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Short-term physiological effects of refeeding 5 to 10-day-old calves after fasting and transport

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ABSTRACT

The short-term physiological responses of 5 to 10-day-old calves to refeeding after 30 hours without food and up to 12 hours of transport were assessed by monitoring packed cell volume and plasma concentrations of total protein, glucose, triglycerides, urea and lactate. Samples were taken immediately before refeeding after three and 12 hours of transport and at the end of 30 hours without food, and in each case three hours later. Refeeding after fasting caused replenishment of plasma glucose concentrations to control levels and in some cases beyond. It was suggested that the overshoot in glucose concentrations, when it occurred, may have been caused by a temporary increase in insulin resistance as a result of metabolic adaptation to starvation. Refeeding after 12 hours transport also resulted in increased glucose concentrations. Thus, refeeding after transport and starvation was beneficial to the calves as it enabled rapid replenishment of depleted energy reserves.

Keywords: Calves; transport; fasting; refeeding, hydration state, energy reserves.

INTRODUCTION

For most domestic animals transportation involves both novel and aversive events including social regrouping, physical injury, food and water deprivation, temperature extremes, humidity, noise and motion (Leach, 1982; Tarrant and Grandin, 1993). The adversity of such stressors influences the ability of the animal to recover following transport. Knowles *et al.* (1993) identified three stages of recovery in lambs. By 24 hours after transport the blood variables associated with stress and dehydration had returned to normal and the levels of lactate had fallen. By 96 hours, live weight and concentrations of parameters associated with energy metabolism had stabilised and by 144 hours a full recovery had occurred (Knowles *et al.*, 1993). Several studies have investigated the effects of transport and starvation on calves of all ages (Soissons *et al.*, 1982; Kent and Ewbank, 1986; Cole *et al.*, 1986, 1988; Tarrant *et al.*, 1992; Warriss *et al.*, 1995), but few have looked at the effects of refeeding as a means of aiding recovery. Knowles *et al.* (1997) found that providing young calves with a mid-journey feed did reduce the effects of food and water deprivation, however, the advantages of disrupting the journey for these minor benefits were questionable. The purpose of the present study was to examine the short-term (3-hour) physiological responses of young calves to refeeding following transport and starvation.

MATERIALS AND METHODS

This study was conducted as part of a larger trial reported by Todd *et al.* (2000). Seventy-six Friesian bull calves, aged one to five days with an average weight of 40 kg when picked up from farms, were used in this study, which was conducted as two identical experiments, two weeks apart. Calves were aged between five and ten days on the first day of the experiment. For each experiment, calves were penned in groups of four or five (1.70 m x 2.43 m) in a well-ventilated shed. Calves had *ad libitum* access to water and pelleted feed (Harvey Farms Topcalf Starter, Levin) and were fed colostrum-free milk.

Calves were allocated to one of eight treatments such that their ages and weights were evenly distributed across groups: C – control, HF – half feed, FF – full feed, 3T – 3 hours transport, 3TR – 3 hours transport then refed, 12T – 12 hours transport, 12TR – 12 hours transport then refed and 12TLD – 12 hours transport at low stocking density.

All groups were fed at 0630 h and again 31 hours later. Calves were fed 50 ml/kg except group HF that received 25 ml/kg at 0630 h. The control group and group 12TR were refed at 13 hours and group 3TR at 4 hours, with 50ml/kg. The stocking density on the truck was 0.2 m^2/calf for groups 3T, 3TR, 12T and 12TR, and 0.4 m^2/calf for group 12TLD (Table 1).

Packed-cell volume (PCV) was estimated by microhaematocrit technique shortly after the blood sample was obtained. The blood was then centrifuged for 20 minutes and the plasma removed and stored at –20 ^{0}C until required for the analysis of total plasma proteins (TPP), glucose, triglycerides (TG), urea and lactate (Todd *et al.*, 2000).

In all groups, blood samples were taken by jugular venipuncture into heparinised vacutainers at zero hours (6 am) before calves were fed. In groups C, HF, FF, 3T, 12T and 12TLD blood samples were taken at 31 and 34 hours for analysis of PCV and plasma concentrations of TPP, glucose, TG and urea. In groups 3T and 3TR, blood samples were taken at 4 and 7 hours and in groups 12T and 12TR at 13 and 16 hours for analysis of PCV and plasma concentrations of glucose and lactate. Blood samples from transported calves were taken immediately before loading and immediately before unloading at the end of the journey. Between samples, calves were transported over a variety of terrains.

Results are either presented as mean values ± the standard error of the mean (SEM) or as mean changes ± SEM over time. A one-way ANOVA and Bonferroni's post-test for equal variances or Tamhane's T2 post-test for unequal variances were used to determine differences between groups after fasting. A two-way factorial ANOVA was used to analyse the effects immediately after transport,

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TABLE 1: Summary of treatment groups for calves that were starved and transported prior to refeeding.

	TREATMENT								
GROUP	fed 50ml/kg at 6.30am	fed 25ml/kg at 6.30am	fed 50 ml/kg every 12h	unfed for 30h	transported for 3h, density 0.2m²/calf	transported for 12h, density 0.2m²/calf	transported for 12h, density 0.4m²/calf	fed 50ml/kg after transport	fed 50ml/kg at 31h
C (n=8)	+	-	+	-	-	-	-	-	+
HF (n=10)	-	+	-	+	-	-	-	-	+
FF (n=9)	+	-	-	+	-	-	-	-	+
3T (n=10)	+	-	-	+	+	-	-	-	+
3TR (n=10)	+	-	-	-	+	-	-	+	+
12T (n=10)	+	-	-	+	-	+	-	-	+
12TR (n=10)	+	-	-	-	-	+	-	+	+
12TLD (n=9)	+	-	-	+	-	-	+	-	+

C - control, HF - half feed, FF - full feed, 3T - 3 hours transport, 3TR - 3 hours transport then refed, 12T - 12 hours transport, 12TR - 12 hours transport then refed, 12TLD - 12 hours transport, low stocking density.

of transport time and refeeding. Differences were considered significant when $P < 0.05$. Statistical analysis was performed using SPSS version 9.0.1.

RESULTS

Effects of refeeding following 30 hours fasting

Mean PCV and plasma concentrations of TPP, glucose, TG and urea were not significantly different between groups at zero hours. Mean concentrations of these parameters at 31 hours following fasting and transport are shown in Table 2. After refeeding at 31 hours, no significant changes were seen in PCV or the concentrations of TPP and TG over the next 3 hours (Table 3) but there were significant increases in urea concentrations from calves in groups FF and 12TLD (Table 3). Plasma glucose concentrations of calves in group C remained unchanged between 31 and 34 hours (Fig. 1) and were significantly greater than the glucose concentrations of calves in fasted groups (HF, FF, 3T, 12T, 12TLD) at 31 hours (Fig. 1). The low glucose concentrations of fasted calves returned to control levels between 1 and 2 hours after refeeding and in some cases were still increasing at 34 hours (Fig. 1).

Effects of refeeding following 3 and 12 hours transport

Mean plasma concentrations of lactate were not significantly different between groups at zero hours. Mean PCV and plasma concentrations of glucose and lactate after transport are shown in Table 4. There was a significant effect of transport duration on PCV but no effect of refeeding (Fig. 2). A decrease in PCV occurred during the three hours after transport in both fed (3TR) and unfed (3T) calves, but no significant change was observed during the three hours after calves were transported for 12 hours (12T, 12TR) (Fig. 2). Plasma glucose concentrations significantly increased in 3T calves during the 3 hours following transport and decreased in 12T calves (Fig. 2). A significant rise in glucose concentrations due to refeeding was seen in calves transported for both 3 and 12 hours (Fig. 2). Plasma lactate concentrations increased significantly in calves that were fed after 3 (3TR) and 12 hours (12TR) of transport (Fig. 2).

TABLE 2: Mean (± SEM) packed cell volume (PCV) and plasma concentrations of total protein (TPP), glucose, triglycerides (TG) and urea in calves at 31 hours following 30 hours fasting and up to 12 hours transport.

Group	PCV (%)	TPP (g/l)	Glucose (mmol/l)	TG (mmol/l)	Urea (mmol/l)
C	33.6 ± 1.54	68.5 ± 4.21	5.7 ± 0.27[a]	0.3 ± 0.04	4.1 ± 0.41
HF	36.7 ± 1.03	68.1 ± 2.91	3.3 ± 0.14	0.2 ± 0.02	3.7 ± 0.24
FF	35.2 ± 1.96	67.6 ± 3.77	3.2 ± 0.19	0.2 ± 0.01	3.9 ± 0.35
3T	35.1 ± 1.21	69.5 ± 4.09	3.2 ± 0.14	0.2 ± 0.02	3.9 ± 0.33
12T	33.7 ± 1.71	63.3 ± 4.27	3.6 ± 0.24	0.2 ± 0.03	3.5 ± 0.52
12TLD	34.0 ± 1.82	68.2 ± 2.21	3.5 ± 0.20	0.3 ± 0.06	3.3 ± 0.33

C - control, HF - half feed, FF - full feed, 3T - 3 hours transport, 12T - 12 hours transport 12TLD - 12 hours transport, low stocking desnity.
[a] significantly different from HF, FF, 3T, 12T, 12TLD

TABLE 3: Mean changes (± SEM) in packed cell volume (PCV) and plasma concentrations of total proteins (TPP), triglycerides (TG) and urea in calves after 30 hours fasting and up to 12 hours transport. The changes were from 31 to 34 hours following refeeding at 31 hours.

Group	PCV (%)	TPP (g/l)	TG (mmol/l)	Urea (mmol/l)
C	0.3 ± 0.28	0.4 ± 1.01	0.0 ± 0.64	0.2 ± 0.12
HF	-0.9 ± 0.68	-0.1 ± 1.48	0.0 ± 0.08	0.5 ± 0.24
FF	0.5 ± 0.72	0.2 ± 0.89	0.1 ± 0.07	0.5 ± 0.14*
3T	-0.2 ± 0.73	-2.9 ± 1.83	0.2 ± 0.14	0.3 ± 0.27
12T	-0.9 ± 0.62	0.2 ± 1.64	0.4 ± 0.34	0.2 ± 0.21
12TLD	-0.1 ± 0.43	-1.2 ± 1.08	0.2 ± 0.23	0.4 ± 0.08*

C - control, HF - half feed, FF - full feed, 3T - 3 hours transport, 12T - 12 hours transport 12TLD - 12 hours transport, low stocking density.
* significantly different from zero

FIGURE 1: Mean changes in plasma glucose concentrations (± SEM) of calves fasted for 30 hours and transported for up to 12 hours. The changes were from 31 to 34 hours following refeeding at 31 hours. C - control, HF - half feed, FF - full feed, 3T - 3 hours transport, 12T - 12 hours transport, 12TLD - 12 hours transport, low stocking density.
* significantly different from HF, FF, 3T, 12T, 12TLD

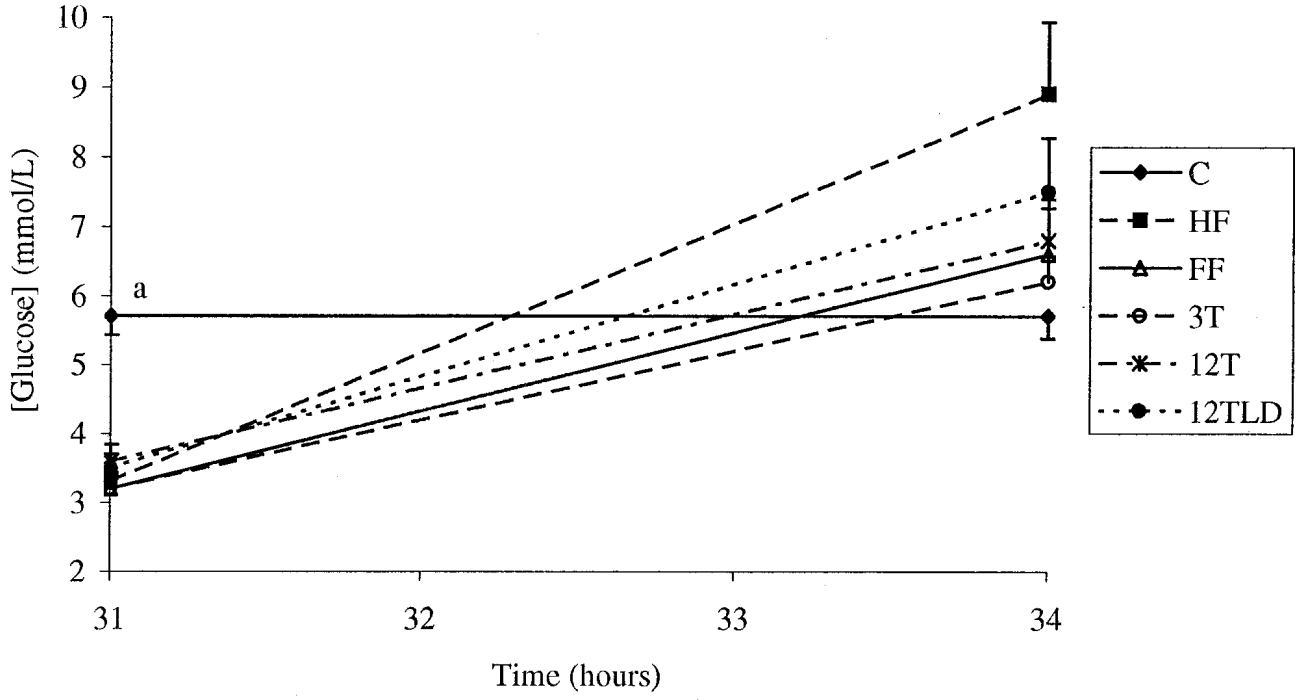
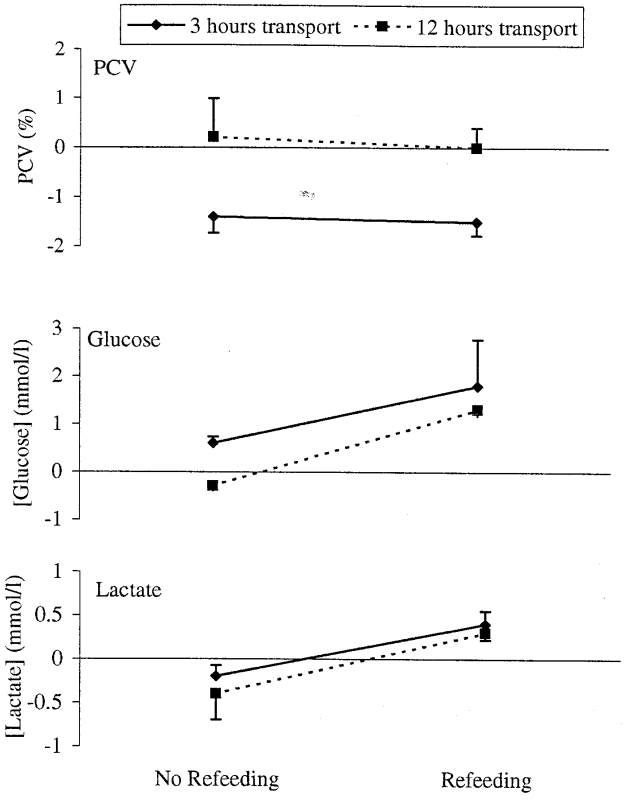


TABLE 4: Mean (± SEM) packed cell volume (PCV) and plasma concentrations of glucose and lactate in calves after 3 and 12 hours transport.

Group	PCV (%)	Glucose (mmol/l)	Lactate (mmol/l)
3T	35.1 ± 0.90	5.2 ± 0.18	1.3 ± 0.13
3TR	37.5 ± 1.71	5.2 ± 0.30	1.1 ± 0.08
12T	35.4 ± 2.12	5.7 ± 0.30	1.5 ± 0.21
12TR	34.8 ± 1.92	5.7 ± 0.24	1.1 ± 0.07

3T - 3 hours transport, 3TR - 3 hours transport then refed, 12T - 12 hours transport, 12TR - 12 hours transport then refed.

FIGURE 2: A comparison of the effects of transport duration (3 or 12 h), and refeeding after transport, on the mean changes (± SEM) in packed cell volume (PCV) and plasma concentrations of glucose and lactate in calves. The changes represented by each point occurred between 4 and 7 hours (3T and 3TR) or between 13 and 16 hours (12T and 12TR). Lines indicate transport duration. Points on the left represent changes in parameters following transport in calves that were not refed (3T and 12T), and points on the right, the changes in calves that were refed (3TR and 12TR).
3T - 3 hours transport, 3 hours transport then refed, 12T - 12 hours transport, 12TR - 12 hours transport then refed.



DISCUSSION

Effects of refeeding following 30 hours fasting

Todd *et al.* (2000) found that 30 hours fasting with or without transport was associated with depleted energy reserves. Low plasma glucose concentrations and increased plasma β-hydroxybutyrate concentrations in fasted calves indicated utilisation or depletion of liver glycogen and mobilisation of lipid. However, a lack of change in plasma urea concentrations suggested there was still a sufficient reserve of energy substrates to minimise the need to catabolise amino acids (Todd *et al.*, 2000).

In the present study, after 30 hours starvation and following refeeding, plasma glucose concentrations of fasted calves showed a very rapid return to normoglycemia and in some calves there was evidence of an overshoot (Fig. 1). The reason for this overshoot in glucose concentrations following refeeding is unknown, but it is possible that a temporary increase in insulin resistance may occur while the animal makes the transition from a starved to a fed state. Insulin concentrations were not measured in the present experiment so we cannot actually associate the change in glucose concentrations with insulin resistance. However,

fasting was found to cause insulin resistance in rat adipose tissue both in vitro (Huber *et al.* 1965) and *in vivo* (Goldman and Cahill, 1964). This may be a mechanism to prioritise the use of available glucose for those tissues and organs that require glucose as their primary energy substrate. Hypothetically, if insulin resistance had developed in the fasted calves in our study, the rate of glucose clearance from the plasma may have been slow, which could explain the overshoot in glucose concentrations in some calves. Huber *et al.*, (1965) reported a similar finding in rat adipose tissue in which insulin sensitivity was rapidly restored to fed levels, often with an overshoot following refeeding.

There was no evidence of lipid mobilisation following refeeding as indicated by changes in plasma TG concentrations (Table 3). Although there were significant changes in plasma urea concentrations in FF and 12TLD calves (Table 3), these increases were minimal. The lack of change in these parameters suggests that lipid and amino acid by-products were not required for use as energy substrates within the 3-hour period of sampling.

Normal levels of hydration were maintained throughout the fasting period (Todd *et al.*, 2000), and no effect of refeeding was seen on hydration state (Table 3).

Effects of refeeding following 3 and 12 hours transport

The increase in plasma glucose concentrations of 3T calves was likely to have been the result of a stress effect caused by disturbances at the beginning of the experiment to which the animals had not yet become accustomed (Todd *et al.*, 2000). This was also reflected in the decreased PCV levels in 3T and 3TR calves (Fig.2). The increase in glucose concentrations in 3TR and 12TR calves after refeeding followed a typical pattern seen in both calves (Siddons *et al.*, 1969) and lambs (Mellor, 1987) in which concentrations increased for up to 3 hours before declining again. The decrease in glucose concentrations of 12T calves was expected (Fig.2) as in fasted calves, glucose concentrations begin to decline after 12 hours without food (Todd *et al.*, 2000). An increase in plasma lactate concentrations occurred only in refed calves (3TR and 12TR) (Fig. 2), implying a lactate response to feeding rather than transport. This transient response has also been seen in lambs (Mellor, 1987) and is probably a metabolic consequence of glucose flooding into the system following hypoglycemia.

Refeeding calves immediately after transport had no apparent effect on their hydration state as indicated by PCV (Fig. 2).

As indicated by the parameters measured in this study, the short-term responses of calves seen here suggest that refeeding following transport and starvation rapidly acts to replace depleted energy reserves and, therefore, may be beneficial to the welfare of the animal by increasing recovery rate.

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