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## Prediction of post-partum anovulatory interval in dairy cows

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### ABSTRACT

Changes in a range of metabolites and body condition parameters in lactating cows were measured during early lactation to determine if any key indicators or combination of key indicators could be used as a predictor of length of post-partum anovulatory interval (PPAI). Fifty-three cows in a range of body condition scores were involved in the trial which ran from calving to 10 weeks post-calving. Plasma non-esterified fatty acids (NEFA) and serum cholesterol, glucose, betahydroxybutyrate (BOH) and urea content were measured in weekly samples. Live weight and body condition score (BCS) were measured weekly.

Cows were categorised into four groups. Three groups were based on the post-partum anovulatory interval (PPAI). PPAI was defined as the interval between calving and the first evidence of oestrus behaviour. The fourth group contained all the two-year-old cows only. There was a calving interval of 31 days from the first to the last cow to calve. The nadir for live weight for all groups occurred one week after calving. Animals with a shorter PPAI had higher live weights on all occasions, with live weight at week 5 having the strongest association with length of PPAI ( $P < 0.001$ ). Mean BCS was higher for animals with a PPAI  $< 60$  days. Plasma urea concentrations were associated with PPAI at weeks 3 and 4 ( $P < 0.01$ ). NEFA and glucose were correlated with PPAI at weeks 3 and 4 only, respectively. There was no association between cholesterol or BOH with PPAI. These results demonstrate that the selected variables do not contribute greatly to prediction of PPAI. Those measures which give the closest association with PPAI are live weight, BCS and age.

**Keywords:** dairy cows; post-partum anovulatory; calving; metabolite; live weight.

### INTRODUCTION

In the New Zealand seasonal dairy farming system, the performance of dairy cows is often impaired by periods of under-nutrition that occur around calving and during the early stages of lactation. This under-nutrition occurs when the energy demands for maintenance and milk production exceed energy supply before the flush of spring pasture growth occurs. The resulting periods of negative energy balance (NEB) have a detrimental impact on cow condition as excessive loss of body reserves occurs. When extreme, this can result in impaired reproductive as well as productive performance.

Investigation of the relationship between energy balance and reproductive performance requires a systematic study of the early post-partum cow, which has not been done previously under New Zealand grazing conditions. A test of "metabolic status" would have relevance to the feeding management of dairy cows in terms of managing anoestrous and periods of limited feed supply (Kolver and Macmillan, 1994). Quantifying these metabolic and physical changes may provide predictor information to identify those animals most affected by extreme underfeeding.

Physical changes in individual cow condition can be assessed by measuring either body weight or body condition scoring (BCS) (Gregory *et al.*, 1998). Metabolites were selected for measurement on the basis that previous authors have identified relationships between these and the return to oestrous cyclicity. Elevated levels of non-esterified fatty acids (NEFA) and betahydroxybutyrate (BOH) in plasma are both associated with adipose tissue mobilisation, with BOH constituting 50% of the major ketone bodies. Verkerk and Guiney (1999) found that elevated BOH levels at weeks 1 and 2, and at weeks 5 and 6 post-partum had value in identifying individuals which subsequently had a prolonged

post-partum anoestrous interval when combined with calving weight, age and calving BCS. Plasma glucose concentrations have been positively correlated with energy balance (EB) (Canfield and Butler, 1990) suggesting that cows in positive EB are better able to maintain blood glucose concentrations. This may be due to increased absorption of glucose precursors (Lean *et al.*, 1992).

Urea and cholesterol have also been linked as indicators of metabolic status in dairy cattle. McDougall (1993) showed that plasma urea concentrations were higher in anoestrous cows which also had lower plasma glucose levels, indicating that anoestrous cows are likely to be in greater energy deficit than their cycling counterparts. A relationship between cholesterol and fertility has also been shown, with the interval from calving to ovulation and to successful pregnancy being longer for cows with a lower nadir of plasma cholesterol after calving, in association with greater loss of body weight during early lactation (Westwood, 1998).

The aim of this current study was to quantify a range of metabolic and physical changes in post-partum dairy cows across a range of BCS at calving, and to determine whether any key indicators or combination of key indicators could be used as a predictor of length of post-partum anovulatory interval (PPAI).

### MATERIALS AND METHODS

This trial was conducted in 53 cows from three spring-calving herds from the No. 2 Dairy, Dairying Research Corporation, Hamilton, New Zealand. The herds were selected to give a range of BCS and were managed as part of an existing farm systems trial at stocking rates of 2.2 (low;  $n=18$ ), 3.1 (medium;  $n=17$ ) and 4.3 (high;  $n=19$ ) cows/ha. Each herd was comprised of equivalent proportions of

2- and 3-year-old cows and mixed-age cows. The trial period ran from calving to 10 weeks post-calving for all cows. Cows were weighed and BCS assessed weekly. Tail paint was applied to all cows and they were observed twice daily for evidence of behavioural oestrus. A blood sample was taken weekly by coccygeal venipuncture into plain and heparinised vacutainers and was analysed using a spectrophotometric auto-analyser Hitachi 717 (Alpha Scientific Ltd, Hamilton, New Zealand) for 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 CV's were 2%, 2%, 1%, 2% and 5% for the analysis of BOH, glucose, urea, cholesterol and NEFA, respectively.

The PPAI in days was calculated for each cow as the interval between calving date and first observed oestrus. Cows were categorised into four groups for graphical presentation based on the PPAI, which was <45 days, 45-60 days or >60 days. The additional group for graphical presentation contained all the two-year-old cows (heifers) only. Data are presented as the mean and maximum s.e.m. through time, weekly from calving to 10 weeks post-partum. The relationship between PPAI and weekly measurements was investigated using Cox's Proportional Hazards regression analysis which enabled cows to be included for which there was only a lower bound for PPAI (the censored data). The relationship of each metabolite with time after calving was determined by fitting a trend to each cow and then calculating the mean and the s.e.m. of these.

## RESULTS

Eight cows were excluded due to late calving dates and two cows were excluded due to the inability to estimate their PPAI. All data are presented according to week post-partum and grouped by PPAI. The interval from first to last calving was 31 days. Table 1 shows the age breakdown within each PPAI period. Nine of the 10 heifers had PPAI > 60 days and half of the cows older than 3 years had PPAI < 45 days.

**TABLE 1:** Number of cows in each post-partum anovulatory interval (PPAI) group by age (years)

Age	PPAI<45 days	PPAI 45-60 days	PPAI>60 days	Totals
2	1	0	9	10
3	1	1	3	5
>3	14	9	5	28

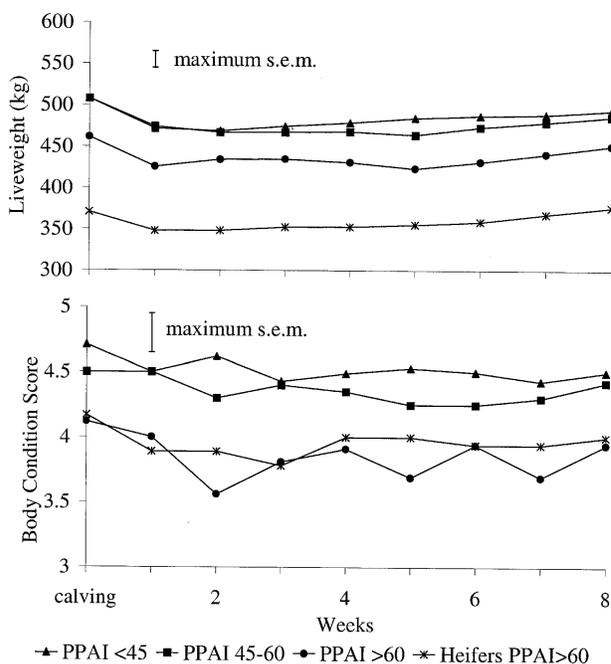
Cox's regression analysis and backward elimination methods were used to derive models for PPAI. An example of such a model is:

Predictor = (0.0147 x live weight week 5) - (2.8119 x BCS week 1) + (3.7555 x BCS week 2) + (0.4207 x urea week 4) + (2.0840 x NEFA week 1) + (1.6096 x NEFA week 4) + (1.3292 x age group (heifers = 0; older cows = 1)) ( $r^2 = 0.76$ ;  $P < 0.001$ ).

A cow with 1 unit increase in this calculated predictor is 2.72 times more likely to show oestrus. This is just one of many models for PPAI that can be derived from this data.

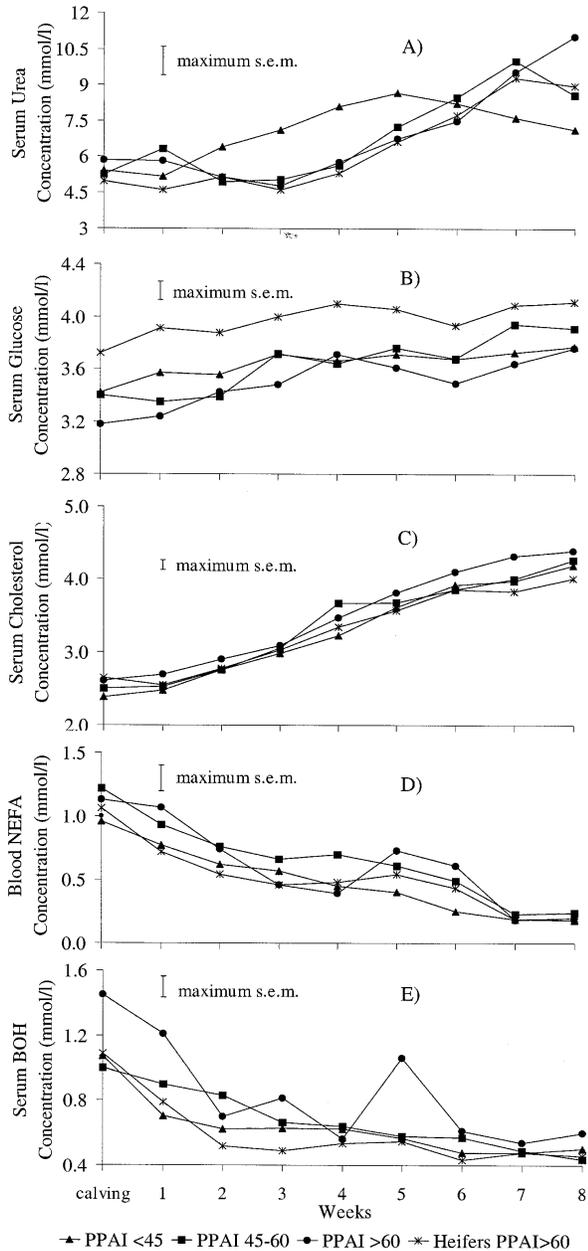
Mean values for physical measurements for each PPAI group are given in Figure 1, with heifers as a separate group. Animals with shorter PPAI had higher live weights on all occasions, with live weight at week 5 post-partum having the strongest association with length of PPAI ( $P < 0.001$ ). Cows with a shorter PPAI had a higher BCS throughout the experimental period ( $P < 0.01$ ). By week 2 post-partum, BCS was lower in cows with an extended PPAI ( $P < 0.0001$ ).

**FIGURE 1:** Mean values for live weight and body condition score (BCS) versus time from calving for each post-partum anovulatory interval (PPAI) group. Maximum s.e.m. for all groups throughout the time period is given by an error bar.



Mean values for metabolite measurements for each PPAI group are given in Figure 2, with heifers as a separate group. Serum BOH and plasma NEFA decreased with time ( $P < 0.001$ ), while serum urea, glucose and cholesterol increased with time ( $P < 0.001$ ). The nadir for live weight for all groups occurred at one week after calving. Serum urea concentrations at weeks 3 and 4 were associated with PPAI ( $P < 0.01$ ; Fig 2A). Serum glucose concentrations were highest in the heifers throughout the time period. The only association between PPAI and glucose occurred at week 4 post-partum ( $P < 0.05$ ; Fig 2B). Serum cholesterol levels had no association with PPAI ( $P > 0.1$  Fig 2C) but plasma NEFA concentrations at week 3 were correlated with PPAI ( $P < 0.1$  Fig 2D). There is no correlation at any time between BOH concentration and PPAI ( $P > 0.4$  Fig 2E).

**FIGURE 2:** Mean values for serum urea (a), serum glucose (b), serum cholesterol (c), plasma non-esterified fatty acids (NEFA) (d) and serum betahydroxybutyrate (BOH) (e) through time from calving for each post-partum anovulatory interval (PPAI) group. Maximum s.e.m. for all groups throughout the time period is given by an error bar.



**DISCUSSION**

The intention of this study was to determine whether some selected variables of dairy cow condition and metabolism, or their combination, could be used as key indicators to predict the length of PPAI. None of these selected variables alone could predict PPAI. The best predictors of length of PPAI were live weight, BCS and age, but only when considered in combination.

Age was an important contributing factor. All except one of the two-year-old animals had a PPAI >60 days and half the three-year-old animals also had a PPAI > 60 days. Of the mature cows, over half had a PPAI < 45 days. The age effect was associated with both live weight and BCS. The two-year-old animals were nearly 100 kg lighter than all other animals throughout the trial period. Those cows with a shorter PPAI had heavier live weights and higher BCS throughout the period. These results are in agreement

with previous studies which identify age, live weight and BCS as risk factors affecting the PPAI (Grainger and McGowan, 1982; Kolver and Macmillan, 1994; McDougall *et al.*, 1995; Verkerk and Guiney, 1999). In this study, the live weight nadir occurred at week 1 post-partum and thereafter all animals showed liveweight gain. This occurred earlier than that reported by Burke *et al.* (1995) at week 4-5 post-partum. Burke *et al.* (1995) reported that animals calving at higher live weights lost more weight during the early post-partum period. The post-partum interval to energy balance nadir is shorter and total energy deficit is smaller for heifers than for older cows (de Vries *et al.*, 1999). Our observations support this as the heifers lost less weight post-partum which may indicate an earlier return to a positive energy balance, but this was not reflected in a shortened PPAI, possibly of their lower BCS at calving. This could be explained because these younger animals were still growing, so that a positive energy balance did not have the same metabolic implications as in older cows.

The pattern of serum urea concentrations in this study is similar to that in previous reports. In this study levels were high during weeks 2-5 post-partum in animals with PPAI <45 days and declined thereafter. Animals with longer PPAIs followed the reverse pattern to be higher after week 5. A study by McDougall (1993), in which urea levels were compared between cycling and anoestrous cows during the week before the start of mating, observed a positive association between urea levels and the probability of the cow being anoestrous. In contrast, these metabolite profiles in the early post-partum period have shown a negative relationship between serum urea levels and time to first ovulation. Eldon *et al.* (1988) suggested low blood urea values during the early post-partum period might reflect excessive stress around parturition and onset of milking, which could in turn result in later ovulation. Urea in plasma or serum has been used as an indicator of protein intake, with low urea levels reflecting lower dietary protein intakes (Kolver and Macmillan, 1994). This could suggest that those cows with PPAI <45 days had higher protein intakes during weeks 2-5 post-partum. To test this hypothesis would require individual feed intakes to be measured.

Serum glucose concentration increased with time post-partum for all animals. High glucose concentrations have been associated with a positive energy balance, as cows in a positive energy balance are better able to maintain blood glucose concentrations, presumably because of increased absorption of glucose precursors (Lean *et al.*, 1992). Findings by Rabiee *et al.* (1999) indicate that glucose is the major source of energy for the bovine ovary. Rhodes *et al.* (1999) found that glucose concentration at 11 days post-partum had a significant negative relationship to length of PPAI, but serum glucose was not a significant indicator in the present study. Interestingly glucose concentration was higher throughout the entire period for the heifers even though their mean PPAI was greater than 60 days. If higher serum glucose levels and less post-partum weight loss both indicate an earlier return to positive energy balance, then this should be reflected in a shorter PPAI. These paradoxical results may be due to essential energy partitioning for growth in the two-year-old heifers.

The positive relationship between cholesterol and time

post-partum was similar for all PPAI groups, with the nadir for cholesterol occurring at week 1 post-partum. Cholesterol concentrations have been positively correlated with energy balance (Lean *et al.*, 1992). While it has been proposed that the supply of cholesterol to the ovary might be rate limiting for steroid synthesis when the animal is in NEB, Rabiee *et al.* (1999) showed there was no relationship between ovarian cholesterol uptake and progesterone output.

Both plasma NEFA and serum BOH concentrations show a negative relationship with time post-partum. Those animals with PPAI > 60 days had more variable levels of both BOH and NEFA particularly at week 5 post-partum. As both NEFA and BOH are products of adipose tissue mobilisation, these cows are likely to have suffered a period of NEB at this time, possibly associated with a “feed pinch”.

In conclusion, this data has demonstrated that no single measure of the variables selected in this study was useful as a predictor of length of PPAI. As reported previously, live weight, BCS and age are all associated with length of PPAI. While the metabolites selected for measurement did provide some additional information when included in a multivariate analysis, they did not contribute greatly to the prediction of PPAI. Overall this suggests that, although the selected variables can be indicative of energy balance state, the homeostatic mechanisms operating in the lactating cow may be sufficiently robust to ensure that concentrations of these metabolites remain relatively stable in peripheral circulation.

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