Short communication: Effects of dietary nonstructural carbohydrates pre- and postpartum on reproduction of grazing dairy cows

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ABSTRACT

The working hypothesis was that postpartum anovulatory intervals (PPAI) of grazing dairy cows are shortened by inclusion of concentrates that increase the nonstructural carbohydrate content of the transition diet. Dietary treatments were arranged as a 2 × 2 factorial, with 68 multiparous cows assigned to isoenergetic diets (114 MJ of metabolizable energy/cow per day) of pasture and pasture silage (PreP) or pasture and pasture silage supplemented with 3 kg of dry matter/cow per day a corn- and barley-based concentrate for 36 d prepartum (PreC). After calving, cows within each prepartum diet group were managed on isoenergetic diets (179 MJ of metabolizable energy/cow per day) of either pasture and pasture silage (PostP) or pasture and pasture silage supplemented with 5 kg of dry matter/cow per day a corn- and barley-based concentrate (PostC) for at least 35 d and until reestablishment of ovulatory cycles. Relative to day of calving (d 0), blood samples were collected at least weekly from d –28 to 35 and milk samples were collected twice weekly for progesterone determination to diagnose ovulatory status. The main variable of interest was PPAI, defined as the interval between calving and the first detected increase in milk progesterone (>3 ng/mL), followed by a pattern of progesterone concentrations consistent with onset of an ovulatory cycle. Subsequent mating records, pregnancy testing, and recalving data were also examined. Prepartum diet did not affect reproduction. The PPAI was 8 d shorter and the 6-wk pregnancy rate was 17% greater in PostC cows compared with PostP cows. Measured indicators of metabolic state and energy balance were poorly related to PPAI. The results support the existence of nutritional signals associated with nonstructural carbohydrates in the postpartum diet, independent of energy balance; these signals benefit the physiological mechanisms underlying the timing of first ovulation and possibly subsequent breeding performance.

Key words: dairy cow transition diet, nonstructural carbohydrate, reproduction

Extended postpartum anovulatory intervals (PPAI) are a major source of reduced fertility in pasture-based seasonal dairy herds constrained to a 365-d intercalving interval (Rhodes et al., 2003). Previous reports indicate that PPAI are influenced by prepartum DMI and BCS at calving (Chagas et al., 2006; Burke and Roche, 2007). These same studies, however, reported that greater pasture DMI after calving was positively associated with milk production but did not affect BCS loss or PPAI when compared with a restricted intake postpartum. This lack of response may be a consequence of the nutritional composition of pasture, with the predominant form of carbohydrates being structural. Diets high in NSC induce an increased insulin profile before first ovulation and have been reported to enhance ovarian follicular development (Garnsworthy et al., 2009) and reduce PPAI (Gong et al., 2002). These previous findings were generated from TMR-fed cows producing up to 45 kg of milk/d. The current objectives were to test whether NSC content in a pasture-based transition diet influences PPAI and to examine potential metabolic mechanisms that could be associated with any observed effects.

This study was conducted at the DairyNZ Lye Farm (Hamilton, New Zealand; 37°46'S, 175°18'E) with prior approval from the Ruakura Animal Ethics Committee. A detailed description of this study is in Roche et al. (2010). Briefly, at 36 ± 8.7 d (mean ± SD) prepartum, 68 multiparous Holstein-Friesian and Holstein-Friesian × Jersey crossbred cows due to calve over a 21-d period were randomly allocated to one of 2 prepartum diets (n = 34/diet); treatment groups were balanced for milk production during early lactation in the previous year, BW, BCS, age, and predicted calving date. Prepartum dietary treatments consisted of feeding cows either pasture and pasture silage (**PreP**) or an isoenergetic comparison (114 MJ of ME/cow per day) receiving 3 kg of DM/cow per day of a corn- and barley-based concentrate (**PreC**). At calving, cows in each prepartum treatment were randomly allocated to one of 2 postpartum dietary treatments (n = 34/diet) in a 2 ×

Received October 29, 2009.

Accepted May 17, 2010.

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Table 1. Mean ($\pm$ SD) DMI and nutrient composition (% DM, unless otherwise stated) of isoenergetic diets differing in the ratio of structural carbohydrate to NSC content offered pre- and postpartum in a 2×2 factorial arrangement ($n = 17$ cows/treatment cell)^{1,2}

Item	Prepartum		Postpartum	
	PreC	PreP	PostC	PostP
Cows (n)	33	33	32	34
DMI (kg/cow per d)	9.8 $\pm$ 1.06	10.3 $\pm$ 1.11	14.1 $\pm$ 1.48	15.2 $\pm$ 2.45
NSC	31.8 $\pm$ 3.20	13.2 $\pm$ 2.21	38.1 $\pm$ 4.00	17.8 $\pm$ 4.25
CP	16.8 $\pm$ 0.94	22.1 $\pm$ 1.52	17.0 $\pm$ 1.57	20.1 $\pm$ 1.18
NDF	39.3 $\pm$ 2.01	52.1 $\pm$ 1.55	33.3 $\pm$ 2.59	47.4 $\pm$ 3.83
ADF	21.1 $\pm$ 1.01	27.9 $\pm$ 1.41	16.7 $\pm$ 1.23	24.7 $\pm$ 2.48
Lipid	3.8 $\pm$ 0.19	4.2 $\pm$ 0.16	3.8 $\pm$ 0.17	4.4 $\pm$ 0.16
Ash	8.2 $\pm$ 0.47	10.3 $\pm$ 0.28	7.8 $\pm$ 0.49	10.3 $\pm$ 0.55
ME (MJ of ME/kg of DM)	11.8 $\pm$ 0.31	11.2 $\pm$ 0.42	12.5 $\pm$ 0.35	11.7 $\pm$ 0.78

¹Prepartum diets (114 MJ of ME/cow per day) were pasture and pasture silage (PreP) or pasture and pasture silage supplemented with 3 kg of DM/cow per day of a barley-corn grain concentrate (PreC). Postpartum diets (179 MJ of ME/cow per day) were pasture and pasture silage (PostP) or pasture and pasture silage supplemented with 5 kg of DM/cow per day of a barley-corn grain concentrate (PostC).

²Data of Roche et al. (2010).

2 factorial arrangement and offered either pasture and pasture silage (**PostP**) or an isoenergetic comparison (179 MJ of ME/cow per day) receiving 5 kg of DM/cow per d of concentrate (**PostC**). The 5 kg of DM of concentrate was introduced gradually over a 3-d period and offered in 2 equal portions at the a.m. and p.m. milkings. Postpartum treatments continued for at least 35 d and until resumption of ovulatory cycling was confirmed as described below. Cows were AI on detection of spontaneous estrus for 7 wk, followed with natural bull mating for 4 wk, during the 11-wk seasonal breeding period beginning d 80 relative to mean calving date.

The detailed methodologies for regulating energy intake and ensuring that the comparison diets were isoenergetic are reported by Roche et al. (2010). Milk yield and milk composition and the blood sampling and assay procedures are also described by Roche et al. (2010). Briefly, all cows grazed adjacently in the same pasture, separated by temporary electric fencing. Pre- and postgrazing herbage masses and pasture area allocations were used to control average daily DMI within treatment groups. Individual milk yields were measured at each of the a.m. and p.m. milkings. Milk composition was determined twice weekly. The nutritive characteristics and mineral concentrations of the feeds offered pre- and postpartum are presented in Table 1. Blood samples were collected weekly from before allocation to prepartum treatment until confirmed detection of first ovulation after calving, as defined below. Plasma samples were analyzed for NEFA, BHBA, glucose, urea, albumin, growth hormone (**GH**), IGF-1, insulin, Ca, and Mg.

Progesterone was measured in a composite of milk samples collected at the Monday p.m.–Tuesday a.m. and Thursday p.m.–Friday a.m. milkings of each week

from calving until each cow was confirmed as having ovulatory cycles. Progesterone content was determined 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 progesterone concentration increased to ≥ 3 ng/mL with subsequent concentrations consistent with the onset of ovulatory cycles. The PPAI estimates for 2 cows (last sampled 39 and 58 d postpartum) were generated using the CENSOR procedure in GenStat version 11.1 (Payne et al., 2008). These 2 cows had not ovulated before onset of the seasonal breeding period, at which time they received a progesterone-based treatment for anestrus.

Data were analyzed in GenStat using linear models and generalized linear models with a binomial error distribution and the logit link for the proportions. The model included pre- and postpartum diet and any interaction as fixed effects and cow as a random effect. Cow was considered the experimental unit. The PPAI were $\log_{10}$ transformed before analyses to reduce the heterogeneity of variance. The $\log_{10}$ PPAI was analyzed using the CENSOR procedure in GenStat to test treatment effects, but the untransformed least squares means are presented in the results. The effect of treatment on PPAI was also tested using Cox regression analysis, which produced the same statistical inferences to the generalized linear model used. Associations between $\log_{10}$ PPAI and the mean value of weekly measured variables for each cow over the first 35 DIM, pooled within treatment, were examined using the CENSOR procedure in GenStat.

Prepartum diet did not affect PPAI, but PPAI of PostC cows (28.7 d) was 8 d shorter than that of PostP cows (36.5 d; Table 2). There was no detectable interaction between pre- and postpartum diets on PPAI. The

Figure 1. Effect of postpartum diet on the proportion of anovulatory cows. Postpartum diets were isoenergetic (179 MJ of ME/cow per day) but differed in the ratio of structural carbohydrate to NSC content as described in Table 1. Diets consisted of pasture and pasture silage (PostP) or pasture and pasture silage supplemented with 5 kg of DM/cow per day of a barley–corn grain concentrate (PostC). Survival curves exclude data of 2 cows for which first ovulation was not detected within the experimental period.

pattern of earlier first ovulations postpartum among PostC cows is depicted in the survival curve (Figure 1).

The effects of treatment on milk production and blood hormones and metabolites are presented in Roche et al. (2010). Briefly, mean concentrations of IGF-1 during the first 35 DIM were greater in PostC

cows than in PostP cows (11.0 vs. 8.6 ng/mL; SE of the difference: 0.7; $P < 0.001$), but average GH and insulin concentrations were not different between these treatment groups. The mean of weekly concentrations of urea, BHBA, and Ca were less but NEFA, glucose, and Mg were greater in PostC cows than in PostP cows during the first 35 DIM.

Pooled within treatment, no significant relationships ($P > 0.2$) were evident between PPAI and mean concentrations of BHBA, glucose, urea, albumin, GH, IGF-1, insulin, or Ca during the first 35 DIM. Mean plasma concentrations of NEFA ($P = 0.055$; $r = -0.28$) and Mg ($P < 0.05$; $r = -0.30$) and BCS ($P < 0.05$; $r = -0.32$) were negatively associated with PPAI, although the proportion of variance accounted for was low ($R^2 = 0.06$ – 0.10). None of the milk yield or composition or BW measures were related to PPAI ($P > 0.05$).

Dietary treatments had no effect on the proportion of cows cycling before the start of the seasonal mating period, the 3-wk submission rate (% cows inseminated in first 3 wk), first service conception rate, final pregnancy rate, or the interval from the start of mating to conception (Table 2). There was, however, a postpartum diet tendency ($P = 0.08$) for 3-wk pregnancy rate to be 21% greater, and 6-wk pregnancy rate to be 17% greater ($P < 0.05$), in PostC cows than in PostP cows (Table 2). There were no detected interactions between pre- and postpartum diets for these measures.

Increasing dietary NSC content postpartum had a notable benefit in reducing PPAI. Whereas many reports fail to show a reproductive benefit from feeding greater levels of concentrate (Patton et al., 2006; McCarthy et

Table 2. Reproductive performance variables in cows offered pre- and postpartum isoenergetic diets differing in the ratio of structural to nonstructural carbohydrate content¹

Variable	Treatment group				SED ²	P-value		
	PP	PC	CC	CP		Pre	Post	Pre × Post
Cows (n)	17	16	16	17				
PPAI ³ (d)	37.7	28.7	28.6	35.3	3.2	>0.1	0.01	>0.1
Cycling ⁴ (%)	94	100	94	100	4.2	>0.1	>0.1	>0.1
3-wk submission rate ⁵ (%)	94	94	94	88	6.4	>0.1	>0.1	>0.1
First-service conception rate (%)	58	81	56	53	11.7	>0.1	>0.1	>0.1
3-wk pregnancy rate (%)	53	81	67	53	11.6	>0.1	0.08	>0.1
6-wk pregnancy rate (%)	82	94	93	71	8.5	>0.1	0.05	>0.1
Final pregnancy rate, (%)	94	100	93	88	5.8	>0.1	>0.1	>0.1
Days to conception ⁶ (d)	21.0	18.1	18.8	25.4	3.8	>0.1	>0.1	>0.1

¹Prepartum diets (114 MJ of ME/cow per day) were pasture and pasture silage (PP and PC) or pasture and pasture silage supplemented with 3 kg of DM/cow per day barley-corn grain concentrate (CP and CC). Postpartum diets (179 MJ of ME/cow per day) were pasture and pasture silage (PP and CP) or pasture and pasture silage supplemented with 5 kg of DM/cow per day barley-corn grain concentrate (PC and CC).

²Standard error of the difference.

³Postpartum anovulatory interval.

⁴Cows having ovulatory cycles before the start of the seasonal mating period.

⁵Cows submitted for insemination within the first 3 wk of the seasonal mating period.

⁶Interval from the start of seasonal mating period to day of conception.

al., 2007; Pedernera et al., 2008), a similar result was reported by Jonsson et al. (1999) where a high level of concentrate feeding within a pasture-grazed diet also shorted the PPAI in multiparous cows. Similar BCS and NE_L with marginally greater levels of plasma NEFA among PostC cows (Roche et al., 2010) indicated that animals of the present study were in a similar, if not greater, level of negative energy balance (NEB) during early lactation compared with PostP cows. A possibly greater NEB, yet reduced PPAI, among PostC cows is interesting given numerous studies demonstrating that increased NEB in early lactation delays timing of first ovulation and estrus (Butler and Smith, 1989; Lucy et al., 1991; Beam and Butler, 1997). Others have also reported that improved feeding levels or metabolic state early postpartum do not necessarily result in enhanced luteinizing hormone secretion or an earlier onset of postpartum ovulation (Hoedemaker et al., 2004; Butler et al., 2006; Burke and Roche, 2007). That a postpartum diet greater in NSC reduced PPAI in the present study indicates the possible presence of a nutrient signal not necessarily associated with energy balance.

Reduced PPAI indicates advanced recuperation of the hypothalamic-pituitary axis to stimulate follicular maturation and advanced capacity of the preovulatory follicle to induce and respond to a preovulatory surge of luteinizing hormone (Jolly et al., 1995; Diskin et al., 2003; Rhodes et al., 2003). Although the nature of this nutrient signal(s) is not obvious from data presented here, reduced PPAI in PostC cows necessarily followed an accelerated postpartum reestablishment of hypothalamic-pituitary-ovarian axis competency. Nutrition or metabolic factors are known to influence the numerous biological mechanisms involved directly and indirectly (Diskin et al., 2003); for example, insulin and IGF-1 enhance estrogenic function in the early-postpartum dominant follicle (Spicer et al., 1993; Gong et al., 2002; Butler et al., 2004). Although no relationships between PPAI and these metabolic hormones were detected in the current study, IGF-1 concentrations were greater in PostC cows. It is possible that transient hormone responses occurred after consumption of concentrates (Sutton et al., 1988) but went undetected because the blood sampling schedule was infrequent and samples were collected just before the a.m. feeding of concentrate in the current study.

The PostC diet increased glucose and NEFA (metabolic fuels) while reducing BHBA and urea (metabolic byproducts). However, differences in these metabolites were small and they had little or no association with variance in PPAI. This implies that these metabolites were unlikely to have played a direct role in the underlying mechanism for a shortened PPAI in PostC cows. The only blood component to be significantly correlated

with PPAI was Mg. Greater concentrations of plasma Mg were expected in PostC cows because of the reduced dietary potassium (antagonist to rumen Mg absorption) content of pasture following dilution by the grain supplement that these cows received. Additionally, the supplementary NSC would be expected to reduce rumen pH, increasing Mg solubility and leading to a more efficient Mg absorption (Dalley et al., 1997). The association of Mg with reduced PPAI was not predicted, and the reason for this link remains unclear. Although Mg is essential for general health and well-being in the periparturient dairy cow (Roche et al., 2002; Lean et al., 2006), there appears to be nothing in the literature linking Mg to PPAI. In general, none of the measured factors, including BCS and Mg, were able to account for more than 10% of the variance observed in PPAI. This was the case despite a reasonable distribution of PPAI among these experimental animals (grand mean: 32.6 d; SD: 13.5 d; range: 13–71 d).

The present study was not designed to investigate dietary effects on binomial reproductive variables. Nonetheless, the data were collected and presented given the interesting outcome. The PostC cows had an improved 6-wk pregnancy rate compared with the PostP cows. This may have been a chance event of binomial data, given the small number of cows involved, and requires cautious interpretation. A similar benefit in pregnancy rate from manipulating dietary NSC content has recently been reported by Garnsworthy et al. (2009) when pregnancy rate was increased for cows fed an NSC-rich diet between calving and resumption of ovulation, before shifting to a reduced NSC-type diet during mating. The PostC cows of the present study would likely have experienced a similar shift as concentrate supplementation was removed beginning at 35 DIM.

The difference in NSC content between treatments is potentially confounded with differences in other dietary nutrient components (Table 1). There remains the possibility, therefore, that the observed differences in reproductive measures between postpartum diets are not entirely attributable to differences in NSC content. For example, daily CP intake for PostC cows (2.4 kg of CP/cow per day) was approximately 600 g less than that for PostP cows (3.0 kg of CP/cow per day). High CP intakes have been identified as a potential impediment to conception (Canfield et al., 1990; Butler et al., 1996) but not to PPAI (Canfield et al., 1990; Ordóñez et al., 2007). Further, there is a general consensus that cows managed within pasture-grazed systems are adjusted to a high protein diet (Westwood et al., 1998; Laven et al., 2007) and fertility is not compromised under these conditions. It is unlikely, therefore, that the difference in CP intake between PostP and PostC cows contributed to the observed 8-d difference in PPAI.

In summary, differences in the NSC content of an isoenergetic diet during the prepartum period did not affect PPAI in pasture-grazed dairy cows. In contrast, PPAI was beneficially influenced by a greater NSC content in an isoenergetic diet during the early postpartum period. This result was associated with a possible improvement to fertility during the breeding period. These benefits were not the result of an improved energy balance, supporting the presence of a nutrient signal mechanism related to dietary NSC content.

ACKNOWLEDGMENTS

This study was funded by DairyNZ Inc. (Hamilton, New Zealand) and the Foundation for Research, Science and Technology (Wellington, New Zealand) through contracts DRCX0204 and DRCX0301. The authors are grateful for technical and animal management support of P. W. Aspin, B. Sugar, A. Napper, and other staff at the DairyNZ Lye Dairy Farm (Hamilton, New Zealand); the statistical support of B. Dow (DairyNZ) is gratefully acknowledged.

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