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# Bioenergetic factors affecting conception rate in Holstein Friesian cows

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## Introduction

Severe negative energy balance (NEB) in early lactation has been associated with impaired ovarian function and delayed resumption of cyclicity. Conception rates have been positively related to the number of ovulatory cycles preceding insemination (Butler and Smith, 1989). Fertility may therefore be reduced by an extended postpartum anoestrus period. Furthermore, Britt (1992) proposed that the developmental capacity of oocytes ovulating 80-100 days post partum is adversely affected by severe NEB in early lactation, leading to reduced conception rates at first service. The metabolic status of the dairy cow during early lactation may thus influence subsequent fertility by various means. The objective of this study was to examine the associations between early lactation energy balance, milk production, milk composition, and dietary energy intake on conception rate to first service.

### **Materials and Methods**

#### **Data Collection**

Milk yield, milk composition, energy intake and fertility data were collated over two energy balance studies of similar design, but conducted in separate years and using a different cohort of cows. The experimental designs of these studies are outlined below.

### Summary of Year1 experiment

Sixty-three spring-calving, pluriparous Holstein Friesian cows were grouped by expected calving date, and allocated from within group according to body condition score (BCS) and bodyweight to one of the following treatments:

- 1. Once daily milking for the first four weeks of lactation.
- 2. Twice daily milking for the entire lactation
- 3. Thrice daily milking for the first four weeks of lactation.

Milking times were 07:30, 15:30 and 22:30 with the once daily cows milked at 07:30 and the twice daily cows milked at 07:30 and 15:30. All cows were milked twice daily from day 29 post-calving. The diet consisted of maize silage, grass silage and concentrate in the proportion 25:25:50 on a DM basis, which was offered *ad-libitum* from calving until turnout to pasture at day 42. Following turnout, cows were offered high quality pasture and 4kg of concentrate, reduced to 2kg at 21 days after first service.

## Summary of Year 2 experiment

Sixty-six spring-calving, pluriparous Holstein-Friesian cows were blocked according to expected calving date, previous lactation yield and BCS, and assigned to one of three treatments:

- 1. Once daily milking on a standard diet for the first four weeks of lactation
- 2. Thrice daily milking on a standard diet for the first four weeks of lactation
- 3. Thrice daily milking on a high-energy diet for the first four weeks of lactation

Milking times were 07:00, 15:00 and 22:00, with the once-daily cows milked at 07:00. The standard diet comprised grass silage with 8kg/day of concentrate. The high-energy diet comprised grass silage/maize silage mixed in the ratio 1:2, with 12kg/day of concentrate. When treatments terminated, all cows were milked twice daily and fed the standard diet until turnout at day 42. Following turnout, cows were offered high quality pasture and 4kg of concentrate, reduced to 2kg at 21 days after first service.

The recording and sampling schedules and laboratory analysis procedures were similar for both studies. Milk yield, bodyweight and dry matter intake (DMI) were recorded daily. Milk samples, representative of the whole milking, were taken twice weekly. Laboratory procedures for analysis of forage and concentrate samples were similar for both years.

The French Net Energy system (Jarrige, 1989) was used to calculate energy balance (EB). This was estimated as the difference between energy intake and the sum of energy required for maintenance and milk production. The net energy (NE) content of the concentrates offered was determined using the NE values (UFL) of ingredients (INRAtion, 1999). The NE value of silage was related to its *in-vitro* dry matter digestibility. The following equations were used to determine the energy required for maintenance and the energy output in milk:

Energy requirement for maintenance: (UFL/day) = 1.4 + 0.6 BW/100

UFL requirements for milk: (UFL/kg of milk) = 0.0054FC+0.0031PC+0.0028LC-0.015 where BW = body weight, FC = fat content, PC = protein content and LC = lactose content all in g/kg.

The breeding policy was identical for both experiments. First insemination was carried out at the first standing oestrus after a voluntary waiting period of 65 days. Conception rate to first service was determined by transrectal ultrasonography at day 30 post-service.

#### Data Handling

Cows on the twice-daily milking treatment in Year 1, having no corresponding milking frequency group in Year 2, were removed from the analysis. Two cows on the once daily milking treatment in Year 1 reappeared in Year 2. Data from these cows in Year 2 were excluded from the data set. Following amendment of the data set, ninetysix cows remained with daily records for each variable over the first 28 days of lactation. The milk production, dietary intake and EB values included in the analysis are mean values for the first 28 days of lactation. The data set was numerically sorted by each individual continuous independent variable and then divided into quartiles.

#### **Statistical Analysis**

The dependent variable investigated was conception rate to first service (Con1) a binary variable (0,1). The probability of Con1 was modeled using PROC LOGISTIC in SAS (SAS system, 1991). A reference quartile was designated for each variable, having and odds ratio of 1 (OR=1). The Con1 for each category of independent variable was compared to Con1 of the reference group for that variable.

Separate logistic regression models for each of the independent EB, intake and milk variables were constructed, comprising the respective independent variable and the adjustment variables (year and milking frequency). Variables with a significance level of < 0.25 for the score chi-square were retained for inclusion into a stepwise

model. The significance level of the Wald chi-square for an effect to stay in the model was 0.05. The adjustment variables of year and milking frequency were also included in the stepwise model. The variance inflation factor associated with each independent variable was determined to test for multicollinearity in the final model.

## **Results and Discussion**

The means, maximum and minimum values, and standard deviations for each of the independent variables are shown in Table 1. No significant associations were observed between either of the adjustment variables and Con1.

Variable	Mean	Std Dev	Min	Max
Milk Yield (kg/day)	27.6	6.26	13.0	40.7
Dietary Energy Intake (UFL/day)	14.57	2.93	7.04	20.1
Milk Energy Output (UFL/day)	12.6	2.62	5.9	18.3
Milk Fat Content (g/kg)	45.0	5.50	34.4	57.4
Milk Protein Content (g/kg)	34.2	3.00	29.0	46.3
Energy Balance (UFL/d)	-3.20	2.22	-8.77	3.69

**Table 1** Descriptive statistics for independent variables<sup>‡</sup>

<sup>‡</sup> Mean values for d1-28 of lactation

The results of the independent variable analysis are outlined in Table 2. Of the milk production variables investigated, only milk protein content was significantly associated with Con1; cows in the highest milk protein content quartile (>35.5g/kg) had an increased likelihood of conception compared to the two lowest quartiles. This is consistent with the findings of Buckley *et al.* (2003), who showed milk protein content and days to nadir of milk protein to be positively associated with reproductive performance. Similarly, in a large field study of Australian dairy herds, milk protein content in early lactation was positively associated with submission rate and pregnancy rate to first service (Morton, 2001).

No significant associations were observed between mean daily milk yield (P=0.98), milk energy output (P=0.71), or mean milk fat concentration (P=0.57), and Con1. A negative genetic correlation between milk yield and fertility has been well established (Pryce *et al.* 2004). Morton (2001) reported no association between milk yield and fertility, while Buckley *et al.* (2003) demonstrated a positive association between conception rate and phenotypic milk yield after adjustment for genetic merit for milk. Milk yields in the current study may have been below the level above which fertility becomes compromised.

The increased risk of reproductive failure associated with genetic selection for milk yield has been widely attributed to the unfavourable effects of NEB on both ovarian function and oocyte quality (Butler, 2001). The results of the present study indicate a tendency for reduced likelihood of conception at first service as the severity of NEB in early lactation increases.

Variable	Quartile	Independent			Stepwise		
		OR	95%CI	P-Value	OR	95%CI	P-Value
				0.03			0.02
Milk	1 (>35.5)	1			1		
Protein	2 (33.5 to 35.5)	1.21	0.30-4.72		1.26	0.30-5.24	
(g/kg)	3 (32.2 to 33.5)	0.18	0.04-0.76		0.13	0.02-0.65	
	4 (<32.2)	0.24	0.06-0.97		0.24	0.05-1.01	
				0.03			0.02
Energy	1 (>16.31)	1			1		
Intake	2 (14.3 to 16.3)	0.08	0.02-0.46		0.06	0.01-0.36	
(UFL/day)	3 (12.2 to 14.3)	0.08	0.01-0.54		0.10	0.01-0.68	
	4 (<12.2)	0.07	0.01-0.51		0.07	0.01-0.55	
				0.09			NS
Energy	1 (>-1.94)	1	1				
Balance	2(-1.94 to -3.45)	0.42	0.11-1.71				
(UFL/day)	3 (-3.45 to -5.37)	0.25	0.07-1.04				
	4 (<-5.37)	0.14	0.03-0.67				

Table2. Associations between milk production, energy intake and energy balance variables, and Con1<sup>†</sup>

<sup>†</sup>Models include the adjustment variables milking frequency and year

There was a significant association between dietary energy intake and conception rate, with cows in the quartile with the greatest energy intake (>16.31 UFL/day) having an increased likelihood of conception compared to the other groups. Dietary intake has previously been associated with reproductive function. Westwood *et al.* (2002) concluded that cows with higher DMI in early lactation had a greater probability of expression of oestrus at first ovulation, and an increased likelihood of conception before day 150 of lactation.

Variation in EB is largely explained by energy intake even when dietary energy availability is not limiting, with milk output having a relatively minor effect (Villa-Godoy *et al.*, 1988). This may explain why energy intake and EB were associated with conception rate in this study while milk yield was not.

The outcome of the stepwise procedure is also detailed in Table 2. The energy intake and milk protein variables were identified as having significant associations with Con1. The final model was significant at the 0.001 level, according to the likelihood ratio statistic. Examination of the variance inflation factors revealed no evidence of multicollinearity among the variables in the final model.

### Conclusions

The results of this study show a positive relationship between nutritional status in early lactation and subsequent conception rate to first service. Mean daily milk yield over the first month of lactation was not associated with likelihood of conception. However, there was a positive association between net energy intake in the early lactation period and the likelihood of conception. There was also a tendency for cows in a more positive energy balance to have improved fertility performance. The results indicate that milk protein content, traditionally considered a benchmark of dietary adequacy, can be utilised to identify cows at risk of poor reproductive performance.

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