

A nutrient-signalling effect of grain feeding on postpartum anovulatory intervals in mature dairy cows

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ABSTRACT

Our previous study with mature dairy cows found that increased pasture intakes in early lactation resulted in more milk being produced but with no change in postpartum anovulatory intervals (PPAI). The present study used a 2 x 2 factorial to determine PPAI in 68 cows with pre- and post-calving diets that were iso-energetic, but varying in the ratio of structural to non-structural carbohydrate. At 36 ± 8.7 days prepartum, cows were assigned iso-energetic diets (114 MJ ME/cow/day) of either pasture/pasture silage (n=34) or the same diet supplemented with 3 kg DM/cow/d barley-maize concentrate (n=34). After calving, cows within each prepartum diet were assigned iso-energetic diets (179 MJ ME/cow/d) of either pasture/pasture silage (n=34) or the same diet supplemented with 5 kg DM/cow/d concentrate (n=34). Postpartum grain feeding reduced ($P < 0.05$) PPAI (28.4 ± 1.8 day) compared to the pasture/pasture silage only diet (36.4 ± 2.5 day). Prepartum diet did not affect PPAI. Body condition score was inversely related to PPAI at all time points ($P < 0.05$), but explained little (5-12%) of the variability in PPAI. Significant relationships to PPAI were also found for blood non-esterified fatty acids, urea and magnesium, but again, r^2 -values were ≤ 0.1 . None of the milk characteristics measured were significantly related to PPAI. This study supports the premise that discrete or specific nutritional cues unrelated to general energy status can favourably target physiological functions in reproductive tissues at the brain and/or ovarian levels.

Keywords: anoestrus; nutrition; dairy cow.

INTRODUCTION

Dairy cows that have extended postpartum anovulatory intervals (PPAIs) take longer to re-establish pregnancy and are more likely to be culled from the dairy herd for reproductive reasons, as compared to cows having a shorter PPAI (Rhodes et al., 1998 and 2003). While some studies have demonstrated a postpartum nutrition effect on PPAI or anoestrous rates (Grainger et al., 1982; McDougall et al., 1995a), others have found no effect of increased dietary energy intakes during the postpartum period in pasture-based feeding systems (McDougall et al., 1995b; Burke et al., 2005). The majority of studies implicate prepartum nutrition and body condition score at calving as having greatest influence on the PPAI (Grainger 1982; Burke et al., 1995 & 2005; Chagas et al., 2001).

Our previous study (Burke et al., 2005) observed that the extra energy afforded to postpartum dairy cows on a greater pasture feeding level was partitioned towards extra milk production, and not towards preservation of body condition or physiological events regulating the PPAI. The compositional nature of pasture (e.g. relatively low non-structural carbohydrate:nitrogen ratio) may have caused this partitioning of additional dietary energy to milk production.

Accordingly, the objective of the present study was to examine reproductive responses to isoenergetic pasture-based diets with high or low carbohydrate:protein compositions.

MATERIALS AND METHODS

Animals, design and treatments

Sixty-eight multiparous Holstein-Friesian cross cows due to calve within the first 21 days of the calving period were used in this 2 x 2 factorial study. At 36 ± 8.7 days before actual calving date, animals were allocated to isoenergetic diets of either pasture/pasture silage (PrePast; n=34) or the same diet with the addition of 3 kg DM/cow/day barley-maize concentrate (PreConc; n=34). Assignment to treatment group was balanced for previous milk production levels, liveweight, body condition score (BCS), age, due-calving date and genetic merit (BW = 115.2). At calving, cows in each pre-calving treatment were randomly (but balanced for calving date) assigned to a diet of either pasture/pasture silage (PostPast; n=34) or an isoenergetic comparison diet of pasture/pasture silage and 5 kg DM/cow/day concentrate (PostConc; n=34). Cows remained on their respective postpartum diets for at least 35 days and until milk progesterone pattern confirmed a resumption of oestrous cycles.

Grazing management and milk production measurements

A companion paper in this proceedings (Roche *et al.*, 2006) details the methodology of grazing management, measurements of feed consumption and milk production characteristics. Briefly, pre- and post-grazing herbage masses and grazing area allocations were used to control average pasture intakes for cows within treatments. Individual milk yields were measured at each milking and compositional characteristics were determined twice weekly. Individual live weight and body condition score (BCS) were measured weekly throughout.

Measurement of postpartum anovulatory intervals (PPAIs) and blood constituents

Progesterone was measured in a composite of milk samples collected at the Monday afternoon-Tuesday morning and Thursday afternoon-Friday morning milkings of each week until cows were confirmed as cycling. Progesterone was measured using an ELISA kit (Ridgeway Sciences, Gloucestershire, UK) validated for use in cattle (Sauer *et al.*, 1986). The PPAI was defined as the interval from calving to the first day that the concentration of progesterone increased to at least 3 ng/ml with subsequent concentrations being consistent with oestrous cycling. Premating oestrous activity and AI dates on detected oestrus were recorded by farm staff using visual observations aided by the tailpainting technique (Macmillan *et al.*, 1988).

Blood samples were collected weekly from all cows until confirmed cycling. Additional samples were collected on the day of calving and for the first four days after calving. Plasma harvested (1,120 g, 10 min) were analyzed for NEFA (colorimetric method), BHBA (BHBA dehydrogenase assay), glucose (hexokinase method), urea (urease method), albumin (citrate buffer reagent), calcium (o-Cresolphthalein complexone) and magnesium (xlidyl blue reaction). All assays were performed on the Hitachi 717 analyzer (Roche) at 30°C by Alpha Scientific Ltd (Hamilton, New Zealand). The inter-assay and intra-assay coefficient of variation was <2% for all assays.

Statistical analyses

Data were analysed as a mixed model using REML procedure in Genstat 5.4.1 with effects of treatment in a 2 x 2 factorial arrangement being fixed and cow as a random effect. The PPAI of two cows that had not ovulated before the end of the experimental period were estimated by the statistical model using censored data procedure. For variables with multiple measures through time, each time point was analysed individually. The mean values over

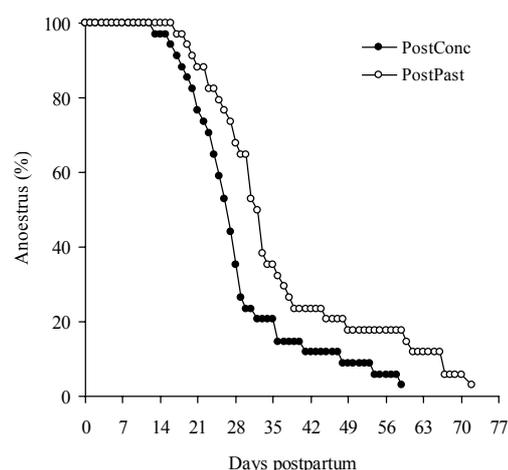
the first 35 days postpartum for each animal were also calculated and analysed in a mixed model as described above. Associations between PPAI and variables measured at individual time points or the mean value for each cow over the first 35 days postpartum were determined using CENSOR procedure in Genstat. The \log_{10} PPAI was used in testing significance of these associations. The associations were analysed initially using all data and secondly, data pooled within treatments. Binary data (% cycling, SR21 and 49dNRR) were analysed using generalised linear models. Data are presented as the mean \pm SEM unless stated otherwise.

RESULTS

Dietary treatments on reproductive performance

Initiation of postpartum oestrous cycles occurred sooner ($P < 0.05$) for cows receiving concentrate postpartum (28.4 ± 1.8 days) as compared with those not receiving concentrate postpartum (36.4 ± 2.5 days; Figure 1).

FIGURE 1: Proportion of anovular cows as a function of time postpartum for diets of either pasture/pasture silage (PostPast; $n = 34$) or pasture/pasture silage with addition of 5 kg DM/cow/day barley-maize concentrate (PostConc; $n = 34$). Data are pooled across prepartum feeding treatments.



There was no significant effect of prepartum diet ($P = 0.7$) or an interaction between pre- and postpartum diet on PPAI ($P = 0.7$). Differences in PPAI did not result ($P > 0.1$) in any differences in the proportion of cows that had been observed in oestrus before the planned start of mating (98%). However, the proportion of cows inseminated during the first 21 days of the breeding period (SR21) was greater ($P < 0.05$) in cows fed concentrate postpartum (97%) compared with those

not receiving concentrate (88%; Table 1). A pre- by postpartum diet interaction was detected ($P < 0.05$) in the 49-day non-return rate (49dNRR) for those cows inseminated in the first 21 days. The interaction is confounded in that changing diets resulted in greater 49dNRR, particularly in cows fed concentrate in the postpartum period as compared with other treatment groups (Table 1).

TABLE 1: Interval from planned start of calving (PSC; 18 July 2005) to mean calving date, postpartum anovulatory intervals (PPAI), proportion observed in oestrus before the breeding period (% cycling), proportion cows submitted for insemination in the first 21 days of the breeding period (SR21), and the 49-day non-return rate (49dNRR) for each of the treatment groups. The 49dNRR is the proportion of these cows inseminated within the first 21 days without a return oestrus within the first 49 days of the breeding period, and therefore assumed to be pregnant.

	PastPast	PastConc	ConcPast	ConcConc
PSC to calving (d) ¹	2.3 ± 8.2	3.4 ± 11.0	3.2 ± 7.1	4.1 ± 9.4
PPAI (days)	37.4 ± 4.2	28.2 ± 2.4	35.3 ± 2.9	28.6 ± 2.6
% cycling	100	100	100	94
SR21 (%)	93	100	88	100
49dNRR (%)	53	87	73	56

¹ Variance is presented as standard deviations

Dietary treatments on energy status

Average daily energy intakes, BCS and milksolids were equivalent ($P > 0.1$) for either diet during the postpartum period (Table 2). Although net energy of lactation (NE_L) was greater ($P < 0.05$) for animals not receiving concentrate, those that were receiving concentrate had higher blood concentrations of NEFA (Table 2).

TABLE 2: Dietary energy intake, body condition scores (BCS), milksolids and net energy of lactation during the first 35 days postpartum in mature Holstein-Friesian cows managed either on a sole diet of pasture/pasture silage (Past) or similar diet supplemented with 5 kg concentrate/cow/day. Diets were held isoenergetic by controlling allowance of pasture/pasture silage.

	Past	Conc	P-value
Cows (n)	34	34	
¹ Energy intake (MJ ME/day)	178 ± 41	181 ± 26	n.s.
BCS at 7 days prepartum	4.8 ± 0.1	4.9 ± 0.1	n.s.
BCS at 21 days postpartum	4.2 ± 0.1	4.3 ± 0.1	n.s.
² Milksolids (kg/cow/day)	1.94 ± 0.06	1.92 ± 0.06	n.s.
² NE_L (MJ/cow/day)	3.52 ± 0.04	3.30 ± 0.04	$P < 0.01$
NEFA at 21 days postpartum (mmol/l)	0.64 ± 0.09	0.95 ± 0.09	$P < 0.05$

¹Variances are standard deviation across days.

²Daily average during first 35 days of lactation.

NE_L (net energy of lactation; MJ/cow) = (0.0929 x fat %) + (0.0547 x crude protein %) + (0.0395 x lactose %) x 4.186 MJ/Mcal x kg milk/cow.

Liveweight, BCS, milk and blood measures on PPAI

Variables for which at least two points in time were significantly ($P < 0.05$) correlated with PPAI across treatments were BCS, NEFA, urea and magnesium. The significance of urea was not evident when the data were pooled within treatments.

The univariate correlations with BCS and magnesium (first week postpartum) were negative (i.e. higher values were associated with shorter PPAI). Every 0.5 BCS unit increase immediately precalving was associated with a 3.5-day reduction in PPAI. A significant and consistent relationship between BCS and PPAI was evident for all measured time points, including the period immediately before treatments were initiated. Even so, BCS accounted for only 5 to 12% of the variance in PPAI. Correlations between PPAI and NEFA were positive at calving but negative at 14 and 28 days postpartum. Again, the degree of variance explained was low ($r^2 < 0.10$).

No significant associations to PPAI were found for liveweight, change in liveweight or change in BCS, milk production variables (milksolids, net energy of lactation, protein %, protein yield, protein:fat ratio) or blood concentrations of albumin, β -hydroxy butyrate, glucose and calcium.

DISCUSSION

Results of the present study are novel in that a clear postpartum nutritional effect on PPAI was establishment by altering composition of carbohydrate and the carbohydrate: protein ratio, while keeping daily energy intakes equivalent. Interestingly, the usual predictors of improved fertility in cattle, such as increased energy intake, greater body condition and more positive energy balance (Rhodes et al., 2003), were not associated with the observed benefit of postpartum concentrate feeding on reducing PPAI. This finding suggests that nutritional cues not necessarily associated with "whole-body" energy status, positively influenced physiological functions at either the hypothalamic-pituitary and/or ovarian level in the early postpartum cow. For this reason, we describe the findings as a "nutrient-signalling" effect.

The "nutrient-signalling" effect is consistent with that reported by Chagas et al. (2003). In this previous study, PPAI was profoundly reduced in poor-conditioned heifers by twice-daily drenching with monopropylene glycol (a glucogenic precursor). Indicators of energy status (BCS and milk production) remained unchanged, but a positive stimulation occurred at

the hypothalamic-pituitary level with increased secretion of luteinizing hormone (LH); and consequently at the ovarian follicular level with earlier ovulation postpartum. While the present study was not designed to examine dietary responses at the hypothalamic-pituitary-ovarian level, it is most likely that the preovulatory conditions within this axis (Smith et al., 2005; Pleasants et al., 2005) were advanced in cows fed concentrate postpartum. The positive benefits of this advancement in reproductive recovery may have carried over with improved submission rate (SR21) and conception rate (49dNRR) compared to cows not receiving concentrate after calving. This is a very cautious interpretation due to the binary nature of the data and that animal numbers are limited.

Relationships between PPAI and constituents in blood and milk that could potentially serve as nutritionally-related signals were examined. These regressions were performed while ignoring treatment, as well as pooled within treatment. The first method may find treatment-associated effects only, whereas the second approach also tested for treatment-independent associations between PPAI and the variable of interest. For example, a significant correlation was found between urea and PPAI when treatment was ignored. However, the significance of this link disappeared when the relationship was explored within treatments. The implication is that concentrate feeding reduced both urea and PPAI, but urea did not influence PPAI.

Body condition score, NEFA and magnesium were correlated with PPAI using either the treatment-independent or within-treatment regression models. The significance of BCS was not surprising since many previous studies demonstrate the importance of BCS on fertility parameters (Rhodes et al, 2003). The magnitude of the influence of BCS on PPAI (ie. approximately 8 days per unit BCS) is consistent with previous studies (McDougall et al., 1995a; Burke et al., 2005).

Elevated NEFA is indicative of enhanced adipose mobilisation and greater degree of negative energy balance. Cows that experience a prolonged period of negative energy balance take longer to ovulate postpartum (Canfield and Butler, 1990). Thus, a positive correlation with PPAI would be expected (i.e. elevated NEFA associated with longer PPAI). In the present study, a positive correlation between NEFA concentrations at calving and PPAI was found as expected. However, significant relationships between NEFA and PPAI on Days 14 and 28 postpartum were inversely correlated. This finding is not consistent with a general belief that prolonged tissue mobilisation

results in extended PPAI (Canfield and Butler, 1990), perhaps further supporting the presence of an overriding nutrient-signal reducing PPAI in cows fed grain postpartum.

Increased blood concentrations of magnesium was expected in cows fed concentrate in the present study, since dietary potassium was reduced and starch-based diets reduce rumen pH, increasing the solubility of magnesium (Dalley et al., 1997). However, involvement of magnesium in influencing PPAI was not expected and the reason is unclear. While magnesium is known to be important for the general health and well being of the parturient dairy cow managed on pasture (Wilson, 2002), there appears to be nothing in the literature linking magnesium to PPAI.

It is clear from the degree of unexplained variance in PPAI that the current measures are inadequate for predicting PPAI. An extended suite of measures (e.g. metabolic hormones, etc) and more complex statistical analyses may provide further understanding. Also, future studies that include measures of LH release patterns and ovarian follicular function would provide a much greater understanding of the physiology for the observed effects in the current study.

CONCLUSION

A pasture-based diet supplemented with a starch concentrate in postpartum dairy cows accelerated the onset of oestrous cycling as compared to a pasture-only type diet that was otherwise iso-energetic. Reduced PPAI in postpartum concentrate fed cows was not associated with improved body condition or energy balance. This finding supports the premise that discrete or specific nutritional cues can favourably target physiological functions in reproductive tissues at the brain and/or ovarian levels.

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