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Associations between milk protein concentration and preceding reproductive performance in Holstein-Friesian heifers and cows in Australia

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ABSTRACT

Milk protein concentration (MP%) has been associated with reproductive performance amongst parous dairy cows. This study aimed to assess whether early lactation MP% of primiparous cows was associated with prior reproductive performance, and to compare the strength of the association between MP% and previous reproductive performance in nulliparous heifers with that in multiparous cows. Primiparous (n = 918 in 35 herds) and multiparous (n = 4,242 in 64 herds) Holstein-Friesian cows with milk production/composition records and sire genetic information in seasonally calving herds were selected from the InCalf database. The date for each herd's planned start of calving (PSC) was calculated as being 282 d after the date breeding started. The interval between a herd's PSC date and each cow's actual calving date (PSC - CI) will therefore reflect time to conception during the previous breeding period. Higher MP% was positively associated with shorter PSC-CI in both primiparous and multiparous cows. Across the range of cows that had a MP% that was $\pm 0.5\%$ of the herd's mean MP%, those with the lowest MP% had an average PSC-CI that was either 13 d or from 28 to 32 d later in primiparous or multiparous cows, respectively. These associations were likely due to biological determinants present before and during the cow's breeding period that were associated with both reproductive performance and subsequent MP%. Since these determinants affected the reproductive performance of non-lactating heifers, some of the associations with MP% were not related to lactation-specific factors even though these same factors may have increased the effect of low MP% on the reproductive performance of the multiparous Holstein-Friesian cows.

Keywords: milk protein concentration; reproduction; Holstein-Friesian; dairy cow.

INTRODUCTION

The variation in milk protein concentration (MP%) among cows within a herd has been associated with differences in their reproductive performance in seasonally calving and year-round calving herds when using a range of parameters of reproductive performance. Those cows with higher MP% have had higher submission rates (Morton, 2000; Buckley *et al.*, 2003), re-submission rates (McDougall, 2003), conception rates (Morton, 2000; McDougall, 2003) and pregnancy rates (Morton, 2000; Buckley *et al.*, 2003). Cows within the lowest quartile based on MP% were more likely to be treated for anoestrus than herdmates within the highest quartile with the difference ranging from 6.8% for Jersey cows to 12% for crossbreeds (Xu & Burton, 2003). Although Patton *et al.* (2007) concluded that MP% in early lactation was a useful indicator of reproductive performance, Haile-Mariam *et al.* (2003) as well as Harris and Pryce (2004) both concluded that including MP% did not increase the accuracy of estimates of sire fertility.

The factors or determinants that result in high MP% and in higher reproduction rates have not been clearly defined. Given that Fahey *et al.* (2003) used data from the InCalf Project (Morton, 2000) to show strong associations between MP% with the preceding calving pattern and with reproductive

performance in the same production season in multiparous cows, we hypothesised that early lactation MP% in primiparous cows may also be associated with their reproductive performance as nulliparous heifers. If so, this would demonstrate that some of the relationship was due to determinants that were influential when the heifers were about 15 months of age and not lactating. The aims of this study were to assess whether the early lactation MP% of primiparous Holstein-Friesian cows was associated with their preceding reproductive performance as nulliparous heifers, and to compare the strength of the association between early lactation MP% and previous reproductive performance in nulliparous heifers with that in lactating cows.

MATERIALS AND METHODS

Milk production data

The InCalf Project involved a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds in nine regions of Australia (Morton, 2000). The Holstein-Friesian cows that were selected for the current study had from one to five milk production records during the first 120 days of lactation as well as individual sire Australian Breeding values and were in seasonally calving

TABLE 1: Mean \pm standard deviation (range) data for milk production and composition during the first 120 days of lactation, and sire genetic details for primiparous and multiparous cows.

Population parameters	Primiparous cows	Multiparous cows
Number of cows per herd	26 (5 to 66)	66 (10 to 175)
Milk volume first 120 days (L)	2,463 \pm 467 (938 to 4,052)	3,221 \pm 619 (1,115 to 5,732)
Milk fat first 120 days (kg)	92 \pm 17 (40 to 184)	120 \pm 24 (44 to 236)
Milk protein first 120 days (kg)	76 \pm 14 (30 to 121)	101 \pm 19 (38 to 173)
Milk fat first 120 days (%)	3.74 \pm 0.43 (2.41 to 5.30)	3.74 \pm 0.51 (2.03 to 6.61)
Milk protein first 120 days (%)	3.08 \pm 0.20 (2.52 to 3.81)	3.16 \pm 0.22 (2.51 to 4.52)
Milk fat % / Milk protein %	1.21 \pm 0.12 (0.79 to 1.63)	1.19 \pm 0.14 (0.62 to 1.88)
Sire Australian breeding value (ABV)		
Number of sires	171	744
Number of daughters per sire	5.3 (1 to 68)	5.7 (1 to 238)
ABV milk volume (L)	1,147 \pm 335 (51 to 1,916)	949 \pm 424 (-603 to 1,924)
ABV milk fat (kg)	37 \pm 12 (5 to 68)	35 \pm 12 (-9 to 68)
ABV milk protein (kg)	30 \pm 7.4 (4 to 62)	25.1 \pm 10 (-7 to 47)
ABV milk fat (%)	-0.14 \pm 0.21 (-0.56 to 0.42)	-0.06 \pm 0.25 (-0.71 to 0.85)
ABV milk protein (%)	-0.11 \pm 0.10 (-0.35 to 0.18)	-0.10 \pm 0.10 (-0.40 to 0.30)
Reliability (%)	96.3 \pm 5.6 (66 to 99)	97.3 \pm 4.2 (55 to 99)

TABLE 2: Associations between the interval from the date for the planned start of calving and actual calving date (PSC-CI) and the independent variables in a multivariable multilevel model. 1st = First lactation etc.; Superscript ^x = Estimate relative to reference category in categorical variables; Superscript ^y = Probability value that refers to the main effect.

Parameter	Parity group	Estimate	Probability
Intercept		30.1 \pm 1.72	<0.01
Cow level estimates (n = 5,160)			
Milk protein (%)		-27.9 \pm 4.70	<0.01
Milk volume (L)		-0.005 \pm 0.007	<0.01
Parity (category)	1st	-15.9 \pm 1.30	<0.01
	2nd	-3.49 \pm 1.08	-
	3rd*	-	-
	4th to 15th	0.88 \pm 0.95	-
ABV milk volume (category)	1st (447 \pm 208)	-0.66 \pm 1.10	0.02 ^y
	2nd (839 \pm 103)	1.50 \pm 0.98	-
	3rd (1,143 \pm 93)	-1.10 \pm 0.89	-
	4th (1,511 \pm 134)*	-	-
ABV milk protein (%)		15.4 \pm 3.86	<0.01
Milk protein (%) x Parity (category)	1st	14.9 \pm 6.32	<0.01 ^y
	2nd	-3.20 \pm 5.58	-
	3rd*	-	-
	4th to 15th	-3.90 \pm 4.63	-
Milk protein (%) x milk volume (L)		0.01 \pm 0.003	<0.01
Herd level estimates (n = 64)			
Herd milk protein (%)		-6.12 \pm 14.1	0.66
Cow milk protein (%) x Herd milk protein (%)		32.8 \pm 26.4	0.21

herds that used artificial insemination for at least the first six weeks of the breeding period. There were 4,242 multiparous cows in 64 herds as well as 918 primiparous cows in 35 herds. Age at first calving was 730 \pm 40 d (range 600 - 901). Multiparous cows were categorised into three sub-groups described as Parity 2 (n = 979); Parity 3 (n = 865); and Parity 4

to 15 (n = 2,398). Milk production variables included cumulative milk volume (L), (Milk 120L), milk fat production (Fat 120kg), milk protein production (Prot 120kg), milk fat concentration (MF%), milk protein concentration (MP%) and MF% to MP% ratio during the first 120 d of lactation. MP% was expressed as true protein on a mass volume (g/L) basis. Details relating to sire Australian breeding value for milk volume (ABV MV), milk fat production (ABV Fatkg), milk protein production (ABV Protkg), milk fat concentration (ABV MF%) and milk protein concentration (ABV MP%) in primiparous and multiparous cows are presented in Table 1.

Reproductive performance data

Pregnancy test data were not available for nulliparous heifers. Instead reproductive performance was assessed for both nulliparous heifers and multiparous cows using calving dates. In seasonally calving herds, the date of the planned start of calving (PSC) for any group of cows will be 282 d after the date breeding commenced. Thus, the interval between a herd's PSC date and each individual animal's actual calving date (PSC - CI) will reflect time to conception. This interval was selected as the dependent variable in this study. Calving dates were available for every cow, but those calving either >48 d before or >160 d after the relevant PSC were excluded from analyses.

Statistical analysis

The relationships between milk production variables during the first 120 d of lactation, sire genetic information, parity and the dependent variable (PSC-CI) were examined using the PROC MIXED procedure of SAS with REML estimation (SAS, 1996). Correlation coefficients between variables were examined using the PROC CORR procedure of SAS (SAS, 1996). Milk protein yield, milk fat yield and MF% to MP% ratio were excluded from analyses as they were highly correlated ($r > 0.75$) with milk volume (MV), MF% and/or MP%.

Cow-level variables that were significant ($P < 0.25$) were included in a multivariable multilevel model. Herd MP% (HMP%) centered at the grand mean to indicate the average MP% for cows within a herd, was tested as a herd-level predictor of PSC-CI (adapted from Singer, 1998). Individual cow MP% (CMP%) was included as a random effect to allow the cow-level relationship (β -coefficient) to vary

across herds (adapted from Singer, 1998). In the model building process, variables that had a probability value of greater than 0.05 were excluded sequentially using backwards elimination. After selection of the main effects, all first order interactions involving main effects were screened for significance ($P < 0.05$) and significant terms were then added to the final multivariable multilevel model in a forward stepwise manner. Selected second order interactions were also tested that included first order interactions involving CMP%.

RESULTS

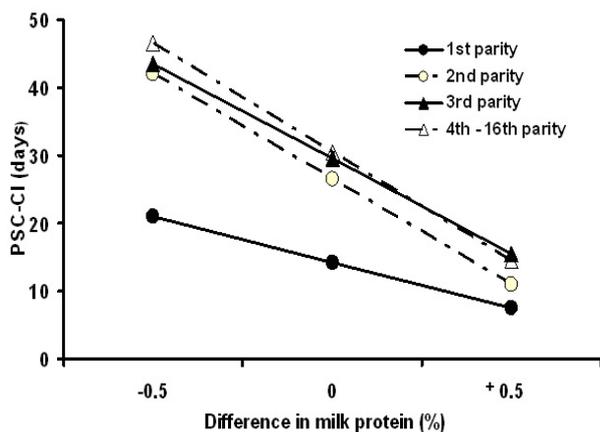
Descriptive statistics (\pm Standard deviation)

The mean PSC-CI for primiparous cows was 15.2 ± 19.1 d (Range -28 to 136). Ninety percent (830/918) of the primiparous cows calved between -10 and 40 d of PSC-CI. This interval was 27.1 ± 25.6 d (Range -48 to 159) for multiparous cows. Seventy four percent of multiparous cows (3,157/4,242) calved between -10 and 40 d of estimated PSC. Yield and compositional averages are summarised in Table 1.

Associations between MP% and PSC-CI (\pm Standard error)

Higher CMP% was associated with shorter PSC-CI using the multivariable multilevel analyses (Table 2). The interaction term for CMP% x HMP% ($P > 0.05$) suggested that the slopes for CMP% did not differ depending upon the average MP% of the herd. The interaction between CMP% and parity indicated that the slopes for CMP% differed depending on parity. The effect of CMP% on PSC-CI was substantially greater in multiparous cows than in primiparous cows (Table 2, Figure 1). For a one percent increase in CMP%, PSC-CI was 13 d shorter ($P < 0.05$) in primiparous cows and between 28 to 32 d shorter in multiparous cows ($P < 0.01$).

FIGURE 1: Least square mean intervals (d) from planned start of calving to calving (PSC-CI) based on the interaction between milk protein (%), (centred at the herd mean) during the first 120 d of lactation and cow parity.



The association between CMP% and PSC-CI also interacted ($P < 0.01$) with MV. The slope of CMP% was stronger in cows with low MV than in those with high MV (Table 2). A second order interaction between MP%, MV and parity was tested and was not significant ($P > 0.05$). PSC-CI was shorter ($P < 0.05$) in cows that had a high sire ABVMV and in cows that had a low sire ABVMP% (Table 2). Removal of either term from the model had very small effects ($< 1\%$ change) on the β -coefficients for CMP%.

The β -coefficients for HMP% (Table 2) represented the herd-level relationship (-6.12 ± 14.1 d, $P > 0.05$) between HMP% and PSC-CI adjusted for the cow-level predictors (CMP%, MV, Parity, ABVMV, ABVMP%, CMP% x Parity, CMP% x MV).

DISCUSSION

MP% could only be measured in nulliparous heifers after the conception date that determined the PSC-CI. For comparability, this approach was also used for multiparous cows. Accordingly, it was not possible to demonstrate cause and effect with this design and potential alternate explanations for the observed association require careful consideration. CMP% may also vary relative to PSC within herds. Only Holstein-Friesian cows were included in the present study and their milk production data was confined to the first 120 days of lactation. With month of calving being related to the dependent variable (PSC-CI), calving date could not be included in the model. Therefore, possible confounding due to absolute calving date could not be removed. However, CMP% varies only modestly with calendar month of calving (White, 2001). In addition, herd was fitted as a random effect in all models and calving periods were relatively compact within herds, with 77% of all cows calving between 10 days before and 40 days after herd PSC. This modest variation in calving date would seem unlikely to have explained the strong associations between CMP% and PSC-CI observed in this study.

Confounding due to gestation length is also unlikely to explain associations of the magnitude observed. Sire of calf, sire breed, natural mating versus artificial insemination and sex of the calf all influence gestation length (Macmillan & Curnow, 1976), but these effects are relatively small with a range of less than six days. Herd was included as a random effect to adjust for regional and herd differences in gestation length (Macmillan & Curnow, 1976).

The observed associations between CMP% in early lactation and previous reproductive performance were most likely due to biological factors present in the individual cow before and during the previous breeding period. The results indicate that these factors were present in both

nulliparous heifers and multiparous cows, and were not entirely limited to factors related to lactation. However, the substantially stronger association in multiparous cows suggests that important additional factors associated with lactation contributed to the observed relationship, or that the causal factors interacted with lactation-specific factors.

Some animals with poor reproductive performance were excluded from analyses. Nulliparous heifers that did not conceive during the previous breeding season could not be included in the data for primiparous cows as lactation was required to determine CMP%. Likewise, lactating cows that did not conceive, or that conceived late and were either culled or induced to calve, were also excluded. These were most likely to have had lower CMP% than cows retained for analyses (Morton, 2000; Fahey *et al.*, 2003). The true association between CMP% and reproductive performance in the previous breeding period is probably even stronger than that observed in the present study.

Identification of factors causing the association between MP% and PSC-CI in both primiparous and multiparous cows will probably contribute to a better understanding of biological factors determining reproductive performance in lactating dairy cows. This understanding may also allow improved genetic selection methods when estimating sire breeding value for reproductive performance. Use of MP% data may not have increased the accuracy of these estimates (Haile-Mariam *et al.*, 2003; Harris & Pryce, 2004) but better knowledge of causal pathways may identify traits more closely associated with reproductive performance.

ACKNOWLEDGEMENTS

This study was supported by funding from the Australian Research Council, the Dairy Research and Development Corporation, Genetics Australia and the National Herd Improvement Association. The statistical advice of Garry Anderson is specifically recognised.

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