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Project Title: Relationship between milk protein percentage and reproductive performance in seasonal calving herds

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Executive Summary

- The objective of this study was to gain a better understanding of the relationship between milk protein percentage and reproductive performance. This information could then be used as a basis for future field studies, with a possible view to using milk protein percentage, alone or in combination with other variables, as an indicator of energy status and reproductive function.

- In this study, a subset of the InCalf Project database was used and then refined to only include Holstein Friesian cows with between one and five milk production records during the first 120 days of lactation in seasonal-calving herds which carried out artificial insemination (AI) for at least the first six weeks of the mating period. This subset then comprised 8,795 cows in 66 herds.

- The mean milk protein percentage for all cows in the first 120 days of lactation was 3.15% (range 2.36 – 4.59). Cows with low milk protein percentage were common in the study herds as 95% of herds (63 of 66) had some cows with low milk protein concentration (less than 3%).

- The mean 3-week submission rate was 19% lower for cows in the lowest (62.5%) compared to the highest (81.5%) quartile for milk protein percent. The mean 6-week incalf rate was 15% lower for cows in the lowest (52.9%) compared to the highest (67.8%) quartile for milk protein percent. The mean 21-week not incalf rate was 5% higher for cows in the lowest (13%) compared to the highest (8%) quartile for milk protein percent. There was only a 4% difference in first insemination conception rates (three-weeks) between the highest (51.3%) and lowest (47.5%) milk protein quartiles. Almost all the variation in 6-week in-calf rate occurred in the first three weeks of mating as the four to six-week in-calf rate varied only slightly (range 21.0 to 23.5%) across milk protein quartiles. There was more variation in four to six-week in-calf rate across milk protein quartiles (range 31.2 to 42.2%) when expressed as a proportion of the cows that were not in calf in the first 3-weeks of mating.

- More than a quarter of all cows in this study were calved less than 42 days at Mating Start Date. Of these cows, 47% were submitted for AI in the first three weeks of mating, 38% were in calf in the first six weeks of mating and 19% were not in calf after 21 weeks of mating. The latter figure represented 50% of all the cows not in calf after 21 weeks of mating. In the cows calved less than three weeks at Mating Start Date, 26.3% were not in calf after 21 weeks of mating.

- The relationship between milk protein and reproductive performance was consistent across lactation number and whether cows are induced or not. Cows with low milk protein percent had a different calving pattern from those with higher milk protein percent. Whereas 92.5% of cows in the high milk protein quartile had calved by at least 3 weeks at mating start date, only 84.3% in the low milk protein quartile had calved by the same date. This indicated that the relationship between milk protein percent and reproductive performance is repeatable across mating periods based on the differences in calving pattern.

- We then tested this hypothesis by assessing whether early lactation milk protein percentage of primiparous cows was associated with their prior reproductive performance (i.e. as nulliparous heifers). We also compared the strength of the association between MP% and previous reproductive performance in nulliparous heifers with that in multiparous cows.

- In seasonal calving herds, cows are mated in groups; the planned start of calving (PSC) date for the group is 282 days after the date that breeding commenced. Thus the interval between the herd PSC date and each animal's actual calving date (PSC-CI) reflects time to conception.

- High milk protein percentage was associated with shorter PSC-CI. However, interactions were detected between milk protein percentage and milk volume and between milk protein percentage and cow age. The interaction between milk protein percentage and milk volume indicated that cows with low milk protein percentage that also had high milk volume had shorter PSC-CI than cows with low milk volume. The interaction between milk protein percentage and cow age indicated that the association between PSC-CI was stronger in multiparous cows than in primiparous cows. For a one percent increase in milk protein percentage PSC-CI decreased by 8 days in primiparous cows and by 31 to 35 days in multiparous cows.

Conclusions

- The association between milk protein percent and reproductive performance was driven mainly by the incidence of non-cycling cows with consequent effects on submission rate in the first 3 weeks of AI as well as in the second 3 weeks. Late calving cows with low milk protein percentage were at the greatest risk of not being submitted for AI and remaining not incalf at the end of a relatively long mating period of 21 weeks.

- Observed associations between PSC-CI and early lactation milk protein percentage were likely due to biological determinants present before and during the cow's breeding period that are associated with both reproductive performance and subsequent milk volume / milk protein percentage.

- Since these associations were present in non-lactating dairy heifers, the biological determinants causing these associations are not restricted to lactation-specific determinants such as post partum negative energy balance. However, the stronger association observed in multiparous cows than in primiparous cows (nulliparous heifers) may be explained by additional effects of lactation-associated factors such as negative energy balance in lactating dairy cows. Furthermore, these biological determinants are operating in addition to milk volume, milk fat percentage, cow-sire ABV's for milk volume and milk protein percentage, precalving liveweight and effects of 'herd' as these variables were fitted simultaneously in the multivariable model.

- A review of the literature indicates that many studies report associations between milk protein percentage and fertility in multiparous cows similar to what we report here. However, as far as we are aware this is the first study to report an association between the conception pattern of non lactating dairy heifers and their future milk protein concentration.

- Further research is required to determine the biological determinants causing the observed relationship in this study.

Publications from this project

- Conference papers (attached) have been presented at the 15th Association for the Advancement of Animal Breeding and Genetics (AAABG) conference in Melbourne in July 2003 and in the proceedings of the 63rd annual conference of the New Zealand Society of Animal Production (NZSAP) in Queenstown in June 2003.
- A manuscript has been submitted to the Journal of Dairy Science (JDS) in March 2004 and a paper will be presented at the 25th biennial conference of the Australian Society of Animal Production (ASAP) in Melbourne in July 2004.
- Articles have also been published in the July/August 2002 Issue and in the May/June 2003 issue of the Australian Dairyfarmer magazine.

15th Conference of Association for the Advancement of Animal Breeding and Genetics

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ASSOCIATIONS BETWEEN MILK PROTEIN PERCENTAGE AND REPRODUCTIVE PERFORMANCE IN HOLSTEIN-FRIESIAN COWS IN SEASONAL-CALVING DAIRY HERDS

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ABSTRACT
The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds throughout Australia. Milk protein percentage was identified as one of 6 factors which had a moderate to large effect on herd reproductive performance. A subset of the original database was used and then refined to only include Holstein-Friesian cows with between 1 and 5 milk production records during the first 120 days of lactation in seasonal-calving herds which carried out AI for at least the first 6 weeks of the mating period. This subset comprised of 8,795 cows in 66 herds. Cows in the subset were categorised into quartiles based on milk protein percentage (low: 2.36 to 2.99 %; med-low: 3.00 to 3.14 %; med-high: 3.15 to 3.29 %; and high 3.30 to 4.59 %) and 5 groups based on interval from calving to mating start date (>12 weeks; 9-12 weeks; 6-9 weeks; 3-6 weeks; ≤3 weeks). Cows with low milk protein percent had a different calving pattern from those with higher milk protein percent. Whereas 92.5% of cows in the high milk protein quartile had calved at least 3 weeks at mating start date, only 84.3% in the low milk protein quartile had calved by the same date. The mean 3-week submission rate and 6-week in-calf rate were lower ($P<0.001$) for cows in the lowest compared to the highest quartile for milk protein percent. Three week non-return rate did not differ ($P>0.05$) between quartile of milk protein percent. The association between milk protein percentage and reproductive performance was due, in part, to the prevalence of non-cycling cows with consequent effects on submission rate in the first 3 weeks of AI. In conclusion, late calving cows with low milk protein percentage were at greatest risk of not being submitted for AI in the first 3 weeks of mating and not being in-calf within 6 weeks of the start of mating.
Keywords: milk protein; fertility; reproduction; Holstein-Friesian; dairy cow.

INTRODUCTION
Morton (2000) reported a strong positive association between milk protein percentage and reproductive performance in Australian herds. Similar relationships have also been reported in Ireland, where Buckley *et al.* (2003) found that low milk protein percentage at and around the time of first AI were associated with low submission and conception rates. The objective of this study was to gain a better understanding of the causes of the relationship between milk protein percentage and reproductive performance in a subset of cows in the InCalf database.

MATERIALS AND METHODS
Data. The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 28,462 dairy cows in 168 herds throughout Australia. A subset of the original database was used and then refined to only include Holstein-Friesian cows with between 1 and 5 milk production records during the first 120 days of lactation in seasonal-calving herds which carried out AI for at least the first 6 weeks of the breeding season. This subset then comprised of 8,795 cows in 66 herds. Cows in the subset were categorised into quartiles (Q) based on the average protein content in milk (expressed as a percentage on a volume basis) from all production records in the first 120 days of lactation, (Q1 low, 2.36 to 2.99 %, n=2199; Q2 med-low, 3.00 to 3.14 %, n=2198; Q3 med-high, 3.15 to 3.29 % n=2199; and Q4 high, 3.30 to 4.59 %, n=2199). Cows were also categorised on interval from calving to mating start date into 5 groups (>12 weeks, n=560; 9-12 weeks, n=3360; 6-9 weeks, n=2541; 3-6 weeks, n=1362; ≤3 weeks, n=972).

Reproductive indices. Submission rate (3-week) was defined as the number of cows with at least one AI during the first 3 weeks of the mating period divided by the total number of cows. Non-return rate (3-week) was defined as the number of cows that received one AI during the first 3 weeks of the mating period and were not inseminated during the second 3 weeks divided by all cows submitted during the first 3 weeks. In-calf rate (6-week) was defined as the number of cows that conceived during the first 6 weeks of the mating period divided by the total number of cows.

Statistics. A multi-level logistic regression model using MLwiN (Goldstein *et al*. 1998) was used to calculate odds ratios and 95% confidence intervals (CI). Herd was included as a random effect with milk protein percent quartile as a fixed effect. Chi squares with 3 degrees of freedom were used to test the significance of associations between milk protein percent and the dependent variable. Intra-class correlation coefficients were calculated using one-way ANOVA (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Reproductive parameters relating to 3-week submission rate, 3-week non-return rate and 6-week in-calf rate were stratified according to calving pattern and milk protein percentage quartiles and are presented in Table 1. As there were different numbers of cows within each category, the averages presented refer to all cows classified according to calving pattern and milk protein percentage. Cows with low milk protein percentage were common in study herds. Ninety five percent of herds (63 of 66) had some cows with low milk protein concentration (less than 3.00%). The mean milk protein percentage for all cows in the first 120 days of lactation was 3.15% ranging from 2.36 to 4.59%. The odds ratios and confidence intervals for 3-week submission rate were Q2 v Q1: 1.43 (1.25, 1.63), Q3 v Q1: 2.02 (1.75, 2.33), Q4 v Q1: 2.66 (2.28, 3.10), Chi square 174.7 ($P<0.001$), for 3-week non- return rate Q2 v Q1: 0.95 (0.81, 1.11), Q3 v Q1: 1.07 (0.91, 1.25), Q4 v Q1: 0.98 (0.84, 1.15), Chi square 2.74 ($P<0.43$) and 6-week in-calf rate Q2 v Q1: 1.24 (1.09, 1.40), Q3 v Q1: 1.50 (1.32, 1.70), Q4 v Q1: 1.90

Table 1. Percentage of cows calving prior to mating start date (calving pattern), 3-week submission rate, 3-week non-return rate and 6-week in-calf rate relative to milk protein percentage quartile

	Q1 2.36 – 2.99%	Q2 3.00 – 3.14%	Q3 3.15 – 3.29%	Q4 3.30 – 4.59%	Average
Calving pattern					
>12 weeks	5.3	5.5	5.6	9.1	6.4
9-12 weeks	31.1	38.9	41.0	41.8	38.2
6-9 weeks	30.1	28.9	29.1	27.5	28.9
3-6 weeks	17.9	15.3	14.6	14.1	15.5
≤ 3 weeks	15.7	11.3	9.7	7.5	11.1
Number of cows	2199	2198	2199	2199	
Submission rate (3-week)					
>12 weeks	83.6	89.2	86.2	93.0	88.8
9-12 weeks	79.9	85.0	88.1	88.4	85.7
6-9 weeks	68.7	73.4	80.6	84.8	76.7
3-6 weeks	57.9	60.8	71.3	76.5	66.0
≤ 3 weeks	14.5	18.9	26.2	26.2	20.2
Average	62.5	70.7	77.4	81.5	73.0
Non-return rate (3-week)					
>12 weeks	57.7	70.1	72.6	72.7	69.2
9-12 weeks	66.8	65.8	69.2	65.0	66.7
6-9 weeks	66.1	60.6	63.2	61.5	62.8
3-6 weeks	55.7	52.7	57.0	50.8	54.1

≤ 3 weeks	42.0	38.3	37.5	46.5	40.8
Average	63.2	62.0	64.9	62.5	63.2
In-calf rate (6-week)					
>12 weeks	69.8	71.7	78.9	77.1	74.8
9-12 weeks	69.5	70.6	72.9	75.8	72.4
6-9 weeks	56.7	59.1	65.3	67.9	62.1
3-6 weeks	47.2	51.0	48.1	59.8	51.2
≤ 3 weeks	13.3	16.9	25.2	26.8	19.1
Average	52.9	58.2	62.8	67.8	60.4

(1.66, 2.17), Chi square 94.7 ($P<0.001$). Intra-class correlation coefficients were 0.08±0.02 and 0.04±0.01 for 3-week submission rate and 6-week in-calf rate respectively with a mean of 133 cows per herd, and 0.02±0.01 for 3-week non-return rate with a mean of 97 cows per herd.

The relationship between reproductive performance and milk protein percent may be driven by energy balance during early lactation. Energy balance has a strong positive influence on resumption of cyclicity (Butler 2000). In turn, submission rates are significantly affected by the prevalence of non-cycling cows (Macmillan 1997). Of the reproductive indices measured in the present study, the largest differences in milk protein quartiles were observed in 3-week submission rate. Heat detection efficiency was about 93% in the study herds (Morton 2000) suggesting that the majority of cows not submitted for AI were most likely to be anoestrus. Furthermore, cows with prolonged periods of anovulatory anoestrus have lower submission, conception and in-calf rates and a greater probability of being culled for failure to conceive compared to cycling herd mates (Macmillan 1997). The results of the present study are consistent with these relationships.

Cows with low milk protein percent had a different calving pattern from those with higher milk protein percent where 92.5% of cows in the high milk protein quartile were calved at least 3 weeks at mating start date compared to 84.3% in the low milk protein quartile. This suggests that the relationship between milk protein percent and reproductive performance is repeatable across mating periods based on the differences in calving pattern. A short interval from calving to mating is negatively associated with reproductive performance (Morton, 2000). More than a quarter (26.6%) of all cows in this study had calved less than 42 days at mating start date. Of these cows, 47% were submitted for AI in the first 3 weeks of mating, 51.7% did not return during the second 3 weeks and only 38% were in-calf in the first 6 weeks of mating. The association between calving date relative to mating start date and early lactation milk protein percent has been independently identified as significantly affecting reproductive performance in seasonal-calving herds. In conclusion, late calving cows with low milk protein percentage were at greatest risk of not being submitted for AI and of not being in-calf within 6 weeks of the start of the mating period. Further studies are required to investigate this phenotypic association and to determine the influence of genotype on the observed association.

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Abstract
The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds throughout Australia. Milk protein percentage was identified as one of six factors associated with herd reproductive performance. In this further study, a subset of the original database was used and then refined to only include Holstein cows with between one and five milk production records during the first 120 days of lactation in seasonal-calving herds which carried out artificial insemination (AI) for at least the first six weeks of the mating period. This subset then comprised 8,795 cows in 66 herds. Cows in the subset were divided into four quartiles based on early lactation milk protein percentage and five intervals from calving to mating start date (MSD). Three-week submission rate and six-week in-calf rate were lower ($P<0.001$) and the 21-week not-in-calf rate was higher ($P<0.001$) for cows in the lowest compared to the highest quartiles for milk protein percent. The association between milk protein percentage and reproductive performance was driven mainly by the incidence of non-cycling cows with consequent effects on submission rate in the first three weeks of AI as well as in the second three weeks. In conclusion, late calving cows with low milk protein percentage were at the greatest risk of not being submitted for AI and remaining not in-calf at the end of a relatively long mating period of 21 weeks.

Keywords: milk protein percentage; fertility; reproduction; Holstein-Friesian; dairy cow.
Short title: Milk protein percentage and fertility

Introduction

Post partum negative energy balance in dairy cows has been associated with an increase in milk fat percentage due to adipose tissue mobilisation and a decrease in milk protein percentage due to a shortage of glucose for milk protein synthesis in the udder (De Vries & Veerkamp, 2000). Negative energy balance, as indicated by a severe loss in body condition and high plasma non-esterified fatty acid concentrations post-partum, is a particular feature of Holstein cows managed under pasture-based milk production systems (Roche *et al*., 2002). Rates of survival in Holstein cows under such systems are low due to high not-in-calf rates of between 25 to 30% at the end of the mating period (Kolver *et al*., 2002; Fulkerson *et al*., 2001).

The InCalf Project (Morton, 2000) reported a strong positive association between milk protein percentage and reproductive performance in seasonal-calving herds in Australia. Moss *et al*. (2002) also showed that low milk protein percentage in the first 120 days of lactation or at first service was a significant risk factor for subfertility in multiparous cows in non-seasonal-calving herds in New South Wales. Similar relationships have also been reported in other international studies. In Ireland, Buckley *et al*. (2003) found that low milk protein percentage at and around the time of AI was associated with lower submission rates and conception rates. In Belgium, Opsomer *et al*. (2000) showed that low mean milk protein percentage during the first 100 days of lactation was associated with an increased risk of anoestrus. A Dutch study (Heuer *et al*., 1999) showed that cows with a fat-protein ratio of >1.5 had higher risks of ketosis, displaced abomasum, ovarian cyst, lameness and mastitis.

The objective of this study was to gain a better understanding of the relationship between milk protein percentage and reproductive performance. This information could then be used as a basis for more complex statistical analyses and future field studies, with a possible view to using milk protein percentage, alone or in combination with other variables, as an indicator of energy status and reproductive function.

Methods and Methods

Data

The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds (124 seasonal-calving and 43 year round calving and one batch calving) throughout Australia. A subset of the original database was used and then refined to only include

Holstein cows with between one and five milk production records during the first 120 days of lactation in seasonal-calving herds that used AI for at least the first six weeks of the mating period. This subset then comprised of 8,795 cows in 66 herds. Cows in the subset were divided into four quartiles based on early lactation milk protein percentage: (low 2.36 to 2.99%, n=2199; med-low 3.00 to 3.14%, n=2198; med-high 3.15 to 3.29%, n=2199; and high 3.30 to 4.59%, n=2199) as well as five intervals from calving-to-MSD (>12 weeks, n=560; 9-12 weeks, n=3360; 6-9 weeks, n=2541; 3-6 weeks, n=1362; <3 weeks, n=972).

Reproductive indices

Submission rate (three-week) was defined as the number of cows with at least one AI during the first three weeks of the mating period divided by the total number of cows. Submission rate (four to six-week) was defined as the number of cows with at least one AI during weeks four to six of the mating period divided by the number of cows that were not inseminated during weeks one to three. Submission rate (one to six-week) was defined as the number of cows with at least one AI during weeks one to six of the mating period divided by the total number of cows. First insemination conception rate (three weeks) was defined as the number of cows that received one AI and conceived during the first three weeks of the mating period divided by the number of cows submitted for AI in the first three weeks of mating. In-calf rate (three week) was defined as the number of cows that conceived during the first three weeks of the mating period divided by the total number of cows. In-calf rate (four to six-week) was defined as the number of cows that conceived during weeks four to six of the mating period divided by the total number of cows. In-calf rate (six week) was defined as the number of cows that conceived during the first six weeks of the mating period divided by the total number of cows. Not-in-calf rate (21-week) was defined as the number of cows that did not conceive during the first 21 weeks of the mating period divided by the total number of cows.

Statistical analysis

A multi-level logistic regression model using MLwiN (Goldstein *et al.*, 1998) was used to calculate odds ratios and confidence intervals (CI) for 3-week submission rate, first insemination conception rate, 6-week in-calf rate and 21-week not-in-calf rate. Herd was included as a random effect with milk protein percent quartile as a fixed effect. Chi squares with three degrees of freedom (3 DF) were used to test the effect of milk protein percent. Intraclass correlation coefficients were calculated using one-way analysis of variance (Snedecor & Cochran, 1980).

Results

Descriptive statistics and odds ratios with confidence intervals of reproductive parameters stratified according to milk protein percentage quartiles in seasonal-calving herds are presented in Table 1. Reproductive parameters relating to submission rates, conception rates, in-calf rates and not-in-calf rates stratified according to calving pattern and milk protein percentage quartiles are presented in Tables 2 and 3. The averages presented in Tables 2 and 3 refer to all cows classified according to calving pattern and milk protein percentage. The intraclass correlation coefficients (95% CI) with a mean of 133 cows per herd were 0.082 (0.051 – 0.112) for 3-week submission rate, 0.02 (0.009 – 0.031) for 6-week in-calf rate and 0.046 (0.027 – 0.065) for 21-week not-in-calf rate. The 3-week submission rate intraclass correlation coefficient was 0.082 (0.051 – 0.112) with a mean of 98 cows per herd.

Prevalence of factor

The mean milk protein percentage for all cows in the first 120 days of lactation was 3.15% (range 2.36-4.59). Cows with low milk protein percentage were common in the study herds as 95% of herds (63 of 66) had some cows with low milk protein concentration (less than 3.00%).

Discussion

The InCalf project (Morton, 2000) identified six key factors which strongly affected herd reproductive performance. They are calving date relative to MSD, milk protein percentage, body condition score at calving, heifer live weight at calving, oestrus detection efficiency and AI competency. These six factors explained more than 70% of the variation in reproductive performance between herds.

Milk protein percentage is associated with reproductive performance but not causal, probably reflecting an underlying biological mechanism. It is hypothesised that this relationship could be mediated by energy balance in the early postpartum cow. Insulin-like growth factor-1 (IGF-1) levels are related to protein synthesis in the mammary gland (Zhao *et al*., 1992) and IGF-1 is also a potent indicator of energy balance (Spicer *et al*., 1990). Severe negative energy balance inhibits reproductive function via reduced LH pulse frequency, growth rate and diameter of the dominant follicle, IGF-1, glucose and insulin concentrations and increased growth hormone concentrations resulting in greater loss of body condition and a higher percentage of anoestrous cows (Roche *et al*., 2000).

The results of the present study are in agreement with the hypothesis, i.e., that the association between milk protein percent and fertility is related to energy balance, as the largest differences between milk protein quartiles were observed in submission rates (Tables 1 and 2) which would be significantly affected by the level of anoestrus (Macmillan, 1997). Although we cannot say with certainty that all cows not submitted for AI were anoestrus, we know that heat detection efficiency was about 93% (Morton, 2000). This suggests that the majority of cows not submitted for AI were anoestrus. Cows with prolonged periods of anovulatory anoestrus have lower submission, conception and in-calf rates and a greater probability of being culled for failure to conceive compared to cycling herd mates (Macmillan, 1997). Cows that display oestrus once or more before first insemination have higher fertility than cows inseminated at first oestrus (Macmillan & Clayton, 1980).

In the present study, there was only a 4% difference in first insemination conception rates (three-weeks) between the highest and lowest milk protein quartiles ($P<0.05$, Table 1). However, a first service conception rate calculated from all first service events could have provided greater differences across quartiles. This is because the cows served in the first three weeks were more likely to be cycling normally and calved longer before AI, based on the positive relationship between calving date and submission rate. It is of interest that almost all the variation in 6-week in-calf rate occurred in the first three weeks of mating as the four to six-week in-calf rate varied only slightly (range 21.0 to 23.5%) across milk protein quartiles (Table 3). There was more variation in four to six-week in-calf rate across milk protein quartiles (range 31.2 to 42.2%) when expressed as a proportion of the cows that were not in calf in the first 3-weeks of mating.

It is well recognised that a short interval from calving-to-mating is negatively associated with conception success. Williamson *et al*. (1980) showed that first service conception rates in eight Victorian herds increased from 18% to 53% as calving-to-service interval increased from 20 to 60 days. Those results are comparable with the calving-to-MSD intervals in the present study where first insemination conception rate was 20.4% at less than 21 days and 48.6% at between 42 and 63 days (Table 3). More than a quarter of all cows in this study were calved less than 42 days at MSD. Of these cows, 47% were submitted for AI in the first three weeks of mating, 38% were in calf in the first six weeks of mating and 19% were not in calf after 21 weeks of mating. The latter figure represented 50% of all the cows not in calf after 21 weeks of mating.

In the cows calved less than three weeks at MSD, 26.3% were not in calf after 21 weeks of mating (Table 3). Although there is a negative relationship between reproductive performance and the interval from calving to mating, this does not explain why such a high proportion of cows were not in calf in this category, considering that such cows had ample opportunity (21 weeks) to be served and conceive. Furthermore, these cows had prolonged exposure to stock bulls, which would potentially overcome any defects in farmer-based heat detection efficiency. It is not clear if the relationship between calving interval and conception rates is due to increasing numbers of oestrous cycles before mating or due to other factors such as energy balance or stage of lactation (Rhodes *et al*., 1999). The results of the present study suggest that the relationship may well be related to energy balance, as indicated by differences in milk protein percentage.

As this is a preliminary analysis of the data at the cow level, there are a number of areas that could potentially bias our interpretation of the results. For example, this analysis has not taken into account factors that may affect milk protein percent such as age, lameness, disease, milk production, forage type or level of concentrate feeding. Age is likely to be a particularly important factor in the relationship between milk protein percent and fertility as heifers are more likely to be anoestrus (McDougall *et al*., 1993).

The association between calving date relative to mating start date and early lactation milk protein percent has been independently identified as significantly affecting the reproductive performance of cows in seasonal-calving dairy herds. The present study has shown that most of the effects involving milk protein percent in Holstein cows occur irrespective of calving date and are most pronounced among later-calving cows.

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TABLE 1: Descriptive statistics and odds ratios of reproductive performance in Holstein cows in seasonal-calving herds based on milk protein percentage during the first 120 days of lactation.

Quartile (Q)	Range in milk protein (%)	3-week submission rate (%)	First insemination conception rate (%)	6-week in-calf rate (%)	21-week not-in-calf rate (%)
Q1 (n=2199)	**2.36 – 2.99**	**62.5**	**47.5**	**52.9**	**13.0**
Q2 (n=2198)	**3.00 – 3.14**	**70.7**	**49.7**	**58.2**	**9.6**
Odds Ratio Q2 v Q1 (95%CI)		1.43 (1.25, 1.63)	1.09 (0.94, 1.27)	1.24 (1.09, 1.40)	0.69 (0.57, 0.85)
Q3 (n=2199)	**3.15 – 3 .29**	**77.4**	**51.2**	**62.8**	**8.5**
Odds Ratio Q3 v Q1 (95%CI)		2.02 (1.75, 2.33)	1.16 (1.00, 1.34)	1.50 (1.32, 1.70)	0.60 (0.49, 0.74)
Q4 (n=2199)	**3.30 – 4.59**	**81.5**	**51.3**	**67.8**	**8.0**
Odds Ratio Q4 v Q1 (95%CI)		2.66 (2.28, 3.10)	1.18 (1.01, 1.37)	1.90 (1.66, 2.17)	0.53, (0.42, 0.66)
Mean	**3.15**	**73.0**	**49.8**	**60.4**	**9.8**
Chi Square (3 DF)		174.7 (P<0.001)	5.3 (P=0.15)	94.7 (P<0.001)	38.3 (P<0.001)

TABLE 2: Percentage of cows submitted for AI in the first three weeks of mating, during weeks four-to-six and during weeks one-to-six relative to calving pattern and milk protein percentage.

	2.36 – 2.99%	**3.00 – 3.14%**	**3.15 – 3.29%**	**3.30 – 4.59%**	**Average**
Submission Rate (three-week)					
>12 weeks	83.6	89.2	86.2	93.0	**88.8**
9-12 weeks	79.9	85.0	88.1	88.4	**85.7**
6-9 weeks	68.7	73.4	80.6	84.8	**76.7**
3-6 weeks	57.9	60.8	71.3	76.5	**66.0**
< 3 weeks	14.5	18.9	26.2	26.2	**20.2**
Average	**62.5**	**70.7**	**77.4**	**81.5**	**73.0**
Submission Rate (four to six-week)					
>12 weeks	94.7	69.2	58.8	78.6	**76.2**
9-12 weeks	78.1	75.0	77.6	85.0	**78.7**
6-9 weeks	66.7	67.5	74.2	75.0	**69.8**
3-6 weeks	68.1	71.2	66.3	74.0	**69.5**
< 3 weeks	34.9	40.6	46.8	44.6	**40.3**
Average	**58.1**	**61.3**	**64.3**	**68.6**	**62.1**
Submission Rate (one to six-week)					
>12 weeks	99.1	96.7	94.3	98.5	**97.3**
9-12 weeks	95.6	96.3	97.3	98.3	**97.0**
6-9 weeks	89.6	91.4	95.0	96.2	**93.0**
3-6 weeks	86.5	88.7	90.3	93.9	**89.6**
< 3 weeks	44.3	51.8	60.7	59.1	**52.4**
Average	**84.3**	**88.7**	**91.9**	**94.2**	**89.8**

TABLE 3: First insemination conception rates, three-week, four-to-six-week, and six-week in-calf rates and 21-week not-in-calf rates relative to calving pattern and milk protein percentage.

	2.36 – 2.99%	**3.00 – 3.14%**	**3.15 – 3.29%**	**3.30 – 4.59%**	**Average**
First insemination conception rate (three-week)					
>12 weeks	49.5	59.8	64.2	62.0	**59.6**
9-12 weeks	54.2	54.8	56.9	55.7	**55.5**
6-9 weeks	48.0	48.6	48.8	48.8	**48.6**
3-6 weeks	35.1	36.6	38.6	37.8	**37.0**
< 3 weeks	22.0	14.9	19.6	25.6	**20.4**
Average	**47.5**	**49.7**	**51.2**	**51.3**	**50.1**
In-calf rate (three-week)					
>12 weeks	44.0	57.5	58.5	62.7	**56.8**
9-12 weeks	45.5	49.6	52.5	51.9	**50.2**
6-9 weeks	35.2	37.1	40.9	43.2	**39.0**
3-6 weeks	21.6	24.3	28.1	31.5	**26.1**
< 3 weeks	3.5	2.8	6.1	7.9	**4.6**
Average	**31.5**	**37.3**	**41.4**	**44.3**	**38.6**
In-calf rate (four to six-week)					
>12 weeks	25.9	14.2	20.3	14.4	**18.0**
9-12 weeks	24.0	20.9	20.4	23.9	**22.2**
6-9 weeks	21.5	22.0	24.4	24.7	**23.1**
3-6 weeks	25.6	26.7	20.0	28.3	**25.2**
< 3 weeks	9.9	14.1	19.6	18.9	**14.6**
Average	**21.4**	**21.0**	**21.4**	**23.5**	**21.8**
In-calf rate (six-week)					
>12 weeks	69.8	71.7	78.9	77.1	**74.8**
9-12 weeks	69.5	70.6	72.9	75.8	**72.4**
6-9 weeks	56.7	59.1	65.3	67.9	**62.1**
3-6 weeks	47.2	51.0	48.1	59.8	**51.2**
< 3 weeks	13.3	16.9	25.2	26.8	**19.1**
Average	**52.9**	**58.2**	**62.8**	**67.8**	**60.4**
Not-in-calf rate (21-week)					
>12 weeks	6.0	2.5	1.6	4.0	**3.6**
9-12 weeks	4.0	5.7	4.3	5.3	**4.9**
6-9 weeks	12.0	8.8	9.7	7.8	**9.6**
3-6 weeks	14.5	11.6	13.1	12.5	**13.0**
< 3 weeks	33.6	26.1	19.2	20.7	**26.3**
Average	**13.0**	**9.6**	**8.5**	**8.0**	**9.8**

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VARIABLES ASSOCIATED WITH THE CALVING PATTERN OF PRIMIPAROUS HOLSTEIN-FRIESIAN COWS IN SEASONALLY CALVING HERDS

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The aim of this study was to identify some variables associated with the calving pattern of primiparous Holstein-Friesian heifers as these variables may help understand determinants of reproductive performance of nulliparous heifers. The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds in 9 regions in Australia. In this further study, a subset of animals from the original study population was selected for further analysis. We selected all primiparous Holstein-Friesian heifers (n=918) in seasonal calving InCalf Project study herds (n=35) that used AI exclusively for the first 6 weeks of breeding and that had milk production, sire Australian Breeding Value (ABV) and precalving liveweight records. In seasonal calving herds, heifers are mated in groups; the planned start of calving (PSC) date for the group is 282 days after the date that breeding commenced. Thus the interval between the herd PSC date and each animal's actual calving date (PSC-CI) reflects time to conception. In herds where PSC date was not known, it was estimated based on gestation length distributions in herds where PSC was known. Heifers were categorised into quartiles for each independent variable and multivariable statistical analyses were performed with herd as a random effect using the PROC MIXED procedure of SAS (SAS, 1996).

Heifers that had high early lactation (120-day) milk volume (MV) and milk protein% (MP%), and low milk fat% (MF%), high precalving liveweights (PLW), (Table 1) and low sire ABV for milk protein% (SABVP%), had shorter PSC-CIs ($P<0.01$). Interactions ($P<0.05$) were detected for MP% x MF%, MP% x PLW and MF% x SABVP%. Observed associations between PSC-CI and early lactation MV, MF% and MP% were likely due to biological determinants present before and during the heifer's breeding period that are associated with both reproductive performance in non-lactating dairy heifers and subsequent milk production/composition in the first lactation. Effects of differences in calving dates of 7-12 days on these variables are likely to be small. The association with SABVP% can be interpreted similarly. Since these associations were evident in non-lactating dairy heifers, the biological determinants causing these associations are not restricted to lactation-specific determinants such as post partum negative energy balance. Furthermore, these biological determinants are operating in addition to MV, MF%, MP%, SABVP%, PLW and effects of 'herd' as these variables were fitted simultaneously in the multivariable model. Further research is required to identify these determinants. High PLW is probably associated with higher liveweight at breeding which results in better reproductive performance.

Table 1. Least square mean±sem PSC-CI (days) by category of 120-day milk volume (kg), milk fat%, and milk protein%, and precalving liveweight (kg)

	Quartile 1 (n=229)	Quartile 2 (n=230)	Quartile 3 (n=229)	Quartile 4 (n=230)
*Milk Volume (kg)	1185	2303	2596	3069
PSC-CI (days)	19.5 ± 2.2a	16.0 ± 2.1b	15.8 ± 2.1b	11.8 ± 2.3c
*Milk Fat (%)	3.22	3.58	3.86	4.30
PSC-CI (days)	13.2 ± 2.2a	13.1 ± 2.1a	16.7 ± 2.1b	20.1 ± 2.4b
*Milk Protein (%)	2.84	3.01	3.14	3.34
PSC-CI (days)	23.0 ± 2.3a	15.6 ± 2.1b	12.9 ± 2.1bc	11.6 ± 2.3c
*Precalving Liveweight (kg)	383	441	480	533
PSC-CI (days)	20.7 ± 2.4a	14.8 ± 2.1b	15.4 ± 2.2b	12.2 ± 2.3b

[a,b,c] Different superscripts within rows are significantly different (P<0.05). *The mean for the quartile of each variable is presented. The quartiles for each independent variable do not necessarily include the same animals.

SAS, (1996) Statistical Analysis System, SAS Inst., Inc., Cary, NC.

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Associations Between Early Lactation Milk Protein Concentration and Reproductive Performance in Holstein-Friesian Cows in Australia

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ABSTRACT

Milk protein concentration (MP%) of dairy cows has been associated with reproductive performance, indicating that one or more biological determinants affect both MP% and reproductive performance. This current study aimed 1) to assess whether early lactation MP% of primiparous cows is associated with their prior reproductive performance (i.e. as nulliparous heifers) and 2) to compare the strength of the association between MP% and previous reproductive performance in nulliparous heifers with that in multiparous cows. The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds in 9 regions in Australia. In this further study, we selected primiparous (n=918) and multiparous (n=4242) Holstein-Friesian cows with cow-sire Australian breeding values (ABV) and milk production records [(milk volume (MV), milk fat concentration (MF%) and milk protein concentration (MP%)] during early lactation in herds having a seasonally concentrated calving pattern. In seasonal calving herds, cows are mated in groups; the planned start of calving (PSC) date for the group is 282 d after the date that breeding commenced. Thus the interval between the herd PSC date and each animal's actual calving date (PSC-CI) reflects time to conception.

High MP% was associated with shorter PSC-CI. However, interactions were detected between MP% and MV and between MP% and cow age. The interaction term between MP% and MV indicated that cows with low MP% that also had high MV had shorter PSC-CI than cows with low MV. The interaction between MP% and cow age indicated that the association between PSC-CI was stronger in multiparous cows than in primiparous cows. For a one percent increase in MP% PSC-CI decreased by 8 d in primiparous cows and by 31 to 35 d in multiparous cows. Observed associations between PSC-CI and early lactation MP% were likely due to biological determinants present before and during the cow's breeding period that are associated with both reproductive performance and subsequent MV/MP%. Since these associations were present in non-lactating dairy heifers, the biological determinants causing these associations are not restricted to lactation-specific determinants such as post partum NEB. However, the stronger association observed in multiparous cows than in primiparous cows (nulliparous heifers) may be explained by additional effects of lactation-associated factors such as NEB in lactating dairy cows.

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

Abbreviation key: **ABV = Australian Breeding Values** (ABV™), **MF%** = milk fat concentration, **MP%** = milk protein concentration, **MSD** = mating start date, **MV** = milk volume (L), **NEB** = Negative energy balance, **PLW** = Precalving liveweight, **PSC** = planned start of calving, **PSC-CI** = estimated planned start of calving to calving interval

Short title: Milk protein concentration and reproduction

INTRODUCTION

Dairy cows in early lactation experience a period of NEB when the energy requirements for milk production and maintenance are greater than those provided by dietary energy intake. In general, this period of imbalance results in the mobilisation of body tissue and is associated with poor reproductive performance (Villa-Godoy et al., 1988, Butler and Smith, 1989, Beam and Butler, 1998, Jorritsma et al., 2003, Wathes et al., 2003). NEB can also be associated with increased MF% due to adipose tissue mobilisation and decreased MP% due to a shortage of glucose for milk protein synthesis in the udder (De Vries and Veerkamp, 2000). Studies show that MP% is positively correlated with energy balance (Grieve et al., 1986) as well as the intake of metabolisable energy yielding carbohydrates (De Peters and Cant, 1992). McGuire et al., (1995) postulated that because concentrations of circulating insulin are directly proportional to energy intake and because the somatotropin and IGF system is modulated by energy status, the basis for the relationship between energy intake and MP% is really a relationship between circulating insulin or the IGF system and milk protein synthesis. Given that NEB can result in decreased MP% (Grieve et al., 1986), decreased IGF-I (Spicer et al., 1990, Wathes et al., 2003) and poor reproductive performance (Butler, 2003, Reist et al., 2003) it is not surprising that a number of studies report positive associations between MP% and reproductive performance.

Higher MP% has been associated with shorter calving to conception intervals (Lampo et al 1963; Kaufmann 1979; Anon 1984; Miettinen and Setala, 1993, Miettinen, 1995), shorter calving to first estrus intervals in primiparous cows (McGowan et al. 1996), shorter calving to first service intervals (Miettinen and Setala, 1993) and higher submission (Morton, 2000), conception (Leaver 1983; Pinto et al. 2000; McDougall, 2003) and pregnancy rates (Buckley et al., 2003). Low MP% has been associated with the incidence of anestrous (Opsomer et al., 2000), a delay in the resumption of postpartum luteal activity in Norwegian cows (Reksen et al., 2002) and more subfertile (requiring more than two inseminations to conceive) primiparous cows (Moss et al., 2002). However, Masilo et al., (1992) found no relationship between MP% and the interval to first ovulation in primiparous cows.

Other studies have reported associations between high MF% to MP% ratios and increased risk of disease including cystic ovarian disease, mastitis and lameness (Heuer et al., 1999) and displaced abomasums (Geishauser et al., 1997) as well as reduced reproductive performance including lower conception rates, increased services per conception, longer calving to conception intervals (Heuer et al., 1999) and lower pregnancy rates (Loeffler et al., 1999a). It is possible that many other associations between MP% and reproductive outcomes have either been observed and not reported or simply not determined in the first place. This is because what is described here is an association and researchers would not intuitively set out to examine such an association. Furthermore it is almost certainly incorrect to suggest that high MP% causes better reproductive performance. It is more likely that the factors or determinants (at this stage unknown) that result in high MP% also result in high reproductive performance.

Given that Morton, (2000) and other studies reported that MP% was associated with reproductive performance in cows we hypothesized that early lactation MP% in primiparous cows was also associated with their reproductive performance as nulliparous heifers. If correct we hypothesised that at least some of the relationship may be explained by determinants other than post partum NEB. The aim of this study was 1) to assess whether the early lactation MP% of primiparous cows was associated with their prior reproductive performance (i.e. as nulliparous heifers) and 2) to compare the strength of association between early lactation MP% and previous reproductive performance in nulliparous heifers with that in lactating cows.

MATERIALS AND METHODS

Data

The InCalf Project included a large, prospective, observational field study that described the reproductive performance of 29,462 dairy cows in 168 herds (124 seasonal-calving and 43 year round calving and one batch calving) in nine regions of Australia (Morton, 2000). A subset of the original database was used and then refined to only include Holstein-Friesian cows with between one and five milk production records during the first 120 d of lactation. These cows were located in seasonally-calving herds that used AI for at least the first six weeks of the breeding period. This subset was then subdivided into primiparous cows (1,797 cows in 60 herds) and multiparous cows (5254 cows in 64 herds). There was cow-sire genetic information available for 1584 primiparous cows in 58 herds and precalving liveweights available for 1049 primiparous cows in 36 herds with information on both cow-sire genetic information and precalving liveweight for 918 primiparous cows in 35 herds. There was cow-sire genetic information available for 4242 multiparous cows in the 64 herds. The precalving liveweight was not recorded for multiparous cows. Only multiparous cows with complete information were used in the present study. The herds were located in five regions in Victoria and Tasmania (Table 1).

Cow Age

Four age categories were created for primiparous (1; 2yr n=918) and multiparous cows (2; 3yr, n=979; 3; 4yr, n=865; 4; 5-16yr, n=2398).

Milk Production

Milk production variables included cumulative milk volume (L), (Milk120), milk fat production (Fat120kg), milk protein production (Prot120kg), milk fat concentration (MF%), milk protein concentration (MF%) and MF% to MP% ratio during the first 120 d of lactation. Milk production records were derived from the Australian Dairy Herd Improvement Scheme (ADHIS) database and the number of milk production records ranged from one to five per cow. MP% was expressed as true protein on a mass volume (g/l) basis.

Cow-Sire Genetic Information

Details relating to cow-sire Australian breeding values (ABV) for milk volume (ABVMV), milk fat production (ABVFatkg), milk protein production (ABVProtkg), millk fat concentration (ABVMF%) and milk protein concentration (ABVMP%) in multiparous and primiparous cows are presented in Table 2. ABV information was not available for individual cows.

Liveweight (Primiparous cows)

Primiparous cows were weighed once prior to calving using electronic scales. Primiparous cows were weighed at 688±1.18 d of age (range 528-875) which was 41±0.70 d (range -1 to -106) prior to calving. Mean PLW was 457±1.9 kg (range 266-652).

Estimated Planned Start of Calving (PSC-CI)

In seasonal calving herds cows are bred in groups; the planned start of calving (PSC) for the group is 282 d after the date that breeding commenced. Thus the interval between the herd PSC date and each individual animal's actual calving date (PSC-CI) reflects time to conception and was the dependent variable in this study. Although calving dates were available for all cows the

PSC date was not known for every herd. However, data from eight herds (n=1667 cows) was available in the year prior to the present study. Based on the distribution of known gestation lengths, 6.1% of cows calved on or before the PSC in herds that did not synchronise (n=7) and 25.2% in the herd that did synchronise (n=1). Herds were deemed to have been synchronised if >55.3% calved within the three weeks following PSC. Therefore, the PSC-CI was taken as the point of the 6.1 or 25.2 percentile of the calving distribution for each herd, depending on whether the herd was deemed to have synchronised or not. The PSC-CI was calculated based on information available for all animals within a herd, including cross breeds and Jerseys but excluding animals that had had twins, aborted, were induced to calve or were milked from one production season to the next without calving. Cows calving >160 d after PSC-CI were also excluded.

Statistical Analysis

The relationships between milk production variables during the first 120 d of lactation, cow-sire genetic information, precalving liveweight, cow age 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). Log transformation of the PSC-CI was considered but this created retransformation problems (Manning, 1998) and did not alter the interpretation of the results. Initially, independent variables were tested one at a time and variables that were significant (P<0.25) were included in a larger multivariable mixed model. Each independent variable was categorised into four sub-groups and either the continuous or categorical variable (CAT) was included in the model, depending on the better fit using Akaike's information criterion (AIC) (Akaike, 1974) and visual appraisal of the graphed variables. Terms that were not significant were excluded individually in a backwards stepwise process until all remaining terms were significant. Herd was included as a random effect. Including herd as a fixed effect did not change the estimates (<8.5% change) or the significance of the other terms in the model. Herd level variables (e.g. mean herd MP%) were also tested but were not significant. Milk protein yield, milk fat yield and MF% to MP% ratio were excluded as they were highly correlated (r > 0.75) with MV, MF% or MP%. All first order interactions were screened for significance (P<0.05) and significant terms were then added to the final model in a forward stepwise manner.

Selected second order interactions were also tested. In order to interpret the intercept continuous variables were mean-centered (subtracted from the mean) such that the estimates corresponded to the mean of each variable in the model. Descriptive statistics (mean±s.e) are presented for the variables of interest. Model estimates (±s.e) for each variable are also presented.

RESULTS

Milk Production and Cow-Sire Genetic information

Descriptive statistics relating to milk production and composition and cow-sire genetic information are presented in Table 2. Cows with low MP% (<3.00%) were common in the study herds as 34/35 of the primiparous and 62/64 of the multiparous herds had cows with MP% less than 3.00%.

Calving Spread

The mean interval from when the first cow calved to when the last cow calved was 22.2±0.62 d (0 to 44) for primiparous cows and 41.2±0.44 d (range 0 to 175) for multiparous cows. Age at first calving was 730±1.31 d for the primiparous cows (range 600-901).

PSC-CI

The mean PSC-CI was 15.2±0.63 d (-28 to 136) for primiparous cows. Ninety percent (830/918) of the primiparous cows calved within -10 and 40 d of PSC-CI. The mean PSC-CI was 27.1±0.39 d (range -48 to 159) for multiparous cows. Seventy four percent of multiparous cows (3157/4242) calved between -10 and 40 d of estimated PSC.

Primiparous and Multiparous cows

Following univariate (Table 3) and then multivariable analysis (Table 4) interactions ($P<0.01$) were detected between MP% and MV and between MP% and cow age. A three way interaction between MP%, MV and cow age was tested and was not significant ($P>0.05$). Primiparous and multiparous cows with MP% had shorter PSC-CI irrespective of the level of milk volume (Table 4, Figure 1). However, the interaction term between MP% and MV indicated that cows with low MP% that also had high MV had shorter PSC-CI than cows with low MV. The interaction between MP% and cow age indicated that the association between

PSC-CI was stronger in multiparous cows than in primiparous cows (Table 4, Figure 2). For a one percent increase in MP% PSC-CI decreased by 8 d (*P*<0.05) in primiparous cows and by between 31 to 35 d in multiparous cows (*P*<0.01). PSC-CI was also shorter (*P*<0.05) in cows that had a high cow-sire ABVMV and in cows that had a low cow-sire ABV MP%. Removal of either term from the model had very small effects (<1% change) on the estimates for MP%. Interaction terms involving MP% also had relatively small effects for multiparous cows (<18% change) but large effects for primiparous cows (100% change) on the estimates for MP%. In a separate model using primiparous cows only PLW was included in the model and although negatively associated with PSC-CI (-0.05±0.02, *P*<0.01) it changed the estimates for MP% by less than 1%.

DISCUSSION

Factors Affecting Milk Protein Concentration and Gestation Length

The main factors affecting MP% in Australian dairy cows are breed (Holstein versus Jersey), location (state and region within state), stage of lactation and month of calving (White, 2001). Only Holstein-Friesian cows were included in the present study as we hypothesized that the association would be strongest in this population due to greater susceptibility to postpartum NEB. However, Morton (2000) reported that the relationship between MP% and reproduction was not unique to Holstein-Friesian cows. Herd was included as a random effect to take account of location differences in the present study.

Milk composition data was confined to the first 120 d of lactation to limit any stage of lactation effects. In the present study month of calving was related to the variable of interest (PSC-CI) and for this reason calving date could not be included in the model. Therefore possible confounding due to absolute calving date could not be removed. On a within herd basis, it could be hypothesised that cows with differing calving dates could have differing MP% due to seasonal changes in pasture supply for example. However, due to the compact nature of the calving pattern (on average all cows in a herd were calved within 28.5±2.02 d (range 0-144) of the first cow in a herd to calve), the effect would have had to occur within this time constraint and this seems unlikely.

Sire of calf (Everitt and Jury, 1972) sire breed (Batra et al., 1982), natural mating versus AI (Macmillan and Curnow, 1976) and sex of the calf (Anderson and Plum, 1965) all influence gestation length. In this analysis, due to lack of data we could not adjust for calf sire, calf breed or calf sex effects on gestation length all of which are a potential source of bias for PSC-CI. However, it was assumed that calf sires were randomly distributed across herds and were not related to the MP%. Breeding policy in commercial herds in Australia usually dictates that cows are exposed to a limited number of either AI or stock bulls and cows are not usually bred based on either phenotypic (MP%) or genotypic MP% (ABVMP%). Furthermore, nulliparous heifers that subsequently have low MP% when lactating, based on their ABVMP%, are not more likely to be mated to a bull with long gestation length. In theory, later calving multiparous cows (i.e. those likely to have lower MP%) may experience greater exposure to stock bulls that would be more likely to be of a beef breed. However, the fact that the association was evident in nulliparous heifers, and also the consistency of the association across all calving periods and not just the later calving cows counteracts this argument. Regional (Macmillan and Curnow, 1976; Batra et al., 1982) and herd (Macmillan and Curnow, 1976) differences that have been reported to affect gestation length were adjusted for in the present study.

Herd mean MP% was included in a univariate analysis and was not significant where herd was also included as a random effect. However, if herd was removed as a random effect (i.e. not included in the model), then mean herd MP% became significant. This indicated that PSC-CI was influenced by herd, but not by mean herd MP%. Including herd as a random effect did not preclude the possibility of confounding due to herd.

MP% increases in pregnant cows from week 24 to the end of lactation (Roche, 2003). Thus, the use of 305-d production data in the present study could potentially bias our interpretation of the results. However, there was a very high correlation between 120-d and 305-d MP% in the current study (r = 0.92 and r = 0.93, $P<0.0001$, for primiparous and multiparous cows, respectively). This indicated that the results should be similar for 120-d and 305-d milk production. Unpublished statistical analyses based on 305-d production figures revealed this to be the case.

Negative Energy Balance

The interaction between MP% and cow age on PSC-CI (Figure 3) in the present study indicated that the strength of the relationship (based on the slope of the line) is much stronger in multiparous cows than in nulliparous heifers. One hypothesis that agrees with this finding is that cows are exposed to severe environmental conditions in the post partum period (e.g. NEB and calving related metabolic disorders). In contrast heifers, although growing, are not subjected to the same intensity of environmental stresses. A number of studies show that NEB as indicated by BCS and BCS loss are related to both reproductive performance and MP% in cows.

Leaver (1983) found that cows with low body condition score (BCS, <2 on a 1-5 scale) in combination with low MP% (<300g/kg) had the poorest conception rates at first service (9/31, 29 %) in comparison to cows with high MP% (>300g/kg) and low BCS (31/65, 48%), high MP% and high BCS (>2 on a 1-5 scale; 75/144, 52%) or cows with low MP% and high BCS (12/16, 75%). Leaver (1983) suggested that the interaction between short term (MP%) and long term (BCS) energy supply was indicative of NEB. A more recent study by Busato et al. (2002) has also shown an interaction between BCS loss and MP%. In that study, MP% was lowest in cows that had a BCS of ≤3.25 (1-5 scale) at one week post calving and that lost >0.75 of a BCS in the first 8 weeks of lactation (2.93%) compared to cows that had a BCS of 3.25 at one week post calving and that lost ≤0.75 of a BCS in the first 8 weeks of lactation (3.17%). Schröder and Staufenbiel (2002) measured the backfat thickness of 46,000 dairy cows by ultrasound and reported that the development of BCS was strongly related to MP% where values below 3.20% reflected an extreme energy deficit in early lactation.

Grieve et al., (1986) reported that estimated energy balance was positively correlated with MP% (0.12 to 0.47). Other studies have also shown that MP% increases with improving energy status of the cow (Journet and Remond, 1980), DMI (Auldist et al., 2000; O'Brien et al., 1997) and energy intake (Emery, 1978; (De Peters and Cant, 1992). The higher DMI and energy intake is presumed to spare amino acids from gluconeogenesis, thus increasing the amino acid supply available for milk protein synthesis (Auldist et al., 2000). This may be as a result of changes in rumen fermentation patterns where starchy diets lead to increased rumen propionate thus sparing amino acids utilisation and allowing more amino acids to be incorporated into milk protein (Moorby et al., 1996).

MP% and MF% in early lactation have been suggested as surrogate measures for determining the nutritional status of cows in early lactation (Nelson and Redlus, 1989; Rathwell, 1990). Loeffler et al. (1999a) reported that MF% to MP% ratio was a significant predictor of pregnancy risk independent of disease, days in milk, farm and seasonal factors. A number of studies have revealed negative correlations between MF% to MP% ratio, dry matter intake and energy balance (Grieve et al., 1986; Gravert, 1991; Hagert, 1992). Similarly, De Vries and Veerkamp (2000) reported that MF% to MP% ratio was positively correlated with nadir of energy balance and negatively correlated with interval to return to positive energy balance and total energy deficit in early lactation in primiparous cows. One of the limitations of using MF% to MP% ratios is that it is impossible to distinguish between low MP% and low MF% and high MP% and high MF%, as ratios can produce exactly the same result even though the MF% and MP% may differ dramatically. For this reason MF% to MP% ratio was not considered for analysis in the present study.

Reduced MP% can be a feature of diets containing high levels of grass silage or grazed grass (Moorby et al., 2001). This may suggest a shortage of suitable amino acid precursors associated with an inefficient utilisation of dietary N in the rumen or an overall shortage of energy (Beever et al., 2001). The differences between cows within the same herd in the present study are difficult to explain. In general individual cows within a seasonally calving herd fed pasture will be managed similarly in terms of access to pasture and are usually fed concentrates at a flat rate rather than according to milk production. Factors that would potentially reduce dry matter intake such as lameness and disease may contribute to reduced MP% and also to poor fertility. However, these would have to appear as year on year effects (i.e. cows lame or diseased in the current lactation resulting in low MP% in the following lactation) and this argument would be less applicable to nulliparous heifers.

Reproductive Performance

In the present study, PSC-CI was an estimate of how soon an animal had conceived relative to the start of breeding. Although the relationship between MP% and reproduction has been reported in both feedlot/indoor/concentrate and seasonal calving pasture feeding systems, there appear to be some differences between milk production systems in how this association is expressed. This may occur because oestrus detection and submission rates to AI are of necessity comparatively high in seasonal production systems (Xu and Burton, 1996).

Buckley et al. (2003) found that cows in herds in Ireland with a higher estimated 200-d MP% had an increased likelihood of being detected in oestrus and being submitted for AI within the first 21 d of the breeding season. Buckley et al. (2003) also found that 305-d MP% was positively associated with the likelihood of pregnancy after 42 d of breeding. In New Zealand, McDougall (2003) reported that MP% in early lactation was associated with the likelihood a cow being detected in estrus between D 14 and 28 post AI, pregnancy rate at D 28 and 56 or final pregnancy rates and the probability of conception over time. Morton (2000) reported that cows in Australian herds with high MP% were more likely to be submitted for AI in the first three weeks of AI, conceive to first service, and be pregnant by six weeks of breeding and 21 weeks of breeding.

According to Pinto et al. (2000), the increase in conception rate to first AI in cows with higher MP% appears to be due to increased fertilisation rates and/or decreased early embryonic mortality. Other studies have shown that postpartum ovarian function may also be involved. Cows that had delayed ovulation for $\geq$45 d post partum as well as those with persistent corpora lutea for $\geq$19 d after the first normal cycle post partum, tended (P<0.06) to have lower MP% than normal cows (Taylor et al., 2003). In all of these studies, it could be argued that postpartum NEB was the underlying mechanism of action. In this case low MP% is a consequence of post partum NEB, and (independently), poor fertility is also a consequence of post partum NEB (Figure 1). However, as the results of the present study indicate post partum NEB only partially accounts for the association between PSC-CI and MP% as evidenced by the nulliparous heifers which were unaffected by postpartum NEB.

Further evidence is provided by Taylor et al., (2004) who reported that metabolic differences in pre-pubertal heifers were later reflected by altered reproductive function (delayed ovulation for $\geq$45 d post partum or persistent corpora lutea for $\geq$19 d) during the first lactation. It is worth noting that the nulliparous heifers in the current study that did not conceive during the previous breeding season could not be included in the data for primiparous cows as lactation is required to determine MP%. Likewise cows that did not conceive or that conceived late and were culled were also excluded. It is a possibility that the association observed in the present study may be even stronger than reported here. Another possibility is that the mechanism of action operates differently for lactating cows (e.g. via NEB) than for nulliparous heifers. Heifers predisposed to having lower MP% may also be predisposed to reaching puberty later resulting in poorer fertility and later conception).

A genotype by environment interaction may also be involved where cows genetically selected for high MV have greater energy deficits, lower MP% and poorer fertility. This could explain why cows in the present study with low MP% and low MV had longer PSC-CI than cows with low MP% and high MV. The interaction between MP% and MV may have occurred where cows with a high genetic potential for milk production are not allowed to express that potential in a seasonal pasture based environment. It could be hypothesised that low MP% combined with low MV is a phenotypic expression of the situation where cows selected for high MV are unable to express that potential in a pasture based environment.

Genetics

Haile-Mariam et al. (2003) found that MP% was negatively correlated both genetically (*rg* -0.27 in early lactation) and phenotypically (*re* -0.04 to -0.06 throughout lactation) with the calving interval in Holstein cows in Australian herds. As the genetic correlation was only favourable in early lactation this indicates that the period of NEB in early lactation is at the basis of the association between MP% and reproductive performance. Arguments against this relationship being either genetic or nutritional in nature are reflected in the results of a study by Roche (2003) where identical twins were managed together on one farm. Although genetics, diet and environment were similar, cows within an identical twin pair that had higher MP% were more likely to conceive.

Grieve et al. (1986) used the breeding value for MP% of the cow-sire as a covariate in an attempt to remove some of the genetic difference for milk components among cows. Including the breeding value improved the prediction of MP% in one of the four datasets analysed. Likewise, the association between pregnancy rate and MP% was evident in a study reported by Buckley et al. (2003) following adjustment for genetic merit for milk production and proportion of Holstein-Friesian genes. A similar approach was adopted in the present study where ABVMP% was fitted to the model in an attempt to take into account some of the genetic differences for MP% among cows. The relatively small effects of cow-sire ABVMP% on the model estimates for MP% could indicate that the relationship does not have a large genetic basis in so far as we were able to adjust for genetic differences using cow-sire ABVMP%. This may because of the small variation in breeding values between sires or of the lack of sensitivity of the ABV's in determining differences between sires. Although the heritability for MP% is higher than for other milk production traits, its coefficient of variation is relatively low. This can limit genetic progress when selecting for MP% (Haile-Mariam et al., 2003).

As discussed by Veerkamp et al. (2003), the associations observed in the present study may run via pleiotropic effects, i.e. via functional pathways or linkages of genes (Falconer and Mackay, 1996). In the case of pleiotropy, the genes that affect fertility may also affect milk volume, milk composition and energy balance or vice versa. For gene linkage, it may be that the alleles for genes affecting fertility, milk volume and milk composition are closely linked at the DNA level (Falconer and Mackay, 1996).

The composition of cow's milk varies depending on the ß–lactoglobulin phenotype of the cow (Hill et al., 1997; McLean et al., 1984). New Zealand cows with the AA ß–lactoglobulin phenotype produced 28% more whey protein, 7% less casein, 11% more fat and 6% less total solids than cows of the BB variant of the ß–lactoglobulin phenotype (Hill, 1993). Differing milk protein genotypes (kappa-casein and beta-lactoglobulin) can affect the phenotypic and genetic variation of milk protein composition while having no significant effect on total MP% (Bobe et al., 1999). In agreement, Auldist et al. (2000) found that cows with differing phenotypes for ß-lactoglobulin did not differ in MP%. The association between MP% and fertility seen in the present study and in other studies is unlikely to be related to individual protein types, as the association was based on true protein of which 80% is casein (MacRae et al., 2000). However, Lin et al. (1987) did find an association between milk protein loci (ß-lactoglobulin) and body weight, gestation length, age at first conception and interval from first service to conception in nulliparous dairy heifers.

CONCLUSIONS

Observed associations between PSC-CI and early lactation MP% were likely due to biological determinants present before and during the cow's breeding period that are associated with both reproductive performance and subsequent milk volume/composition. Since these associations were evident in non-lactating dairy heifers, the biological determinants causing these associations are not restricted to lactation-specific determinants such as post partum NEB. The stronger association observed in multiparous cows than in primiparous cows (nulliparous heifers) may be explained by additional effects of lactation-associated factors such as NEB in lactating dairy cows. Furthermore, these biological determinants are operating in addition to MV, MF%, ABV's for MF% and MP%, PLW and effects of 'herd' as these variables were fitted simultaneously in the multivariable model. Further research is required to identify these biological determinants.

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Table 1: Distribution of herds and cows within region.

State	Location	Number of Herds		Number of Cows	
		Primiparous	Multiparous	Primiparous	Multiparous
Victoria	Northern Victoria	9	11	298 (32%)	857 (20%)
	East Gippsland		12		864 (20%)
	South Gippsland	16	18	417 (45%)	1108 (26%)
	South Western Victoria	3	16	56 (6%)	1024 (24%)
Tasmania	North West Tasmania	7	7	147 (16%)	389 (9%)
Total		35	64	918	4242

Table 2: Mean (range) milk production during the first 120 d of lactation and cow-sire genetic details in primiparous and multiparous cows.

	Primiparous	**Multiparous**
Number of cows per herd	26 (5 to 66)	66 (10 to 175)
MV(L)	2463±15.4 (938 to 4052)	3221±9.5 (1115 to 5732)
Fatkg	91.6±0.58 (40 to 184)	119.6±0.36 (44 to 236)
Protkg	75.7±0.45 (30 to 121)	101.4±0.29 (38 to 173)
MF%	3.74±0.01 (2.41 to 5.30)	3.74±0.01 (2.03 to 6.61)
MP%	3.08±0.01 (2.52 to 3.81)	3.16±0.01 (2.51 to 4.52)
MF% : MP%	1.21±0.01 (0.79 to 1.63)	1.19±0.01 (0.62 to 1.88)
	Cow-sire Australian Breeding Values (ABV)	
Number of sires	171	744
Number of daughters per sire	5.3 (1 to 68)	5.7 (1 to 238)
ABVMV	1147±11.1 (51 to 1916)	949±6.5 (-603 to 1924)
ABVFatkg	37.1±0.38 (5 to 68)	34.9±0.18 (-9 to 68)
ABVProtkg	29.9±0.25 (4 to 62)	25.1±0.15 (-7 to 47)
ABVMF%	-0.14±0.01 (-0.56 to 0.42)	-0.06±0.01 (-0.71 to 0.85)
ABVMP%	-0.11±0.01 (-0.35 to 0.18)	-0.10±0.01 (-0.40 to 0.30)
Reliability%	96.3±0.19 (66 to 99)	97.3±0.06 (55 to 99)

Table 3: Association (univariate mixed method procedure) between the independent variables and the dependent variable (PSC-CI) in primiparous and multiparous cows.

		Primiparous + multiparous cows (n=5160)	
		Estimate	**P value (main effect)**
Milk Protein (%)		-20.0±1.59	<0.0001
Milk Volume (L)		0.004±0.001	<0.0001
Age (category)	1	-10.0±1.04	<0.0001
	2	-1.67±1.09	
	3*	–	
	4	-0.61±0.93	
ABV Milk Volume (category)	1	2.78±0.93	<0.0001
	2	3.66±0.93	
	3	-0.32±0.93	
	4*	–	
ABV Milk Fat (%) (category)	1	-3.93±0.93	0.0002
	2	-3.07±0.93	
	3	-2.66±0.93	
	4*	–	
ABV Milk Protein (%)		5.61±3.34	0.09

*Estimate is relative to reference category for each variable.

Table 4: Association (multivariable mixed method procedure) between the independent variables and the dependent variable (PSC-CI) in primiparous and multiparous cows.

		Primiparous + multiparous cows (n=5160)	
		Estimate	**P value (main effect)**
Intercept		30.4±1.74	<0.0001
Milk Protein (%)		-34.8±3.70	<0.0001
Milk volume (L)		-0.005±0.007	<0.0001
Age (category)	1	-15.6±1.30	<0.0001
	2	-3.75±1.09	
	3*	–	
	4	0.99±0.95	
ABV Milk Volume (category)	1	-0.73±1.10	0.03
	2	1.31±0.98	
	3	-1.25±0.90	
	4*	–	
ABV Milk Protein (%)		14.3±3.87	0.0002
Milk Protein (%) x Age	1	26.7±5.42	<0.0001
	2	2.20±4.84	
	3*	–	
	4	4.19±4.05	
Milk Protein (%) x Milk Volume (L)		0.011±0.002	<0.0001

*Estimate is relative to reference category for each variable.

Figure 1: The proposed dynamics of the observed association between milk protein concentration and reproductive performance (PSC-CI).

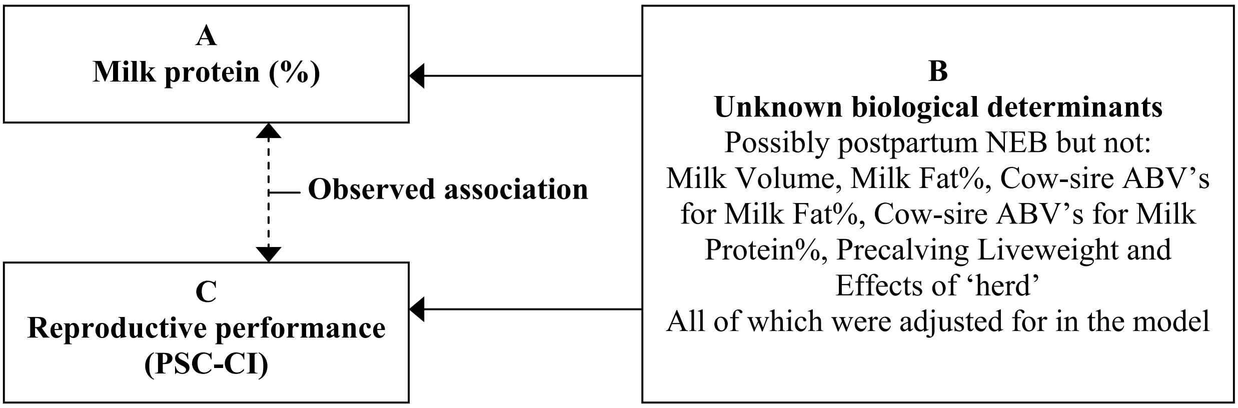

Figure 2: Least square mean planned start of calving to calving interval (PSC-CI) based on the interaction between milk protein (%) and milk volume (L), (both mean-centered) in the first 120 d of lactation.

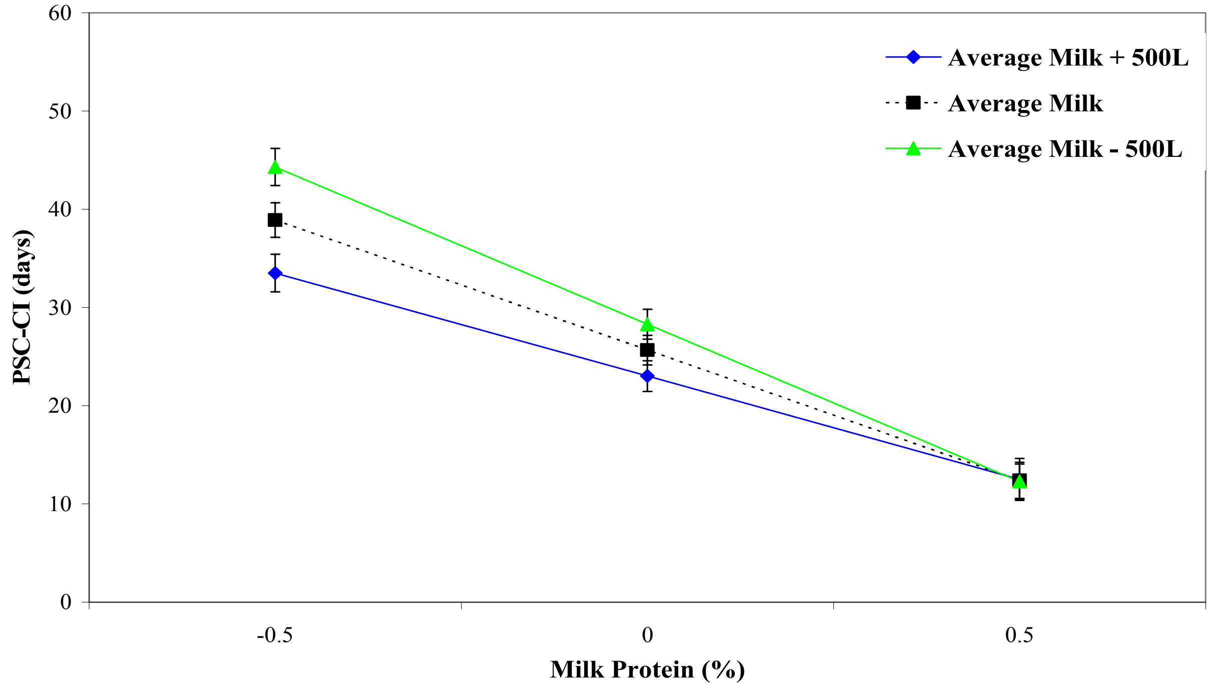

Figure 3: Least square mean planned start of calving to calving interval (PSC-CI) based on the interaction between milk protein (%), (mean-centered) and cow age in the first 120 d of lactation.