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Relationships between milk protein percentage and reproductive performance in Australian dairy cows

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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.

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.

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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 this 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

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**

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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