
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

An attempt to create an 'anoestrous cow' model by restricting feed allowances in non-lactating cyclic cows

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ABSTRACT

This experiment aimed to characterise the ovarian response to reducing live-weight (LWT) with the view of creating nutritional anoestrus in cyclic cows. The ovaries of 8 non-lactating 3 yr old Friesian cows were ultrasounded daily during 5 synchronised oestrous cycles (Rounds 1 to 5) over 12 months. An initial live-weight of 445 ± 3.3 kg at Round (R) 1 was reduced by 10% to R2, and by a further 10% to R3 by restrictive feeding. Respective LWT gains of 12 and 10% occurred between R3, R4 and R5 ($P<0.05$). Blood samples were collected 3 times weekly to monitor plasma progesterone concentrations (PPC) and metabolites.

The proportion of cows detected in oestrus at the start and end of R3 (6 of 16) was less than in other rounds ($P<0.05$) where an overt oestrus was usually observed. Only one animal ceased ovulating. Dominant follicles and corpora lutea were reduced in size at R2 following the first decline in LWT ($P<0.05$). The size of ovarian structures was more normal at R3, but mid-luteal PPC were reduced, indicating a decline in luteal activity ($P<0.05$). Low levels of serum albumin (hypoproteinemia) and Mg (hypomagnesaemia) during R3 prevented further dietary restrictions and LWT reductions. It was concluded that ovarian activity is responsive to changing LWT. However, the level of dietary restriction required to induce nutritional anoestrus in pasture-fed Friesian cows may be greater than the level required to maintain reasonable health.

Keywords: Nutrition; ovary; bovine.

INTRODUCTION

Prolonged periods of anovulatory anoestrus is the major form of infertility in the New Zealand dairy herd where dairy production is seasonally based. Opportunities for studying postpartum anoestrus (PPA) are limited to a short period in Spring. However, prolonged periods of restricted dietary intake have been used to induce a state nutritional anoestrus in cyclic beef cattle (Imakawa et al., 1986; Richards *et al.,* 1989; Rhodes *et al.,* 1995). Imakawa *et al.* (1986) incorporated ovariectomy in a nutritional anoestrous animal model to demonstrate that the hypothalamus of this animal is particularly sensitive to oestradiol negative feedback. The negative effects of inadequate nutrition on the ovary appear to be manifested at the level of the central nervous system and involve reduced release of GnRH and LH (Schillo, 1992).

The objective of the present study was to examine ovarian responses to restricted feeding and reduced live-weights (LWT) in pasture fed dairy cows with the view to creating an "anoestrous cow" model in the non-lactating animal.

MATERIALS AND METHODS

The 8 animals used in this study were 4 year old non-lactating Friesian cows weighing 445 ± 3 kg which had failed to re-establish pregnancy after calving during the previous Spring as 3 year olds. Their oestrous cycles were synchronised by intravaginal insertion of a CIDR™ (InterAg, Hamilton) device for 10 days. A gelatine capsule containing 10 mg oestradiol benzoate was inserted with each CIDR device, and a full dose of prostaglandin (PGF_{2a}: 5 ml Lutalyse™, Upjohn, Auckland) was injected 3 days before device removal. Detection of oestrus was aided by the use of tail paint.

The ovaries of every cow were ultrasounded from 48 h after device removal (Day 0) until the day after ovulation of the subsequent oestrous cycle, or until 2 complete anovulatory follicle waves had been monitored. All follicular and luteal structures greater than 3 mm in diameter were sketched and the video image recorded on cassette. Initiation of an ovarian follicle wave was defined as the day on which a large dominant follicle (DF)(>8 mm) was retrospectively traced to being 4-5 mm in diameter.

Blood samples were collected from a coccygeal vessel 3 times weekly for determination of plasma progesterone concentrations (PPC; Coat-a-Count, DPC, California). Additional blood samples were collected on Day 7 of the oestrous cycle for determination of serum concentrations of blood urea nitrogen (BUN), albumin, glucose, non-esterified fatty acids (NEFA), β-OH butyrate, total thyroxine (TT4) and magnesium (Mg) by the Ruakura Animal Health Laboratory, Hamilton.

These procedures were repeated on 4 further occasions (Rounds 2 to 5) during the period of a year beginning in November, 1993. LWTs were recorded twice weekly. These data were used to constantly adjust pasture feed allowances to initially create incremental reductions in LWT between R1, R2 and R3; followed by incremental increases of similar magnitudes between R3, R4 and R5.

The effects of period (Round) and animal on the size of ovarian structures, P4 and metabolites in blood were

TABLE 1: Average (sem) live-weights (LWT), proportion of cows with overt oestrus, inter-ovulatory intervals (IOIs), number follicular waves (FW) per cycle, plateau diameters of the first (DF1) and second dominant follicles (DF2), plateau diameter of the corpora lutea (CL) and mid-luteal plasma progesterone concentration (PPC) during Rounds 1 to 5.

Detail	R1	R2	R3	R4	R5
Start date	24/11/93	2/2/94	20/4/94	22/6/94	26/10/94
Day of trial	0	70	147	210	336
LWT (kg)	445 (3.3)^{a}	399 (2.3)^{b}	357 (2.0)^{c}	411 (1.7)^{b}	454 (2.7)^{a}
Overt oestrus (n= 16)	12^{a}	14a	6b	16a	16a
IOI (days)	22.1 (0.5)	21.9 (1.1)	24.1 (1.8)	22.0 (0.8)	22.8 (1.1)
FW per cycle	2.6 (0.2)	2.8 (0.3)	2.9 (0.4)	2.6 (0.2)	2.8 (0.3)
IOI of Cow 1740	23	29	33	25	27
FW/cycle of Cow 1740	3	4	5	3	4
DF1 plat. diam. (mm)	12.2 (0.3)^{a}	11.2 (0.5)^{b}	12.7 (0.3)^{a}	12.7 (0.2)^{a}	12.3 (0.1)^{a}
DF2 plat. diam. (mm)	11.5 (0.8)^{ab}	9.8 (0.7)^{a}	10.8 (0.9)^{ab}	12.5 (0.5)^{b}	12.1 (0.6)^{b}
CL plat. diam. (mm)	24.4 (0.7)^{ab}	22.9 (0.7)^{a}	23.9 (1.0)^{ab}	25.1 (0.7)^{bc}	26.6 (0.8)^{c}
PPC mid-luteal (ng/ml)	5.4 (0.6)^{ab}	5.9 (0.7)^{ac}	4.0 (0.4)^{b}	6.2 (0.5)^{ac}	7.0 (0.6)^{c}

^{abc} Values with different letter superscript within rows are different (P<0.05).

tested by analyses of variance using a general linear model and by paired Student's t-tests. The proportion of animals with overt oestrus was tested by Chi-square analysis. All data are expressed as the mean (± sem).

RESULTS

Two incremental losses of 10% LWT were achieved between R1 and R3 followed by stepwise increases of similar magnitude (12 and 10%) to R4 and R5 (Fig. 1). The lowest average LWT occurred during R3 following a mean loss of 88 kg (20%) from R1 (P<0.05; Table 1). Individual losses in LWT ranged from 16 to 25% over this period (Fig. 1). Serum concentrations of glucose and BUN were lowest during R2 (Fig. 2). Reductions in serum albumin indicating hypoproteinemia were delayed until after R2 and remained suppressed until after R4 (Fig. 2). Blood levels of Mg followed a similar trend, declining to 0.60 ± 0.02 mmol/l during R3, i.e. 0.05 mmol/l below the normal reference range set by the Ruakura AHL. Levels recovered after Mg supplementation. There was a significant effect of round on concentrations of NEFA which indicates that fat stores were initially mobilised (i.e., R2) then deposited (i.e., R5). There was no round or animal effect on concentrations of β-OH which remained between 0.2 and 0.3 mmol/l.

FIGURE 1: Mean and individual live-weight (LWT) changes (LWT at Round 1 (R1) equivalent to 100%) for 8 animals subjected to restricted feeding from R1 to R3, and then allowed to regain LWT from R3 to R5.

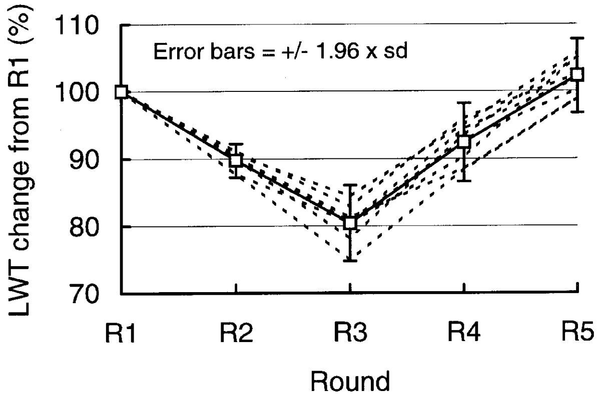
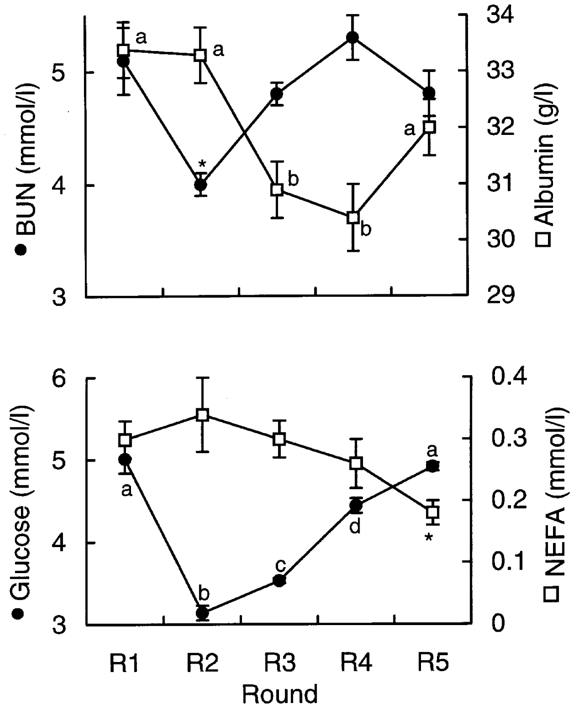


FIGURE 2: Mean serum concentrations of β-urea nitrogen (BUN), albumin, glucose and non-esterified fatty acids (NEFA) in blood of the 8 animals during the mid-luteal phase in each round. Differences (P<0.05) are denoted with letters or * when only one value in the series is different.



The proportions of animals detected in oestrus at the beginning and end of R3 (6/16 cases) were less than other rounds (Table 1; P<0.05). The reduced behavioural responses in R3 were associated with the occurrence of anovulation in 2 animals. One of these (#1822) had a single anovulatory follicle wave followed by a normal oestrous cycle. The other (#1819) had 4 consecutive anovulatory follicle waves before a dominant follicle finally ovulated. The maximum diameters of these anovulatory DFs were either 10 or 11 mm, while the maximum diameter of the ovulatory follicle was 13 mm. The average inter-ovulatory interval (IOI) of 22.5 ± 0.5 d did not vary between rounds ($P>0.1$). However, the average IOI of one animal (#1740; 27.4 (1.7 d) was greater than for the others ($P<0.05$). The IOIs and the number of follicle waves per cycle increased with reduced live-weight ($P<0.05$), and then declined as live-weight increased (Table 1). The average IOI of animals with oestrous cycles of 2 follicle waves (2-wave) was 19.9 ± 0.5 d, and of those with 3 follicle waves (3-wave) was 23.4 ± 0.3 d ($P<0.05$).

The mean plateau diameter of the DF of the first wave (DF1max) was smaller at R2 compared to other Rounds ($P<0.05$; Table 1). The smallest DF2 were also measured during R2 (Table 1). Neither growth rate (1.8 ± 0.1 mm/d) nor regression rate of the DF1 (1.4 ± 0.1 mm/d) was affected by Round. Similarly the growth and regression rates of the DF2 in 3, 4 or 5-wave oestrous cycles were not different (1.7 ± 0.1 mm/d and 1.5 ± 0.1 mm/day respectively; $P>0.1$). The average plateau diameter of corpora lutea (CL; Days 8 to 11) was smallest at R2 and largest at R5 (Table 1; $P<0.05$). The maximum mean PPC measured during R3 was lower than in R2, R4 and R5 (Table 1; $P<0.05$). There was a significant animal effect where #1740 had a higher maximum PPC (8.7 ± 0.8 ng/ml) compared to other individuals which ranged from 4.7 ± 0.8 ng/ml (#1755) to 6.9 ± 0.6 ng/ml (#1822).

DISCUSSION

This study has shown that the ovarian activity of cyclic pasture fed cows is affected by changing nutritional status. A 20% reduction in initial LWT did not uniformly induce a state of anoestrus in these cows, but did raise concerns for their health and well being. Low blood concentrations of albumin (hypoproteinemia) and Mg (hypomagnesaemia) were factors in deciding not to further reduce LWT, or to prolong the low LWT during R3.

The approach of restricting dietary energy has been used to induce anoestrus in cyclic cattle. Imakawa *et al.* (1986) reported that nutritional anoestrus was induced in nulliparous beef heifers which lost 20% of initial live-weight during a period of 186 days. Nutritional anoestrus was induced in multiparous beef cows over a similar time, but required a 24% loss in LWT (Richards *et al.,* 1989). In *Bos indicus* cattle, Rhodes *et al.* (1995) found that oestrous cycles could cease as soon as 48 d (average: 93 d) after initiation of a restricted feeding regime by which time the reduction in initial LWT could be only 10% (average: 22%). The present study, which used primiparous 4 year old Friesian cows, resulted in a reduction in LWT by an average of 20% over a period of about 150 d from the initial LWT. The only animal to become anovulatory was one which lost 25% of its initial LWT. This may indicate that in general, there were insufficient losses in LWT to induce nutritional anoestrus in these animals. Other possibilities include an insufficient period of feed restriction (i.e. <150 d), differences due to the type of animal (dairy *vs* beef), breeds and type of feed (pasture *vs* high-fibre/low digestibility rations).

Restricted feeding is reported to reduce both diameter and persistency of DF's (Rhodes *et al.,* 1995; Murphy *et al.,* 1991). The present study suggests that the development of ovarian structures may be sensitive to nutritional factors associated with changes in dietary energy, and LWT, rather than LWT loss *per se*. The changes in diameter of DF1, DF2 and the CL were greatest following the first 10% decrease in LWT, than following the further reduction of 10% at R3 (Table 1). Development of these structures is dependent on gonadotrophic support from the pituitary gland. The suppressive effect of feed restriction on the GnRH "pulse generator" and hence pulsatile release of LH can be dramatic in many mammals, but links between LWT loss and the factors that regulate ovulation remain unresolved (Shillo, 1992).

In conclusion, ovarian structure and function are influenced by nutrition in cyclic cows. The level of dietary restriction required to induce nutritional anoestrus in the non-lactating pasture-fed Friesian cow may be greater than the level required to maintain reasonable health.

ACKNOWLEDGEMENTS

This research was funded under a contract (93-DRC-301-01) with the Foundation for Research, Science and Technology.

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