Associations of uterine pathology with milk production and effects of treatment with a non-steroidal anti-inflammatory drug in dairy cows

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Abstract

Subclinical endometritis (SCE) is a form of uterine pathology characterised by an increased proportion of polymorphonuclear cells (PMN) in the uterus post-calving. Previous studies demonstrated a negative association between SCE and lower milk production, with indications that systemic inflammation underlies this link. Accordingly, the hypothesis tested was that treatment with a non-steroidal anti-inflammatory drug (NSAID) would increase milk yield in cows with ≥ 14% PMN at Day 14. In this study, ≥ 14% PMN in the cytological sample collected at Day 14 postpartum was defined as SCE. To test this hypothesis, 213 dairy cows were paired by calving date and Day 14 PMN %, then randomly assigned to three doses of a NSAID (Carprofen 1.4 mg/kg, n = 103) between 21 and 31 days postpartum or left as untreated controls (n = 108). The effect of the NSAID treatment on milk production was analysed using mixed models. While the results supported previous evidence of a negative association between SCE and milk production, NSAID had no effect on either PMN % at Day 42 or milk production (P > 0.1) in this study. The lack of a NSAID effect was possibly because SCE spontaneously resolved in more than 90% of the cases.

Keywords: subclinical endometritis; anti-inflammatory; milk production; dairy cow

Introduction

Subclinical endometritis (SCE) is characterised by an increased proportion of polymorphonuclear cells (PMN) in the uterus post-calving (Barlund et al. 2008; Dubuc et al. 2010; Sheldon et al. 2009). It has been previously reported that SCE has a negative association with milk yield (Burke et al. 2010; McDougall et al. 2011) and milk fat and protein percentage (Green et al. 2009) in pasture-based systems. These three studies indicate that systemic inflammation, as measured by the concentration of circulating acute phase proteins such as albumin and globulin, has a key role in this association. A proposed mechanism is that the inflammation associated with SCE, through pro-inflammatory cytokines, may be reducing dry matter intake in cows with SCE, thus lowering milk production (Bell & Roberts 2007; Bertoni et al. 2008; Johnson & Finck 2001). This hypothesis was supported by the reduced dry matter intake and milk production in cows with a uterine infection reported by Bell and Roberts (2007).

Inflammation can be reduced using a non-steroidal anti-inflammatory drug (NSAID), which inhibits the action of the cyclooxygenase enzyme (COX) and prevents the secretion of prostaglandins, a pro-inflammatory molecule (Erdem & Guzeloglu 2010; Heuwieser et al. 2011; Sordillo et al. 2009). Therefore, the general hypothesis proposed is that treatment with a NSAID early postpartum would increase milk production in cows with high PMN%. The timing of NSAID treatment is an important consideration, however, because prostaglandin-mediated inflammation is a normal part of the uterine involution process after calving (Barlund et al. 2008). Therefore, the specific hypothesis tested was that treatment with a NSAID between 21 and 31 days postpartum will increase milk production in cows with ≥ 14% PMN at Day 14.

Materials and methods

Experimental design

This study used 213 multiparous cows (Holstein-Friesian (n = 136), Holstein-Friesian x Jersey (n = 77)) aged 5.4 ± 2.2 (standard deviation) years and with a mean live weight of 445 ± 56 kg. The cows were enrolled at DairyNZ, Scott Farm, Hamilton, between May and October 2011. Prior approval for animal use was obtained from the Ruakura Animal Ethics Committee, Hamilton, New Zealand. The number of cows required for the NSAID and control groups (n = 100) to detect a 30% reduction in PMN% from Day 14 to Day 42 cytology at 80% power with 5% significance was calculated using a standard deviation of 0.45 ($\log_{10}$ PMN%) (S Meier, Unpublished data) and a detectable difference of 0.18 ($\log_{10}$ PMN%). The experiment was commenced with sufficient cows to allow for ~10% of the cows to be removed from the experiment due to illness during the transition period.

To allow time for involution to proceed without interference, the NSAID treatment was delayed until between Day 21 and Day 31 postpartum. To ensure that the Control and NSAID treatment groups were balanced for PMN%, a uterine cytology sample was taken on between Days 14 and 17 postpartum. Using a randomised block design, cows were paired by calving date and Day 14 to 17 PMN% before being randomly allocated to either the Control or NSAID treatment group. Where pairs were not able to be created due to uneven numbers of cows on the day of sampling, the unpaired cow was randomly allocated to either the Control or NSAID treatment, with the un-allocated pair, being of comparable calving date and PMN%, randomly selected from the next group of sampled cows where possible. Cows with calving difficulty (n = 1), retained foetal membranes (n = 4) and milk fever (n = 12) were retained in the trial. Two cows were excluded from the trial as they had received a systemic antibiotic during the trial.

Uterine cytology

A uterine endometrial cytology sample was collected from each cow between Day 14 and 17 and between Day 42 and 45 postpartum as described by Burke et al. (2010). The vulva of the cow was cleaned with a paper towel and a double-guarded, modified artificial insemination pipette was passed through the cervix and into the uterus. A clean stylette with an attached clean cytology brush (Pap endocervical sample brush, EBOS Group Ltd., Christchurch, New Zealand) was used to collect the sample from the uterine wall. The stylette was then retracted into the artificial insemination pipette sheath and all sampling equipment removed from the cow. Cells recovered from the uterus was rolled onto a microscope slide and air-dried. When completely dry the slides were stained with Diff-Quick (Dade Behring, Newark, Delaware, USA) to differentiate cytoplasmic detail. The slides were then examined at Massey University for determination of the percentage of PMN present. Areas of each slide that contained small clusters of epithelial cells in the order of 5 to 20 per cluster, were preferentially selected and all identifiable nucleated cells counted. Based on Day 14 PMN% results, cows were retrospectively grouped into quartiles and cows in the upper quartile (≥ 14% PMN) were classified as having SCE. We defined SCE at Day 42 using a PMN of ≥ 7%, based on the results reported by Burke et al. (2010).

Anti-inflammatory treatment

The NSAID used was Carprieve LA with an active ingredient of Carprofen 50 mg/ml and a plasma half-life of 70 hours (Norbrook NZ Ltd, Auckland, New Zealand). Cows selected for treatment with NSAID were given three doses over a nine day period between 21 and 31 days postpartum. The drug dose was 1.4 mg/kg (1 mL/35 kg live weight). There are few reports on the use of NSAIDs in the early postpartum period. An extended period of coverage for the treatment was achieved by administering three doses of (1.4 mg/kg) over a nine day period. The most recent live weight of the cow was used to calculate the correct drug dose. The drug was administered subcutaneously above the last long rib.

Milk production

Individual milk yields (kg/cow/day) were measured twice daily (ALPRO, DeLaval, Tumba, Sweden) and recorded. Milk protein, fat and lactose per cent were determined weekly by Fourier-transfer infrared spectroscopy (FT120, Foss Electric, Hillerød, Denmark) and recorded. Milk somatic cell count (SCC) was determined fortnightly for every cow by Fourier-transfer infrared spectroscopy (Fossomatic, Foss Electric, Hillerød, Denmark) and recorded.

Grazing management

Cows were maintained as two separate dry (pre-calving) and milking herds, until all cows were calved. Both herds received a generous allowance of fresh ryegrass and white clover pasture supplemented with maize silage and grass silage.

Body condition scoring and live weight

The cows were weighed and had their body condition scored (BCS) on a 10 point scale: (Roche et al. 2004) fortnightly until 1 June, then weekly until the end of the trial.

Statistical analysis

Associations between uterine PMN% and milk production parameters were determined using mixed models in GenStat 14 (Payne et al. 2011). Breed (Holstein-Friesian vs. Holstein-Friesian cross), age (3 vs. 4+ year olds), their interaction, and Day 14 PMN% (based on quartiles) were included in the model as fixed effects. Cow was included as a random effect. Milk production data for 211 cows for the first 21 days postpartum were included in the analysis. Body condition score data for the pre-calving (Day -28 to the day before calving) and pre-treatment periods (Days 6 to 19 postpartum) were analysed using the same method. The data were split into three groups based on PMN% quartiles: High PMN group (top 25%, n = 53, PMN ≥ 14%), Medium PMN group (middle 50%, n = 105, PMN 1–14%), and Low PMN group (bottom 25%, n = 53, PMN ≤ 1%). Somatic cell count data were $\log_{10}$ transformed prior to analysis.

To investigate the effect of NSAID on milk production, the data from 211 cows for the 21 days after the second NSAID treatment were analysed by GenStat 14 using mixed models (Payne et al. 2011). Breed (Holstein-Friesian vs. Holstein-Friesian cross), age (3 year olds vs. 4+), their interaction with Day 14 PMN% (based on quartiles) and treatment, and the interaction of Day14 PMN% with treatment were included in the model as fixed effects. Cow was included as a random effect. A within-age group, within-breed group, within-PMN group covariate was

included in the analysis. This was obtained by using the residuals from a linear model including age, breed, and Day 14 PMN%, fitted to the cow means for the week before treatment. Body condition score for the treatment period was analysed using the same method.

The association between Day 14 and Day 42 PMN% were analysed by regression. Day 14 PMN% was initially included as a covariate for Day 42 PMN%, but then excluded when not significant. The within cow change from Day 14 PMN% to Day 42 PMN% was also investigated, with PMN% as a continuous variable. The PMN% was analysed using an angular transformation of PMN%, with the raw means presented to help with interpretation.

Results

The effect of a non-steroidal anti-inflammatory drug on proportion of polymorphonuclear cells

Day 14 PMN% was 9.9 (range 0–61%) and did not differ between the Control (10.2%) and NSAID treated cows (9.7%). At Day 42 the PMN% for the Control (1.4%) and the NSAID (1.7%) group were not different (SED = 0.5, P > 0.1). Incidence of SCE (PMN% ≥ 7%) at Day 42 for the Control (4.6%), and NSAID (3.9%) group did not differ (P > 0.1).

Association between the proportion of polymorphonuclear cells on Day 14 and milk production

For the first 21 days postpartum, milk protein yield was 300 g/d lower (P < 0.05) in the High PMN group than the Low PMN group (Table 1). There was a tendency for milk yield (P = 0.07) and lactose (P = 0.08) to also be lower in the High PMN group. Milk fat yield and SCC did not differ (P > 0.1) between High and Low PMN groups. There were no milk production differences between the PMN groups beyond the first 21 days of lactation (Table 1).

The observed differences in milk production were confounded with differences in BCS. The High PMN group had a lower (P < 0.05) BCS than the Low PMN group for the pre-calving period (4.6 vs. 4.8, SED = 0.09), the pre-treatment period (4.3 vs. 4.5, SED = 0.09) and the treatment period (4.0 vs. 4.2, SED = 0.07).

The effect of a non-steroidal anti-inflammatory drug treatment on milk production

The NSAID treatment had no effect (P > 0.1) on any of the milk production variables measured (Table 2), nor was there any difference in BCS (4.1, SED = 0.03, P > 0.1) between the Control and NSAID groups.

Discussion

Previous studies have reported a negative association between SCE with milk production (Burke et al. 2010; McDougall et al. 2011) and milk composition (Green et al. 2009) with indications that inflammation is involved in this association.

This led to the hypothesis that treatment with a NSAID between 21 and 31 days postpartum will increase milk production in cows with ≥ 14% PMN at Day 14. Timing of the NSAID treatment, between Day 21 and Day 31, was considered the earliest time postpartum to administer an anti-inflammatory drug, because uterine involution is a necessary inflammatory process. In this study, NSAID treatment had no effect on any of the milk production, BCS or PMN% variables measured. The results do not support the hypothesis that a NSAID treatment from Day 21 is beneficial for reducing PMN% and increasing milk production of cows with ≥ 14% PMN at Day 14.

Table 1 Mean milk yield and composition, and somatic cell count (SCC) for 53 cows with a low proportion of polymorphonuclear (PMN) cells (≤ 1%) and 53 cows with a high proportion of PMN cells (≥ 14%) at Day 14 postpartum. P value in bold indicates significance at P < 0.05. P value in italics indicates significance between P = 0.05 and P = 0.10.

Days in milk	Measurement	Low PMN%	High PMN%	Standard error of difference	P value
0 to 21	Milk yield (kg/d)	18.9	18.0	0.5	*0.07*
	Milk fat (kg/d)	0.92	0.87	0.03	0.13
	Lactose (kg/d)	0.92	0.87	0.02	*0.08*
	Milk protein (kg/d)	0.69	0.66	0.02	**0.04**
	Log_{10} SCC (cells/mL)	1.96	1.93	0.07	0.63
	Back transformed SCC (cells/mL)	91,200	85,100		
22 to 42	Milk yield (kg/d)	19.8	19.1	0.7	0.34
	Milk fat (kg/d)	0.84	0.81	0.03	0.43
	Lactose (kg/d)	0.97	0.95	0.04	0.52
	Milk protein (kg/d)	0.63	0.62	0.03	0.27
	Log_{10} SCC (cells/mL)	1.74	1.67	0.09	0.41
	Back transformed SCC (cells/mL)	55,000	46,800		

Table 2 Mean milk yield and composition and somatic cell count (SCC) for cows in the Control and non-steroidal anti-inflammatory drug (NSAID) groups 21 days after the second NSAID treatment.

Measurement	Control group	NSAID treatment group	Standard error of difference	P value
Milk yield (kg/d)	19.6	19.6	0.3	0.98
Milk fat (kg/d)	0.83	0.84	0.01	0.99
Lactose (kg/d)	0.97	0.97	0.01	0.91
Milk protein (kg/d)	0.64	0.64	0.01	0.74
Log_{10} SCC (cells/mL)	1.72	1.72	0.06	0.85
Back transformed SCC (cells/mL)	52,500	52,500		

Effect of non-steroidal anti-inflammatory drug treatment on the proportion of polymorphonuclear cells, milk production and body condition score

The NSAID treatment did not affect PMN% change from Day 14 to Day 42, any of the milk production variables, nor was there any difference in BCS between the Control and NSAID groups.

The PMN% at Day 14 in this study was much lower than expected with an average of 9.9% PMN, and fewer than 20% of cows having a PMN% equal to or above18%. This low Day 14 PMN%, in conjunction with a high self-cure rate of more than 90% in the Control group, means that the incidence of inflammation within this study group may have been too low to detect a treatment response.

It is also possible that the treatment did not have the intended anti-inflammatory effect. It has not been established that there was a reduction in prostaglandin production, and therefore inflammation, which was the intended treatment response of the NSAID. To establish if there was a prostaglandin response to treatment is beyond the scope of this paper, but is being investigated. Additionally, the prostaglandin pathway is not the only pathway to produce inflammation; therefore, even if NSAID resulted in the intended response, inflammation from other sources may still be present.

The NSAID treatment may have been applied too late. The treatment was administered between Days 21 and 31 postpartum so that the NSAID treatment would not interfere with the normal uterine involution process. However, as there was a tendency for a negative association between SCE as assessed by Day 14 PMN% in this study, and milk production to 21 days postpartum, the treatment may have had a beneficial effect if it had been applied earlier in the postpartum period. To support this hypothesis, cows treated with aspirin, which is also an NSAID, for the first five days postpartum, had a higher peak milk yield and reached peak milk production earlier than untreated cows (Trevisi & Bertoni 2008).

In summary, the failure to detect an effect of NSAID in the current study may have been due to a lower level of inflammation than expected when measured as PMN%, a high self-cure rate, or the delayed timing of the NSAID treatment.

Association between the proportion of polymorphonuclear cells on Day 14 and milk production

The tendency for a negative association of SCE as assessed by Day 14 PMN% in this study, with milk production is consistent with the results from previous studies (Green et al. 2009; Burke et al. 2010; McDougall et al. 2011). However this association is confounded with BCS, as pre-calving BCS was lower in the High PMN group in both the Burke et al. (2010) and the McDougall et al. (2011) studies as well as in the current study. This reduced BCS in High PMN cows could account for the lower milk yield as pre-calving BCS is known to affect milk production (Roche et al. 2007; Roche et al. 2009). There was no indication that NSAID treatment between 21 and 31 days postpartum ameliorated this negative association between SCE and milk production. This suggests that inflammation at this stage of lactation is not involved with the negative associations between PMN%, milk production or BCS in the cows studied.

Conclusion

Results support a negative association between SCE as assessed by Day 14 PMN% in this study, and milk production but not the hypothesis that NSAID treatment from Day 21 will reduce PMN% or ameliorate the effect on milk production. This may be a consequence of lower than expected levels of inflammation and a greater than 90% self-cure rate by Day 42 in the animals studied or that the treatment was applied to late.

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