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Can β-hydroxybutyrate levels predict the post-partum anoestrous interval of dairy cows?

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ABSTRACT

Concentrations of β-hydroxybutyrate in plasma (pBOH) and milk (mBOH) were measured post-partum (pp). Mean pBOH levels were highest in Weeks 3&4 pp (1.1 ± 0.1 mmol/L). Cows were grouped for length of post-partum anoestrous interval (PPAI) for the statistical analyses. Of those cows which required hormonal treatment because they were anoestrous at the planned start of mating, 77% had pBOH >1 mmol/L during Weeks 1&2 pp, compared to 52% of other cows ($P < 0.1$). Levels of pBOH alone could not predict the length of the PPAI ($r^2 = 2\%$). Logistic regression analyses confirmed age, calving live-weight and calving condition score as risk factors for prolonged PPAI. When these factors were considered with pBOH at Weeks 5&6 pp, a predictive relationship was found ($P < 0.05$). Levels of mBOH and pBOH were related ($r^2 = 0.68$; $P < 0.001$). These results support negative energy balance in early lactation as a risk factor for prolonged PPAI, along with age and live-weight and body condition score at calving.

Keyword: Dairy cows; post-partum anoestrus; ketones; milk; plasma.

INTRODUCTION

Excessive negative energy balance and associated loss of body condition during the post-partum period is correlated to the length of the post-partum anoestrous interval (PPAI; Grainger and McGowan, 1982; McDougall *et al.*, 1995). Prolonged PPAI in individual cows has a negative impact on the overall reproductive performance of dairy herds in the New Zealand seasonal dairy system because submission and conception rates during the first three weeks of the mating period will be reduced. Since the calving pattern during the following spring is less compact, with a longer interval from the start of calving to the mean calving date, there is an impact upon the efficiency of feed utilisation which may, in turn, reduce economic efficiency (Macmillan *et al.*, 1990; Xu and Burton, 1996). Objective methods to identify those cows in which loss of body condition is of sufficient magnitude that the PPAI is lengthened would be of practical value, since these animals could be candidates for strategic nutritional supplementation. An objective screening method for practical application should ensure that cows can be sampled easily and non-invasively. Milk, therefore, would be a useful medium with which to work.

Physiological markers of negative energy balance in ruminants include elevated levels of plasma ketone bodies, generated when fat deposits in the animal are mobilised. Levels of β-hydroxybutyrate (BOH) in plasma of 1 mmol/L or greater are considered indicative of mobilisation of fat reserves (Lean, 1987). It was hypothesised that a predictive relationship exists between plasma BOH levels and length of the PPAI. This paper describes an experiment to investigate this hypothesis, and also to examine the relationship between BOH concentrations in milk and plasma.

MATERIALS AND METHODS

Friesian (n = 54) and Jersey (n = 39) cows from herds at the Nos. 2 and 5 Dairies of the Dairying Research Corporation which calved during July 1998 were enrolled for this project. The group comprised mainly 2 and 3 year old cows (n = 46 and 24, respectively) with the balance ranging from 4 to 6 years old. Cows were weighed and body condition scores were assessed weekly. Animals were observed twice daily for evidence of behavioural oestrus.

Blood samples were taken by coccygeal venipuncture into vacutainers containing sodium heparin. Composite milk samples were collected using a proportioning milk meter (Tru Test Co, Auckland, New Zealand). Sampling occurred at two weekly intervals from calving, either until there was evidence of ovulation having occurred (milk progesterone concentration greater than 1.5 ng/ml, indicative of the presence of an active corpus luteum; McDougall, 1994), or until the cow was 9 or 10 weeks post-partum (pp). The sampling periods were denoted as Periods 1-5 (Pers. 1-5), being pp Weeks 1&2, 3&4, 5&6, 7&8, and 9&10, respectively.

Blood samples were stored in iced water following collection and analysed within 3 hours for plasma BOH using a spectrophotometric auto-analyser Hitachi 717 (Alpha Scientific Ltd, Hamilton, New Zealand). This system has a detection limit of 0.1 mmol/L in plasma. Milk samples were also analysed for BOH with this auto-analyser, but with process changes which increased the sensitivity such that the detection limit was 0.02 mmol/L. The intra-assay coefficients of variation (CV's) for these procedures were 0.6% and 42% for blood and milk samples, respectively. Concentrations of progesterone in milk were determined using a commercial RIA kit (Coat-A-Count™, DPC, CA, USA). Inter-assay CV's were 6.5%, 9.3%, and 3.7% while the intra-assay CV's were 9.8%, 7.8% and 18.8% for standard concentrations of 4.5, 3.0 and 0.4 ng/ml, respectively.

Results are reported as mean ± SEM. Cows were categorised into 4 groups according to the length of their PPAI. Three groups which cycled spontaneously had PPAI of either <40 days, 40-59 days, or >60 days while the fourth group (treated) were still anoestrous at the start of herd mating. Treated cows received a standard hormonal treatment using a progesterone-releasing CIDR device (CIDR-B, InterAg NZ Ltd, Hamilton, NZ) for 6 days followed by an intramuscular injection of 1 mg oestradiol benzoate (CIDIROL, InterAg NZ Ltd, Hamilton, NZ). Data were analysed using Minitab v. 10Xtra (Minitab Inc., State College, PA, USA). Linear and logistic regression analyses examined for effects associated with age, breed, body condition score and live-weight at calving, and plasma BOH concentrations at each sample (Pers. 1-5). Categorical variables were tested by Chi square analysis. The relationship between milk and plasma levels of BOH was determined by regression analysis.

RESULTS

Twenty-eight cows had PPAI of <40 days, 28 had PPAI of 40-59 days, while 14 cows took 60 or more days to recommence oestrous cyclicity. The overall mean length of the PPAI in these cows was 47.3 ± 1.6 days. Twenty-three cows, of which 10 were 2 years old and 7 were 3 years old, were in the treated group. At the time of treatment, the mean PPAI for this group was 72.5 ± 1.7 days (range: 63-94 days).

Mean plasma and milk BOH concentrations for each group at each sample period are given in Tables 1 and 2, respectively. Overall mean plasma BOH levels were highest during Pers. 1 and 2 (1.0 ± 0.1 and 1.1 ± 0.1 mmol/L, respectively) with ranges of 0.3 – 5.6 and 0.3 – 4.6 mmol/L, respectively. Levels of plasma BOH during the remaining Pers. 3, 4, and 5 were 0.6 ± 0.1, 0.5 ± 0.1 and 0.4 ± 0.1 mmol/L, respectively, with maximum levels of 2.3, 1.7 and 1.2 mmol/L, respectively.

TABLE 1: Mean (±SEM) plasma BOH concentrations (mmol/L) in cows with varying lengths of post-partum anoestrous intervals and sampled at fortnightly intervals (Period 1 = Weeks 1&2 post-partum, Period 2 = Weeks 3&4, and so on).

	Period				
	1	2	3	4	5
≤ 40 days (n=28)	0.83 (0.14)	0.88 (0.16)	0.54 (0.06)		
41-60 days (n=28)	1.01 (0.10)	1.25 (0.22)	0.55 (0.07)	0.47 (0.08)	
> 60 days (n=14)	1.15 (0.33)	1.61 (0.41)	0.45 (0.05)	0.40 (0.05)	0.39 (0.05)
Treated (n=23)	1.21 (0.15)	0.99 (0.18)	0.72 (0.10)	0.53 (0.06)	0.53 (0.10)

TABLE 2: Mean (±SEM) milk BOH concentrations (mmol/L) in cows with varying lengths of post-partum anoestrous intervals and sampled at fortnightly intervals (Period 1 = Weeks 1&2 post-partum, Period 2 = Weeks 3&4, and so on).

	Period				
	1	2	3	4	5
≤ 40 days (n=28)	0.08 (0.01)	0.06 (0.02)	0.03 (0.01)		
41-60 days (n=28)	0.09 (0.02)	0.08 (0.01)	0.05 (0.01)	0.03 (0.01)	
>60 days (n=14)	0.12 (0.03)	0.11 (0.03)	0.04 (0.01)	0.03 (0.01)	0.04 (0.01)
Treated (n=23)	0.14 (0.05)	0.04 (0.01)	0.03 (0.01)	0.02 (0.01)	0.04 (0.01)

Linear regression analyses failed to establish significant predictive relationships between plasma BOH concentrations and PPAI group (r^2 = 2%). Logistical regression analysis identified age, calving live-weight and body condition score at calving, but not breed, as alternative indicators of PPAI group, although the relationships were tenuous (r^2 = 21%, 16% and 10%, respectively). When the analysis included these factors along with plasma BOH variables from Pers 1, 2 and 3, the predictive relationship become significant (P < 0.5), with plasma BOH in Per. 3 being a significant indicator of PPAI group (P < 0.02).

Plasma BOH concentrations of ≥1 mmol/L are considered diagnostic of subclinical ketosis. When cows were classified to this criterion, 33% of cows in Per. 1 and 35% of cows in Per. 2 had evidence of subclinical ketosis compared to 10%, 2% and 1% in Pers. 3, 4 and 5 respectively. Of those cows which were subsequently treated for anoestrus, 77% had plasma BOH ≥1 mmol/L in Per. 1, compared to 54%, 48% and 56% of cows with a PPAI <40 days, 40-59 and ≥60 days, respectively (P < 0.1). The proportions of cows with plasma BOH >1 mmol/L in Per. 2 were not different (35%, 30%, 39% and 50% for treated, <40 days, 40-59 and >60 days, respectively). When plasma BOH at Per. 3 was considered in the context of the animal having subclinical ketosis, it predicted 22% of cows which subsequently needed treatment compared to 12% 7% and 0% with PPAI <40 days, 40-59 and ≥60 days, respectively (P<0.001).

Levels of BOH in milk and plasma were related (r^2 = 0.68; P<0.001).

DISCUSSION

The intention of this study was determine whether levels of BOH in the plasma of dairy cows, which become elevated as body fat depots are mobilised during early lactation, could be used as a predictor of the length of the PPAI. The results have demonstrated that plasma BOH concentrations during the first six weeks after calving, and particularly during the Weeks 5&6 pp, can provide predictive information, but that they are not of practical value when considered alone.

Previous studies which identified age and condition score at calving as being risk factors (Grainger and McGowan, 1982; McDougall *et al.*, 1995) were confirmed, and when these characteristics were included in the prediction of the risk of prolonged PPAI, a significant relationship was established. Levels of BOH in plasma during Per. 3, corresponding to Weeks 5&6 pp, were the most valuable predictor in this relationship.

That Weeks 5&6 pp is the best time to assess plasma BOH levels for prediction of risk of prolonged PPAI is perhaps not surprising. The negative energy balance of early lactation in dairy cows has been well characterised, as has the capacity of high yielding cows to mobilise body tissues (Lean, 1987). This effect may be exacerbated in New Zealand grazing systems because grass growth peaks often do not occur until cows are several weeks into lactation. Since feed requirements will exceed pasture production during

this time, decisions to allocate pasture often lead to limited energy intakes (Penno, 1998). Thus many high-producing cows in the NZ pasture-based system will have subclinical ketosis during early lactation, and evidence of this will consequently be of less predictive value for prolonged PPAI; rather, risk will associated more with those individuals who experience elevated plasma BOH for a longer than usual period, i.e., into Weeks 5&6 pp.

The strong relationship between plasma and milk BOH concentrations provide good evidence that milk could be a useful medium for routine estimation of BOH levels. One advantage is that individual cows can be sampled easily and by non-invasive means. The possibility exists that samples collected routinely for herd improvement records could be analysed; however it will be important to develop a method with improved accuracy compared to that reported here, in which the intra-assay CVs were very high even though the sensitivity was satisfactory.

CONCLUSION

The results reported support negative energy balance in early lactation as a risk factor for prolonged PPAI, but age and condition score at calving are also important. Concentrations of plasma BOH during the post-partum period in dairy cows, and in particular during Weeks 5&6 post-partum, may be combined with information about age, calving live-weight and condition score at calving to determine a predictive index which could identify those cows at greatest risk of suffering a prolonged periods of post-partum anoestrus.

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