

The Effects of a Short-term Increase in Supplementation on the Reproduction of Crossbred Dairy Cows

Abstract

The experiment investigated the hypothesis that crossbred dairy cows, rearing their calves by partial suckling, would ovulate following the removal of their calves if restored to positive energy balance by a short-term increase in supplementation. The five treatments, involving 65 cows, were *Control* Restricted forage; 3.5 kg concentrate per day (total weight of concentrate = 588 kg); *Treatment 2* Restricted forage; 2.5 kg concentrate per day from calving to week 9; 7 kg concentrate per day from week 10 to 15; 2.5 kg concentrate per day from week 16 to 24 (total weight of concentrate = 609 kg); *Treatment 3* Restricted forage; 3.5 kg concentrate per day from calving to week 9; 7.0 kg concentrate per day from week 10 to 15; 3.5 kg concentrate per day from week 16 to 24 (total weight of concentrate = 735 kg); *Treatment 4* Restricted forage; 3.5 kg concentrate per day from calving to week 8; 7.0 kg of concentrate from week 9 to 13; 3.5 kg of concentrate from week 14 to 24 (total weight of concentrate = 710.5 kg); *Treatment 5* Restricted forage; 3.5 kg concentrate per day from calving to week 8; 6 kg of concentrate from week 9 to 15; 3.5 kg of concentrate from week 16 to 24 (total weight of concentrate = 710.5kg). Calves were allowed to suck residual milk to 12 weeks of age. Energy balance was estimated by measuring intake, milk yield and determining the digestibility of organic matter. The occurrence of ovulation was determined by the analysis of progesterone concentration in milk. All four treatments receiving additional supplementation were restored to positive energy balance while the Control treatment remained in negative energy balance. The percentage of cows ovulating was 36, 92, 92, 90 and 60 for Control and treatments 2,3,4 and 5, respectively (P=0.026). However, there was no particular association between the timing of ovulation and weaning. Nonparametric analysis comparing the timing of ovulation for combined results from Control + treatment 2 and combined results from treatments 3,4 and 5 estimated mean time to fail to ovulate as 10 ± 9.0

and 87 ± 7.6 days, respectively ($P=0.023$). The percentage of cows showing oestrus was 9, 33, 33, 40 and 40 for Control and treatments 2,3,4 and 5, respectively ($P=0.197$). Short-term increases in supplementation are unlikely to be an attractive means of reducing calving intervals.

Introduction

Maintaining good indices of fertility in cows of smallholder dairying schemes appears to be a widespread difficulty. Calving intervals of over 500 days are commonly reported, with delays in conception being in part the result of long periods of post partum anovulation and anoestrus. The cattle used in smallholder dairying are generally *Bos taurus* x indigenous breeds, that are often permanently housed to protect them from tick-borne and other diseases. As all forage must be cut and carried to the cows, they are frequently inadequately nourished and in poor body condition. In addition, it is not uncommon for cows to suckle calves during early to mid lactation, the calves either taking first and residual milk or being allowed to suck a teat throughout milking. The result of this suckling stimulus may well inhibit the hypothalamic-pituitary-ovarian axis, delaying follicular development and ovulation (Carter *et al*, (1980). However, the removal of the sucking stimulus at weaning should result in the rapid resumption of gonadotrophin release and follicular growth. Continuing ovarian quiescence may be a consequence of the cow being in negative energy balance or unduly emaciated (Williams, 1990). The experiment described here investigated the efficacy of a relatively brief period of enhanced supplementation of cows around the time their calves were weaned. The hypothesis tested was that cows restored to energy balance when the suckling stimulus was removed would ovulate in the immediately succeeding period.

Materials and Methods

Treatments

The experiment investigated the effects on the reproductive performance of crossbred dairy cows of a short-term increase in supplementation applied around the time that their calves were weaned at 12 weeks of age. The five treatments were as follows:

Control: Restricted forage: 3.5 kg concentrate per day (total weight of concentrate = 588 kg).

Treatment 2: Restricted forage: 2.5 kg concentrate per day from calving to week 9: 7 kg concentrate per day from week 10 to 15: 2.5 kg concentrate per day from week 16 to 24 (total weight of concentrate = 609 kg).

Treatment 3: Restricted forage: 3.5 kg concentrate per day from calving to week 9: 7.0 kg concentrate per day from week 10 to 15: 3.5 kg concentrate per day from week 16 to 24 (total weight of concentrate = 735 kg).

Treatment 4: Restricted forage: 3.5 kg concentrate per day from calving to week 8: 7.0 kg of concentrate from week 9 to 13: 3.5 kg of concentrate from week 14 to 24 (total weight of concentrate = 710.5 kg).

Treatment 5: Restricted forage: 3.5 kg concentrate per day from calving to week 8: 6 kg of concentrate from week 9 to 15: 3.5 kg of concentrate from week 16 to 24 (total weight of concentrate = 710.5kg).

Experimental design

Crossbred dairy cows (various *Bos taurus* breeds x Tanzanian Zebu) were blocked according to genotype (<75% *B. Taurus*; 75% *B.Taurus*; >75% *B. taurus*), parity (1st lactation; 2nd-5th lactation; 6th lactation and above), calving year and calving season . Cows were randomly allocated from blocks to treatments as they calved.

Management

Cows were tethered in a barn where they could be individually fed. Milking was carried out by hand twice daily (at 06.00 and 15.00 h) and calves were allowed to suck their dams for 30 min following each milking session until weaning at 12 weeks.

Feeds and feeding. Freshly-cut napier grass (*Pennisetum purpureum*) was offered to provide maintenance (E_M) for live weight at calving (W) as estimated by

$$E_M(\text{MJ/day}) = [0.53(W/1.08)^{0.67} + 0.0091W]/0.713$$

with a 15% safety margin allowed. The dry matter content of the napier grass was determined by oven drying at 105°C for 24h at the beginning of each plot. It was assumed that the average ME value for forage was 9.9 MJ/kg DM (FAO, 1991). The concentrate (67 parts maize bran: 33 parts coconut cake per kg) plus 100g dried bone

meal was given twice a day at the two milking sessions. Calves were provided with green forage and concentrate from three weeks of age until weaning.

Health care. Cows were dipped against ticks once weekly using Steladon®. Prophylactic treatments against trypanosomiasis was done using Samorin® at 3-month intervals.

Measurements

All feeds offered were weighed. Refusals were collected, DM content determined and intakes established. Milk yield was recorded daily at each milking. Calf weight gain was used to calculate milk intake by the calf, assuming that 1 kg of gain is equivalent of 9 kg of milk consumed (Drewery *et al*, 1959; Msanga, 1997). Calves were weighed at weekly intervals and their gain established by linear regression. Cows were weighed on the day of calving and thereafter on a weekly basis. Body condition scoring of cows was carried out weekly using a system derived from Lowman *et al*, (1973) and Pulan (1978).

Digestibility of the diets was estimated using eight cows on each dietary treatment. A separate digestibility value was established for each period of the study (i.e. calving to 8-9 weeks, 9-10 weeks to 13-15 weeks, 14-16 weeks to 24 weeks) approximately half way through each period. Total faeces were collected immediately after voiding for 24-h per day for eight consecutive days. Daily faeces production was bulked, mixed, weighed, and a sample dried at 105°C for 24 hours. The dried sample was then ashed (Association of Official Analytical Chemists (AOAC), 1985) to determine organic matter content.

Samples of the morning milk were taken three times weekly. A 10ml aliquot was mixed with 0.5 ml of 1% potassium dichromate solution, centrifuged for 15 minutes at 2000G, then left for 15 minutes to allow the fat to harden. The fat layer was pierced and the skim milk withdrawn to storage tubes. Progesterone concentrations were determined a self-coating radioimmunoassay procedure according to FAO/IAEA (1997). Sensitivity of the assay was 0.05 nmol/l while the intra and inter assay coefficients of variation were 7.8 and 12%, respectively.

Milk progesterone concentrations above 1 nmol/l for two consecutive samples were regarded as indicative of the presence of a corpus luteum. A penile deviated bull was allowed to walk through the cows after each milking session to detect oestrous cows.

A separate aliquot of the milk sample (not centrifuged) was used to determine milk protein and fat using procedures described by AOAC (1985).

The energy balance of each cow was computed using the expression

$$\text{Total ME}_{\text{intake}} = \text{ME}_{\text{maintenance}} + \text{ME}_{\text{milk synthesis}} \pm \text{ME}_{\text{liveweight change}}$$

and the equations and efficiency factors presented in McDonald *et al*, (1995).

Statistical analysis

Most data was analysed by analysis of variance according to the general linear model procedure in the Statistical Analysis System Release 612 (1996). The model adopted for most normally distributed datasets was

$$Y_{ijklm} = U + T_i + G_j + P_k + C_l + S_m + e_{ijklm}$$

where:

- Y_{ijklm} = the performance of an individual for a given variable
- U = the overall mean
- T_i = the i^{th} dietary treatment effect
- G_j = the j^{th} genotypic effect
- P_k = the k^{th} parity effect
- C_l = the l^{th} year of calving effect
- S_m = the m^{th} season of calving effect
- e_{ijklm} = the effect of a random error term

Body condition score at calving and at weaning were also included as factors in the model in the analysis of some lactation and reproduction variables. The live weights of the cows at calving and birth weights of calves were used as covariates in the analysis of liveweight changes.

The control and treatment 2 were pooled to form L group and treatments 3,4 and 5 pooled to form H group of treatments. Contrasting between L and H groups of supplementation was done using a contrast estimate statement in a general linear

model procedure which had a provision for not only contrasting between levels but also within each level.

Body condition score and condition score changes were analysed using a logistic regression using the probit procedure of SAS (1996). The GLM procedure was employed to estimate mean body condition and condition score change at different times of the study.

Because all cows did not ovulate during the 24-weeks of observation, a non-parametric survival technique, the Kaplan-Meier method (MINITAB Release 13.20 for Windows98, 2000) was employed. Chi-square analysis was also used to test the differences in the number of cows showing reproductive events between treatments.

Results

It was originally intended that 85 cows should be represented in the experiment but for various reasons only 65 cows were recruited. Of these, five cows died and 5 cows were removed because of milk yields below 3 litres per day. The five cows died from trypanosomiasis (1), mixed infection of east coast fever and pyometra (1), mastitis (1), anaplasmosis (1) and milk fever (1). Three calves died during the study.

Energy balance

Table 1 shows the estimated energy balances ($ME_{\text{intake}} - [ME_{\text{maintenance}} + ME_{\text{milk synthesis}}]$) of the cows during the three experimental periods.

Table 1: Effect of dietary treatment on estimated energy balance ($ME_{\text{intake}} - [ME_{\text{maintenance}} + ME_{\text{milk synthesis}}]$) during the three periods of the experiment

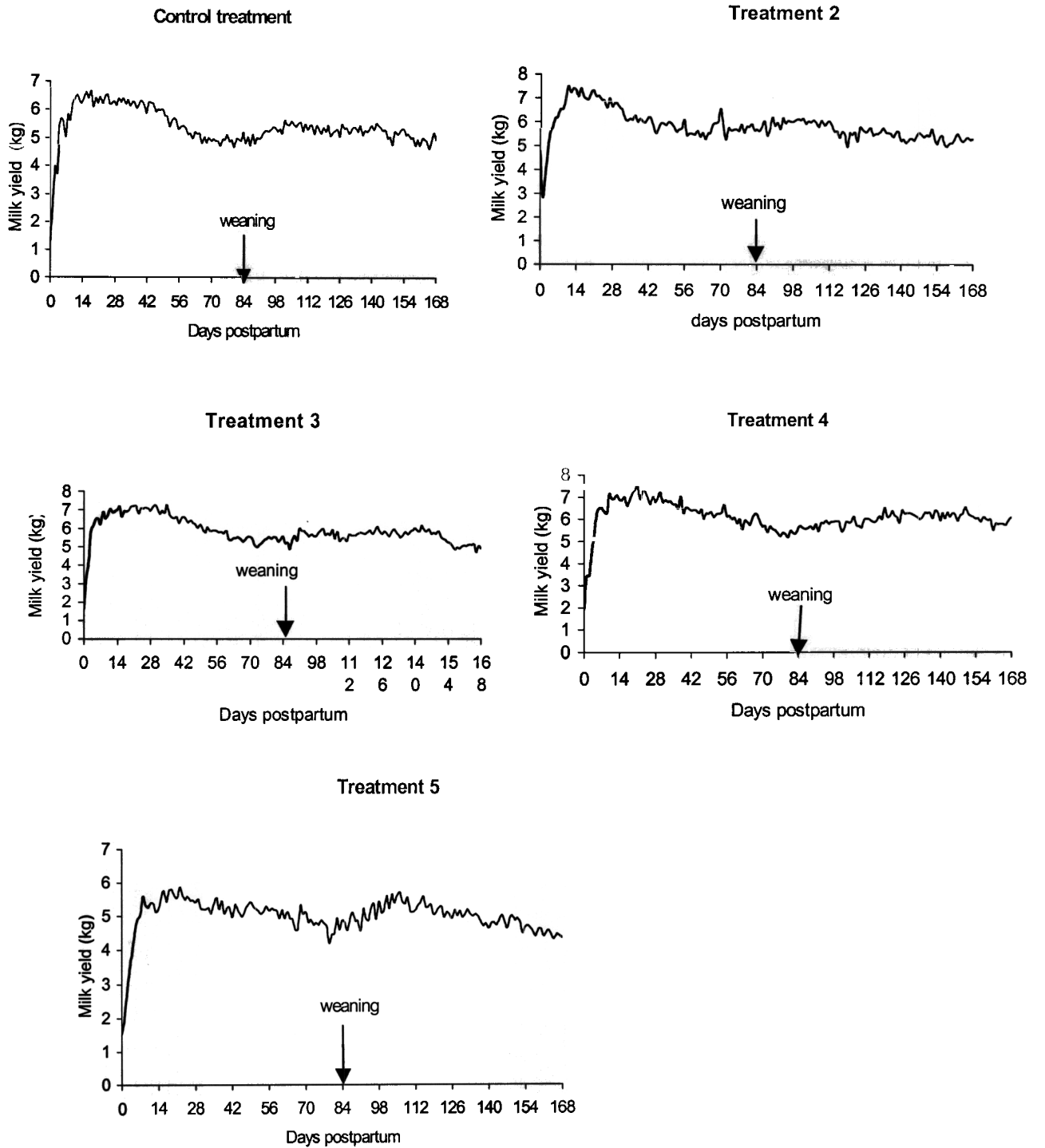
	Low level		High level			SED
	T1	T2	T3	T4	T5	
No. of cows	11	12	12	10	10	
Energy balance (MJ/day) calving to 8-9 weeks	4.2	-9.2	-7.7	-3.5	5.8	12.51
Energy balance (MJ/day) 9-10 to 13-15 weeks	-9.4 ^a	10.6 ^b	13.2 ^b	7.0 ^{ab}	5.2 ^{ab}	9.20
Energy balance (MJ/day) 14-16 to 24 weeks	13.2	5.6	22.6	12.1	15.2	7.45

Means with different subscript letters within a row are statistically significantly different ($P < 0.05$)

Overall, energy balance improved as lactation progressed, mainly as a result of increased ME intakes. During the first 8-9 weeks of lactation, the control and treatment 5 were estimated to be in positive energy balance, apparently because of lower milk production than other treatments. However, there were no statistically significant differences between dietary treatments. During the period of supplementation, all cows receiving the additional supplement achieved positive energy balance compared to the negative energy balance of control cows. Significant differences occurred between control cows compared to treatments 1 and 2 ($P < 0.05$). In the final period of the project all treatments were estimated to be in positive energy balance and there were no significant differences between treatments.

The mean daily milk yield over the 24-week experiment was 6.7 (SD = 1.33) kg. Dietary treatment had no significant effect, neither on mean milk yield nor on yield during the supplementation period. The only factor included in the model to contribute significantly to variation in milk yield was the parity of the cows. Figure 1 shows the lactation curves recorded for the five treatments over the 24-week period. A common feature is the secondary peak in yield occurring following the weaning of the calves.

Figure 1: Mean daily milk yield for 168 days of lactation.



Although estimates of energy balance indicate energy retention as a result of supplementation, this could not be detected in either liveweight changes (Figure 2) or body condition score changes (Figure 3). Indeed, both live weight and body condition score tended to decline throughout much of the 24-week experiment. Dietary treatment made no significant contribution to variation in either liveweight change or body condition score change in any of the experimental periods.

Reproduction

Table 2 shows a summary of the number of cows that ovulated, showed oestrus and conceived during the 24-week study period. Of the 55 cows, 67% ovulated, 26% showed oestrus and 7% conceived. The number of cows that ovulated was significantly different between the L (48%) and H (81%) dietary treatment groups (P=0.009). Comparison between individual dietary treatments also showed significant differences (P=0.026), treatments 2, 3 and 4 being particularly successful in comparison to the control. Similarly, the number of cows showing oestrus was significantly different between the L (22%) and H (38%) dietary treatment groups (P=0.016) although there were no significant differences (P=0.197) between individual dietary treatments.

Table 2: The effect of dietary treatment on the number of cows ovulating, showing oestrus and conceiving during the first 24 weeks of lactation

	Low level		High level			Total	F value	df	Pr>F
	T1	T2	T3	T4	T5				
No.of cows	11	12	12	10	10	55			
No.(%)ovulated	4(36)	11(92)	11(92)	9(90)	6(60)	37(67)	11.039	4	0.026
No.(%) showed oestrus	1(9)	4(33)	4(33)	4(40)	4(40)	14(26)	6.029	4	0.197
No.(%) conceived	0(0)	2(17)	2(17)	1(10)	1(10)	4(7)	3.595	4	

There was no striking evidence that weaning had any causative role in the occurrence

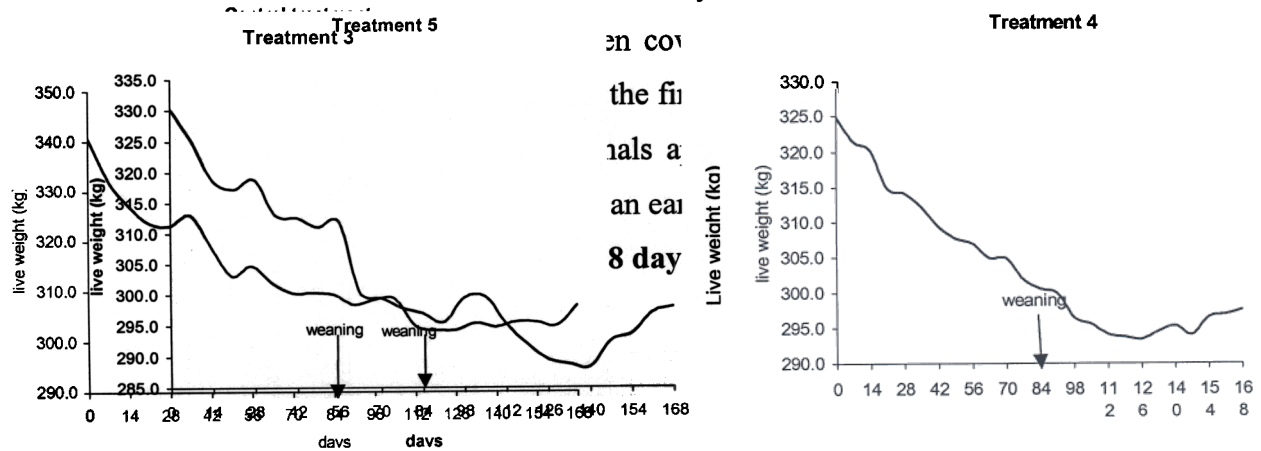
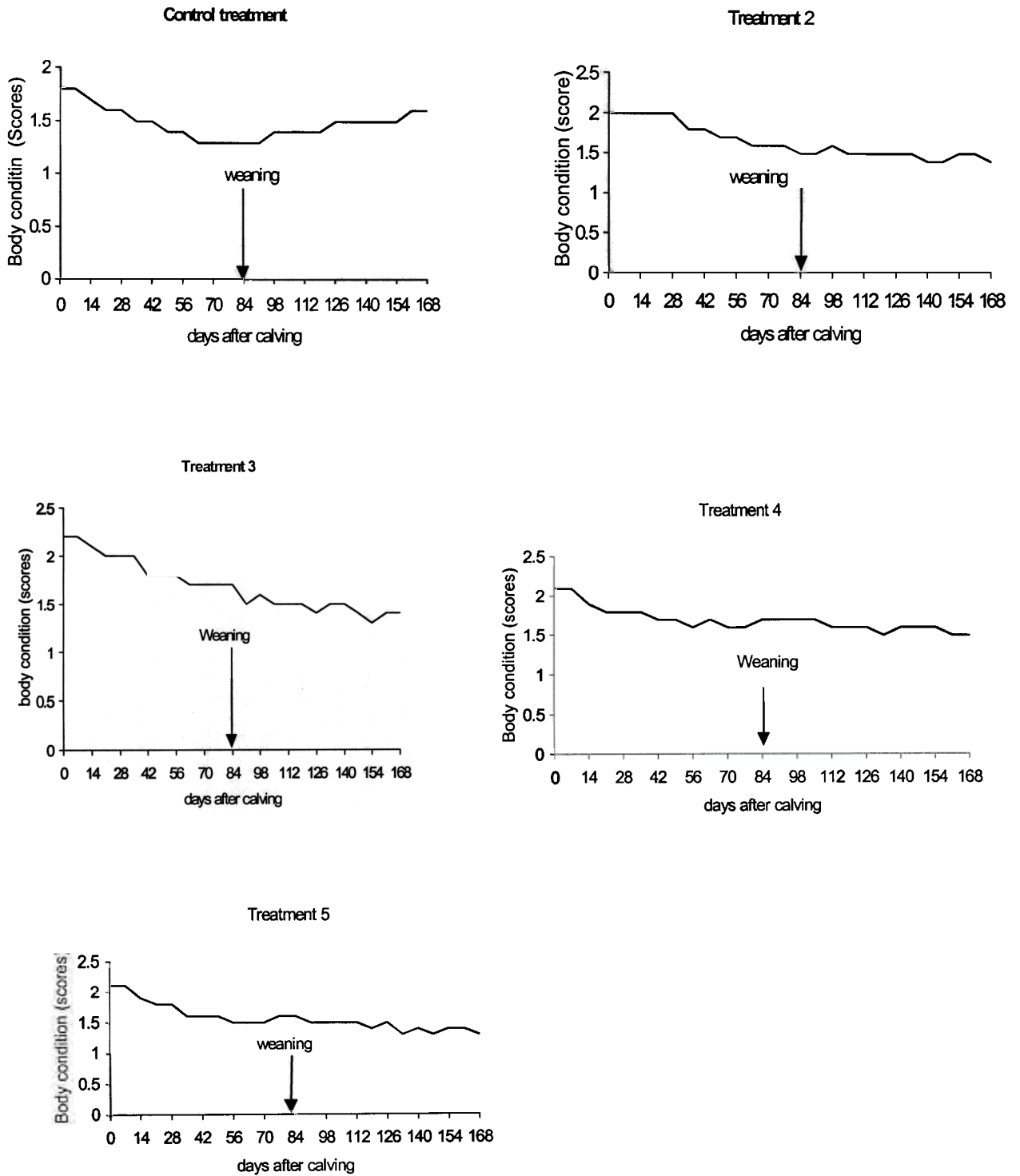


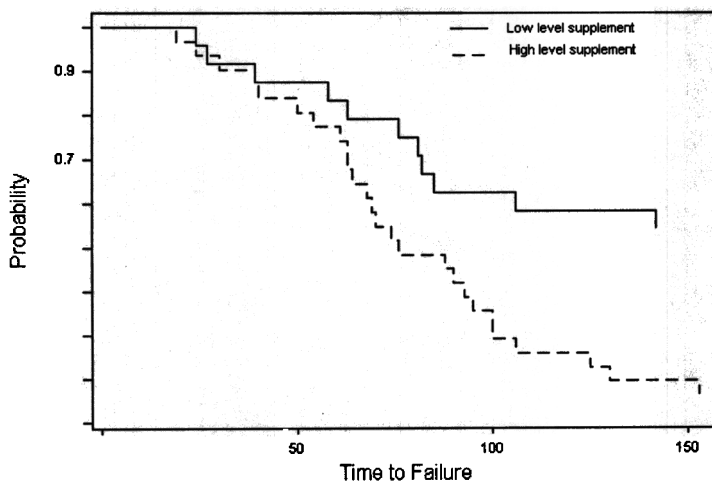
Figure3: Mean body condition score of cows for 168 days of lactation



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Data on days to first rise in plasma progesterone concentration did not differ significantly from a normal distribution. Results show that the mean number of days to first rise was 74 (SD=33.0). Neither dietary treatment nor any other factor included in the model contributed significantly to the variation. Non-parametric survival analysis comparing individual dietary treatments also showed no significant differences but when L and H groupings were compared, the Wilcoxon comparison of survival plots showed there was a significant difference ($\chi^2 = 5.193$; $df = 1$; $P = 0.023$). The survival plots are shown in Figure 4. The mean time for a cow to fail to ovulate was 110 ± 9.0 days in the L grouping compared to 87 ± 7.6 days in the H grouping.

Figure 4: Nonparametric survival plots for the probability of failure to ovulate within 168 days for treatment 1&2 (L) and treatments 3,4 and 5 (H).



Discussion

Increasing supplementary feeding from 8-9 weeks after calving was successful in increasing energy retention in cows. Over the course of this enhanced period of energy intake, ME appeared to be channelled towards body tissue rather than stimulating increased milk yield. The event of weaning the calf appeared to be of greater significance to milk yield and may have masked any small effects of

supplementation. Bryant and Msanga (1999) have also reported enhanced daily yields following weaning, resulting in significant improvements in the persistency of the lactation curve.

The failure to detect increased energy retention either by liveweight change or changes in body condition score is surprising. For example, the cows of treatment 3 were given an additional supplement of 122.5 kg of concentrate from weeks 9-13. Assuming a DM concentration of 0.85 and a ME concentration of 11 MJ/kg DM, the animals would have consumed an additional 1250 MJ ME. Assuming 1 kg of gain is equivalent to 26 MJ ME, then the cows on average would have gained 48 kg of live weight. In practice the cows lost weight, although the estimates of energy retention suggested a positive energy balance of 13.2 MJ per day. This would have been equivalent to a gain of approximately 18 kg over the 5-week period. Liveweight changes may not reflect energy retention as gains or losses in live weight may be achieved by changes in gut fill, changes in fluid retention, or transfers between tissues differing in energy concentration. All of these are possible in lactating cows.

The results do suggest that increased supplementation and the resulting positive energy balance was effective in promoting the occurrence of ovulation. The importance of cows being in positive energy balance or at least being in a stage of transition from negative to positive energy balance for the resumption of ovulatory activity is stressed by Butler (2000). The results from treatment 2 are of particular interest. In this treatment the strategy was to redistribute a fixed input of concentrate supplement rather than give additional supplement. The strategy appears to have been successful in that the results obtained were similar to those achieved by giving more concentrate. However, few cows showed oestrus on any treatment during the experimental period and even fewer conceived. A feature of the ovulatory activity of some cows was a series of cycles where ovulation was not accompanied by oestrus. Of 25 cows that ovulated before day 100 post partum, 17 failed to show oestrus before the end of the experiment. These cows commonly showed a series of 5-6 silent ovulations. Mukasa-Mugewa *et al* (1997), working with zebu cattle in Ethiopia, recorded similar observations. Almost all their cows had between one and eight silent ovulations before first oestrus. The interval to the first silent ovulation was 44 days, some three months prior to the first observed oestrus. In the present experiment, the

higher level of concentrate offer did significantly improve the numbers of cows showing oestrus, suggesting that energy balance is implicated in moderating the occurrence of oestrous behaviour. However, the relationship between energy balance and oestrous behaviour appears to operate at a higher threshold than the relationship between energy balance and ovulation. Alternatively, as the first ovulations following parturition are normally not accompanied by oestrous behaviour (Peters and Ball, 1995), oestrous behaviour as an event can be seen as temporally lagging behind ovulation. In a situation of short term supplementation, it could be that oestrous behaviour is less likely to benefit as the period of supplementation may have ceased before the event is due to take place.

While it would seem that short term supplementation may promote earlier ovulation in post partum crossbred dairy cows, it may well be of little advantage in reducing calving intervals because of what appears to be a continuing inhibition to the occurrence of oestrous behaviour. This may be a consequence of the withdrawal of the additional supplementation or of the continuing poor body condition score of the cows. Since the additional supplementation does not manifest itself in increased milk yields, there are no reasons for recommending short-term supplementation as a practical nutritional strategy.

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