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Postpartum anoestrous interval and metabolic changes in heifers supplemented with pasture prepartum

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ABSTRACT

The aim of this study was to determine whether supplementation with pasture from 6 weeks before calving would alter the postpartum anoestrous interval (PPAI) in heifers calving with low body condition score (BCS). Metabolic hormones and metabolites were measured during the pre- and postpartum period. Forty Friesian heifers were managed during the last five months of gestation to achieve a BCS (1-10) of 4.0 (restricted; R; n=27) or 5.0 (fully fed; FF; n=13) by 6 weeks prepartum. One group with low BCS then received pasture *ad libitum* until calving (restricted+fully fed; R+FF; n=12). Live weight (LW) and BCS were assessed weekly. Blood samples were collected weekly from 10 weeks prepartum to 10 weeks postpartum to measure glucose, urea, non-esterified fatty acids (NEFA), betahydroxybutyrate (BOH) and cholesterol content. The patterns of metabolites measured differed between groups at different times, future work to investigate the relationship these profiles and PPAI would be of value. The percentage of cows cycling as measured by an elevated milk progesterone concentration was 69%, 75% and 8% for FF, R+FF and R groups, respectively. Fully feeding heifers, with low condition score, 6 weeks before calving decreased PPAI when compared to heifers receiving restricted feeding.

Keywords: dairy cows; postpartum anoestrous; metabolites; body condition score; live weight.

INTRODUCTION

The postpartum anovulatory interval (PPAI), which extends from parturition to the time of the ovulation, has a large influence on the probability of cows becoming pregnant during the breeding season. Prolonged periods of anovulatory anoestrus is a major form of infertility in New Zealand dairy cows (Macmillan, 1980). It is important to understand the physiological mechanisms controlling the resumption of ovarian activity. Nutrition is a major factor determining reproductive efficiency in a pasture-based system. Reduced nutrient intake delays the onset of puberty in heifers (Day *et al.*, 1986), and increases the PPAI and calving-to-conception interval in cows (reviewed by Short *et al.*, 1990). Young heifers bred to calve as 2-year-olds have a longer PPAI than mature cows and PPAI is longer in Friesian than Jersey cows (Burke *et al.*, 1995; McDougall *et al.*, 1995). This difference in PPAI between mature cows and heifers may be associated with increased stress from calving and lactation, as well as the nutrient requirements for growth. Negative energy balance (NEB) during the early postpartum period in cows occurs as energy requirements for production and maintenance exceed energy intakes, with body tissues mobilised to provide substrates for energy production (Butler *et al.*, 1981; Butler, 2000). Negative energy balance is associated with reduced blood glucose and increased plasma non-esterified fatty acids (NEFA) and ketone bodies. This suggests that insufficient gluconeogenesis and enhanced fat and muscle mobilisation occurs during periods of NEB. The relationship between NEB and PPAI in heifers may be associated with reduced blood glucose and increased blood NEFA and ketone body content. In first-lactation dairy cows, energy balance and live weight are significantly correlated with the length of the PPAI and with subsequent fertility (Butler *et al.*, 1989).

It has been shown that heifers with a low BCS at calving are more likely to have a longer PPAI than heifers with a high BCS (McDougall, 1993; Clark *et al.*, 2000).

The objectives of this study were to determine whether supplementation with pasture, for 6 weeks before calving, would alter PPAI in heifers calving with low condition score

and to measure levels of metabolic hormones and metabolites in these heifers both pre- and postpartum and relate these to PPAI.

MATERIALS AND METHODS

This trial was conducted at the Dexcel Limited, Hamilton, New Zealand. Forty Friesians heifers (calving at 2 years of age) were managed during the last five months of gestation to achieve a body condition score (BCS 1-9) of 4.0 (restricted; R; n=27) or 5.0 (fully fed; FF; n=13) by 6 weeks prepartum. One group with low BCS then received *ad libitum* pasture feeding until calving (restricted+fully fed; R+FF; n=12). After calving all heifers were managed as a single herd and offered generous pasture allowance (average post-grazing residuals of 2087 kg dry matter/hectare).

Live weight and BCS were assessed weekly. Blood samples were collected weekly from 10 weeks prepartum (-10) to 10 weeks postpartum (+10) by coccygeal venipuncture into heparinised vacutainers and were analysed using a spectrophotometric auto-analyser Hitachi 717 (Alpha Scientific Ltd, Hamilton, New Zealand) to measure BOH, glucose (hexokinase method), urea nitrogen (urease method), cholesterol (cholesterol esterase method), and NEFA (plasma; colorimetric method). The inter-assay coefficients of variation (CVs) were 3%, 2%, 2%, 2% and 3%, and the intra-assay CVs were 2%, 2%, 1%, 2% and 5% for the analysis of BOH, glucose, urea, cholesterol and NEFA content, respectively.

To monitor the time of first ovulation, progesterone concentration was measured in milk samples collected three times each week. Concentrations of progesterone in milk were determined using a previously validated commercial RIA kit (Dieleman & Bevers, 1987; Coat-A-Count, DPC, CA, USA). Inter-assay CVs were 7.3 %, 5.7 % and 12.8 % while intra-assay CVs were 5.0 %, 4.7 % and 8.7 % for standard concentrations of 4.4, 3.0 and 0.4 ng/ml, respectively. When more than two consecutive measurements of progesterone in milk were > 1.0 ng/ml the animal was considered to have ovulated. The live weight,

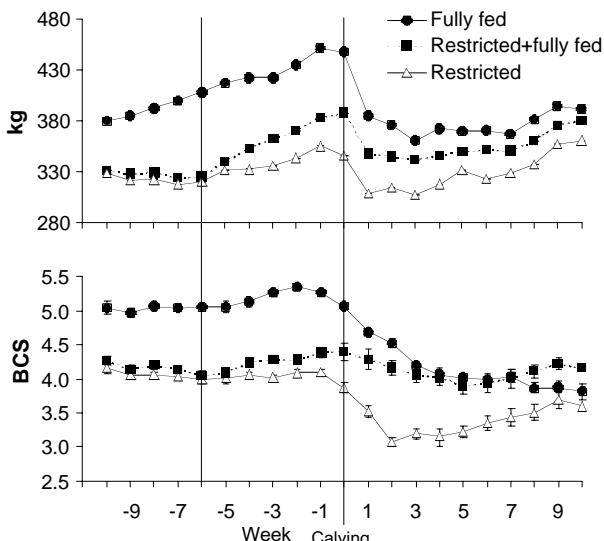
BCS and metabolic hormones and metabolites levels were analysed using analysis of variance for each individual week in Genstat 4.1. The percentage of heifers ovulating was analysed using generalised linear models with binomial error distribution. All the differences discussed in this paper are significant at $P < 0.05$.

RESULTS

Figure 1 shows mean live weight (LW) and BCS for each group during the trial period. The R and R+FF groups were approximately 80 kg lighter than the FF group at 6 weeks prepartum. Between weeks -10 and +2 the R and R+FF groups had lower LW than the FF group. Seven weeks before calving, just prior to the period of supplementation for the R+FF group, mean live weights were 407, 320 and 325 kg for FF, R+FF, R, respectively, with BCS of 5.0, 4.0 and 4.1, respectively. One week before calving, LW was different among all groups (346, 387 and 448 kg for the R, R+FF and FF groups, respectively). The differences in LW between R and FF were observed until week +10. Between R and R+FF groups LW differed from weeks -3 to +4 and during week +6. Between R+FF and FF, LW differed between weeks -10 to +2 and during week +4.

The differences in BCS between R and FF were observed from weeks -10 to +7. Between R and R+FF, BCS differed between weeks -3 and +10. There were no significant differences in BCS between R+FF and FF after week +3.

FIGURE 1: Live weight and body condition score (mean \pm SEM) from week -10 to +10 postpartum for the restricted, restricted+fully fed and fully fed groups.

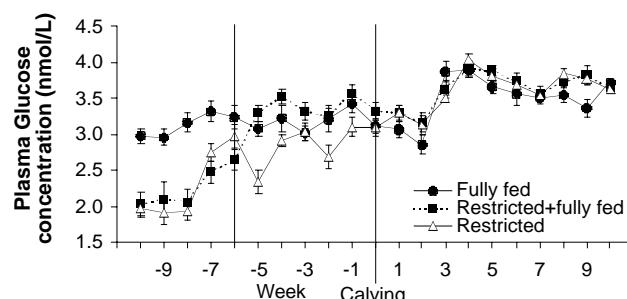


The mean blood glucose concentration (Fig 2) differed between FF and both R+FF and R from weeks -10 to -6 and week +9 but not between R+FF and R. Groups FF and R differed during the weeks -4 and -2 and between the R and R+FF from weeks -4 to week -1.

Plasma concentrations of urea were similar across all groups. The restricted group had higher urea levels during the week +2 (5.11 ± 0.24) when compared with the R+FF (3.58 ± 0.24 nmol/L) and FF (3.75 ± 0.20 nmol/L) groups.

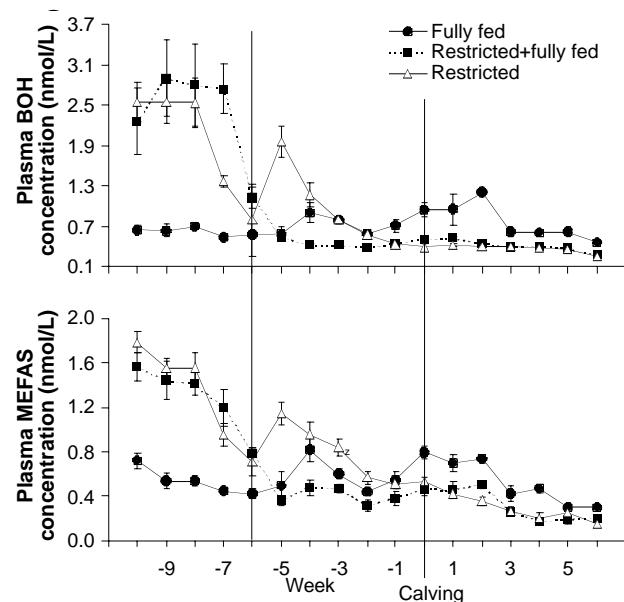
Plasma concentrations of BOH (Fig 3) were similar in FF than both R and R+FF from weeks -9 to -7. Plasma

FIGURE 2: Mean values (\pm SEM) for plasma glucose concentration from week -10 to week +10 postpartum for the restricted, restricted+fully fed and fully fed groups.



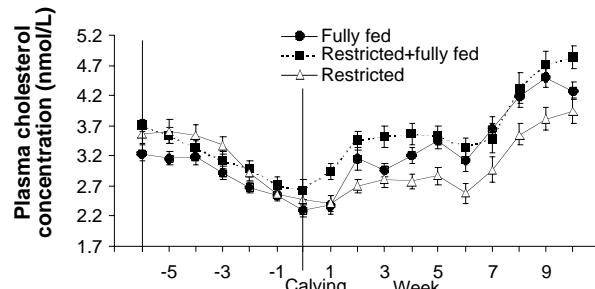
concentrations of BOH were also lower in FF than R+FF at week -6, and from calving to week 6 FF was greater than R. Groups R+FF and R also differed during weeks -6, -4, -3 and -1. Plasma concentrations of NEFA (Fig 3) were lower in FF than the other two groups between weeks -10 to -6 and higher in FF between weeks +1 to +5. Plasma concentrations of NEFA were also different between the R and R+FF group for 4 weeks before calving.

FIGURE 3: Mean values (\pm SEM) for plasma BOH and NEFAS concentration from week -10 to week +6 postpartum postpartum for the restricted, restricted+fully fed and fully fed groups



Plasma cholesterol concentrations (Fig 4) were similar across all groups from weeks -6 and +1 but differed from weeks +2 to +10 between the R and R+FF groups and at weeks +2, +4 and +10 between the R+FF and FF groups.

FIGURE 4: Mean values (\pm SEM) for plasma cholesterol concentration from week -6 to week +10 postpartum postpartum for the restricted, restricted+fully fed and fully fed groups.



The percentage of animals that ovulated by the start of mating (77 days after calving for each animal) was 69% (9/13), 75% (9/12) and 8% (1/15) for the FF, R+FF and R groups respectively. The first animal that ovulated on days 39, 47 and 69 postpartum in the FF, R+FF and R groups, respectively. After 61 days postpartum more than 50% of the cows had ovulated in the FF, R+FF groups. There was no difference in the percentage of animals that had ovulated before the start of mating between the R+FF and the FF groups. The mean PPAI amongst those cows that ovulated by the start of mating did not differ between the FF and R+FF groups (56 and 59 days).

DISCUSSION

In this study, heifers with low BCS that received *ad libitum* pasture six weeks before calving had a similar PPAI to cows calving in high BCS. Heifers with low BCS that did not receive supplementation had a longer PPAI.

McDougall (1993) also showed observed that heifers calving at body condition score of 5.1 are more likely to ovulate by 40 days after calving than heifers with BCS of 3.9. The relationship between prepartum BCS and nutrition concurs with previous relationships reported between PPAI, live weight and energy balance (Butler *et al.*, 1989). The current study supports the hypothesis that low BCS and restricted feed intake in the prepartum period has the potential to reduce subsequent fertility via an extended PPAI.

Both BCS at parturition and postpartum nutrition influenced blood/plasma concentrations of glucose, insulin, and NEFA and also the timing of onset of luteal activity in primiparous beef cows (Vizcarra *et al.*, 1998). Plasma levels of glucose and urea at 10–15 days postpartum have been suggested as indicators of the interval until the onset of postpartum ovarian activity. There was a negative correlation between the level of urea and the time of first postpartum ovulation (Eldon *et al.*, 1988). In the current experiment only urea levels during the second week postpartum were lower in the R group that had a long PPAI. Energy balance and low levels of plasma glucose affect postpartum reproductive performance in dairy cattle (Butler *et al.*, 1981). This was not observed by Clark *et al.* (1992), heifers with longer PPAI had higher glucose concentration than older cows with shorter PPAI. These results might be due to a requirement for energy partitioning for growth in two-year-old heifers. In the current experiment, blood glucose concentrations postpartum did not differ among the groups.

Reduced feed intake led to increased concentrations of NEFA through increased lipolysis (Richards *et al.*, 1989). Levels of BOH are also associated with adipose tissue mobilisation. Increased plasma concentrations of NEFA and BOH combined with age, calving live-weight and condition score at calving have been suggested as indicators of energy status and could be used to identify cows with greatest risk of having a prolonged PPAI (Verkerk & Guiney 1999). In this experiment, the concentrations of NEFA and BOH before calving were different between the R and the R+FF groups; however, the fully-fed group had higher levels of these metabolites after calving when compared to the other groups but had lower PPAI only when compared with the R group. Heifers with higher BCS have greater fat

reserves than heifers with low BCS. This may explain the greater loss in live weight and body condition of these animals after calving as they mobilise these reserves for milk production. Milk production was higher in this group than the others (data not reported). These results agree with observations of multiparous dairy cows in which individuals that experienced the greatest negative energy balance ovulated later in the postpartum period after losing more body condition and body weight, eating less feed and producing less milk than those that ovulated earlier (Butler *et al.*, 1981; Canfield & Butler 1990).

Plasma cholesterol levels in the heifers that had longer PPAI were lower than the other groups, FF or R+F. Plasma cholesterol concentrations were similar in all groups before calving. After calving, the R group had lower plasma cholesterol concentration than the FF and R+F groups. Plasma cholesterol concentration is positively correlated with energy balance (Lean *et al.*, 1992). Studies using diets containing approximately 8% lipid increased serum cholesterol concentrations and were associated with higher mean progesterone concentration *in vivo* (Williams, 1989). Granulosa cells from preovulatory follicle of heifers fed a high lipid diet (8% total lipid) secreted 2.1- to 3.5-fold greater quantities of pregnenolone and progesterone *in vitro* (Wehrman *et al.*, 1991). Ryan *et al.* (1992) observed an increased follicular development, and increased total cholesterol and progesterone concentration in follicular fluid of heifers fed a high lipid diet (5.4% added fat). However, cholesterol uptake and progesterone production in the ovary were not correlated, suggesting that cholesterol uptake does not limit steroidogenesis (Rabiee *et al.*, 1995; 1999). Studies in rats have shown that only 50% of the total progesterone secreted following administration of radiolabelled cholesterol was labelled (Swann & Bruce, 1986). Schuler *et al.* (1981) found no alteration in ovarian steroid output in the rat after serum cholesterol concentrations were increased six fold. Clark *et al.* (2000) observed no relationship between cholesterol concentration and PPAI. These studies indicate that cholesterol supply to the ovary does not appear to be limiting steroidogenesis. In the current experiment the R group had a lower nadir of plasma cholesterol after calving, they also had a longer the interval from calving to ovulation, low body condition and bodyweight pre- and postpartum. Westwood (1998) observed that the interval from calving to successful pregnancy was longer for cows with a lower nadir of plasma cholesterol after calving. These cows ovulated later and lost more bodyweight during early lactation. Cholesterol concentrations may have important long-term regulatory effects on ovarian activity, but they probably operate through, and reflect, improved energy balance rather than through limiting steroidogenesis.

CONCLUSIONS

The use of pasture supplementation, six weeks before calving, in animals with low body condition and live weight improved their reproductive performance. The severity of postpartum negative energy balance and the delay in the initiation of normal postpartum cyclicity may be associated with live weight and body condition. Body condition score could be used as the basis for selecting animals for which supplementation 6 weeks prepartum would improve

subsequent reproductive performance. Live weight and body condition score were only different between the animals supplemented with pasture six weeks and the restricted group three weeks before and after calving. It is possible that the rising plane of nutrition before calving had a stronger effect at the hypothalamus-pituitary axis than the body weight and condition before calving. The patterns of changes in the metabolic profiles differed between groups at different times, future work to investigate the relationship these profiles and PPAI would be of value.

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