pregnancy was confirmed via an ultrasound scan carried out by a veteri-

narian at approximately d 30 post-insemination and by elevated milk progesterone concentrations. Animals on the control, individual cow, and low-protein treatments were inseminated as required beginning 56 d postcalving. Those on the high-starch/high-fat treatment were inseminated from no earlier than 71 d postcalving to allow at least 1 estrous cycle to occur on the high-fat diet before insemination. Breeding commenced no earlier than 56 or 71 d postcalving (according to treatment) and did not occur before November 30 to maintain a compact autumn-calving pattern. All inseminations were performed following the first observed estrus after the commencement of breeding. All fertility-related events were recorded. Artificial insemination was carried out 12 h after an observed estrus by a trained technician.

Progesterone Parameter Definitions

A full description of the methodology used to interpret and analyze the progesterone data was provided by McCoy et al. (2006). Briefly,

- the OLA is indicated by the first of at least 2 consecutive progesterone concentrations ≥ 3 ng/mL in whole milk.
- the luteal phase (LP) of an individual estrous cycle is defined as the period between the first progesterone concentration ≥ 3 ng/mL and the last consecutive milk progesterone concentration ≥ 3 ng/mL in whole milk.
- the inter-ovulatory interval (IOI) is defined as the period between the first progesterone rise (above

3ng/mL) of one cycle to the first progesterone rise (above 3 ng/mL) in the next cycle

- the inter-luteal interval (**ILI**) is defined as the period between the demise of one corpus luteum and the rise of the next, and is the interval from the first milk progesterone concentration <3ng/mL to the last consecutive milk progesterone <3 ng/mL in whole milk.

Abnormal Progesterone Patterns

Progesterone data was assessed according to Lamming and Darwash (1998) to characterize abnormal progesterone profiles which were:

- delayed ovulation type I (**DOV I**) was defined as progesterone concentration <3 ng/mL in whole milk for ≥ 45 d (prolonged ovulation).
- delayed ovulation type II (**DOV II**) was defined as progesterone concentrations <3 ng/mL in whole milk for ≥ 12 d after the OLA (prolonged inter-luteal interval).
- 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).

Several measures of reproductive performance were calculated for each cow. These included conception rate to first, second, and combined inseminations. Additionally, in-calf rates at 100 d and at the end of the breeding period were calculated.

Milk yield (**MY**) and DMI were recorded daily for each cow and average daily MY and DMI were calculated on a weekly basis for each animal. Milk composition, live weight, and BCS on a scale from 0 to 5 (Edmonson et al., 1989) were recorded weekly. Milk composition (fat, protein, and lactose content and SCC) was determined from refrigerated preserved samples (Lactab Mark III; Thompson and Cooper Ltd.) collected during 2 consecutive milkings (a.m. and p.m.) from each animal. The a.m. and p.m. samples were analyzed separately for milk fat, protein, and lactose content by infrared milk analyzer (Milkoscan FT 120, Foss UK Ltd., Warrington, UK) and milk SCC was determined using a Fossomatic 360 (Foss Electric, Hillerød, Denmark). A weighted milk composition was calculated for each week.

Table 2. The scoring scale used for observed symptoms of estrus (Van Eerdenburg et al., 1996)

Symptoms of estrus	Score ¹
Mucous vaginal discharge	3
Cajoling	3
Restlessness	5
Sniffing the vagina of another cow	10
Chin-resting	15
Mounting (or attempting to mount) other cows	35
Mounting headside of another cow	45
Mounted but not standing immobile	10
Standing immobile on being mounted	100

¹The symptom scores are cumulative.

Samples of grass silage and maize silage were analyzed daily for oven DM content. Weekly samples were subjected to near infrared reflectance spectroscopy (Park et al., 1998) for estimation of ME content. Twice weekly, fresh samples of maize silage and grass silage were analyzed for gross energy (Porter, 1992) and pH. The same samples were also analyzed for nitrogen and ammonia nitrogen concentrations, as described by Steen (1989), and for lactic acid and VFA, ethanol, and propanol concentrations, as described by Porter and Murray (2001). Dried silage samples were prepared twice weekly and a composite 2-wk sample was analyzed for NDF, ADF, and ash contents, as described by Cushnahan and Gordon (1995). Samples of maize silage were dried at 60°C twice weekly and composited 2-wk samples were analyzed for starch using the Megazyme kit (K-TSTA; Megazyme International Ireland Ltd., Co. Wicklow, UK; procedure detailed at <http://www.megazyme.com/booklets/KTSTA.pdf>). Each batch of concentrate was sampled and composite 2-wk samples were analyzed for oven DM content (at 60°C). Dried samples were analyzed for nitrogen (Steen, 1989), ADF, NDF, and ash concentrations, as described by Cushnahan and Gordon (1995) and were bulked for starch analysis by the Megazyme kit.

Blood samples were collected weekly between 0930 and 1130 h from the coccygeal vein of all cows using heparin-coated evacuated tubes (Becton Dickson, Oxford, UK) from calving until d 100 of lactation, and then every 2 wk thereafter. Plasma was recovered and stored at -20°C pending urea analysis using an Olympus AU640 autoanalyzer (Olympus Life and Materials Science, Hamburg, Germany).

Calculation of Energy Balance

The average DEB for each animal was calculated for each week of lactation according to Thomas (2004) as follows:

$$\text{DEB} = \text{ME intake} - \text{ME requirement} [-10 + (\text{ME}_{\text{preg}} + \text{ME}_{\text{maintmilk}} \times \text{LW}^{0.75})] + [(0.0013 \times \text{LW})/\text{Km}],$$

where ME_{preg} is the ME requirement for pregnancy, $\text{ME}_{\text{maintmilk}}$ is the combined ME requirement for maintenance and milk production, and Km (efficiency of energy use for maintenance) = $0.35 \times \text{ME}/\text{GE} + 0.503$. Milk yield, DMI, milk composition, LW, and feed composition data were all used in the calculations. Missing values (fewer than 2 percent of all data) were estimated from data for the week before, and the week following.

Statistical Analysis

All statistical analysis was performed using Genstat (Payne et al., 2007). Data were analyzed by a repeated measures approach using the residual maximum likelihood (REML) procedure. The model fitted fixed effects for parity, postcalving treatment, and week of lactation for each parameter and included all second- and third-level interactions between these variables. Within the model, individual animals represented subjects and week of lactation represented time points that were equally spaced and the same for all animals. As the correlations between successive measurements on the same subject were assumed to decrease with time with the interval between successive time points being 1 wk, an autoregressive error correlation model of order 1 was used.

Linear regression analysis was performed to examine the effects of production and metabolic parameters (independent variables) on all reproduction-related variables. The independent variables used in the multiple linear regression analysis were MY, milk energy concentration, daily EB, cumulative EB, average DEB in wk 1 to 3 of lactation, average DEB in wk 1 to 6 of lactation, and the number of weeks to the EB nadir. A logistic regression model was used to analyze conception rate (CR) to first service, CR to second service, CR to first and second services, 100 d in-calf rate, DOV types I and II, PCL types I and II, and whether or not more than 1 abnormal progesterone profile was observed. When assessing treatment effects, voluntary waiting period was included as a covariate in the analysis. Animals on the control, individual cow, and low-protein treatments had a voluntary waiting period of 56 d postcalving, whereas animals on the high-starch/high-fat treatment had a voluntary waiting period of 71 d.

Estrous behavior data were not normally distributed and, therefore, a square root transformation was applied before analysis to normalize the data.

In total, 16 animals were removed from the study over the duration of the experiment due to ill health, injury, or death.

RESULTS

Nutritional Composition

The nutritional composition of the diets offered in the current study are presented in Table 3. The control diet had a starch content of 177 g and a protein content of 185 g of CP/kg of DM. The starch contents of the basal (0–35 d postpartum), high- (35–50 d postpartum), and low-starch diets offered as part of the high-starch/high-fat treatment were 177, 273, and 97 g of starch/kg of DM respectively. The dietary protein contents of the rations offered to animals on the individual cow treatment were 205, 181, and 156 g of CP/kg of DM for high-, medium-, and low-protein rations. The CP content of the low protein diet was 163 g/kg of DM and the starch content was 187 g of CP/kg of DM. Metabolizable energy contents for the rations were similar across all treatments (12.2–12.5 MJ/kg of DM).

Production Performance

Animals allocated to the individual cow treatment had higher average DMI [$P < 0.001$; standard error of differences (SED), 0.32], total ME intake ($P < 0.001$; SED, 0.08), grass silage DMI ($P < 0.001$; SED, 0.05), and maize silage DMI ($P < 0.001$; SED, 3.69) compared with those allocated to the other treatments (Table 4). Mean concentrate DMI was not different between treatments ($P > 0.05$). Milk yield and milk fat and milk protein concentrations were not affected ($P > 0.05$) by dietary treatment. Across all treatments, the average milk yield was 32.8 kg/d, milk fat concentration was 38.4 g/kg, and milk protein concentration was 33.7 g/kg during the first 210 d of lactation. Neither LW nor BCS during the first 210 d of lactation were affected by dietary treatment ($P > 0.05$). A statistically significant ($P = 0.015$; SED 0.675) interaction was observed between stage of lactation and treatment effects for milk protein content; however, no statistically significant relationships were identified for other production variables.

Plasma Metabolites

Milk urea concentrations were lower in animals offered the low-protein treatment ($P < 0.001$) compared with those offered the control, individual cow, and high-starch/high-fat treatments (133.6 g/kg vs. 164.3, 174.0, and 158.2 g/kg, respectively). Mean plasma urea concentrations differed ($P < 0.001$) between the low-protein treatment (11.0 mg/dL) and control, individual cow management, and high-starch/high-fat treatments (14.1, 14.1, and 14.7 mg/dL, respectively).

Table 3. Key nutritional components of the total diet for each nutritional treatment¹

Item	Treatment								
	Control	Individual cow				High-starch/high-fat			
		High-protein	Medium-protein	Low-protein		Basal	High	Low	Low-protein
Starch (g/kg of DM)	177.0	136.2	168.7	201.3		177.3	273.7	97.3	187.1
CP (g/kg of DM)	185.7	205.6	181.1	156.6		186.0	165.7	169.6	164.0
ME (MJ/kg of DM)	12.5	12.3	12.3	12.4		12.2	12.5	12.2	12.2
Fat (g/kg of DM)	48.9	38.1	53.0	38.1		48.9	38.1	71.8	48.9

¹The treatment diets refer to the TMR and include any concentrates fed in the parlor.

Energy Status

Overall, mean daily EB during the first 210 d of lactation was higher ($P < 0.001$) in animals allocated to the individual cow treatment (31.7 MJ/d) than in cows offered the control, high-starch/high-fat, or low-protein treatments (18.5, 18.8, and 13.1 MJ/d, respectively; Figure 2). Animals allocated the individual cow treatment had established a positive EB by wk 4 postpartum, whereas animals on the control, high-starch/high-fat, and low-protein treatments entered positive EB only in wk 5, 8, and 8 postpartum, respectively. Cumulative EB was higher ($P = 0.001$) in animals allocated to the individual cow treatment (6657 MJ) than in those allocated the control, high-starch/high-fat, or low-protein treatments (3,874, 3,958, and 2,740 MJ, respectively).

Fertility Parameters

Conception rate to first or second, or first and second service combined was not affected ($P < 0.05$) by nutritional treatment (Table 5) at 30.5% (SED, 9.5), 41.7% (SED, 12.2), and 58.8% (SED, 10.2) respectively. Animals allocated to the individual cow treatment had a lower ($P > 0.05$) CR to first insemination (24%) than did those receiving the control, high-starch/high-fat,

and low-protein diets (33.3, 36.0, and 28.5%, respectively). Conversely, the CR to second service of animals allocated to the individual cow treatment was higher ($P > 0.05$) than in those allocated to the other treatments (52.6 vs. 42.9, 31.3, and 40.0%, respectively). No statistically significant correlation between EB and CR in the current study. Voluntary waiting period had no significant effect on any of the parameters analyzed.

Nutritional treatment had no effect on CR during the first 100 d of the breeding period or throughout the entire 6-mo breeding season (both $P > 0.05$; Table 5). However, animals offered the individual cow treatment had slightly improved 100 d in-calf rate and higher CR at the end of the breeding season, although neither of these variables was statistically significant. The 100 d in-calf rate and overall CR of animals offered the individual cow treatment, the control treatment, the high-starch/high-fat treatment, and the low-protein treatments were 79.2 and 95.8%, 71.4 and 85.7%, 68.0 and 88.0%, and 71.4 and 81.0%, respectively.

Luteal Activity Characteristics (by Milk Progesterone Concentrations)

Nutritional treatment had no effect ($P > 0.05$) on the interval to the OLA, or on the durations of the

Table 4. Treatment effects on milk production and energy balance between 1 and 210 d of lactation

Item	Treatment					P-value	
	Control	Individual cow	High-starch/ high-fat	Low-protein	SED ¹	Treatment	Treatment × DIM
Milk yield (kg/d)	32.8	32.7	33.3	32.6	1.14	0.707	0.108
Milk fat (g/kg)	37.7	39.4	38.6	38.1	0.72	0.198	0.494
Milk protein (g/kg)	33.8	33.9	33.6	33.7	0.38	0.984	0.015
Milk urea (mg/kg)	164	174	158	134	2.9	0.001	0.285
Mean live weight (kg)	563	557	565	566	15.4	0.861	0.959
DMI (kg/d)	19.7	21.0	19.7	19.3	0.32	0.001	0.752
Concentrate DMI (kg/d)	12.2	12.3	12.2	11.9	0.17	0.458	0.495
Grass silage DMI (kg/d)	4.5	5.3	4.5	4.4	0.82	0.001	0.166
Maize silage DMI (kg/d)	3.0	3.5	3.0	3.0	0.05	0.001	0.166
Total ME (MJ/d)	249	265	251	244	3.7	0.001	0.717
DEB ² (MJ/d)	18.4	31.7	18.8	13.1	3.62	0.001	0.429

¹Standard error of differences.

²Daily energy balance.

Figure 2. Effects of treatments on daily energy balance between calving and 210 d of lactation ($P < 0.001$, standard error of differences = 3.62). Color version available in the online PDF.

luteal phase, inter-ovulatory interval, or inter-luteal interval (Table 5). However, although not statistically significant, the low-protein and high-starch/high-fat treatments had the greatest proportions of animals cycling by 30 d postpartum (81.0 and 73%, respectively), whereas the control treatment and individual cow treatment achieved only 54 and 67%, respectively. By 40 d postpartum, 85.7 and 84.6% of cows on the low-protein and high-starch/high-fat treatments were cycling compared with 75.0 and 77.8% of those on the control and individual cow treatments.

Parity had a statistically significant effect on the duration of the IOI with multiparous animals having a longer IOI than primiparous animals (24.2 vs. 21.3 d; $P < 0.05$). However, parity did not affect ($P > 0.05$) either the interval to OLA, or the duration of the LP or ILI. The LP was shorter ($P < 0.001$) in the first cycle postpartum than in either the second or third cycles (10.9 d vs. 14.2 and 14.8 d, respectively) but was unaffected by parity or nutritional treatment (Table 6). The IOI and ILI were not different between cycles ($P > 0.05$).

In the first cycle postpartum, the duration of the LP was greater ($P < 0.05$) in animals that did not become pregnant at either the first or second services when compared with animals that became pregnant (12.6 vs. 9.5 d; SED, 1.43). The length of the second cycle postpartum was greater ($P < 0.05$) in animals that did not become pregnant at the first AI postpartum than in those that became pregnant (25.3 vs. 21.3

d, respectively; SED, 2.70). The length of the second cycle postpartum tended to be greater ($P = 0.062$) in animals that were not pregnant by 100 d after the commencement of breeding than in those that were pregnant (26.4 vs. 22.9 d, respectively; SED, 2.62). In the third cycle postpartum, the ILI was greater ($P < 0.05$) in animals that were not pregnant by 100 d after the commencement of breeding than in animals that were pregnant (6.0 vs. 4.6 d, respectively; SED, 0.68). Compared with pregnant animals, those that were not pregnant by the end of the breeding period had a longer third cycle IOI (26.3 vs. 22.3 d; $P < 0.05$; SED, 3.78) and ILI (5.8 vs. 4.7 d; $P < 0.01$; SED, 1.30).

Atypical Progesterone Profiles

Across all treatments, 62.2% (SEM 14.0) of animals displayed at least one atypical progesterone profile (Table 5). Within this average figure, animals subjected to the individual cow treatment had the highest proportion displaying one or more atypical profiles (81.5%), whereas 57.6, 58.3, and 47.6% of cows on the high-starch/high-fat, control and low-protein treatments, respectively, displayed 1 or more atypical profiles, but treatment did not significantly affect the proportion of animals exhibiting at least 1 atypical cycle.

No effect of treatment was observed on the incidences of DOV I or DOV II ($P > 0.05$), which were observed in 12.5 and 16.3% of cycles, respectively, across all nutritional treatments. The incidences of PCL I or PCL

Table 5. Treatment effects on conception rate and estrous cycle characteristics

Item	Treatment				SED ¹	<i>P</i> -value
	Control	Individual cow	High-starch/ high-fat	Low-protein		
Voluntary waiting period (d)	56	56	71	56	—	—
Interval from calving to conception (d)	134.0	127.9	150.0	142.0	13.5	0.548
Conception rate to first insemination (%)	33.3	24.0	36.0	28.5	9.5	0.805
Conception rate to second insemination (%)	42.9	52.6	31.3	40.0	12.2	0.641
Conception rate to first and second inseminations (%)	61.9	60.0	56.0	57.1	10.2	0.977
In-calf rate at 100 d of the breeding period (%)	71.4	79.2	68.0	71.4	9.3	0.841
In-calf rate at the end of the breeding period (%)	85.7	95.8	88.0	81.0	6.9	0.430
Onset of luteal activity (d)	31.5	26.1	21.7	25.5	6.54	0.648
Proportion of animals cycling by 30 d postpartum (%)	54.2	66.7	73.1	81.0	18.3	0.756
Proportion of animals cycling by 40 d postpartum (%)	75.0	77.8	84.6	85.7	15.4	0.648
Proportion of animals cycling by 50 d postpartum (%)	87.5	81.5	92.3	85.7	12.9	0.715
Luteal phase (d)	12.6	14.1	14.3	12.4	1.21	0.252
Inter-ovulatory interval (d)	22.6	23.9	22.3	22.2	1.74	0.552
Inter-luteal interval (d)	6.2	5.8	5.0	5.8	1.55	0.595
Delayed ovulation type I ² (%)	12.5	18.5	8.0	17.0	8.58	0.979
Delayed ovulation type II ² (%)	25.0	15.4	8.0	16.7	8.01	0.170
Persistent corpus luteum type I ² (%)	4.2	26.9	16.7	17.6	5.61	0.089
Persistent corpus luteum type II ² (%)	25.0	46.2	44.0	22.2	8.93	0.819
Animals with one or more atypical cycles (%)	58.3	81.5	57.6	47.6	18.5	0.281

¹Standard error of differences. ²Percentage of cycles that were atypical.

Table 6. Effects of cycle number postcalving on estrous cycle parameters

Item	Cycle number postcalving			SED ¹	P-value
	1	2	3		
Luteal phase (d)	10.94	14.4	14.78	0.911	0.001
Inter-ovulatory interval (d)	21.07	24.16	23.04	1.725	0.177
Inter-luteal interval (d)	6.08	6.05	5.01	1.169	0.476

¹Standard error of differences.

II were not affected ($P > 0.05$) by nutritional strategy and occurred in 16.4 and 34.4% of cycles, respectively (Table 5).

Estrous Behavior

In the current study, an estrus detection rate of 85.5% was achieved when recording commenced at the start of breeding and continued to 150 d of lactation. Dietary treatment had no statistically significant effect on the expression of individual estrous behaviors or the total estrous score for each animal (Table 7). An increase in the size of the sexually active group (the number of animals in estrus simultaneously) increased ($P < 0.01$) the total estrous score (163, 356, 307, 381, and 445 for sexually active group sizes of 1, 2, 3, 4, or 5 or more animals, respectively; SED, 77.1; Table 8). No statistically significant effect of nutritional treatment on the size of the sexually active group was found. The size of the sexually active group had an effect on the incidence of mounting or attempted mounting of other cows ($P < 0.05$; SED, 1.95) and the incidence of standing immobile on being mounted ($P < 0.01$; SED, 2.47). Chin-resting and sniffing the vagina of another cow were expressed most frequently during the observation periods (89.0 and 88.1%, respectively). The behavior of mounting, or attempted mounting of another cow was expressed in 81.6% of all heats. Standing immobile on being mounted was observed in only 47.4% of all heats.

A parity $\times$ nutritional treatment interaction was observed for standing immobile on being mounted ($P <$

0.01; SED, 1.22). Standing immobile on being mounted was expressed more frequently by primiparous animals in the high-starch/high-fat and low-protein treatment groups than in either the control or individual cow management groups. However, standing immobile on being mounted was expressed more frequently in multiparous animals offered the control and individual cow treatments compared with the high-starch/high-fat and low-protein treatments.

The total estrous score was associated with conception success ($P < 0.001$; Table 9), with animals that conceived having a higher total estrous score than animals that did not conceive (406 vs. 201; SED, 39.9) and multiparous animals had a tendency to achieve higher total estrous scores than did primiparous animals ($P = 0.062$).

Logistic regressions were performed on variables, including daily EB, cumulative EB, and blood metabolite concentrations against conception rates but no statistically significant differences were observed.

DISCUSSION

The aim of the current study was to assess the effectiveness of 4 nutritional strategies in improving the fertility of high-yielding dairy cows.

High-Starch/High-Fat (Treatment 2)

The high-starch/high-fat treatment was designed to influence plasma insulin concentrations such that, in

Table 7. Treatment effects on the expression of estrous behaviors and total score (following a square root transformation)

Item	Treatment				SED ¹	P-value
	Control	Individual cow	High-starch/high-fat	Low-protein		
Chin resting	6.575	8.061	7.501	8.226	1.158	0.660
Mounting or attempting to mount another cow	6.661	6.488	8.07	6.984	1.277	0.420
Sniffing the vagina of another cow	5.61	6.483	5.078	5.778	0.8729	0.403
Standing immobile on being mounted	4.485	6.964	7.963	6.183	2.236	0.381
Total score ²	241.6	372.7	380.7	325.5	67.91	0.170

¹Standard error of differences.²Total score did not undergo square root transformation.

Table 8. Effects of the size of the sexually active group on the expression of estrous behaviors (following a square root transformation)

Behavioral activity	Size of the sexually active group (n)					SED ¹	P-value
	1	2	3	4	5+		
Chin resting	6.563	7.264	6.599	9.383	8.144	1.32	0.086
Mounting or attempting to mount another cow	3.993	6.894	8.019	7.255	9.094	1.952	0.023
Sniffing the vagina of another cow	6.509	5.426	5.345	5.826	5.579	0.959	0.406
Standing immobile on being mounted	0.795	8.344	7.408	6.996	8.452	2.467	0.007
Total score ²	162.9	356.0	306.7	380.7	444.5	77.07	0.006

¹Standard error of differences.
²Total score did not undergo square root transformation.

early lactation (calving to 50 d postcalving), the high-starch option would favor elevated insulin, thereby enhancing ovarian follicle development and decreasing the interval to OLA. Gong et al. (2002) illustrated that including 260 g of starch/kg of diet DM (high-starch) increased the proportion of dairy cows ovulating by 50 d postpartum and decreased the interval from calving to first ovulation. van Knegsel et al. (2007a) also found that multiparous cows offered a high-starch diet tended to have an earlier OLA than animals offered diets with lower starch contents. However, Garnsworthy et al. (2009) reported a delay in OLA in cows offered a high-starch diet (182 g of starch/kg of DM) compared with those offered a low-starch diet (98 g of starch/kg of DM) although, in this study, the time difference was not statistically significant. In the current study, the low-protein and high-starch/high-fat treatments tended to have the greatest proportions of cows cycling by 30 d postpartum (0.81 and 0.73, respectively). In the current study also, the average interval to OLA was 33 d in animals offered the high-starch/high-fat treatment, which is comparable to the values of 31.6 d reported for multiparous cows fed high-starch diets by Garnsworthy et al. (2009). van Knegsel et al. (2007a) reported an interval to OLA of 20.4 and 31.7 d in multiparous and primiparous cows, respectively, whereas Gong et al. (2002) reported an interval to OLA of 41.4 and 27.5 d in cows of high genetic merit and low genetic merit, respectively.

High plasma insulin levels are detrimental to oocyte quality in cows (Fouladi-Nashta et al., 2005) and heifers (Adamiak et al., 2005, 2006), explaining the reason for the switch from a high-starch (insulinogenic) formu-

lation to a low-starch (non-insulinogenic) formulation within treatment 2. The low-starch formulation was made lipogenic by supplementation with 750 g/d of protected fat in an effort to further encourage follicular development and corpus luteum formation between d 50 to 120 of lactation, as suggested by Gong et al. (2002). A decrease in oocyte quality will suppress the potential for oocyte development and decrease the blastocyst count post-fertilization (Fouladi-Nashta et al., 2005). Recently, Garnsworthy et al. (2009) reported higher pregnancy rates by d 120 postpartum in cows offered a high-starch diet until the time of the first rise in milk progesterone and then switched to a low-starch diet than in cows offered a low-starch diet followed by a high-starch diet, a high-starch diet throughout, or a low-starch diet throughout. In the current study, the high-starch/high-fat treatment had no effect on conception rate to first service, 100 d in-calf rate, or the incidence of atypical progesterone profiles. One explanation for the different results between studies could be that a high-starch diet was continued after the OLA in the current experiment, which may have had detrimental effects on the potential for oocyte development post-insemination.

In the current study, no statistically significant effect of treatment on conception rate to first service existed when data from multiparous animals were analyzed separately. However, a tendency existed for multiparous animals on the high-starch/high-fat treatment to have a higher conception rate to first service (52.9%) relative to controls (25.0%). This was greater also than the 38% conception rate to first service observed by Garnsworthy et al. (2009) for a similar experimental treatment.

Table 9. Effects of total estrous score on conception

Parity	Pregnant	Not pregnant	SED ¹	P-value
All	406.4	201.3	39.88	0.001
Primiparous	329.9	199.6	54.65	0.062
Multiparous	483.0	203.1		

¹Standard error of differences.

In the current study, treatment groups consisted of 27 cows. Due to reasons unrelated to experimental procedures, 16 animals were removed at various stages during the study. Using the calculations of Morris (2002) for the analysis of continuous data with a normal distribution, and assuming a coefficient of variation of 17%, 26 animals per treatment would be required to detect a significant difference of 12% between treatments (at the 95% confidence level). However, for data with a binomial distribution, the assessment of treatment effects would have required 267 animals per treatment to detect a 12% difference in CR at the 95% confidence level and with an experimental power of 0.8 (Thrusfield, 2005). Such numbers of cows were beyond the resources available for the current study.

Individual Cow Management (Treatment 3)

In the individual cow treatment, the variation in EB within treatment was significantly decreased between wk 2 and 8 postcalving (Figure 3). In wk 2 postpartum, no difference in average DEB between diets was observed; however, by wk 8 postpartum, all animals on this treatment had reached positive DEB and the

degree of variation in DEB within the treatment was much less than that in other treatments, resulting in a more energetically uniform group of animals.

Overall, animals on the control treatment had the greatest variation in EB. Although the effect was not statistically significant, cows on the individual cow treatment tended to have better reproductive performance (conception rate to first and second service and 100 d in-calf rate) which may have been due to a higher proportion of animals being in positive EB earlier in lactation. It is hypothesized that the developmental and steroidogenic competence of follicles is decreased by exposure to a metabolically challenging period such as that presented by NEB during the long period of follicular growth (Britt, 1992; Roth et al., 2001). Furthermore, the oocytes within such follicles, which ovulate 60 to 80 d later, may be inferior (Leroy et al., 2010). However, animals on the individual cow treatment tended to have a lower conception rate to first insemination despite their generally improved energy status. The observed tendency for an improved conception rate to second insemination in animals on the individual cow treatment may be a reflection of successful ovulation of a good quality oocyte that has been recruited

Figure 3. Average daily energy (EB) balances during wk 2 and 8 postpartum. Ind = individual.

during a period of increasing EB. Animals allocated to the individual cow treatment attained a positive EB at wk 4 postpartum, whereas those allocated to the control, high-starch/high-fat, and low-protein treatments attained a positive EB in wk 5, 8, and 8, respectively. Beam and Butler (1997, 1999) suggested that follicles emerging after, rather than before, the EB nadir are more likely to ovulate because of the increase in LH pulse frequency, which is evident after the EB nadir (Diskin et al., 2003). Despite the apparent advantages and benefits, it is acknowledged that managing cows on an individual basis to improve EB may prove difficult to implement in practice on-farm.

Onset of Luteal Activity

There were no statistically significant treatment effects on the interval to OLA in the current study. However, the mean interval (35.3 d) was longer than previously reported: 27.0 d by Darwash et al. (1997); 27.9 d by Royal et al. (2000); 30.1 d by McCoy et al. (2006); and 32.2 d by Law et al. (2009c). This chronological progression suggests that the interval to OLA is continuously increasing. Increases in time to OLA may reflect the inability of the ovulatory follicle to produce sufficient estradiol to facilitate ovulation in the early postpartum period and may be related to the degree of negative DEB (as previously stated). Short intervals from calving to OLA increase the potential number of estrous cycles preceding insemination and improves conception rate (Butler, 2003). Conception rate to first service in the current study (mean 30.5%) was lower than reported previously. Lamming and Darwash (1998), found a CR of 60.9% in cows with normal progesterone profiles and 43.7% in those with atypical profiles. Royal et al. (2000) reported a CR of 39.7%, whereas Mayne et al. (2002) reported a value of 37.1%. The current value was close to that of 30.7% reported by Law et al. (2009c). Trends observed between 1998 and 2009 suggest a continuation in the decrease in CR to first service of 1% per year, as calculated by Royal et al. (2000).

Progesterone Profiles

In the current study, 62.2% of cows exhibited at least one atypical progesterone profile. This figure is higher than that (42.1%) reported by McCoy et al. (2006) but similar to the figure of 62% reported by Law et al. (2009c). Similarly, Royal et al. (2000) reported that the proportion of cows exhibiting at least 1 atypical cycle had increased from 31.7 to 43.7% between 2 databases collated for the periods 1975 to 1982 and 1995 to 1998, respectively. Delayed ovulation occurred in 28% of all

cycles, as reflected in a prolonged ILI (Royal et al., 2000). In the current study, the incidence of DOV I (12.5%) was lower than reported in many recent studies but within the range of values reported since 1998 (11% by Lamming and Darwash, 1998; 12.9% by Royal et al., 2000; 17.4% by McCoy et al., 2006; and 20.7% by Law et al., 2009c). The incidence of DOV II (16.3%) was comparable to previously published values of 13% by Lamming and Darwash (1998), 16% by Royal et al. (2000), and 28% by Law et al. (2009c). Delayed ovulation may be caused by a delay in, or failure of, the surge in circulating concentrations of LH, which causes thinning and subsequent rupture of the follicle wall, leading to ovulation. In their review, Peter et al. (2009) attributed DOV II to the insensitivity of the hypothalamus to positive feedback by estradiol or to altered follicular responsiveness to gonadotropic support, mediated via metabolic hormones such as IGF-1 and insulin (Beam and Butler, 1999). Delayed ovulation type II also may occur when incomplete luteolysis of the corpus luteum from the previous cycle occurs or where a continuing luteinized ovarian structure is present (Lee et al., 1985; Sirois and Fortune, 1990; Silvia et al., 2002; Sartori et al., 2004).

The incidence of PCL I in the current study was 16.4%, which is comparable to values of 16.0, 19.0, and 19.0% from Taylor et al. (2003), McCoy et al. (2006), and Law et al. (2009c), respectively. The incidence of PCL II (34.4%) was unexpectedly high when compared with previously published work, which showed values of 6.35% (Lamming and Darwash, 1998), 6.4 and 16.8% in 1975 to 1982 and 1995 to 1998, respectively (Royal et al., 2000), 11% (Taylor et al., 2003), 11.9% (McCoy et al., 2006), and 20.7% (Law et al., 2009c).

Persistent corpora lutea are mostly associated with 3 occurrences: 1) when embryonic loss occurs following maternal recognition of pregnancy; 2) when luteolysis is delayed in the absence of pregnancy, and 3) when an estradiol-producing dominant follicle is absent at the time of luteal regression (Peter et al., 2009). Delayed luteolysis may occur as a result of a suboptimal uterine environment that disrupts normal hormonal and luteolytic mechanisms (Kindahl et al., 1999; Sheldon et al., 2006). Sheldon et al. (2006) illustrated that uterine infection can increase prostaglandin E₂ (PGE₂) as a result of a stimulatory effect of LPS found on the uterine pathogen *Escherichia coli*. Elevated PGE₂ has a luteotrophic effect, which increases progesterone secretion and, subsequently, prolongs the luteal phase. Under normal, optimal uterine conditions, prostaglandin F₂α from the uterus 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 (Law et al., 2009c). The

Table 10. Dependability of estrous behaviors

Item	Sniffing	Chin resting	Mounting	Standing heat
Reliability ¹	84.6	85.2	87.6	100.0
Percentage expression ²	88.1	89.0	81.6	47.4
Dependability ³	7,456	7,587	7,149	4,736

¹Percentage of cows that expressed this behavior and were judged to be in estrus according to their milk progesterone concentration profile.

²Percentage of estrous cycles in which behavior was expressed.

³Function; reliability $\times$ percentage expression.

absence of an estradiol-producing dominant follicle at the time of luteal regression results in the suppression of the pulsatile release of PGF₂ α and, subsequently, luteolysis (Knickerbocker et al., 1986; Thatcher et al., 1989; McCracken et al., 1999).

Extended IOI is evident in animals with cycles exhibiting PCL II or DOV II. In the current study, no statistically significant treatment effects on the incidence of IOI were observed and the overall mean was 22.7 d. However, the mean IOI in animals allocated to the individual cow management treatment was 23.9 d. According to Royal et al. (2000), IOIs outside the range 19 to 23 d are associated with decreased fertility. In the current study, 36% of cycles had an IOI greater 23 d, whereas 27% were shorter than 19 d.

In the current study, cows failing to conceive to first or second inseminations had a longer luteal phase in the first cycle postpartum. The luteal phase is generally shorter in the first cycle than in subsequent cycles due to the premature release of PGF₂ α (Peter et al., 1989) in response to the increased estradiol produced from formation of the post-ovulatory dominant follicle (Crowe, 2008). Furthermore, animals failing to conceive to the first insemination or not pregnant by 100 d after commencement of breeding, had a longer IOI in the second cycle postpartum and a longer ILI in the third cycle postpartum. Using the IOI limits set by Royal et al. (2000), 63% of the cycles in the current study were associated with decreased fertility. In concurrence, the results from the current study confirm that irregularities in estrous cycles have detrimental effects on fertility.

The Expression and Detection of Estrous Behavior

Efficient estrous detection is essential to increasing the reproductive performance of dairy cows. In the current study, treatments had no statistically significant effect on behavioral expression during estrus or on the total estrous score calculated for successive observations. The most commonly expressed estrous behaviors were chin-resting (89.0%), sniffing the vagina of another

cow (88.1%), and mounting or attempting to mount another cow (81.6%; Table 10). The most reliable estrous behaviors, when expressed, were standing immobile upon being mounted by another cow (100.0%), mounting or attempting to mount another cow (87.6%), chin-resting (85.2%), and sniffing the vagina of another cow (84.6%).

Van Vliet and Van Eerdenburg (1996) conducted 30 min of observation of cows every 2 h and found that only 37% of estrous cycles were characterized by standing immobile on being mounted. Using the same protocol, Lyimo et al. (2000) observed that 53% of estrous cycles were characterized by standing immobile on being mounted. Standing immobile on being mounted was expressed in 47.4% of cycles in the current study when observed twice daily for 30 min. This is lower than previously reported (51.7%) by Law et al. (2009b), who also observed animals twice daily for 30 min. Standing immobile on being mounted has long been recognized as the primary and most reliable sign of estrus and the best indicator of the cow's preovulatory state (Hafez, 1969). However, the expression of standing immobile on being mounted appears to have decreased in the modern high-yielding dairy cow and may no longer be a dependable behavior in defining estrus on modern dairy herds where labor and time resources are limiting (Law et al., 2009b). In the current study, dependability was calculated according to Law et al. (2009b). Our data suggest that the most dependable behaviors as indicators of estrus are chin-resting and sniffing the vagina of another cow, whereas standing immobile on being mounted was ranked only fourth in terms of dependable behavior.

The number of animals in estrus simultaneously (the sexually active group) is known to affect the expression of estrous behavior (Van Eerdenburg et al., 1996; Law et al., 2009b). In the current study, as the size of the sexually active group increased, the expression of mounting or attempting to mount another cow, and the total estrous score, also increased. The behavior of standing immobile on being mounted was expressed significantly more when 2 or more animals were in es-

trus simultaneously. Roelofs et al. (2005) found that chin-resting and mounting or attempting to mount another cow was expressed less frequently when one animal was in estrus compared with when 2 or more animals were in estrus simultaneously.

The expression of estrus is positively correlated with, and is largely controlled by, plasma estradiol concentration (Lyimo et al., 2000). Larger pre-ovulatory follicles have been associated with higher estradiol concentrations, which potentially increase the intensity of estrous expression (Lyimo et al., 2000). However, Lopez et al. (2004) reported that high-yielding dairy cows had lower estradiol concentrations compared with lower-yielding dairy cows despite having larger preovulatory follicles. All animals in the current study were high-yielding dairy cows and ovarian activity was not measured. However, animals that became pregnant had a significantly higher total estrous score in the cycle of conception, which may result from increased estradiol concentrations and the subsequent LH surge, inducing ovulation. Parity had no statistically significant effect on the total estrous score, but multiparous cows tended to achieve higher total estrous scores than did primiparous cows.

CONCLUSIONS

None of the nutritional regimens or interventions applied in the current study had a statistically significant effect on the reproductive performance of the high-yielding Holstein-Friesian dairy cows. However, with larger numbers, significance may have been achieved and verified the observed trends: improved ovarian activity in animals offered the high-/low-starch diet and improved energy balance and subsequent fertility in cows managed on an individual basis. Managing the nutrition of cows so as to adjust individual milk yields resulted in a more energetically uniform group of cows at the commencement of breeding. Attention to detail is needed in detecting estrus in high-yielding dairy cows, as standing heat (the most commonly used pre-ovulatory indicator) was expressed in only 47.4% of the estrous cycles. Secondary behaviors, such as sniffing the vagina and chin-resting, were more frequently expressed and we suggest that these behaviors should be incorporated into heat detection protocols. The findings from this study re-emphasize the complex and multifactorial nature of the reproductive cycle in modern high-yielding Holstein-Friesian cows.

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