THE EFFECTS OF FASTING AND TRANSPORT ON CALVES.

A Thesis presented in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE

at Massey University

Sarah Elizabeth Todd

February 1998

ACKNOWLEDGEMENTS

Many people have provided invaluable input to this thesis. Their contributions of knowledge and inspiration, time, encouragement, and support - financial and otherwise, are gratefully acknowledged.

My supervisors Professor David Mellor, Dr Kevin Stafford and Professor Neville Gregory equipped me with the necessary skills to complete this thesis, maintained my focus and invested much time providing knowledge, encouragement and patience.

Ricci Wesselink, who provided moral support and empathy, together with Noel Rutherford, contributed many hours of assistance caring for calves and running experiments.

Robert Bruce provided practical advice on calf husbandry and much entertainment during experiments. Cheryl McMeekan, Kate Littin, Mark Simpson, Jo Robins, Chris Rogers, Rebecca Osborne, David Simcock and Geoff Orbell offered companionship and support and contributed generous amounts of time during experiments.

Neil Ward organised equipment, provided logistical advice, technical support and practical assistance.

Barry Parlane, Martin Chesterfield and Shaun Wilson provided the experimental site and animals. Bruce Cann provided the video recorders and Tony Fox took the photos. Glen Oroua Livestock provided the vehicle and driver for transportation of calves during the experiments.

Keith Thompson, Rosalind Power and Sheryl Bayliss analysed the samples at Massey University. Mariette Komene and Mary Lorier analysed the samples at the Animal Health Laboratory, Ruakura.

This thesis would not have been possible without the financial support of the Ministry of Agriculture (MAF Policy). The funding for this work and the support given by David Bayvel are gratefully acknowledged.

I would like to thank my family who, in different ways, have provided support and reassurance whenever it was needed, and Dave, who with much patience and understanding has been my mainstay during this time.

And finally, I would like to thank AWAC for providing the opportunity to establish an acceptable standard of living for bobby calves and all domestic animals in our care.

Prior approval was obtained (from the Massey University Animal Ethics Committee) to conduct all experiments described in this thesis.

ABSTRACT

The welfare of domestic animals is becoming increasingly important in New Zealand. Consequently, Codes of Recommendations and Minimum Standards are produced by the Animal Welfare Advisory Committee to maintain adequate standards of welfare for animals in our care. These Codes are updated to incorporate new knowledge which may improve welfare standards further. There is one such Code for the welfare of bobby calves. In New Zealand, calves born to dairy cows that are not required for replacements in the herd are slaughtered for human consumption. These 'bobby calves' are exposed to a number of factors which have the potential to compromise welfare. Work in this thesis is based on the recommendations and minumum standards given in the bobby calf code. The general aim of this work was to investigate the combined effects of transport and nutrition on bobby calves, and to assess the ability of the calves to operate within their physiological capacity without significant welfare compromise during the bobby process.

In this study the metabolic effects of feeding and fasting hand-reared dairy calves aged 1-2 weeks were examined over a period of 30 hours. Parameters used to assess the response to feeding and fasting included PCV and plasma concentrations of total proteins, glucose, triglycerides, beta-hydroxybutyrate and urea. In mild climatic conditions and with access to water at all times, it was found that feeding calves the recommended volume of colostrum or milk at 12 hourly intervals was sufficient to maintain high glucose concentrations between feeds. A period of 30 hours without food had minimal adverse effects on calves as they were able to maintain energy levels during this time without excessive use of endogenous energy reserves. There was no evidence to suggest that significant dehydration had occurred.

Work in this study included examination of the metabolic effects of transport duration and stocking density in calves that were deprived of food for 30 hours. PCV and plasma concentrations of total proteins, glucose, triglycerides, beta-hydroxybutyrate, urea, creatine phosphokinase and lactate were measured. Three hours of transport at the recommended stocking density ($0.2m^2$/calf) caused minimal adverse effects in food-

deprived calves. Food-deprived calves transported for 12 hours at the recommended density maintained normoglycemia for 6 hours longer than non-transported food-deprived calves. This was thought to be caused by a mild increase in physical activity resulting from the need to maintain balance during transport. Thus the physical activity probably produced a glucose-sparing effect by mobilising muscle glycogen. The response of food-deprived calves transported for 12 hours at half the recommended density ($0.4m^2$/calf) was similar to that of non-transported, food-deprived calves. This suggests minimal physical activity occurred at the lower stocking density and this was attributed to the fact that most of these calves lay down during transport.

In this study the initial metabolic responses of calves to feeding were evaluated after 30 hours of food-deprivation in transported and non-transported calves, and immediately after transport of 3 or 12 hours duration. Parameters measured included PCV and plasma concentrations of total proteins, glucose, triglycerides, urea and lactate. Feeding after 30 hours without food apparently caused a decrease in glucose clearance. It is thought that this may have resulted from a metabolic overcompensation due to delayed adjustment of hormones and metabolites from the starved state to the fed state. Feeding immediately after transport restored plasma glucose levels to be within the normal range within 3 hours.

As indicated by the parameters measured in this study, hand-reared dairy calves appear to tolerate the combined effects of transport and food-deprivation quite well. However the present experiments were conducted in mild climatic conditions. Air temperatures ranged from 7-13 °C and there was little wind or no rain. In situations of climatic extremes, the physiological capacity of calves to withstand the bobby process may not be as great. At higher temperatures there is a risk of dehydration. At lower temperatures, especially combined with wind and rain, an increased metabolic rate may be required to fuel heat production so that endogenous body reserves may not last as long during times of food-deprivation.

TABLE OF CONTENTS

THE EFFECTS OF FASTING AND TRANSPORT ON CALVES.....i
ACKNOWLEDGEMENTS.....ii
ETHICAL APPROVAL.....iv
ABSTRACT.....v
TABLE OF CONTENTS.....vii
LIST OF FIGURES.....x
LIST OF TABLES.....xv

CHAPTER 1: GENERAL INTRODUCTION.....1
1.1 WELFARE OF THE BOBBY CALF.....1
1.2 METABOLIC TRANSITIONS IN PRE-RUMINANT CALVES.....3
1.3 USE OF METABOLIC PARAMETERS.....9
1.4 OUTLINE OF THESIS.....10

CHAPTER 2: THE EFFECTS OF FEEDING AND FASTING HAND-REARED DAIRY CALVES AGED 1-2 WEEKS.....12
2.1 SUMMARY.....12
2.2 INTRODUCTION.....13
2.3 MATERIALS AND METHODS.....16
2.3.1 Animals.....17
2.3.2 Groups.....20
2.3.3 Experimental Procedures.....21
2.3.4 Methods.....22
2.3.5 Blood Plasma Measurements.....23
2.3.6 Presentation of Results.....23
2.4 RESULTS.....28
2.4.1 General Clinical State At Pick-Up.....28
2.4.2 Indices Hydration State.....29
2.4.3 Indices of Metabolic State.....30

2.4.4 General Clinical State Throughout the Experiment....31
2.5 DISCUSSION....39

CHAPTER 3: THE EFFECTS OF ROAD TRANSPORT ON UNFED, HAND-REARED DAIRY CALVES AGED 1-2 WEEKS....47
3.1 SUMMARY....47
3.2 INTRODUCTION....48
3.3 MATERIALS AND METHODS....49
3.3.1 Transportation....49
3.3.2 Groups....50
3.3.3 Experimental Procedures....52
3.3.4 Blood and Plasma Measurements....52
3.3.5 Presentation of Results....53
3.4 RESULTS....58
3.4.1 Duration of Transport....58
3.4.2 Stocking Density During Transport....60
3.4.3 Noise Level During Transport....62
3.5 DISCUSSION....76

CHAPTER 4: INITIAL PHYSIOLOGICAL RESPONSES OF HAND-REARED DAIRY CALVES AGED 1-2 WEEKS TO REFEEDING AFTER FASTING AND TRANSPORT....85
4.1 SUMMARY....85
4.2 INTRODUCTION....86
4.3 MATERIALS AND METHODS....88
4.3.1 Groups....88
4.3.2 Experimental Procedures....90
4.3.3 Blood and Plasma Measurements....91
4.3.4 Presentation of Results....91
4.4 RESULTS....94
4.4.1 Refeeding After 30 hours Without Food....94
4.4.2 Refeeding After Transport....95

4.5 DISCUSSION.....104

CHAPTER 5: GENERAL DISCUSSION.....111

5.1 GENERAL CONCLUSIONS.....111

5.2 ASSESSMENT OF PARAMETERS.....113

5.3 FUTURE RESEARCH.....118

REFERENCES.....120

LIST OF FIGURES

		PAGE
CHAPTER TWO		
Figure 2.1	Pens in the calf shed.	25
Figure 2.2	Calf teat feeders.	25
Figure 2.3	Obtaining a blood sample by jugular venipuncture.	27
Figure 2.4	Taking rectal temperatures and recording behaviours before sampling.	27
Figure 2.5 a-e	Subjective observations of calves at pick-up.	32
Figure 2.6	Weight of calves at pick-up.	33
Figure 2.7	Rectal temperature of calves at pick-up.	33
Figure 2.8	Plasma gamma-glutamyl transferase concentration in calves at pick-up.	33
Figure 2.9	Mean PCVs in fed calves and calves that were unfed for 30 hours.	34
Figure 2.10	Mean total plasma protein concentrations in fed calves and calves that were unfed for 30 hours.	34
Figure 2.11	Mean plasma glucose concentrations in fed calves and calves that were unfed for 30 hours.	36
Figure 2.12	Mean changes after 31 hours in plasma concentrations of beta-hydroxybutyrate, triglycerides and urea in fed calves and calves that were unfed for 30 hours.	36
Figure 2.13 a	Change in weight after the experiment of individual fed calves and individual calves that were unfed for 30 hours.	37
Figure 2.13 b	Mean change in weight after the experiment of fed calves and calves that were unfed for 30 hours.	38
Figure 2.14	Mean rectal temperatures of fed calves and calves that were unfed for 30 hours.	38

CHAPTER THREE

Figure 3.1	Layout of the truck.	50
Figure 3.2	Observing behaviour of the calves in pens on the truck before sampling. The noise meter is secured to the top of a gate.	54
Figure 3.3 a&b	Calves in pens on the truck at different stocking densities.	55
Figure 3.4	Obtaining blood samples by jugular venipuncture from calves on the truck.	56
Figure 3.5	Unloading calves from the truck after transport.	56
Figure 3.6	Mean PCV in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	65
Figure 3.7	Mean concentration of total plasma proteins in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	65
Figure 3.8	Mean plasma concentrations of glucose in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	66
Figure 3.9	Mean changes (0 to 31h) in plasma concentrations of beta-hydroxybutyrate, triglycerides and urea in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	67
Figure 3.10 a	Mean plasma concentrations of creatine phosphokinase in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	68
Figure 3.10 b	Mean plasma concentrations of creatine phosphokinase in unfed calves (12T) transported for 12 hours at the recommended stocking density ($0.2m^2$/calf).	68
Figure 3.11	Mean plasma concentrations of lactate in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	68

Figure 3.12	Mean change in weight (0 to 31h) of unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	69
Figure 3.13	Mean rectal temperatures of unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).	69
Figure 3.14	Mean PCV in unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	70
Figure 3.15	Mean concentration of total plasma proteins in unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	70
Figure 3.16	Mean plasma concentrations of glucose in unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	71
Figure 3.17	Mean changes (0 to 31h) in plasma concentrations of beta-hydroxybutyrate, triglycerides and urea in unfed calves transported for 12 hours at high ($0.2m^2$/calf) or low ($0.4m^2$/calf) stocking densities.	72
Figure 3.18	Mean plasma concentrations of creatine phosphokinase in unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	73
Figure 3.19	Mean plasma concentrations of lactate in unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	73
Figure 3.20	Mean change in weight (0 to 31h) in unfed calves transported for 12 hours at high ($0.2m^2$/calf)and low ($0.4m^2$/calf) stocking densities.	74
Figure 3.21	Mean rectal temperatures of unfed calves transported for 12 hours at high ($0.2m^2$/calf) and low ($0.4m^2$/calf) stocking densities.	74
Figure 3.22 a-c	Noise levels (dB) in the truck during transport expressed as the percentage of time spent at different	75

noise levels.

CHAPTER FOUR

Figure 4.1 Mean change (31 to 34h) in PCV after refeeding at 31h following 30h without food in transported and non-transported calves. 96

Figure 4.2 Mean change (31 to 34h) in total plasma protein concentration after refeeding at 31h following 30h without food in transported and non-transported calves. 96

Figure 4.3 a Mean change (31 to 34h) in plasma glucose concentrations after refeeding at 31h following 30h without food in transported and non-transported calves. 98

Figure 4.3 b Mean plasma glucose concentrations after refeeding following 30h of food deprivation in transported and non-transported calves. 99

Figure 4.3 c Mean incremental changes in plasma glucose concentrations after refeeding following 30h without food in transported and non-transported calves. 99

Figure 4.4 Mean change (31 to 34h) in plasma triglyceride concentrations after refeeding at 31h following 30h without food in transported and non-transported calves. 100

Figure 4.5 Mean change (31 to 34h) in plasma urea concentrations after refeeding at 31h following 30h without food in transported and non-transported calves. 100

Figure 4.6 Mean change in PCV in calves that were refed after transport (3TR & 12TR) and not refed after transport (3T &12T). 102

Figure 4.7 Mean change in plasma glucose concentrations in calves that were refed after transport (3TR & 12TR) and not refed after transport (3T &12T). 102

Figure 4.8 Mean change in plasma lactate concentrations in calves that were refed after transport (3TR & 12TR) 102

and not refed after transport (3T &12T).

LIST OF TABLES

		PAGE
CHAPTER TWO		
Table 2.1	Summary of treatments.	21
Table 2.2	Summary diagram of experimental protocol.	26
Table 2.3	Summary of the number of calves with diarrhoea.	28
Table 2.4	Initial mean values of metabolic parameters.	35
CHAPTER THREE		
Table 3.1	Summary of treatments.	51
Table 3.2	Summary diagram of experimental protocol.	57
Table 3.3	Initial mean values of metabolic parameters.	64
Table 3.4	The amount of time spent above certain noise levels.	63
CHAPTER FOUR		
Table 4.1	Summary of treatments.	88
Table 4.2	Summary diagram of experimental protocol.	93
Table 4.3	Mean concentrations of hydration parameters after refeeding at 31h and 3h later at 34h in transported and non-transported calves following 30h of food deprivation.	97
Table 4.4	Mean concentrations of metabolic parameters after refeeding at 31h and 3h later at 34h in transported and non-transported calves following 30h of food deprivation.	101
Table 4.5	Mean concentrations of parameters in calves that were refed after transport (3TR & 12TR) and not refed after transport (3T & 12T).	103

CHAPTER 1: GENERAL INTRODUCTION.

1.1 WELFARE OF THE BOBBY CALF

The majority of people care about the general well-being of domestic animals, especially that of newborns. A young animal left to starve would be likely to generate a greater degree of concern from a passer-by than an adult animal found in the same situation. This concern, which is based on innate instincts and knowledge gained from personal experience, stems from an understanding of the vulnerability of young animals to physical challenges. Newborns have immature physiological systems, they are dependent on their mothers for food and protection, they are physically weak and are susceptible to disease. For these reasons neonatal animals are more likely to experience welfare compromise than adults.

An area of concern unique to the New Zealand dairy industry is the welfare of bobby calves. Some calves born to New Zealand dairy cows are kept as replacements for the herd and some are reared for dairy beef. The remaining 'bobby calves' are surplus to requirements and are slaughtered for human consumption. A typical bobby calf is removed from its mother at less than a day after birth and is housed with other bobby calves. They are usually fed stored colostrum or milk twice daily from buckets or teat feeders. Calves are transported to the freezing works when they reach a minimum of 4 days of age. On the day of transport, calves are given their morning feed then placed in pens on the roadside. Trucks collect the calves and transport them to the works where they are unloaded and placed in lairage. The calves are slaughtered that afternoon or on the following morning. There are a number of factors involved in this bobby process which have the potential to cause welfare compromise to the animal. These include lack of food and water, exposure to environmental extremes and susceptibility of the calf to disease, illness, injury and fatigue. For these reasons and because of the general public's attitude toward young animals, the bobby calf industry has become a sensitive issue.

The general public may feel concern for animals in this situation, but it is the attitude and knowledge of the farmers, truck-drivers and meat-workers who are directly involved with bobby calves that determines the immediate welfare status of these animals. Many of these people care about the calves welfare but are not aware of their needs and limitations. Others have grown accustomed to the bobby calf practice and feel no concern for the animals, while for some people the welfare of bobby calves is of no consequence.

In 1989 the Minister of Agriculture established an Animal Welfare Advisory Committee (AWAC) which produces Codes of Recommendations and Minimum Standards. These codes provide guidelines on farming procedures and other activities involving animals with the aim of minimising potential welfare compromise. One such code, for 'the welfare of bobby calves' (which will be referred to as 'the Code') provides advice to people involved with the calves on how the animals should be handled to operate within their physiological capabilities. The Code is also an instrument of compliance to, and proceedings against, those who allow an animal to reach an unsatisfactory state of welfare. The content of the Codes is based on the Five Freedoms which were formulated by the Farm Animal Welfare Council (FAWC) in the U.K as a framework for the analysis of welfare. The Five Freedoms were expressed most recently (FAWC, 1997) as:

Freedom from hunger and thirst
Freedom from discomfort
Freedom from pain, injury and disease
Freedom to express normal behaviour
Freedom from fear and distress

The Five Freedoms define ideal states of welfare rather than practical welfare standards. They have recently been modified by Mellor and Reid (1993) to the 'five domains of potential welfare compromise'.

Guidelines in the bobby calf code are based on the most up to date information available at the time of its development. This information was obtained from scientific literature

and knowledge gained from practical experience. Although there is literature on some aspects included in the Code, the combined effect of these factors and the ability of the calf to operate within its physiological capacity without significant welfare compromise during the bobby process had not been examined.

Work in this thesis explores the effects of nutrition and transport on pre-ruminant, hand-reared dairy calves (bobby calves). Nutrition and transport are two main aspects of the bobby process which have the potential to cause welfare compromise to the calf. Criteria for experimental treatments were based on the recommendations and minimum standards given in the previous bobby calf code (AWAC, 1993). Results of the present work were used to modify related sections of the recently updated Code (AWAC, 1997). The ability of the calves to tolerate the process was indicated by their metabolic responses to treatments. As background to these studies, discussion in the remainder of this chapter will include an outline of the physiological characteristics of the bobby calf during the two main stages of its life: the transition from fetus to neonate and the period of milk dependency. The physiological consequences of starvation during the period of milk dependency will also be discussed.

1.2 METABOLIC TRANSITIONS IN PRE-RUMINANT CALVES.

The transition from fetus to neonate.

When an animal is born it undergoes a transition from a fully dependent to a physically autonomous mode of existence which involves a number of adaptive physiological changes (Edwards, 1970). The newborn must maintain adequate oxygen saturation of the blood, regulate acid-base balance, establish endogenous metabolic pathways for energy production and maintain body temperature within critical limits (Kasari, 1994). To help prevent infection, the calf must also absorb maternal antibodies from colostrum (Kasari, 1994). The major metabolic adaptations which must be made by the calf at birth result from cessation of the thermoneutral environment and the placental supply of nutrients.

In utero the fetus exists in a thermoneutral environment. When it is born, the neonate is usually exposed to cold challenge resulting from a relative decrease in air temperatures. Consequently most newborns must employ thermogenic mechanisms and maintain high rates of heat production to avoid hypothermia (Mellor and Cockburn, 1986). Cold air temperatures combined with wind and rain produce a potentially lethal situation for a newborn (Alexander, 1979). Rain removes heat by either a direct cooling action of the cold water on the animal, or by evaporation, or both. Wind increases both evaporative and conductive heat loss. Energy-rich substrates are required to fuel heat production and to balance any heat loss. These substrates are derived from body reserves deposited before birth and from colostrum consumed after birth (Mellor, 1992).

The fetus is provided with a continuous supply of nutrients from the placenta, the balance of nutrients being rich in carbohydrates and amino acids and poor in fat (Slater and Mellor, 1981; Battaglia and Meschia, 1986). Immediately after birth, the newborn is subjected to a brief period of starvation until it is suckled by its mother. When suckling occurs the newborn must adapt to a change in nutrient supply and composition. In contrast to the continuous placental supply of nutrients, colostrum ingested by the newborn during its first few days of postnatal life is especially high in fat (Mellor and Cockburn, 1986) and is obtained intermittently.

The metabolic changes required for the newborn to adapt to postnatal life are regulated by hormonal actions. Hormones may be categorised as stable background hormones, reactive labile hormones or active labile hormones, depending on the way they act or react. Stable background hormones such as thyroid hormone control the basaal rate of metabolism. They generally respond to external inputs such as metabolic adjustments associated with the physiological state or an environmental influence. Reactive labile hormones such as insulin and glucagon maintain metabolic equilibrium by rapidly responding to internal inputs. Active labile hormones, such as the glucocorticoids and catecholamines, respond to external stimuli (eg - stressors) and cause, amoung other things, changes in metabolism which prepare the body to cope with such stimuli. These three levels of hormonal action maintain basal metabolic functions and metabolic equilibrium, and facilitate responses to more extreme conditions faced by the animal.

The transition from fetus to neonate is thought to begin with a prepartum rise in fetal plasma cortisol concentrations. This cortisol rise provides the main fetal stimulus to initiate the hormone changes which induce parturition (Challis and Lye, 1986). Body stores of liver and muscle glycogen accumulate in the fetus as term approaches (Shelley, 1961) and in fetal lambs the prepartum cortisol surge has been associated with increased concentrations of liver glycogen (Barnes *et al.* 1977) and circulating levels of insulin (Fowden, 1980). This prenatal deposition of glycogen provides a source of energy for the calf during the postnatal period prior to sucking.

Immediately after birth there are increases in plasma glucose and insulin concentrations and marked changes in circulating levels of catecholamines (Kasari, 1994). Increased concentrations of catecholamines have been reported to occur in newborns in response to hypoxia, cold exposure or cord cutting (Girard *et al.*, 1992). However plasma concentrations of catecholamines decrease rapidly after 30 minutes of postnatal life (Mayor and Cuezva, 1985). During the first two hours after birth, hypoglycemia develops as a result of cessation of the placental supply of nutrients and the limited amount of circulating glucose. Hypoglycemia initiates a reduction in the insulin/glucagon molar ratio which favours catabolic processes and therefore the provision of energy from endogenous stores. It is thought that postnatal hypoglycemia stimulates increased plasma catecholamine concentrations resulting in liver glycogenolysis (Mayor and Cuezva, 1985). Muscle glycogen is mobilised during muscular activity causing unmetabolised lactate to be released into the blood (Alexander, 1979). Lactate is used as a substrate for gluconeogenesis, the rate of which increases soon after birth. Thus between 3 and 6 hours after birth, the rates of glycogenolysis and gluconeogenesis increase and plasma glucose levels in the newborn become normoglycemic (Mayor and Cuezva, 1985).

Apart from the limited amount of carbohydrate available to the newborn between birth and sucking, lipids are the only other major source of fuel for heat production in newborn infants, piglets and lambs (Mellor and Cockburn, 1986). There is minimal amino acid catabolism during at least the first day after birth in these species which rules out significant contributions from protein to the newborns energy consumption (Mellor and Cockburn, 1986). It is likely that this also applies to newborn calves as they are

physiologically similar to lambs. Heat production is fuelled simultaneously by carbohydrate and lipid, but at high rates of heat production carbohydrate is the predominant fuel (Mellor, 1992). Carbohydrate is the limiting energy substrate because once it is exhausted high rates of heat production cannot be sustained, and when lipid supply fails first, high rates of heat production can only be sustained for as long as carbohydrate supply lasts (Mellor and Cockburn, 1986).

Thus to maintain metabolic reactions and thermogenesis during the transition from fetal to neonatal life, the calf must adapt from having a continuous placental supply of carbohydrate and amino acids, to a brief period of starvation immediately after birth, and then to an intermittent supply of nutrient-dense colostrum which is especially rich in fat and proteins, but where protein catabolism is minimal as a source of energy.

The period of milk dependency.

The combined contributions of endogenous liver and muscle glycogen could meet the carbohydrate requirements of lambs for up to 10 hours after birth (Shelley, 1960; Alexander, 1979; Mellor and Cockburn). Mellor and Cockburn (1986) reported from the work of others that exhaustion of endogenous glycogen reserves coincides with hypothermia in unfed lambs. It would therefore be of benefit to the newborn calf to ingest food as soon as possible after birth, but certainly within 10 hours.

The first feed a newborn calf should receive is nutrient-dense colostrum. As well as containing immunoglobulins which help establish immunity, colostrum is rich in lipid which corrects any deficit the newborn may have and extends the availability of glycogen (Mellor and Cockburn, 1986). The carbohydrate component of colostrum, lactose, is only sufficient to supply energy requirements for about half a day, thus glycogen reserves are again required to make up the energy difference (Mellor and Cockburn, 1986). During the first day the composition of colostrum gradually changes to that of milk which is also high in fat and low in carbohydrate (Girard and Ferre, 1982), but mature milk is characterised by a higher lactose, lower fat and lower protein content than colostrum. As sucking in the newborn calf becomes established, milk

becomes the only source of carbohydrate, lipid and protein (Mellor and Cockburn, 1986).

The physiology of a postnatal calf is distinct from that of an adult ruminant as from birth to the age of 1-2 months the reticulo-rumen is poorly developed (Bauchart *et al.*, 1989). In contrast to the adult ruminant, the young calf has no fermentation process and no microbial modification of the chemical structure of dietary substances occurs (Bauchart *et al.*, 1989). During the first month the calf is entirely dependent on its mother's milk which by-passes the non-functional rumen by means of an oesophageal groove and enters the abomasum (Leat, 1970). Once in the intestines, digestion is similar to that of a neonatal non-ruminant. Consequently, in terms of function, the pre-ruminant calf may be considered a monogastric animal (Bauchart *et al.*, 1989).

Mellor and Cockburn (1986) reviewed the ways in which milk-derived substrates replace colostrum and endogenous glycogen as energy supplies. They reported that the concentrations of lactose and lipid available for ingestion increase, digestion of protein increases, there is rapid growth of the neonatal gut to accommodate increased absorption and intake of larger volumes, and an improved thermoregulatory ability to lower the rate of heat production.

The high-fat diet of milk requires oxidation of fatty acids for the provision of energy (Mayor and Cuezva, 1985). One way in which fatty acids are oxidised results in increased plasma concentrations of ketone bodies (Girard and Ferre, 1982) which are utilised as energy substrates by extrahepatic tissues during this period (Mayor and Cuezva, 1985). The sucking calf also requires a constant supply of glucose which is partly obtained from the supply of lactose in milk and partly by gluconeogenesis (Girard and Ferre, 1982). The hormonal environment of the newborn during the period of milk dependency is linked to the type of diet and is therefore characterised by high plasma glucagon and low plasma insulin concentrations. In lambs during this time, elevation in plasma concentrations of catecholamines are important for maintenance of lipolysis and glucose production (Martinez *et al.*, 1990).

A high growth rate occurs during the perinatal period and this is associated with a rapid rate of protein turnover and rapid protein accretion in most tissues of the body (Girard *et al.*, 1992). Although protein digestion increases during the period of milk dependency, the protein component of milk provides only enough substrate for accretion of body protein, therefore rates of amino acid catabolism and urea excretion are low during this period (Cresteil and Leroux, 1977).

Towards the end of the period of milk dependency when weaning begins to occur, milk intake is gradually replaced by solid food, so that the diet becomes higher in carbohydrate and lower in fat (Girard *et al.*, 1992).

Starvation.

During starvation there is no exogenous supply of energy substrates. Energy must be derived from endogenous body reserves, which therefore creates a negative energy state (Clarenburg, 1992). In adult animals the metabolic response to starvation has three stages based on the energy substrate used: carbohydrates tend to be used first, then lipids are mainly utilised, and finally protein. The brain depends primarily on glucose as an energy substrate, therefore there are two mechanisms employed to maintain the supply of glucose to the brain.

The first mechanism for maintaining cerebral glucose supply involves increasing plasma glucagon concentration and the decreasing insulin concentration which occur when plasma glucose concentrations decline. These changes cause rapid hepatic glycogenolysis together with and eventually replaced by glucose provision by hepatic gluconeogenesis. Substrates for gluconeogenesis include lactate from skeletal muscle glycogen, alanine from muscle protein and glycerol from adipose tissue (White and Baxter, 1994). By 36 hours of starvation gluconeogenesis accounts for over 75% of hepatic glucose production in adult animals (White and Baxter, 1994).

To allow hepatic glucose production to adequately supply the oxidative needs of the brain, a glucose-sparing mechanism which reduces glucose utilisation by other tissues would be advantageous. Thus, the second mechanism for maintaining blood glucose

supply to the brain involves a decrease of glucose oxidation by muscle and other tissues capable of using alternative fuels.. This is achieved by a decreased rate of glucose entry into adipose tissue due to the fall in insulin and glucose concentrations; and the provision of free fatty acids and ketone bodies as alternative fuels which occurs at the onset of starvation (White and Baxter, 1994). During this stage lipids become the primary energy substrate. The decrease in insulin concentrations causes an increase in the rate of adipose tissue lipolysis such that fatty acids are released into the plasma. The rate of lipolysis increases with the duration of starvation, but the rate of utilisation of fatty acids matches their rate of appearance. Some tissues use fatty acids as direct substrates for metabolism. Also, the high glucagon/insulin ratio enables increased hepatic beta oxidation of fatty acids, leading to ketone body formation (White and Baxter, 1994). By the time ketone body oxidation by the brain occurs, there has been a progressive reduction in the rate of gluconeogenesis. In adult animals by four weeks of starvation, ketone bodies provide 50-60% of the brain's energy requirements (White and Baxter, 1994).

In extreme situations of starvation when both carbohydrate and lipid supplies have been exhausted, structural protein becomes the primary energy source which results in degradation of muscle tissue.

The above description outlines metabolic adaptations that would occur in an adult animal. Energy reserves of newborn animals subjected to starvation would be exhausted more rapidly due to their under-developed stores of endogenous body reserves and their immature physiological systems. Therefore newborns subjected to starvation would not be expected to fare as well as an adult in the same situation.

1.3 USE OF METABOLIC PARAMETERS.

Measurement of blood metabolites can provide information about the physiological state of an animal. Work in this thesis involved assessing the metabolic responses of calves to transport and to various nutritional states.

As an indication of hydration state, packed cell volume (PCV) and concentrations of total plasma proteins (TPP) were measured. If there has been no loss of erythrocytes or protein, changes in PCV and TPP reflect changes in plasma volume (Boyd, 1981) such that an increase in these parameters would be observed if the animal became dehydrated.

Blood metabolite concentrations reflect a balance between entry of the metabolite into the blood and and its utilisation rate thereby allowing short or long term changes to nutritional levels to be measured (Mellor, 1987). Plasma concentrations of glucose, triglycerides, beta-hydroxybutyrate and urea were measured to represent changes in carbohydrate, lipid and protein metabolism. If the animal has been fed, an increase in plasma concentrations of these parameters (apart from urea) may indicate absorption of these substrates from the gut. If the animal had not been fed, increased concentrations may reflect mobilisation of the substrate from endogenous stores or de novo synthesis from precursors. Conversely, decreased concentrations of these metabolites is suggestive of uptake by the tissues for storage or oxidation. Urea is a waste product resulting from catabolism of amino acids, so that an increase in urea concentrations represents increased protein catabolism provided kidney function is not compromised.

As indicators of physical exercise, plasma concentrations of lactate and creatine phosphokinase (CPK) were measured. Lactate is produced from pyruvate within muscles under anaerobic conditions (Guyton, 1991), so that high levels of lactate may indicate vigorous muscle use. CPK is an enzyme which catalyses the reversible phosphorylation of creatine by ATP. Creatine is the major storage form of high-energy phosphate required by muscle (Kaneko, 1989), thus increased concentrations of CPK may indicate muscular contraction. CPK also leaks out of the cell if the muscle has been damaged but it is generally so sensitive to muscle damage that large increases in its plasma activity indicate this (Lewis, 1978).

1.4 OUTLINE OF THESIS

A study of the effects of feeding and fasting on hand-reared dairy calves is outlined in Chapter 2. It is recommended in the Code (AWAC, 1993) that hand-reared dairy calves

be fed 5 % of their body weight (50ml/kg) twice daily, so control calves in this study were treated in this way. If calves subjected to pre-slaughter transport and lairage are fed on the morning of transport as recommended by the Code (AWAC, 1993), they may be without food for a period of up to 30 hours before being slaughtered. Therefore we investigated the metabolic responses of calves to withholding food for a period of 30 hours following a morning feed of the recommended volume. In normal farm practice, several calves are usually fed from one feeder, so it is possible that weaker or less dominant calves receive less than the recommended volume. Consequently we assessed the metabolic effects of feeding calves half the recommended volume (25 ml/kg) prior to 30 hours without food.

As well as being deprived of food for up to 30 hours, bobby calves are also subjected to road transport. It is recommended in the Code that calves be transported for no more than 12 hours at a stocking density of $0.2m^2$/calf. Thus experiments described in Chapter 3 were designed to evaluate the metabolic responses of hand-reared dairy calves to transport of 3 or 12 hours duration at the recommended stocking density during a period of 30 hours without food. We also examined the responses of such calves to 12 hours transport at half the recommended density ($0.4m^2$/calf) which enables the calves to lie down.

Experiments described in Chapter 4 were designed to investigate the short-term metabolic responses of calves to refeeding. Transported and non-transported calves were refed following 30 hours without food to determine the ability of the animals to recover metabolically from the process of pre-slaughter transport and lairage. Calves were also refed immediately after transport to investigate whether feeding during the pre-slaughter transport and lairage process increased their ability to tolerate potential welfare compromise.

The general conclusions of this work are summarised in Chapter 5. There is also a critique of the physiological parameters employed in this work. This critique involves discussion of how suitable the chosen parameters were for representing accurately the metabolic status of 1-2 week old hand-reared dairy calves. Finally, possibilities for future research are discussed in relation to the findings obtained from this work.

CHAPTER 2: THE EFFECTS OF FEEDING AND FASTING HAND-REARED DAIRY CALVES AGED 1-2 WEEKS.

2.1 SUMMARY

Beef calves are born at pasture with minimal interference from humans. They are left with their mothers and feed from them until they are weaned. This is a more natural situation than that of the dairy calf which is removed from its mother at one day of age, housed with other calves and fed by artificial means. Many hand-reared dairy calves surplus to requirements are slaughtered about a week after birth, therefore feeding these calves on a high plane of nutrition is not a priority. Instead they are usually fed a maintenance ration which provides sufficient nourishment for the calf until slaughter. A number of potential problems associated with feeding regimes of surplus dairy calves may cause the animal to become nutritionally compromised. The nutritional status of a calf prior to preslaughter transport and lairage influences its ability to cope with potential physiological challenges occurring at this time.

In this study we examined the physiological and metabolic responses of calves to feeding high or low volumes of food prior to 30 hours of food deprivation. Observations regarding the general health of calves were made at the time the animals were obtained. Throughout the experiment PCV and total plasma protein concentrations of calves were measured to indicate the hydration state of the animal. Plasma glucose, triglyceride, beta-hydroxybutyrate and urea concentrations were measured to provide information about the metabolic status of the calves. Weight and rectal temperatures were also obtained during the experiment. There were 3 groups involved: control (C), in which calves were fed the recommended volume twice a day; full feed (FF), where calves were fed the recommended volume (50 ml/kg or 5% of body weight) before food was withdrawn for 30 hours; and half feed (HF) where calves were fed half the recommended volume before food was withdrawn for 30 hours. Results indicated that the recommended feeding regime used on dairy farms provided calves with adequate nourishment. In mild weather conditions which were present

during this experiment, calves were able to withstand a 30 hour fast without significant metabolic compromise provided they were sufficiently nourished to begin with.

2.2 INTRODUCTION

Nutrition of the pre-ruminant calf.

The body requires energy to fuel metabolic reactions and heat production. This energy is derived from exogenous and endogenous sources. When an animal is born it has a reserve of energy in body stores that are laid down during gestation (Mellor and Cockburn, 1986). As a newborn starts to feed, nutrients obtained from the diet are utilised in conjunction with body reserves. Energy rich exogenous nutrients obtained from the diet in the form of carbohydrates, lipids and proteins are used immediately for catabolic reactions or growth and repair of tissues, or are stored in the body for later use by becoming an endogenous reserve. If an animal is not fed, its exogenous supply of energy is no longer available and it must rely on endogenous sources of energy to maintain bodily functions.

The physiology of a calf less than 2 weeks of age is different from that of older calves as a result of the type of food the calf ingests. At birth, although anatomically a ruminant, functionally the calf is a monogastric animal with abomasal function predominating (Thickett *et al.*, 1986). As newborn animals can no longer obtain nutrients from their mothers via the placenta, their survival in the period immediately after birth and before the onset of successful sucking depends upon the stores of nutrients laid down before birth.

For continued survival the newborn requires food shortly after birth. A newborn calf exhibits a strong drive to stand and seek the teat in the first hour or so after birth (Webster, 1984). The first milk a calf will obtain is colostrum which accumulates in the udder during the final few days of pregnancy. Colostrum continues to be available during the first 12 to 24 hours after birth but it is diluted progressively as milk production increases (Mellor, 1990). Colostrum has at least three main functions: it

contains nutrients to fuel heat production and to maintain metabolic reactions; it contains immunoglobulins to provide protection against disease; and it contains substances which promote gut growth and differentiation (Mellor, 1990). It is recommended that the calves receive colostrum within 6 hours of birth (AWAC, 1993) to help provide early protection against environmental pathogens and to provide an energy source.

During the first week of postnatal life the calf will drink from its mother 7-10 times a day and will seldom consume more than 1 litre on any one occasion (Webster, 1984). Also, the digestive process in the abomasum is such that the flow of nutrients to the small intestine is regulated at a steady rate, therefore under normal circumstances the calf is unlikely to overload the abomasum. As calves get older and stomach size increases, the frequency of feeding decreases and they are able to ingest a larger volume at each feed.

By two weeks the calf starts to eat solids including grass and other plants containing cellulose (Webster, 1984). As the calf begins to eat solid food, development of the forestomach is initiated (Thickett *et al,* 1986). To cope with the transition from liquid to solid food, the pre-ruminant calf has the ability to direct food to where it is best utilised: milk to the abomasum and solid food to the reticulo-rumen. Milk is channelled straight from the oesophagus to the abomasum via the oesophageal groove (Thickett *et al,* 1986). When the calf sucks milk from its mother a nervous reflex stimulates a specialised muscle to contract. The contraction closes the roof of the oesophageal groove converting it to a tube which delivers milk into the abomasum (Webster, 1984). This is known as the oesophageal groove reflex.

Although milk is balanced to meet the nutrient requirements of young calves, it cannot sustain normal growth for long periods without problems occurring as it is deficient in magnesium, iron and copper (Webster, 1984). Thus in the young calf, the diet of milk is supplemented by grass, hay and grain. The increasing intake of solid food, and particularly, the end products of rumen fermentation, volatile fatty acids, stimulate the physical and functional development of the rumen from an initial size of about 2 litres capacity (Owens *et al,* 1986; Thickett *et al,* 1986). Rumen volume and dry feed intake

are positively related (Thickett, *et al,* 1986) and the rumen is fully functional by three months of age (Webster, 1984). Thus as the calf progresses from drinking milk to eating solid feed, the need for glucose as the primary energy substrate in the pre-ruminant calf decreases, the rumen develops and the calf becomes increasingly capable of processing solid foods.

A major use for energy substrates is heat production. At birth the newborn may experience a marked decrease in environmental temperature. A large surface area to body mass ratio and the evaporation of amniotic fluid predispose the newborn calf to significant heat loss during the early postnatal period (Carstens, 1994). Rectal temperatures of newborn calves maintained at air temperatures of 10 ^{0}C decreased on average from 39.2 ^{0}C to 38.5 ^{0}C during the 90 minutes following birth and stabilised at about 38.8 ^{0}C five hours later (Vermorel *et al.*, 1983). It is important that the core temperature of the animal provides optimum conditions for metabolism. In order to maintain core temperature at this level the newborn calf must generate large quantities of heat. This is achieved through shivering thermogenesis in muscle tissue and non-shivering thermogenesis in brown adipose tissue (Carstens, 1994). The ability of both mechanisms to generate heat is dependent on the rate of energy oxidation.

Problems associated with nutrition in the young, hand-reared dairy calf.

Dairy cows are selectively bred to produce milk and many have low hanging udders with large teats. In addition they have not been selected for their maternal behaviour as their calves are usually removed from them a day after birth (Edwards, 1982). These characteristics may act to the detriment of a calf during its first few critical hours after birth. In contrast to beef calves, a high prevalence of delayed first suckings has been reported among dairy calves (Webster, 1984; Ventorp and Michanek, 1991).

Calves that do not receive sufficient colostrum within 6 hours of birth have a decreased ability to withstand infection and must continue to utilise body reserves, mainly of carbohydrates and lipids. Consequently they are likely to become hypoglycemic. In lambs this leads to cerebral compromise in warm conditions, or to hypothermia in cold conditions (Mellor and Cockburn, 1986).

When dairy calves are removed from their mothers at 1 day of age, they are usually housed with other calves and fed colostrum or milk obtained from the cows in the dairy shed. The milk is usually fed from large containers with several teats (calfaterias) which enable a number of calves to feed from one source. Beef calves of this age usually feed every few hours in contrast to hand-reared dairy calves which are usually fed every 12 hours. Consequently by the time each feed is offered, dairy calves are often very hungry and tend to drink volumes which may be greater than the volume of their abomasum. If a calf less than one week old consumes more than about 1.5 litres of fluid, overflow into the omasum is apparently likely occur (Webster, 1984). As these calves are not usually fed individually, the smaller or less dominant calves may not receive sufficient volumes of milk as a result of competition from the other calves. During pre-slaughter transport and lairage calves may be without food for up to 30 hours (see Chapter 1: Introduction). The ability of a calf to withstand this process may be influenced by the nutritional circumstances imposed on it both before and during this process.

Aims of this study.

To evaluate some physiological and metabolic responses caused by feeding regimes occurring during pre-slaughter transport and lairage in young, hand-reared dairy calves, the following situations were examined:

- Feeding calves at the recommended rate twice daily.
- Withholding food from calves for 30 hours.
- Feeding calves different volumes before withholding food for 30 hours.

2.3 MATERIALS AND METHODS

This research was conducted as one trial which was performed as 2 identical experiments on separate days (21-22/8/95 and 4-5/9/95).

2.3.1 Animals

Eighty Friesian bull calves with a mean weight of 40 kg (range 32 - 51 kg) and aged between 1 and 5 days were collected as they became available from 12 dairy farms in the Manawatu district. The Code (AWAC, 1993) recommends that calves be a minimum age of 4 days before being sent for slaughter. Calves were aged between 5 and 10 days on the first day of the experiment.

The calves were transported to the experimental site in a trailer and were placed in groups of five, ear-tagged (Allflex NZ Ltd; Palmerston North) and large numbers for video identification were drawn on cardboard and glued (ADOS) on both sides of each calf's rump.

On arrival at the experimental site, a jugular blood sample was taken for plasma gamma-glutamyl transferase (GGT) determination and the following observations and measurements were made on all calves.

Gum Colour. Gum colour is an important clinical observation as it illustrates how well the circulatory system is functioning. It is therefore one of the 'vital signs' which are used to give an immediate indication of how serious an animals condition is. A healthy pink gum colour indicates normal circulation whereas a pale or dark gum colour may indicate some degree of circulatory compromise. The colour of gums was recorded - it varied from white to purple (white/pink, pink, pink/red, red, purple).

Scour Scale. Infection and nutritional disorders are the two common causes of diarrhoea in the young calf. Nutritional diarrhoea results from undigested material meeting the normal microbial flora of the small intestine and may be caused by a change in food or feeding time, overconsumption or some other upset to routine (Webster, 1984). Calves that are removed from their mothers at an early age and are housed and fed together are subjected to many changes. It is common for a calf in this situation to develop nutritional diarrhoea. Until their immune system is functioning, newborn calves are highly susceptible to infectious diarrhoea caused by bacterial or viral pathogens. The immature calf is also particularly prone to septicemia via the gut wall (Webster,

1984). Symptoms of diarrhoea depend on the severity of the condition and include voiding of fluid faeces, abdominal pain, lethargy, and elevated temperature (Webster, 1984). We recorded the occurrence of diarrhoea when calves arrived at the experimental site using a subjective scale which rated the consistency of the faeces as 1-fluid, 2-sloppy, 3-firm.

Belly Fill. Belly fill gives a subjective indication of how much food the calf has ingested recently. In a sick calf or a calf that has not fed, the abdomen may appear very hollow, whereas a calf that has been fed may have a belly that is round in appearance. Belly fill was rated on a scale: 1 - hollow flanks, 2 - straight, flanks, 3 - round flanks.

State of Umbilical Cord. After birth, one part of the umbilical cord remains with the calf. In a healthy calf this remnant dries up, usually from the severed end up, and drops off over the period of a dew days. We recorded the condition of the remaining umbilical cord as wet, wet/dry (ie - top part wet, bottom part dry), dry or absent.

Rectal Temperature. Metabolic activity in mammals requires an optimum temperature to proceed properly and a healthy animal will have a temperature within the optimum temperature range for its species. Marked temperature increases above normal (hyperthermia) are often an indication of a pathological process. Fever is an elevated body temperature where mehanisms that control body temperature have an elevated set point. High environmental humidity, temperature, and exercise, will cause elevation of body temperature (Blood and Radostits, 1989). Hypothermia, subnormal body temperature, occurs in shock, circulatory collapse and prior to death in many diseases (Blood and Radostits, 1989). The body temperature of healthy ruminants is subject to diurnal variations of around 1 ^{0}C, rising during the day and falling at night (Siegmund, 1973). In calves the normal range of rectal temperatures has been reported as being between 38.0 ^{0}C and 39.5 ^{0}C (Webster, 1984), with young animals having more labile temperature levels than older animals and exhibiting greater diurnal fluctuations (Siegmund, 1973). The young respond to infections with a much greater temperature elevation (Siegmund, 1973), so that rectal temperatures in the young calf may not be indicative of disease unless greater than 40 ^{0}C.

Weight. Weight indicates the size of an animal and is dependent on breed, sex and age. A consistent increase in weight should be evident during the first few weeks in healthy postnatal animals. This is a result of rapid structural growth as the animal increases in size and also from the gradual accumulation of reserves of energy which are stored predominantly as body fat. The weight and ages of calves at pickup were measured. Allocation to different groups achieved similar weight and age distributions.

Tooth Eruption. Recording the number of teeth the young calf has may give some indication of the age of the animal. At birth the calf does not require teeth as it relies solely on the colostrum and milk which are usually obtained from its mother. However the digestive system of the calf changes from the pre-ruminant state to that of a ruminant, which is associated with the consumption of solid food. Calves have 8 incisors which erupt during the first two weeks after birth. About 75% of full term calves have all incisors erupted at birth (Sisson and Grossman, 1953). A record was made of the number of teeth which had broken through the gums and also the number of teeth that could be felt emerging.

Gamma-Glutamyl Transferase (GGT) Concentration. Newborn calves are highly susceptible to disease. Acquisition of passive immunity is achieved by the ingestion of colostrum from the dam. Colostrum is rich in immunoglobulins which are proteins that help provide protection against disease. It is necessary for the newborn calf to receive immunoglobulins shortly after birth for two reasons. Firstly, the cow only produces immunoglobulins in high concentrations in colostrum for a short period of time after birth (MacKenzie, 1984). Secondly, from the time of birth the ability of the calf's intestines to absorb immunoglobulins decreases due to a process called 'gut closure' where the gut wall no longer allows the absorption of immunoglobulin molecules. This process is completed between 24 and 48 hours post partum (Stott et al, 1979). GGT is an enzyme found in the cell membrane of most tissues. GGT activity has been related to the presence of immunoglobulins. Thompson and Pauli (1981) found low gamma globulin levels concurrent with low serum GGT activity. It has been suggested that serum GGT levels are a reliable indicator of whether a calf has sucked colostrum and therefore acquired an immune status sufficient to protect it from neonatal disease (Thompson and Pauli, 1981). Perino *et al.* (1993) reported that a plasma GGT

concentration of less than 200 u/l indicated failure of passive transfer of immunoglobulins. Thus GGT activities in plasma obtained from the calves at pick up were analysed to indicate the degree of sucking that had occurred.

Feeding. Calves were at least one day old at pick-up. Their colostrum intakes were unknown. The adequacy, or otherwise, of this was assessed by reference to their plasma GGT concentration. After arrival at the experimental site, calves were given 0.5 litres of electrolytes (Lectade Plus - *SmithKline Beecham* or Reviver - *Techvet*). Calves that developed diarrhoea at later stages were also treated with electrolytes. When calves had faeces of normal consistency they were fed cold, stored colostrum every 12 hours and their intakes were recorded. Calves were initially given 0.5 litres of colostrum to enable them to become accustomed to the different feed composition. All calves were fed the recommended amount of 50 ml/kg (5% of body weight) twice daily for at least 1 day prior to the beginning of the experiment. Colostrum feeding equipment was washed thoroughly and sterilised with chloride of lime between feeds. Calves had ad libitum access to water and pelleted feed (Harvey Farms Topcalf Starter; Levin).

Housing. Calves were housed in a shed with a concrete floor and eight pens in two rows of four (Fig 2.1). The pens (1.70 m x 2.43 m) had wire netting walls (0.83 m high) and grid floors with rubber mats covering a drainage system. Each pen had troughs for water, and solid feed and attachments for 5 litre calf feeders (Mother Udders; Skellerup Industries Ltd, Palmerston North) (Fig 2.2). The shed had skylights and sufficient windows to allow natural lighting. Fluorescent bulbs provided artificial illumination when necessary. Mean temperatures during spring in New Zealand ranged from 7-13 ^{0}C (Dirks, 1995). Four video cameras were set up to monitor behaviour, each camera covering two pens.

2.3.2 Groups

Ten calves (5 per experiment) were allocated to each group such that ages and weights were evenly distributed. The average weight of the calves on the day before the experiment was 41 kg. Three of the groups are discussed in this chapter (Table 2.1) and the other 5 (3T, 3TR, 12T, 12TR, 12TLD) will be discussed in chapters 3 and 4.

	TREATMENTS			
GROUP n=10	fed 50ml/kg at 6am feed	fed 25ml/kg at 6am feed	fed 50ml/kg every 12h	unfed for 30h
C	√	-	√	-
FF	√	-	-	√
HF	-	√	-	√

Table 2.1 Summary of treatments.

Control (C). Calves were fed 50 ml/kg body weight twice daily (as recommended by the Code) at 12 hourly intervals beginning on the morning of the first experimental day at 6.30 am. They also had ad libitum access to water and calf pellets.

Full Feed (FF). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water.

Half Feed (HF). Calves were fed 25 ml/kg body weight (half the recommended amount) on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had had access to water.

2.3.3 Experimental Procedures

A 9 ml blood sample was taken at 0 hours (6 am) then calves were fed according to their group. Another blood sample was obtained 1 hour after this feed (7 am) following which blood samples were taken at 4, 7, 10, 13, 16, 19, 22, 25, 28 and 31 hours. All animals were weighed prior to the experiment and again after 31 hours. Rectal temperatures were also obtained following blood sampling at 0, 13, 31 hours. The experimental protocol is summarised in Table 2.2.

2.3.4 Methods

Blood Sampling. Blood samples were taken by jugular venipuncture using 9 ml sodium-heparin plastic vacutainers (Fig 2.3). Immediately after obtaining the sample, the packed cell volume (PCV) of each sample was measured using the capillary tube centrifugation method. The remaining blood was centrifuged for 20 minutes then the plasma was extracted and stored frozen (-20 ^{0}C) for future analysis.

Behavioural Observations. Without disturbing the animals, the activity (standing or lying; head position; eyes open or closed) of each calf was noted immediately before each blood sample was taken. A calf vigour score was also assigned by the person bleeding the calf whilst blood sampling was occurring. General vigour scale: 1 - no resistance, 2 - some resistance, 3 - struggling. Vigour scores were assigned by several different people which resulted in inter-operator variation and caused obvious inconsistencies in the recordings. Accordingly, these results are not presented.

Rectal Temperature. A flat type digital clinical thermometer (Lesters Veterinary Imports, 94B Malden St, Palmerston North) was used to determine rectal temperature (Fig 2.4). The thermometer was held against the wall of the rectum for 1 minute or until a stable reading was obtained.

Body Weight. Calves were weighed to the nearest 500 g using a weighing platform on loadbars attached to a meter (Tru-test, AG500 series).

Skinfold Thickness. Skinfold thickness was measured using vernier callipers (minimum reading of 0.1 mm). The neck of the calf was held level and straight while a fold of skin from the surface of the neck was measured between the callipers. Several readings were taken in order to obtain an average value. The method we used produced inconsistencies due to inter-operator variation and making measurements at different sites. It would have been more accurate to shave the area of neck to be measured and to mark two lines on the skin to indicate placement of the callipers. Accordingly these results are not presented.

2.3.5 Blood Plasma Measurements

Packed cell volume was estimated by the microhaematocrit technique shortly after the blood sample was obtained. At Ruakura Animal Health Laboratory, plasma samples were analysed for concentrations of triglycerides which were determined on an Hitachi 717 Autoanalyser (30 ^{0}C) using the enzymatic colorimetric test (modified according to Wahlefeld, 1974). At Massey University plasma samples were analysed on an Hitachi 704 Multi-channel analyser for concentrations of gamma-glutamyl transferase (Persijn and Van der Slik, 1976), using the Boehringer Mannheim kit no.1489224; glucose, using the enzymatic colorimetric method (Trinder, 1969) Boehringer Mannheim kit no. 1448668; total plasma protein, using the Biuret method (Weichselbaum, 1946) Boehringer Mannheim kit no. 1553836; urea, using the kinetic UV test (Neumann and Ziegenhorn, 1977) Boehringer Mannheim kit no. 1489364; and beta-hydroxybutyrate (Li, 1985) Sigma, catalogue no. 310-A, procedure no. 310-UV.

2.3.6 Presentation of Results

Subjective observations of tooth eruption, gum colour, belly fill, state of umbilical cord and occurrence of diarrhoea, in calves at pick up have been presented as histograms showing the percentage or number of calves at each observation. PCV and plasma concentrations of total proteins and glucose, and rectal temperatures were expressed graphically as mean ± standard error of the mean (SEM) for each group at each sample time. Calf liveweight and plasma concentrations of beta-hydroxybutyrate, triglycerides and urea were presented graphically as the mean (± SEM) change in concentration during the experiment.

Analysis of these data was performed using GraphPad Prism™ version 2.0. When the significance of differences between the three groups at a particular sample time were required, a one-way analysis of variance (ANOVA) was conducted when data had equal variances, otherwise a Kruskal-Wallis test for non-parametric data was used. ANOVA post-tests were used to establish which differences were significant between the pairs. When the significance of differences between two sample times was required for one group, a paired students t-test was used. Where data were presented as mean changes a

one-sample t-test was used to determine whether these changes were significantly different from zero.

Fig 2.1 Pens in the calf shed. Five calves (1 group) were allocated to each pen for the duration of the experiment.

Fig 2.2 Calf teat feeders. The black plastic feeders were placed in rings on the wall of the pen and secured by attaching the teat to the feeder.

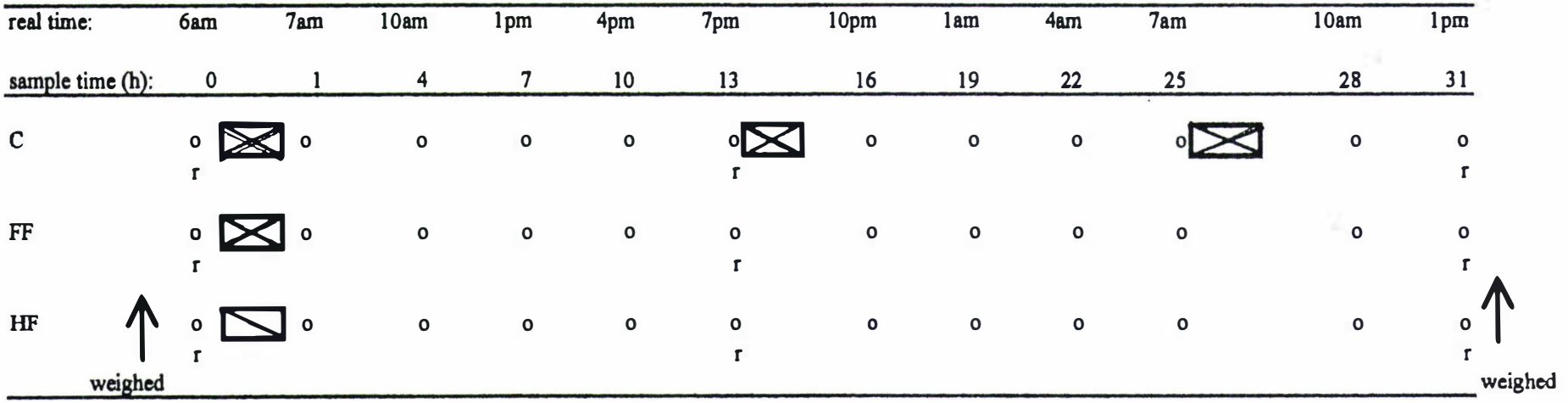


Table 2.2 Summary diagram of experimental protocol

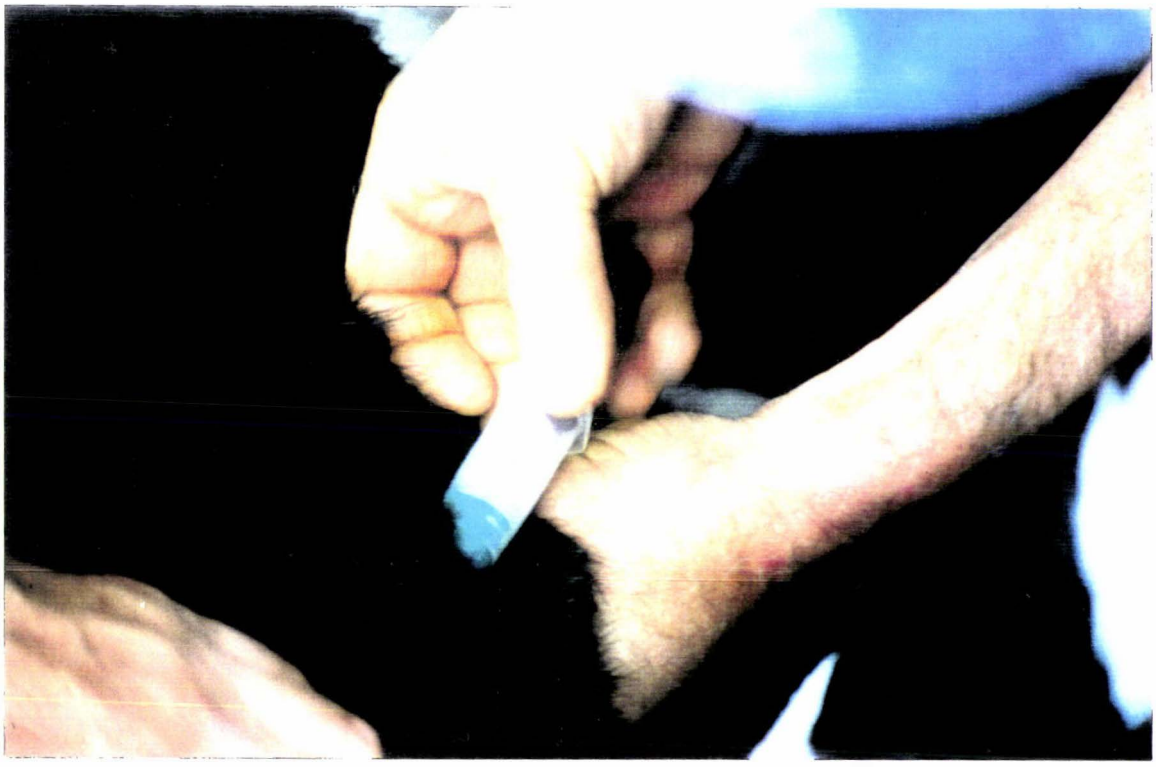


Fig 2.3 Obtaining a blood sample by jugular venipuncture.

Fig 2.4 Taking rectal temperatures and recording behaviours before blood sampling.

2.4 RESULTS

2.4.1 General Clinical State At Pick-Up

Observations. The following observations were from all 80 calves included in the experiment. The calves had 4, 6 or 8 erupted teeth t pick-up; 84% had all 8 incisors (Fig. 2.5). Most calves (82.5%) had healthy pink coloured gums, and the rest showed darker colours of red or purple or had gums that were light in colour. Of the calves that still had a remnant of the umbilical cord (65%), 73% of these had shrivelled cords. The belly fill scale produced a relatively normal distribution with most calves having straight abdomens and a small percentage of calves having hollow or round abdomens. Diarrhoea was evident in many calves at pickup, with 58% having fluid faeces and only 4% having firm faeces. After treatment with electrolytes, all calves (except four) recovered from diarrhoea before the experiment began, and were therefore fed milk during the experiment. Four calves were diagnosed as having rotavirus (a viral pathogen which causes infectious diarrhoea), and were treated with electrolytes during the experiment. These animals were excluded from the statistical analysis. Thirteen calves developed diarrhoea on the second day of the experiment but were not treated with electrolytes until after the experiment.

group	# calves at each scour level at pick-up					day 1 of exp	day 2 of exp	day after exp
	1	1.5	2	2.5	3			
C	8	1	0	0	1	2 x rotavirus (withdrawn)	1	0
FF	5	2	3	0	0	1 x rotavirus (withdrawn)	1	0
HF	6	1	2	1	0	0	3	2
3T	7	2	1	0	0	0	1	0
12T	6	2	2	0	0	0	0	0
12TLD	5	2	2	0	1	1 x rotavirus (withdrawn)	1	0
3TR	5	0	4	0	1	0	4	0
12TR	4	4	2	0	0	0	1	0

Table 2.3 Summary of the number of calves with diarrhoea. (Groups 3T, 12T, 12TLD, 3TR and 12TR are discussed in chapters 3 and 4).

Weight. Calves had a mean weight at pick-up of 40.1 ± 0.4 kg with a range of 31.7 - 51.4 kg (Fig. 2.6). The distribution of weights around the mean was normal with 50% of calves weighing between 37 and 42 kg.

Rectal Temperature. The calves had a mean rectal temperature of 39.1 ± 0.1 ^{0}C at pick-up and a range of 29.4 - 40.3 ^{0}C (Fig. 2.7). Distribution of weights around the mean was skewed to the right as the majority of calves had temperatures around the mean and a small number had low temperatures.

Gamma-glutamyl transferase (GGT). At pick-up, calves had a mean plasma GGT concentration of 568 ± 65 u/l and a large range of 9 - 2507 u/l (Fig. 2.8). The distribution around the mean was skewed to the left with more than 50% of calves having GGT levels less than 600 u/l. 19% of calves had GGT levels less than 200 u/l.

2.4.2 Indices of Hydration State

PCV. At 0 hours (before calves were fed) the initial mean PCV for group C was 35 ± 2 % which although numerically lower, was not significantly different from mean PCVs in FF and HF calves (Fig. 2.9). PCVs in all three groups changed little throughout the experiment, apart from a small decline during the first 4 hours which was significant in the HF group ($p<0.005$). There were no significant differences in PCV between the HF and FF calves. Group C had lower PCVs than the HF and FF groups, although these were only significantly different at 4 and 22 hours ($p<0.05$).

Total plasma proteins. Mean total plasma protein concentrations ranged from 51.4 to 89.6 g/l (Fig. 2.10). Control animals had a mean concentration of 70.6 ± 4.1 g/l at 0 hours. This concentration did not change significantly over the 31 hours and there were no significant differences between mean total plasma protein concentrations of calves in different groups.

2.4.3 Indices of Metabolic State

Glucose. The initial mean plasma glucose concentration of control calves was 5.8 ± 0.1 mmol/l (Table 2.4). This concentration had decreased ($p<0.05$) after the first 13 hours during which time plasma glucose concentrations were not significantly different from those of unfed calves (Fig 2.11). After feeding at 13 hours, glucose concentrations of C calves increased over 3 hours and then declined until they were fed again at 25 hours which initiated a repetition of this pattern. During the 31 hours control calves maintained mean plasma glucose concentrations of between 5 and 6 mmol/l. The concentrations of glucose in unfed calves were significantly lower than those of the controls after 13 hours. They continued to decrease, becoming significantly different from the initial concentrations after 16 hours in HF calves ($p<0.05$) and after 19 hours in FF calves ($p<0.005$). After 24 hours the concentrations remained fairly constant. Concentrations in HF calves were not significantly different from those in FF calves throughout the experiment.

Beta-hydroxybutyrate. Mean plasma concentrations of beta-hydroxybutyrate were not significantly different between groups at the initial sample (Table 2.4). Control calves had a mean concentration of 0.09 ± 0.01 mmol/l at 0 hours. Calves in the unfed groups exhibited significant increases in plasma beta-hydroxybutyrate concentrations by 31 hours ($p<0.0001$). There were no differences seen in concentrations of plasma beta-hydroxybutyrate between calves in the two unfed groups at the end of the experiment (Fig 2.12).

Triglycerides. Initial mean plasma triglyceride concentrations were similar between calves of the three groups (Table 2.4). Control animals had mean plasma triglyceride concentration of 0.3 ± 0.7 mmol/l. Changes in triglyceride concentration by 31 hours were not significant and there were no differences in concentrations of calves from different groups (Fig 2.12).

Urea. Control calves had initial mean plasma urea concentrations of 3.1 ± 0.3 mmol/l (Table 2.4). There were no significant differences between the initial plasma concentrations in calves of the three groups. There was a numerical increase in urea

concentrations in all groups but this was not significant. Mean plasma urea concentrations did not differ significantly between calves of different groups (Fig 2.12).

2.4.4 General Clinical State Throughout the Experiment

Weight. Calves were weighed on the day before the experiment. Apart from 2 calves in group C (19 and 3), the calves in this group showed changes in body weight of less than 2 kg (Fig 2.13a). The majority of calves in the unfed groups lost body weight during the experiment. HF calves lost up to 6 kg of body weight and FF calves up to 3.5 kg (Fig 2.13a). Mean weights before the experiment ranged from 31.4 to 50.4 kg and there were no significant differences between groups at this time. Calves in group C did not show significant changes in mean weight over the duration of the experiment (Fig 2.13b). Mean weight loss did occur in unfed calves (HF and FF) but this was only significant in FF calves ($p<0.05$).

Rectal temperature. The mean initial rectal temperature for control animals was 38.6 $\pm$ 0.1 ^{0}C (Fig. 2.14). Calves in HF and FF groups had an initial temperature of 39.0 $\pm$ 0.1 ^{0}C and the range in temperature for all groups was 37.7 to 40.3 ^{0}C. The mean rectal temperature of all calves did not change significantly during the 31 hours. Temperature in the control calves tended to be lower than unfed calves at 0 and 13 hours, but this difference was only significant between C and HF calves at 13 hours ($p<0.05$).

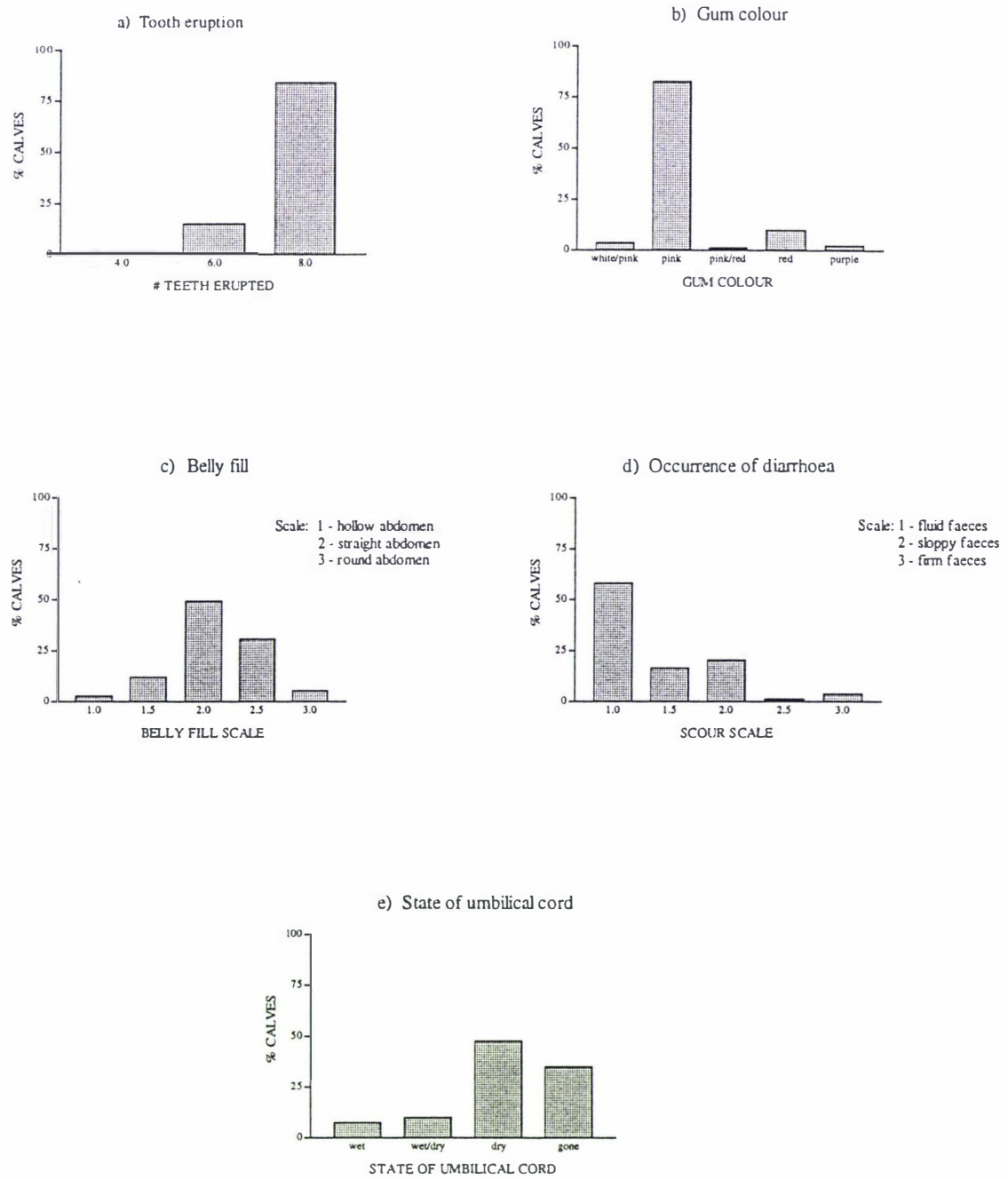


Fig 2.5 Subjective observations of calves at pick-up.

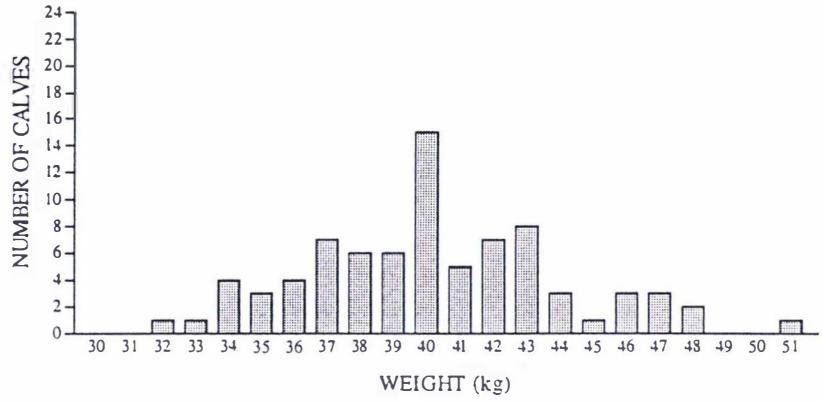


Fig 2.6 Weight of calves at pick-up.

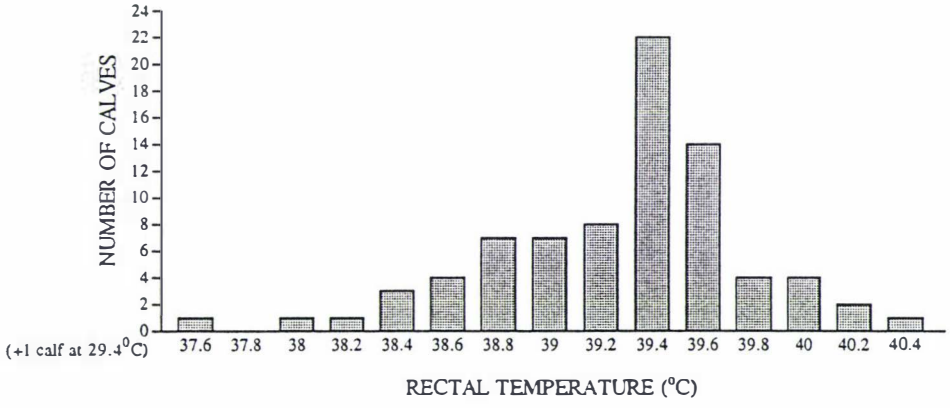


Fig 2.7 Rectal temperature of calves at pick-up.

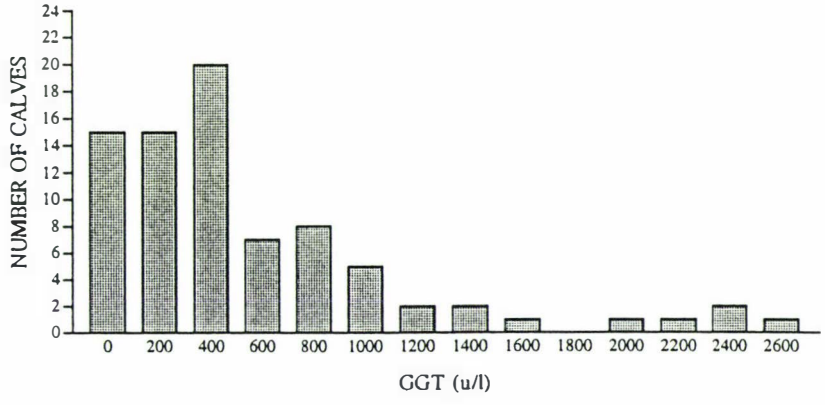


Fig 2.8 Plasma gamma-glutamyl transferase (GGT) concentration in calves at pick up.

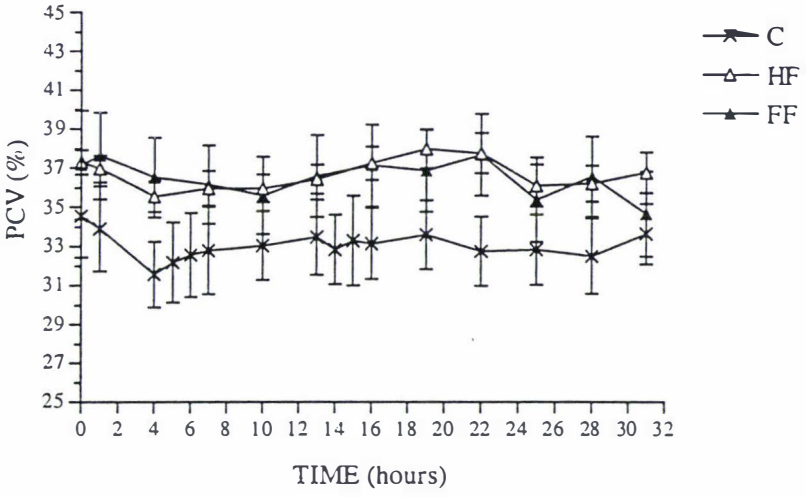


Fig 2.9 Mean PCVs (± SEM) in fed calves and calves that were unfed for 30 hours.

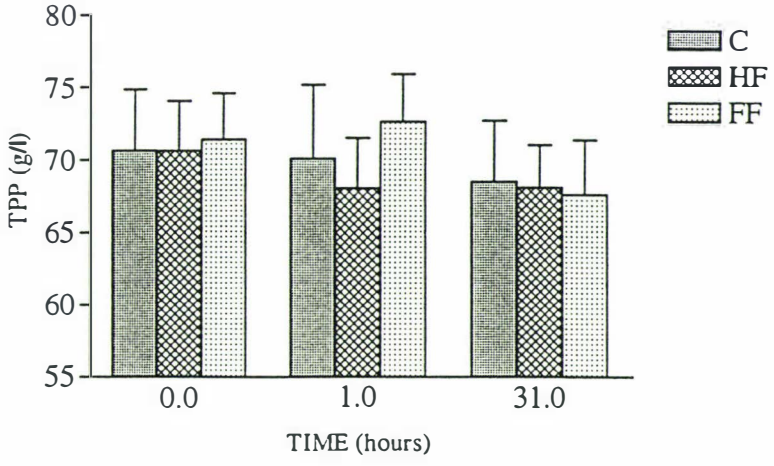


Fig 2.10 Mean total plasma protein (TPP) concentrations (± SEM) in fed calves and calves that were unfed for 30 hours.

Plasma concentration at 0 hours ±SEM (mmol/l)	C	HF	FF
glucose	5.85 ± 0.14	5.41 ± 0.26	5.04 ± 0.36
beta-hydroxybutyrate	0.09 ± 0.02	0.12 ±0.26	0.14 ± 0.03
triglycerides	0.34 ± 0.07	0.34 ± 0.09	0.30 ± 0.11
urea	3.13 ± 0.34	3.06 ± 0.18	3.61 ± 0.55

Table 2.4 Initial mean values of metabolic parameters.

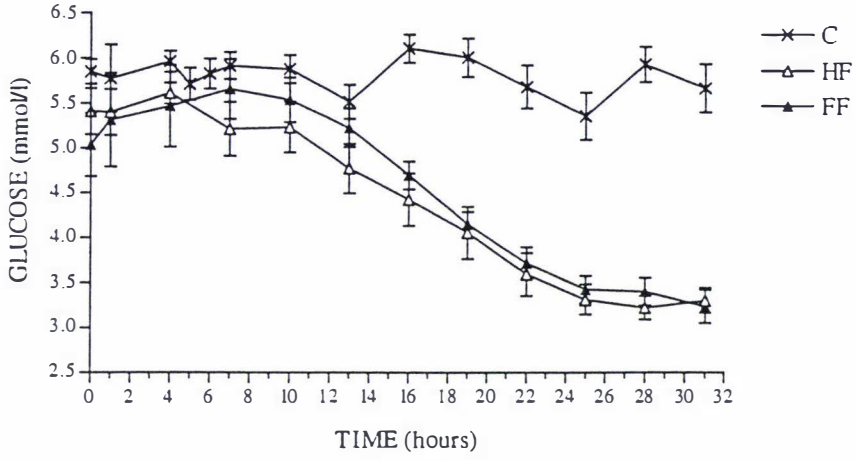


Fig 2.11 Mean plasma glucose concentration (±SEM) in fed calves and calves that were unfed for 30 hours.

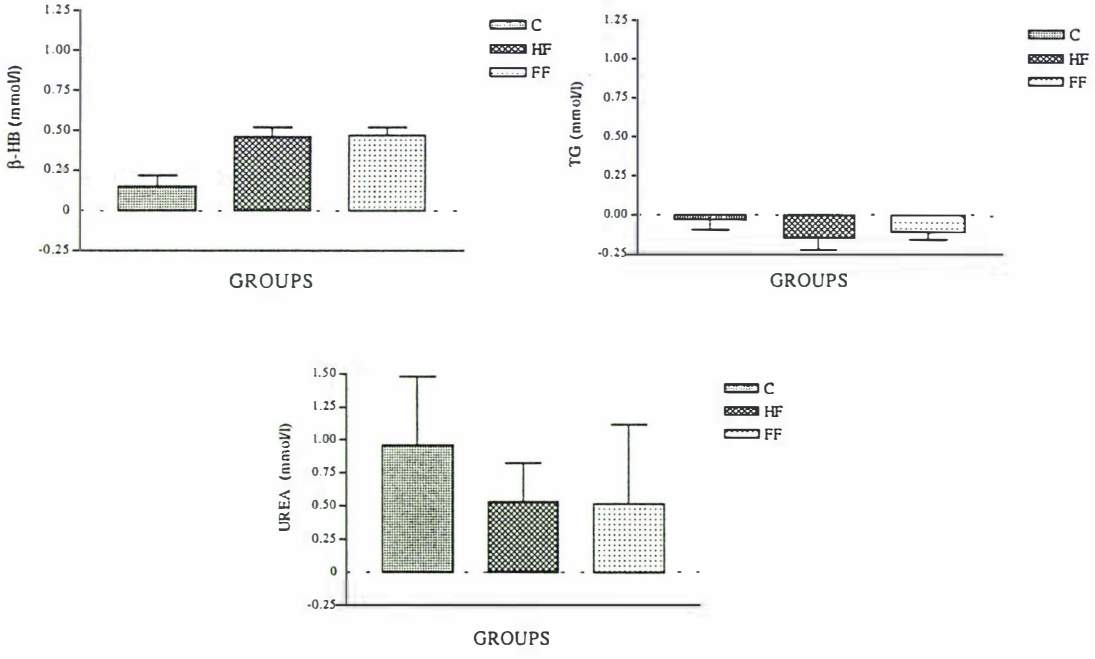


Fig 2.12 Mean changes after 31 hrs in plasma concentrations of beta-hydroxybutyrate (β-HB), triglycerides (TG) and urea (±SEM) in fed calves and calves that were unfed for 30 hours.

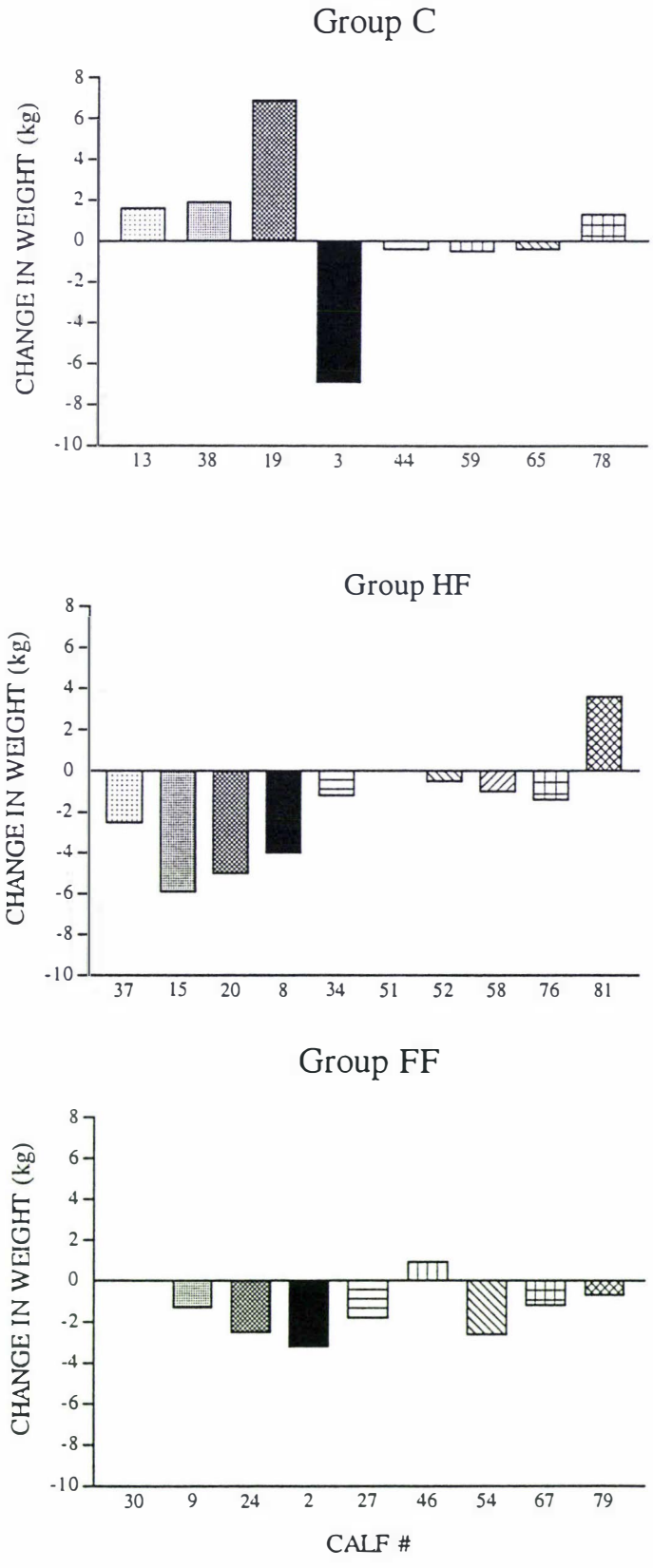


Fig 2.13a Change in weight after the experiment of individual fed calves and individual calves that were unfed for 30 hours.

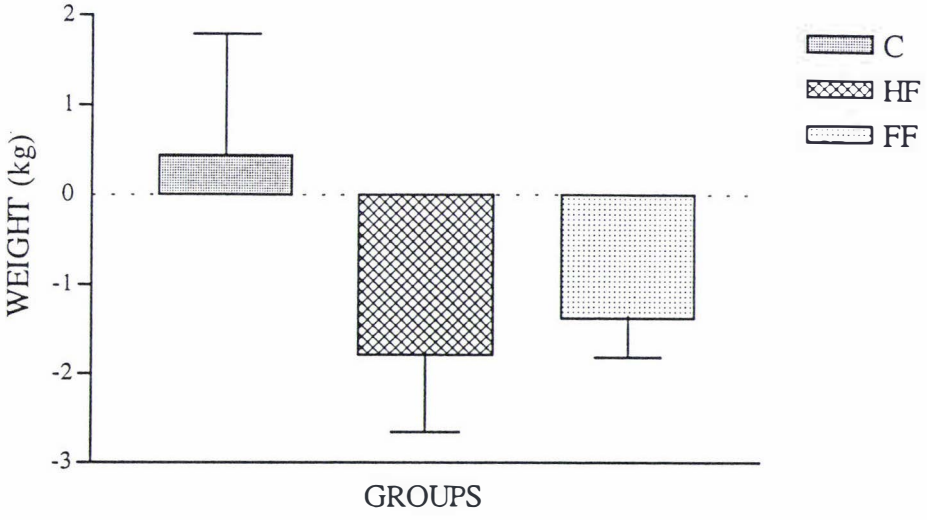


Fig 2.13b Mean change in weight (±SEM) after the experiment of fed calves and calves that were unfed for 30 hours.

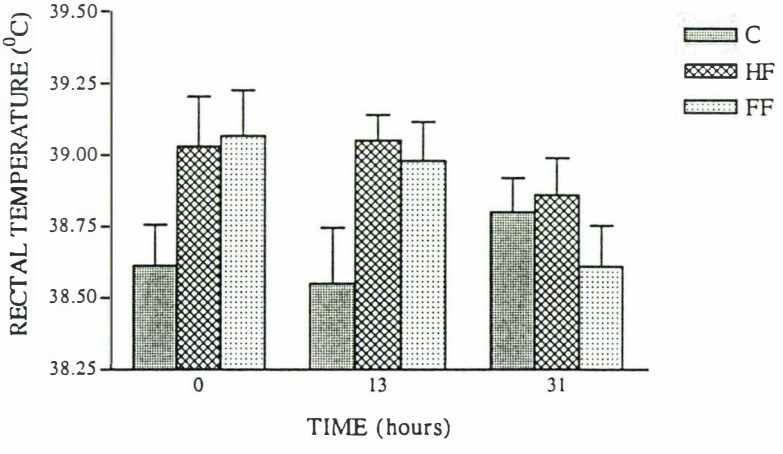


Fig. 2.14 Mean rectal temperatures (±SEM) of fed calves and calves that were unfed for 30 hours.

2.5 DISCUSSION

Calves at pick-up.

Results obtained from the subjective observations (tooth eruption, gum colour, umbilical cord remnant, belly fill) and from measurements of liveweight, rectal temperature and GGT concentrations carried out at pick-up were as would be expected for normal healthy full term calves (see 2.2 - Introduction).

GGT concentrations in calf plasma are largely determined by the calf receiving an adequate intake of immunoglobulins. This can be influenced by the age of the calf at first sucking, the amount of colostrum ingested by the calf, and the concentration of immunoglubulins in colostrum from the mother (Stott *et al.*, 1979). The concentration of GGT in the blood may also be influenced by the time at which the blood sample was taken after the first feed of colostrum. Plasma GGT concentrations in the calf are directly dependent on the ability of the calf to absorb immunoglobulins (Fey, 1971), so that the large range of plasma GGT concentrations found in these calves was normal. Braun *et al.*, (1982) analysed plasma concentrations of GGT and found that in newborn calves before feeding, concentrations ranged from 10-31 u/l. Concentrations reached maximum levels (370 - 5000 u/l) at 1-2 days after birth during which time calves had received colostrum, then decreased to a stable concentration of 100 u/l on the 20th day. Using 200 u/l as a 'cut-off' (Perino *et al.*, 1993), the concentrations of GGT in most calves from our study indicated that they had received sufficient colostrum to provide passive transfer of immunoglobulins, but 19% of the calves did not. Taking into account the variables which influence plasma GGT concentrations and the fact that 85% of calves in this study were older than 2 days (in which case 'gut closure' should have occurred), the mean GGT concentration of 568 ± 65 u/l obtained from these calves would suggest that concentrations had peaked.

The occurrence of diarrhoea at pick-up was high, as indicated by 58% of the calves having fluid faeces. It is likely that most of these calves had nutritional diarrhoea as a result of a number of factors including stress, change of environment and change of feed. This type of diarrhoea is common in neonatal calves on dairy farms and they

usually recover quickly when treated with electrolytes. The development of diarrhoea in the thirteen calves on the second day of the experiment may have resulted from additional disturbances experienced during the experiment.

Clinical state.

Rectal temperature may provide an indication of the overall well-being of an animal. The causes of decreased body temperature (hypothermia) have been well outlined in newborn lambs (Eales *et al.*, 1982). Eales *et al.* described the two major causes of hypothermia in newborn lambs as being excess heat loss and inhibited heat production. Excess heat loss usually occurs soon after birth as a result of exposure of the wet newborn lamb to inclement weather conditions (Eales and Small, 1980). The causes of inhibited heat production in newborn lambs have been summarised as starvation (Alexander, 1962); chronic and acute hypoxaemia due to problems with placental insufficiency and problems occurring during birth (Eales and Small, 1980); and immaturity, resulting in a low heat production capacity (Eales et al., 1982). It may be that parallel causes of hypothermia occur in newborn calves. The calves in our study were housed at mild environmental temperatures and were not exposed to inclement weather, so that excess heat loss would not be a major of hypothermia in these calves. As our calves were at least 5 days old during the experiment, hypoxaemia and immaturity as causes of hypothermia can also be ruled out. Thus if calves in this study were suffering from hypothermia, the most likely cause would be starvation or depletion of energy reserves. Mean rectal temperatures were in the normal range for healthy calves throughout the experiment. The fact that no hypothermic calves were observed suggests that sufficient energy reserves were available for heat production.

Mean changes in body weight of control calves were minimal. Unfed calves lost a mean of 1.5 kg (FF) and 2 kg (HF) over the 2 days. This weight loss was similar to that observed by Kirton and Paterson (1973) where calves between 1 and 2 weeks of age were starved for up to 3 days: starvation for 1 day resulted in a mean live weight loss of 1.53 kg and for 2 days a loss of 2.86 kg. Estimations of weight loss in the present calves due to carbohydrate and lipid utilisation were calculated using the summit metabolic rate of Holstein calves (Carstens, 1994) and the average respiratory quotient of lambs,

piglets and infants (Mellor and Cockburn, 1986). Assuming the calves did not lose weight in the form of faeces or urine, it was estimated that at the maximum metabolic rate and a respiratory quotient of 0.85, during the experiment, weight loss due to carbohydrate utilisation would have been about 1.2 kg, while lipid utilisation would have accounted for 0.5 kg. These figures represent an overestimate of substrate utilisation as the calves would not have exhibited maximum metabolic rates during this period (Mellor and Cockburn, 1986). A more realistic estimation of substrate utilisation may occur at 40% of summit metabolic rate. At this level of metabolism carbohydrate utilisation would account for around 0.5 kg of weight loss and lipid utilisation for 0.1 kg. At a low metabolic rate (10% of summit metabolic rate) carbohydrate utilisation may account for 0.1 kg and lipid 0.05 kg of weight loss. Thus it would appear that weight loss occurring in these calves may have resulted to some extent from catabolism of glycogen and fat reserves. The remaining weight loss is likely to have been due to water loss through excretion. In our study, although mean losses of body weight seen in unfed calves after the experiment were not significant, individual calves in these groups showed obvious weight loss during this time (Fig 2.13a). Weight losses in HF calves appeared to be slightly greater than those in FF calves as may be expected when fed half the volume.

There is some evidence that calves were initially disturbed by the proceedings of the experiment but recovered during the first few hours. This was indicated by initially higher PCVs and plasma glucose concentrations. All calves had higher PCVs at 0 hours (Fig 2.9) followed by a decrease until 4 hours. There are two possible explanations for this. It may be that the calves were slightly dehydrated from not having received any food during the night and PCV recovered after feeding between 0 and 1 hours. This reasoning is not justified as control calves were fed again at 13 and 25 hours and a similar change in PCV was not observed. Alternatively, calves may have been stressed by activities occurring at the beginning of the experiment. Stress can cause an increase in ACTH secretion which initiates secretion of cortisol. In some animals, cortisol secretion is associated with mobilisation of erythrocytes (Persson, 1967), resulting in an increased PCV. Stress may also activate the hypothalamus causing mass sympathetic discharge. Excitation of the sympathetic nervous system causes splenic contraction and therefore release of red blood cells. These factors may explain the high initial PCVs in

our calves. It would appear that calves rapidly adapted to the handling and other disturbances they were subjected to as values then returned to basal levels by 4 hours and no further significant changes were observed. This theory is supported by the observations in calves seen by Kinsbergen *et al.*, (1994) who found high initial concentrations of cortisol which had returned to basal levels by 4 hours. These authors also attributed their high initial PCVs to handling stress. Stress acting via cortisol and/or catecholamines also promotes gluconeogenesis and glycogenolysis and therefore increased glucose concentrations in the blood. This was observed in our experiment as calves had somewhat elevated initial plasma concentrations of glucose compared to the values at 13 hours. (Fig 2.11).

Hydration state.

In general minimal effects of the present treatments on hydration state were seen in the calves as indicated by PCV and total plasma protein concentrations. These results may be as expected because although the calves were not fed colostrum, they did have ad libitum access to water and were observed to drink. Although control calves had numerically lower PCVs than unfed calves during the experiment, this can be attributed to natural variation as the PCVs of control calves remained consistently lower than those of control calves throughout the experiment. There was no indication from the PCV of dehydration in any group. Similarly, the concentration of total plasma proteins indicated little change in hydration state during the 31 hours. The mean total plasma protein concentration in control animals was similar to those given by Shannon and Lascelles (1966), Naylor *et al.* (1977) and Katunguka-Rwakishaya *et al.* (1985) in calves.

Metabolic state.

From birth until sucking occurs, the newborn must derive metabolic fuel from its own energy reserves which are deposited during gestation. Protein catabolism during the first day after birth is minimal, therefore glycogen and lipid are the main energy reserves at this time (Mellor and Cockburn, 1986). Liver glycogen is the major source of circulating glucose in the newborn until sucking occurs (Girard *et al.*, 1992) and in

lambs, represents about 15% of the available body glycogen (Mellor and Cockburn, 1986). Plasma glucose concentrations of unfed calves at birth are low for the first few hours, averaging 3.4 mmol/l (Daniels *et al.,* 1974).

Glucose concentrations rapidly increase after colostrum intake to reach a peak around 12-14 hours after birth (Daniels *et al.,* 1974; Ronge and Blum, 1988;). Apart from the immediate hours after birth, fed, pre-ruminant calves have much higher glucose concentrations in plasma than do mature cattle (Oltner and Berglund, 1982). Control calves (fed, pre-ruminant animals) in this study had a mean range of glucose concentrations similar to those reported by other authors in fed calves at 4-13 days of age (5 to 7 mmol/l: Shannon and Lascelles, 1966; Wood *et al.,* 1971; Oltner and Berglund, 1982; Ronge and Blum, 1988).

The glucose response to feeding showed two phases: an increase in plasma glucose concentrations after feeding over 2 - 3 hours, followed by a gradual decline. The transient increase in glucose concentration which followed the second and third feeds in control calves (Fig 2.11) was also seen by Siddons *et al.*, (1969). The first phase (glucose increase) represents replenishment of blood glucose dominating after feeding. The young calf is able to readily utilise glucose, galactose and lactose (Siddons *et al.*, 1969) and the major carbohydrate in colostrum is lactose (Mellor and Cockburn, 1986). When calves are fed, lactose is digested to release glucose and galactose which are absorbed in the blood and contributes to plasma glucose directly or indirectly after conversion of galactose to glucose in the liver. Glucose concentrations reached a maximum level 2-3 hours after feeding. The second phase of the glucose response to feeding, which is representative of glucose utilisation predominating, begins when the rate of glucose utilisation exceeds the rate of replenishment. Concentrations of plasma glucose then decline as this readily available source of carbohydrate is delivered to peripheral tissues for utilisation in metabolic reactions.

The decrease in concentration of plasma glucose seen in unfed calves after 13 hours indicates that glycogen reserves were becoming depleted (Fig 2.11). The three major sources of glucose include the diet, liver glycogen and muscle glycogen. Glucose or its direct precursors absorbed from the gut enters the circulation where it remains available

for metabolic needs. In fasted animals this readily available carbohydrate supply is rapidly utilised and the consequent limited supply of exogenous fuel substrates necessitates a switch to mobilisation of the body's fuel reserves. Hormonal action promotes glycogenolysis in the liver which helps to maintain adequate levels of glucose in the blood, but this supply of liver glycogen is limited. In an adult fasted sheep the supply of liver glycogen lasts for approximately 24 hours, whereas in the newborn lamb the supply of liver glycogen could only meet the entire carbohydrate requirements for up to 2 hours (Mellor and Cockburn, 1986). The other source of carbohydrate is muscle glycogen, but products of this store are mainly metabolised in the muscle (see Chapter 3). After 30 hours without food the calves had mean plasma glucose concentrations of 3.4 mmol/l which was not particularly low when compared with the glucose concentration of calves that had acute diarrhoea. In such calves glucose concentrations of 2.2 mmol/l have been reported (Lewis *et al.*, 1975). As there were no differences between glucose concentrations of FF calves and HF calves, it appears that feeding half the volume (25 ml/kg) before the onset of food deprivation did not induce a detectably poorer metabolic state. It should be noted however that the HF calves were fed at the full recommended rate prior to the start of the experiment. Had that not been so they may have exhibited less tolerance metabolically to the 30 hours of feed withdrawal.

As carbohydrate reserves become depleted, lipids are utilised as an alternative energy source. In our study, plasma triglyceride concentrations were measured at the beginning and end of the experiment and no significant changes of concentration were seen in any of the calves during this time. Measuring triglyceride concentrations more frequently would have provided a better indication of the status of plasma triglycerides during the course of the experiment. Due to the dynamic nature of triglyceride metabolism, their presence or absence in plasma is difficult to interpret. A more accurate impression of lipid metabolism may be gained from combining the responses of plasma triglyceride, free fatty acid and beta-hydroxybutyrate concentrations. In one study where calves were starved for 24 hours (Kinsbergen *et al.*, 1994), a gradual decrease in triglyceride concentration and a corresponding increase in free fatty acid concentration was observed. This suggests that triglycerides were being degraded in the liver to release fatty acids into the circulation, thereby providing a metabolic fuel. It is likely that a similar response would have been observed in the unfed calves in our study and there

was evidence to support this possibility. The ketone, beta-hydroxybutyrate, is produced as a result of high rates of fatty acid degradation in the liver. An increase in mean plasma beta-hydroxybutyrate concentration was seen in unfed calves in our experiment after 30 hours. Thus although we could not use triglyceride concentration as an accurate indicator of lipid utilisation in this study, the increase in beta-hydroxybutyrate concentration does suggest that our unfed calves needed to utilise body reserves of lipid to fuel metabolic reactions. This is supported by the findings of Kinsbergen *et al.* (1994). They reported that young calves have a high metabolic rate and low fat storage and that although fat reserves in 1 week-old calves are relatively small, such animals were able to rapidly mobilise fat to meet their energy needs.

The mean concentrations of plasma urea were similar to those found in other one week old calves (Kinsbergen *et al.*, 1994), and there was little change in concentration. There are two possible explanations for the lack of change in urea concentration of fed and unfed calves observed here: either calves of this age were not capable of catabolising amino acids as an energy substrate for metabolism, or the need to catabolise protein was limited. Amino acid catabolism is minimal during the first day in unfed newborns representing only 4-8% of the rate of heat production (Mellor and Cockburn, 1986) but there is evidence to suggest that calves between 1 and 2 weeks old are capable of catabolising significant amounts of protein if required (Blaxter and Wood, 1951; Kinsbergen *et al.*, 1994; R. Wesselink, pers. comm.). One week-old calves fed twice daily on milk for 24 hours showed no change in plasma urea concentrations, but when starved for 24 hours, an increase in urea concentration occurred the following day (Kinsbergen *et al.*, 1994). Similarly, the rate of endogenous urea nitrogen excretion in 1 week-old calves was 33 mg/kg/d and increased to 178 mg/kg/d in starved calves (Blaxter and Wood, 1951). The rate at which protein catabolism occurred in the fed and unfed calves in our study was estimated. At maximum metabolic rates (from Carstens, 1994) and using an average weight of 40 kg, protein catabolism was estimated to represent about 0.5% of the total energy consumption in fed calves and about 3% in unfed calves. Thus the contribution of protein catabolism to the overall metabolic rate in these calves appeared to be low.

The calves in this study did not become hypothermic, suggesting that there was enough fuel for heat production throughout. This was supported by observed metabolic parameters which also indicated that the supply of energy reserves had not been exhausted in FF and HF calves. The concentrations of plasma glucose were greater than 3 mmol/l, mobilisation of lipid was still occurring as indicated by elevated beta-hydroxybutyrate concentrations, and there was apparently no additional protein catabolism. Thus, in energetic terms, the welfare status of these calves had apparently not been significantly compromised.

Conclusions.

The following conclusions may be drawn from this work:

- Feeding calves at 12 hourly intervals is sufficient to maintain acceptably high glucose concentrations.
- At mild temperatures of between 7 and 13 ^{0}C, and with access to water at all times, calves that were without food for a 30 hour period showed no evidence of significant dehydration.
- Calves were able to maintain their energy levels during 30 hours of food deprivation by utilising glucose from endogenous stores which became depleted but not exhausted. This necessitated the use of lipid reserves, but protein catabolism other than for maintenance was apparently minimal.
- The responses of calves which were fed 25 ml/kg on the morning of feed withdrawal and were then starved for 30 hours were no different to those of calves treated in the same way but fed twice this volume. Thus it would not necessarily be detrimental to the calf if it did not receive the recommended volume of feed on the morning before feed is withheld (assuming its previous intakes had been at the recommended level).

CHAPTER 3: THE EFFECTS OF ROAD TRANSPORT ON UNFED, HAND-REARED DAIRY CALVES AGED 1-2 WEEKS.

3.1 SUMMARY

Animal transportation is a common and necessary feature of farming practice both in New Zealand and overseas. Animals of all ages are transported by road, rail, sea and air. There are many variables involved in transport and often one, or a combination of these may have a detrimental effect on the animals involved.

In this chapter the effects of transport duration and stocking density were examined in 1-2 week old calves that were also deprived of food for 30 hours. PCV and plasma concentrations of total proteins, glucose, beta-hydroxybutyrate, triglycerides, urea, creatine phosphokinase and lactate were measured throughout the experiment. Liveweight and rectal temperatures were also obtained. Groups included the two control groups C and FF (as in the previous chapter) which were not transported, and 3 transport groups. Calves in the transport groups were transported for 3 or 12 hours (3T or 12T) at a stocking density of $0.2m^2$/calf, and for 12 hours at a stocking density of 0.4 m^2/calf (12TLD). It was found that in the mild weather conditions that occurred during this experiment, 3 hours of transport had little apparent metabolic or physiological effect, whereas 12 hours transport at the high stocking density was more physically demanding. It was suggested that this was primarily due to the muscular activity required by the calves to maintain balance on the moving truck, but shivering could also have been a contributing factor. In contrast, calves that were transported for 12 hours at the lower stocking density responded in a similar manner to FF and 3T calves. This was attributed to the fact that they could lie down which was probably less demanding physically.

3.2 INTRODUCTION

Transport of domestic animals is required for the purposes of breeding, finishing and slaughter (Tarrant, 1990). For most domestic animals transportation involves both novel and aversive events which can be stressful to the animal (Fraser, 1979). The transport process involves assembling the animals prior to transport and includes loading, confinement with and without motion, unloading and penning in a new environment (Tarrant and Grandin, 1993). During this time, animals may be exposed to a number of potential stressors such as social regrouping, physical injury, lack of food and water and environmental stresses including heat, cold, humidity, noise and motion (Leach, 1982; Tarrant and Grandin, 1993). These physical and emotional stimuli have been recognised as a common cause of distress in transported animals (Fraser, 1979).

Responses of cattle to transport vary depending on the situation. Changes in various biochemical and physiological systems have been used to indicate the development of stress reactions during and after transport (Leach, 1982). Physiological and behavioural signs of stress do not necessarily indicate detriment to the animal but may represent a normal adaptation to an uncertain and threatening situation (Tarrant and Grandin, 1993). Conversely, if these physiological and behavioural signs become more intense, the pathological changes that result may compromise the welfare of the animal.

Many dairy calves are slaughtered because they are surplus to requirements. These calves are usually transported from the farm to a slaughterhouse. Within New Zealand calves are most commonly transported by road and the journey may last up to 12 hours (AWAC, 1993). During the journey and the time spent in lairage until slaughter, food is not available so calves may spend up to 30 hours without food (Chapter 1). In the previous chapter, some of the physiological and metabolic responses of 1 - 2 week old calves to withholding food for 30 hours were discussed. It was found that under the conditions of the experiment (see Chapter 2), no significant dehydration occurred. Calves utilised carbohydrate and lipid reserves, but minimal additional protein degradation was apparently required to fuel metabolism. Calves were also normothermic, indicating an adequate availability of substrates for heat production. These findings imply that the calves had energy reserves to spare following 30 hours

without food and therefore that these animals were not significantly compromised with regard to energy status (Chapter 2).

Aims of this study.

To evaluate the effect of road transport on 1-2 week old calves that were also deprived of food for 30 hours. Some physiological and metabolic responses to the following situations were examined:

- Transporting unfed calves by road for durations of 3 or 12 hours
- Transporting unfed calves by road for 12 hours at stocking densities of 0.2 m^2/calf or 0.4 m^2/calf.

3.3 MATERIALS AND METHODS

This research was conducted as one trial which was performed as 2 identical experiments on separate days (21-22/8/95 and 4-5/9/95). The materials and methods for this chapter with regard to animals (2.3.1) and methods (2.3.4) are as described in chapter 2.

3.3.1 Transportation

Calves were transported by truck for periods of 3 or 12 hours. Pen dimensions on the truck (1750 mm x 1300 mm) were such that with 10 calves (equivalent to 2 groups in one experiment) an average area of 0.2 m^2/calf was available as recommended in the Code (AWAC, 1993). With 5 calves (equivalent to 1 group in one experiment) the available area averaged 0.4 m^2/calf or half the recommended stocking density.

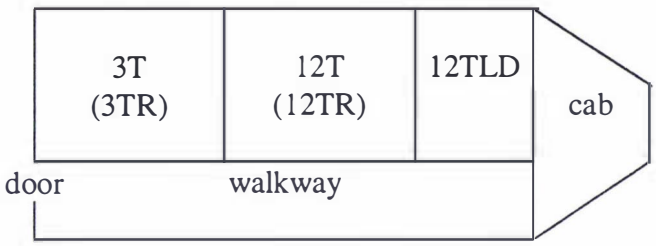


Fig 3.1 Layout of the truck. Groups 3TR and 12TR are to be discussed in chapter 4. (See Table 3.1 for summary of treatments).

Noise levels during transport were recorded using a noise logging dosimeter (Quest M-28) which was attached to the top of one of the gates (Fig 3.2). It was operated continuously for the first six hours of transport giving readings every 5 minutes. The data were printed out on return and provided decibel readings as histograms and peak values.

Between each 3 hourly blood sample, the calves were transported continuously for approximately two and a half hours over a variety of terrains, then returned to the experimental site for the next sample. The odometer readings were recorded each time the truck returned.

3.3.2 Groups

Ten calves (5 per experiment) were allocated to each group such that ages and weights were evenly distributed. The mean weight of calves on the day before the experiment was 41 kg. Five of the groups will be discussed in this chapter, the other 3 (HF, 3TR, 12TR) will be discussed in chapter 4 (Table 3.1).

GROUP n=10	TREATMENT					
	fed 50ml/kg	fed 50ml/kg	unfed for 30h	trans. 3h, density 0.2m^2/calf	trans. 12h, density 0.2m^2/calf	trans. 12h, density 0.4m^2/calf
C	√	√	-	-	-	-
FF	√	-	√	-	-	-
3T	√	-	√	√	-	-
12T	√	-	√	-	√	-
12TLD	√	-	√	-	-	√

Table 3.1 Summary of treatments.

Control (C). Calves were fed 50 ml/kg body weight twice daily (as recommended in the Code) at 12 hourly intervals beginning on the morning of the first experimental day at 6.30 am. They also had ad libitum access to water and calf pellets. These calves were not transported and remained in their pens during the experiment.

Full Feed (FF). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water. These calves were not transported and remained in their pens during the experiment.

3 Hours Transport (3T). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 3 hours starting immediately after the second blood sample was taken. During transport the available area was 0.2 m^2/calf (Fig 3.3a).

12 Hours Transport (12T). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 12 hours starting immediately after the second blood sample was taken. During transport the available area was 0.2 m^2/calf (Fig 3.3a).

12 Hours Transport, Low Density (12TLD). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 12 hours starting immediately after the second blood sample was taken. During transport the available area was 0.4 m^2/calf (Fig 3.3b).

3.3.3 Experimental Procedures

For all animals a 9 ml blood sample was taken at 0 hours (6 am) then calves were fed according to their group. Another blood sample was obtained after this feed at 1 hour (7 am) following which blood samples were taken at 4, 7, 10, 13, 16, 19, 22, 25, 28 and 31 hours.

Those animals being transported were bled on the truck (Fig 3.4) which returned every 3 hours for this purpose. Following transport the calves were bled before being unloaded and returned to their respective pens (Fig 3.5).

All animals were weighed prior to the experiment and again at the end of 31 hours. In groups C and 3T rectal temperatures were also obtained following blood sampling at 0, 4, 13, 31 hours. In groups FF, 12T and 12TLD rectal temperatures were taken at 0, 13 and 31 hours. A video recording of the behaviour of calves after transport was made between 11 am - 1 pm, 2.30 pm - 5 pm and 8.30 pm - 9 pm on day 1, however the recordings did not provide sufficient detail for analysis. The experimental protocol is summarised in Table 3.2.

3.3.4 Blood and Plasma Measurements

PCV was estimated by the microhaematocrit technique shortly after the blood samples were taken. At Ruakura Animal Health Laboratory, plasma samples were analysed for concentrations of triglycerides which were determined on an Hitachi 717 Autoanalyser (30 ^{0}C) using the enzymatic colorimetric test (modified according to Wahlefeld, 1974); and lactate using the UV method (Noll, 1974). At Massey University plasma samples were analysed on an Hitachi 704 Multi-channel analyser for concentrations of glucose,

using the enzymatic colorimetric method (Trinder, 1969) Boehringer Mannheim kit no. 1448668; total plasma protein, using the Biuret method (Weichselbaum, 1946) Boehringer Mannheim kit no. 1553836; urea, using the kinetic UV test (Neumann and Ziegenhorn, 1977) Boehringer Mannheim kit no. 1489364; beta-hydroxybutyrate (Li, 1985) Sigma, catalogue no. 310-A, procedure no. 310-UV; and creatine phosphokinase using the optimised standard method conforming to the recommendations of the Deutsche Geselischaft fur Klinische Chemie (Gruber, 1978), Boehringer Mannheim kit no. 763870.

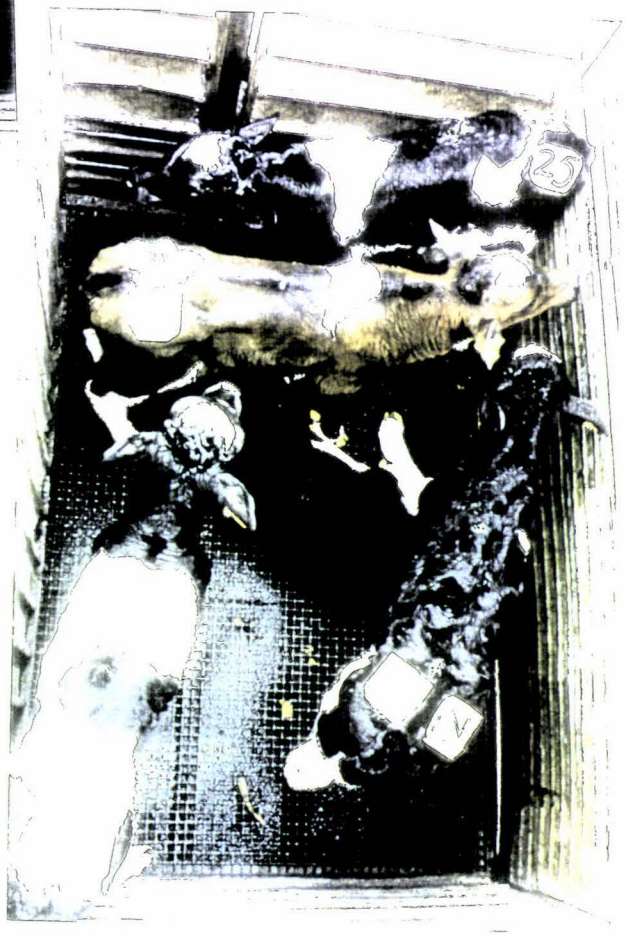
3.3.5 Presentation of Results

PCV and plasma concentrations of total proteins, glucose, creatine phosphokinase and lactate, and rectal temperatures were expressed graphically as mean ± standard error of the mean (SEM) for each group at each sample time. Liveweight and plasma concentrations of beta-hydroxybutyrate, triglycerides and urea were presented graphically as the mean change in concentration during the experiment ± SEM. Noise data was presented graphically as the amount of time (%) spent at each noise level.

Analysis of these data was performed using GraphPad Prism™ version 2.0. When the significance of differences between more than two groups at a particular sample time were required, a one-way analysis of variance (ANOVA) was conducted when data had equal variances, otherwise a Kruskal-Wallis test for non-parametric data was used. ANOVA (Bonferronis) and Kruskal Wallis (Dunns) post-tests were used to establish which differences were significant between the pairs. When the significance of a difference between two sample times was required for one group, a paired Student's t-test was used. Where data were presented as mean changes, a one-sample t-test was used to determine whether the changes were significantly different from zero.

Fig 3.2 Observing behaviour of calves in pens on the truck before blood sampling. The noise meter is secured to the top of a gate.

a) 0.2m^2/calf



b) 0.4m^2/calf

Fig 3.3 a) and b) Calves in pens on the truck at different stocking densities.

Fig 3.4 Obtaining blood samples by jugular venipuncture from calves on the truck.

Fig 3.5 Unloading calves from the truck after transport.

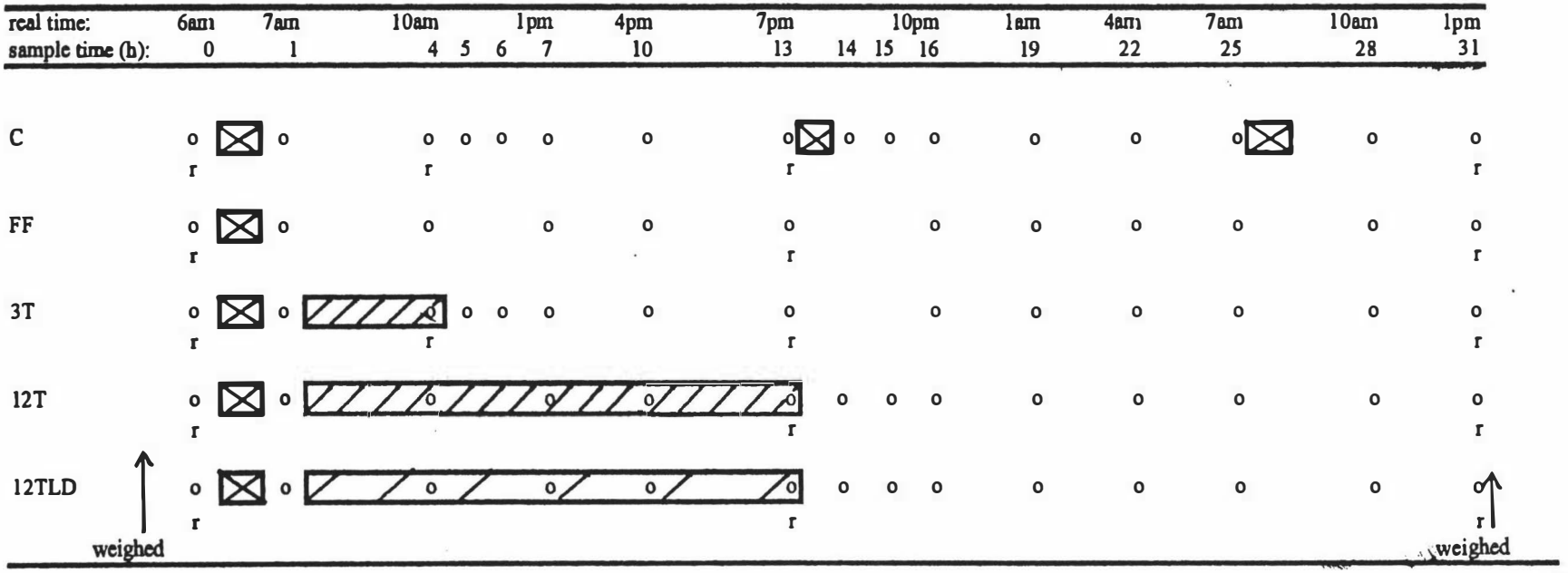


Table 3.2 Summary diagram of experimental protocol

3.4 RESULTS

3.4.1 Duration of Transport

Indices of hydration state.

Initial values of mean PCV ranged from 35 to 37 % and between groups there were no significant differences in mean PCV (Fig 3.6). A small, non-significant decrease in mean PCV of calves in all groups occurred during the first four hours. Mean PCVs in all groups changed little throughout the experiment and remained within the range of 31 to 38 %.

Mean concentrations of total plasma proteins ranged from 64 to 72 g/l at 0 hours and remained within this range throughout the experiment (Fig 3.7). There were no between-group differences in mean concentrations during this period.

Indices of metabolic state.

Mean plasma glucose concentrations of calves from all groups were not significantly different at 0 hours (Table 3.3) and did not differ significantly during the first 13 hours (Fig 3.8).

Concentrations of plasma glucose in control calves decreased after the first 13 hours ($p<0.05$). These concentrations increased after feeding at 13 hours and reached a peak 3 hours later. Glucose concentrations in control animals then declined until calves were fed again at 25 hrs which initiated repetition of this pattern. During the 31 hours, control calves maintained mean plasma glucose concentrations of between 5 and 6 mmol/l.

Calves from group 3T exhibited an increase in glucose concentrations after transport between 4 and 7 hours ($p<0.05$). After this time (7 hours) plasma glucose concentrations of FF and 3T calves were very similar during the experiment. Glucose concentrations in FF and 3T decreased at a steady rate until 25 hours at which time a

nadir was reached. Concentrations of glucose in these animals were significantly lower than in control calves after 16 hours ($p<0.01$).

Glucose concentrations of calves from 12T did not begin to decline until 19 hours and became significantly different from values from control calves after this time. Concentrations continued to decline at a steady rate until 31 hours.

Mean initial concentrations of plasma beta-hydroxybutyrate ranged between 0.08 and 0.14 mmol/l (Table 3.3) and there were no significant between group differences in concentrations. Beta-hydroxybutyrate concentrations significantly increased in FF ($p<0.001$), 3T ($p<0.001$) and 12T ($p<0.001$) calves by 31 hours (Fig 3.9). The concentration of beta-hydroxybutyrate in FF calves was significantly higher than those in control calves at this time ($p<0.05$).

Mean plasma concentrations of triglycerides in all calves were similar at 0 hours (Table 3.3). Changes in triglyceride concentration by 31 hours were not significant and there were no between-group differences in mean plasma triglyceride concentrations (Fig 3.9).

Mean initial concentrations of plasma urea were similar in all calves (Table 3.3). There were no significant changes in mean concentrations by 31 hours (Fig 3.9) and these concentrations did not vary significantly between calves of different groups.

Indices of physical state.

Mean plasma concentrations of creatine phosphokinase were similar in all groups at 0 hours and ranged from 73 to 95 u/l. Only calves in group 12T showed significant changes in concentrations during the experiment (Fig 3.10a). After 3 hours there was a steady increase the concentration of mean creatine phosphokinase in these calves until the end of transport at 12 hours (Fig 3.10b). After this time concentrations rapidly declined then stabilised at slightly higher than initial values.

Mean concentrations of plasma lactate ranged from 0.9 to 1.15 mmol/l at 0 hours. These concentrations were not significantly different between groups. Calves in groups C and 3T showed similar patterns in mean lactate concentrations during the experiment (Fig 3.11) and there were no significant differences in concentrations between these groups. During the first 4 hours C and 3T calves exhibited an increase (although not significant) in mean lactate concentrations. This was followed by a significant decrease ($p<0.05$) until 13 hours. Concentrations then increased again over the next 3 hours ($p<0.01$) followed by another decline until 31 hours. Plasma concentrations of lactate in 12T calves increased until after transport at 12 hours. Following this, concentrations of plasma lactate in 12T calves declined. Lactate concentrations in 12T calves were significantly different from concentrations in 3T and C calves at 12 hours.

Indices of clinical state.

Mean change in body weight during the 30 hours of observation was not significant in control animals (Fig 3.12). Calves in groups FF, 3T and 12T which were deprived of food for 30 hours, exhibited a mean weight loss of 1 to 1.5 kg ($p<0.05$) and there were no significant differences in weight loss between these groups.

Initial mean rectal temperatures ranged between 38.6 and 39.2 ^{0}C (Fig 3.13). Temperatures of calves in groups C, FF and 12T did not change significantly during the 31 hours. Group 3T calves exhibited a decrease in temperatures between 13 and 31 hours ($p<0.005$). Control calves had significantly different rectal temperatures from 3T calves at 13 hours, otherwise there were no differences in temperatures between groups.

3.4.2 Stocking Density During Transport

To avoid repetition, only results from the 12TLD calves will be discussed here as the other three groups were described in section 3.4.1 above.

Indices hydration state.

Calves in 12TLD exhibited a similar pattern of change in PCV to the other groups and the PCV remained within the range of 31 to 38 % during the experiment (Fig 3.14). No significant changes in PCV were observed during this period. Similarly, the mean concentrations of total plasma proteins in these calves were no different from those described in the other groups (Fig 3.15).

Indices of metabolic state.

Initial mean plasma glucose concentrations of 12TLD calves were similar to those of calves in the other groups (Table 3.3). 12TLD calves exhibited the same pattern of change in mean plasma glucose concentrations during the 31 hours as occurred in FF calves (Fig 3.16), and concentrations were significantly lower than those of control calves from 16 hours ($p<0.05$).

Mean concentrations of plasma beta-hydroxybutyrate were not significantly different from those of the other groups at 0 hours (Table 3.3). By 31 hours, there was a significant increase in the mean concentration of beta-hydroxybutyrate in 12TLD calves ($p<0.05$) which was similar to those seen in FF and 12T groups (Fig 3.17).

12TLD calves exhibited mean plasma triglyceride concentrations that were within the range of those seen in the other groups (Table 3.3). No changes in these concentrations were observed during the experiment (Fig 3.17).

Initial mean concentrations of urea in 12TLD calves were similar to those of other groups (Table 3.3). A numerically smaller change in urea concentration occurred in 12TLD calves compared with the change seen in calves in the other groups (Fig 3.17).

Indices of physical state.

Throughout the experiment, mean plasma concentrations of creatine phosphokinase in 12TLD calves remained within the range of 90 to 115 u/l (Fig 3.18) which was not significantly different from concentrations in the other calves at 0 and 31 hours. At 13 hours, creatine phosphokinase concentrations were significantly higher in 12T than in C and 12TLD ($p<0.001$).

Concentrations of mean plasma lactate in 12TLD calves declined between 4 and 7 hours ($p<0.005$) (Fig 3.19) during which period concentrations in 12TLD calves were significantly lower than those in C and 12T calves ($p<0.05$). Concentrations then increased over the next 6 hours ($p<0.005$) and thereafter declined again until 31 hours ($p<0.05$).

Indices of clinical state.

Mean weight loss in 12TLD calves was not significant (Fig 3.20) although 6 of the 9 calves in this group lost between 1 and 4 kg during the experiment. No significant changes in rectal temperature occurred in 12TLD calves over the 31 hours (Fig 3.21) and these calves exhibited rectal temperatures similar to those seen in other groups.

3.4.3 Noise Level During Transport

Noise levels on the truck during transport reached a maximum of 120 dB (Fig 3.22 a,b,c). When expressed as the amount of time (%) spent at each dB level, transport caused a biphasic response. The percentage of time spent above a certain noise level is illustrated in the table below.

Transport time (h)	% time spent at or above each noise level (dB)						
	≥50	≥60	≥70	≥80	≥90	≥100	≥110
3	100	94	80	62	53	29	3
12	100	87	65	49	40	17	2

Table 3.4 The amount of time spent above certain noise levels.

Mean plasma concentration ± SEM (mmol/l)	C	FF	3T	12T	12TLD
glucose	5.85 ± 0.14	5.04 ± 0.36	5.16 ± 0.19	5.55 ± 0.23	5.59 ± 0.17
beta-hydroxybutyrate	0.09 ± 0.02	0.14 ± 0.03	0.10 ± 0.02	0.09 ± 0.01	0.08 ± 0.01
triglycerides	0.34 ± 0.07	0.30 ± 0.11	0.27 ± 0.04	0.24 ± 0.03	0.26 ± 0.05
urea	3.13 ± 0.34	3.61 ± 0.55	2.98 ± 0.12	2.87 ± 0.27	2.81 ± 0.25

Table 3.3 Initial mean values of metabolic parameters.

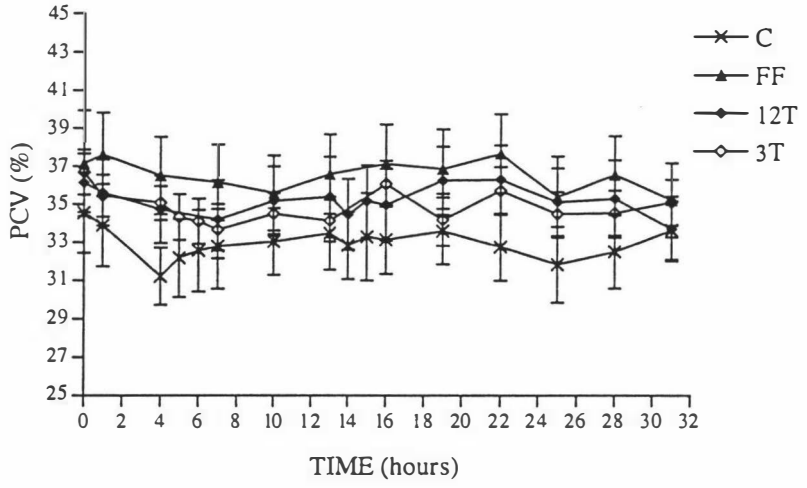


Fig 3.6 Mean packed cell volumes (PCV) (±SEM) in unfed calves transported for 3 or 12 hours at the recommended stocking density (0.2 m^2/calf).

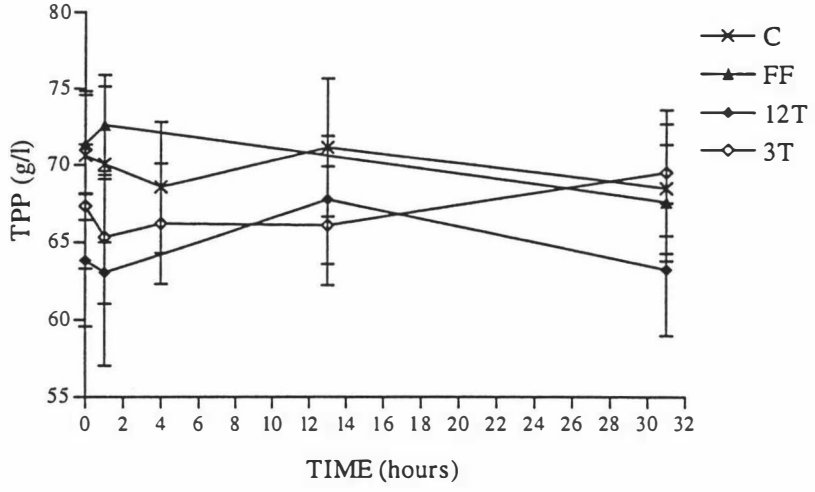


Fig 3.7 Mean concentration (±SEM) of total plasma proteins (TPP) in unfed calves transported for 3 or 12 hours at the recommended stocking density (0.2 m^2/calf).

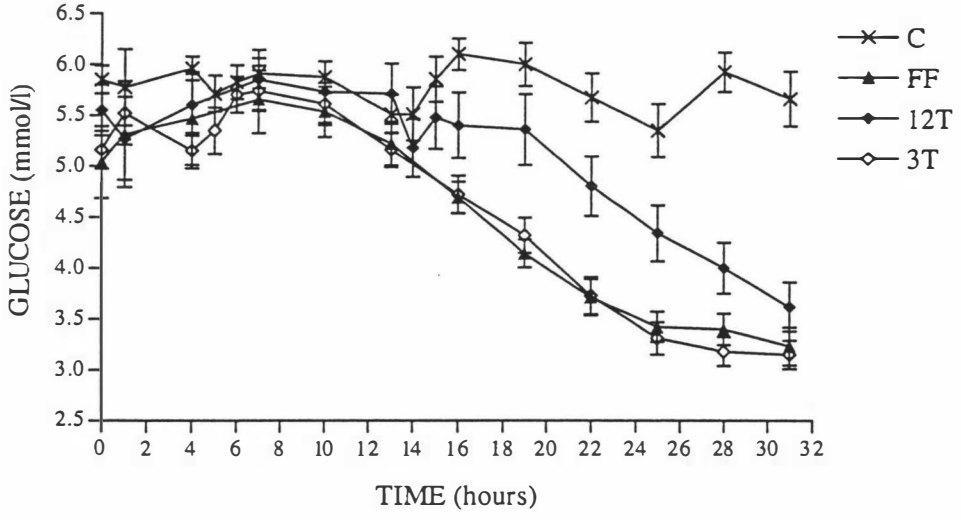


Fig 3.8 Mean plasma concentrations (±SEM) of glucose in unfed calves transported for 3 or 12 hours at the recommended stocking density (0.2 m^2/calf).

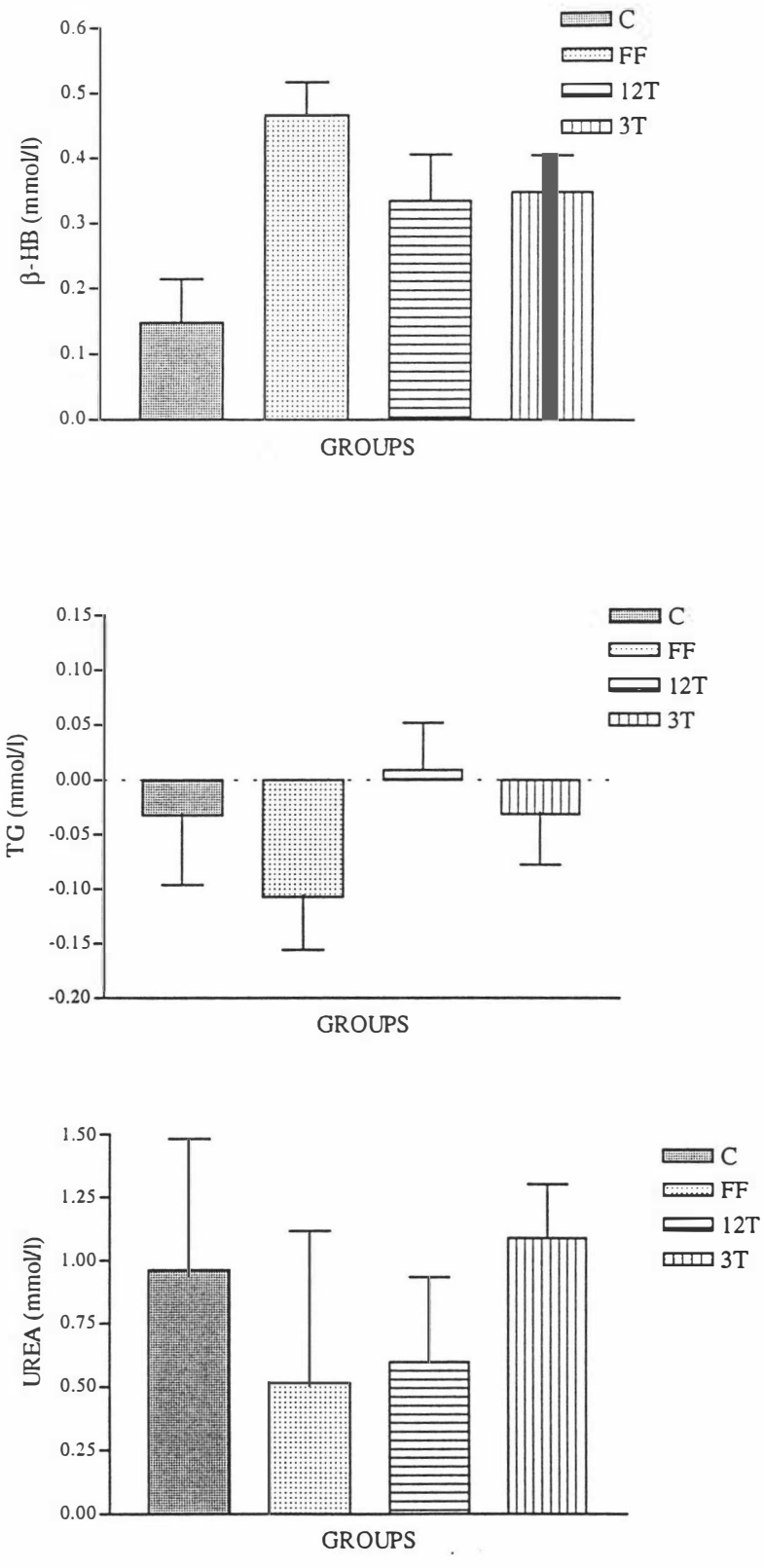


Fig 3.9 Mean changes (0 to 31h) (±SEM) in plasma concentrations of beta-hydroxybutyrate (β-HB), triglycerides (TG), and urea in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).

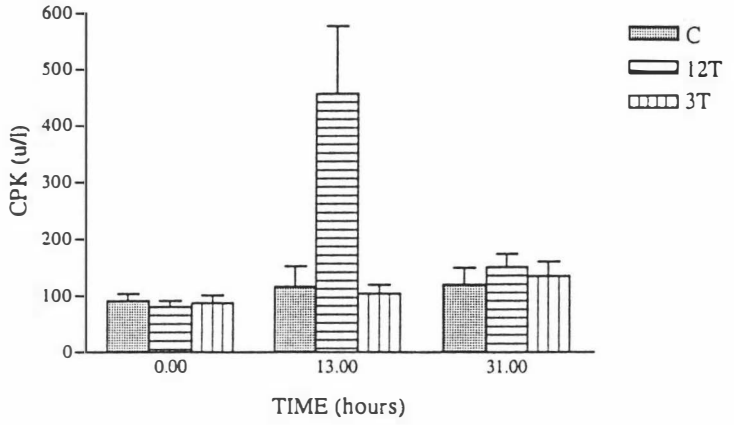


Fig 3.10a Mean plasma concentrations (±SEM) of creatine phosphokinase (CPK) in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).

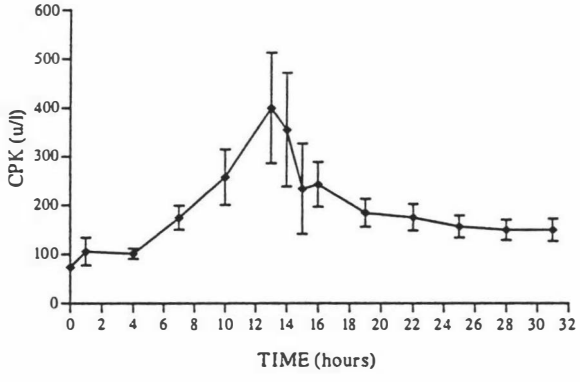


Fig 3.10b Mean plasma concentrations (±SEM) of creatine phosphokinase (CPK) in unfed calves(12T) transported for 12 hours at the recommended stocking density ($0.2m^2$/calf).

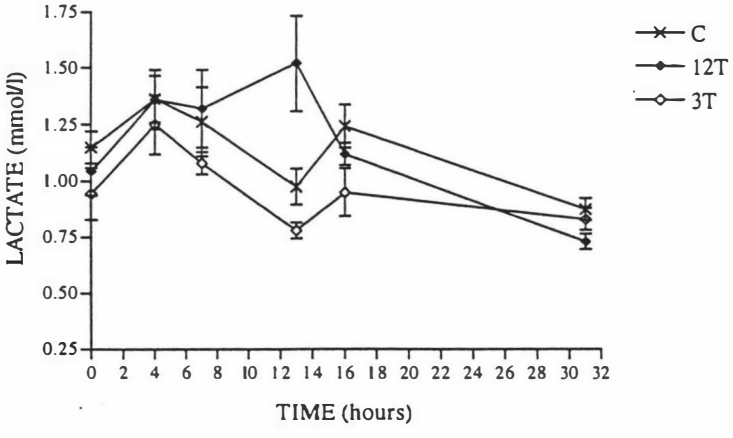


Fig 3.11 Mean plasma concentrations of lactate (±SEM) in unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).

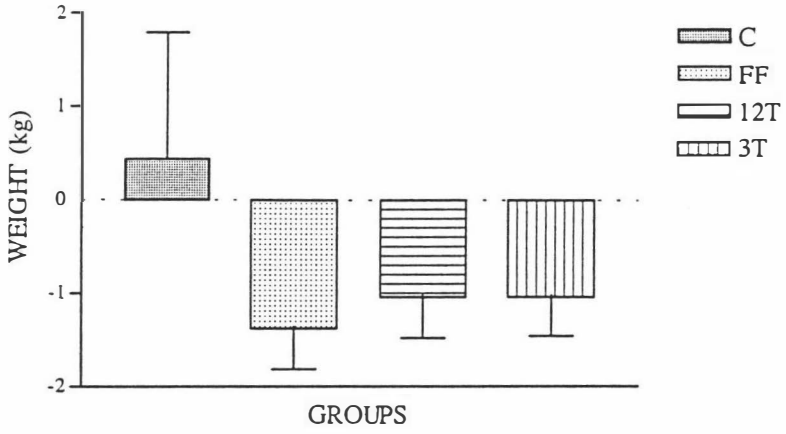


Fig 3.12 Mean change (±SEM) in weight (0 to 31h) of unfed calves transported for 3 or 12 hours at the recommended stocking density ($0.2m^2$/calf).

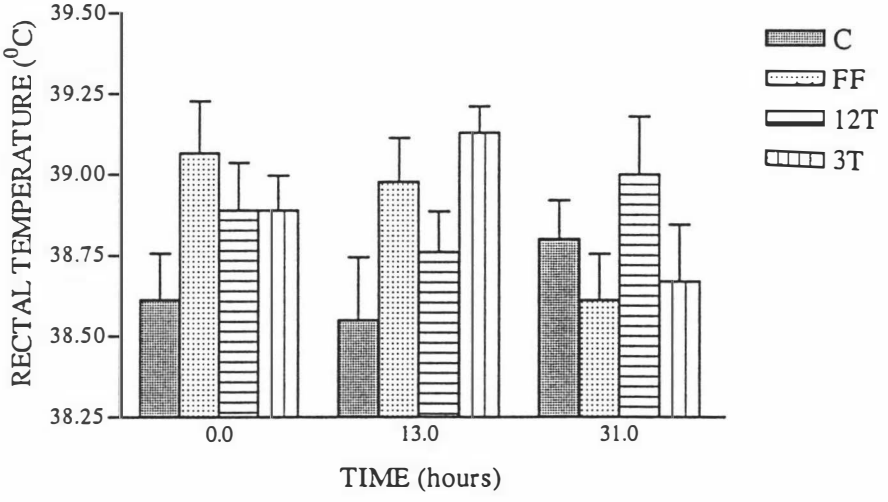


Fig 3.13 Mean rectal temperatures (±SEM) of unfed calves transported for 3 or 12 hours at the recomended stocking density ($0.2m^2$/calf).

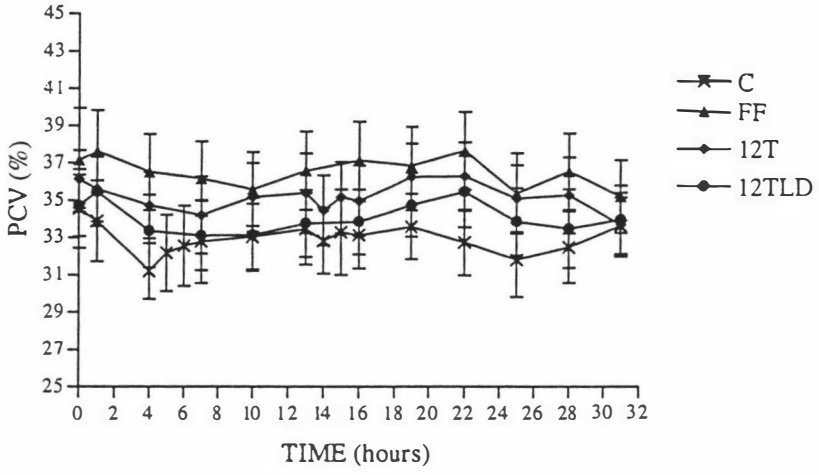


Fig 3.14 Mean packed cell volume (PCV) (±SEM) in unfed calves transported for 12 hours at high (0.2 m^2/calf) and low (0.4 m^2/calf) stocking densities.

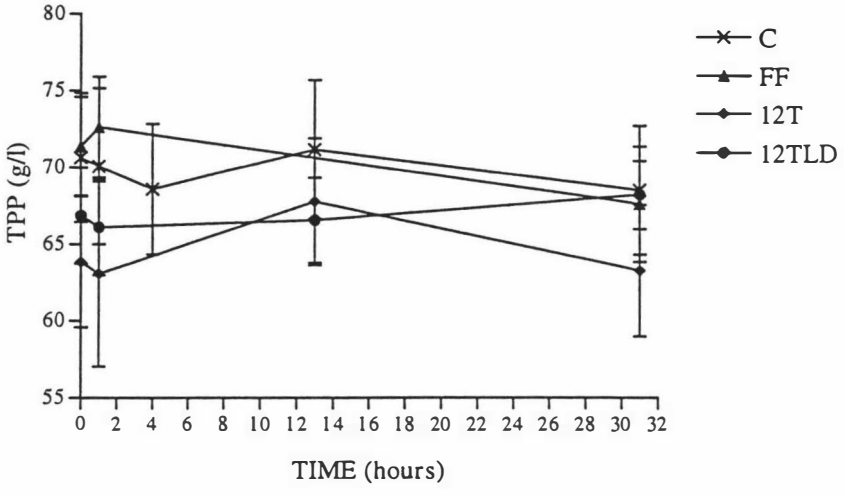


Fig 3.15 Mean concentration (±SEM) of total plasma proteins (TPP) in unfed calves transported for 12 hours at high (0.2m^2/calf) or low (0.4m^2/calf)stocking densities.

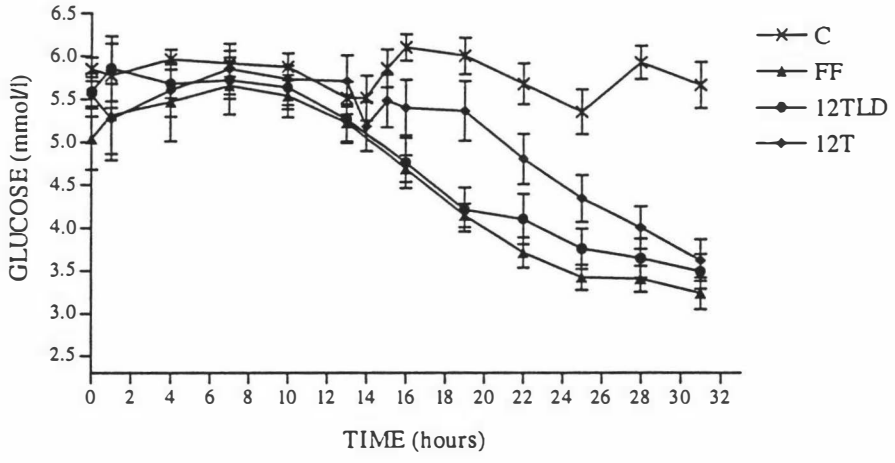


Fig 3.16 Mean plasma concentrations (±SEM) of glucose in unfed calves transported for 12 hours at high (0.2 m^2/calf) and low (0.4 m^2/calf) stocking densities.

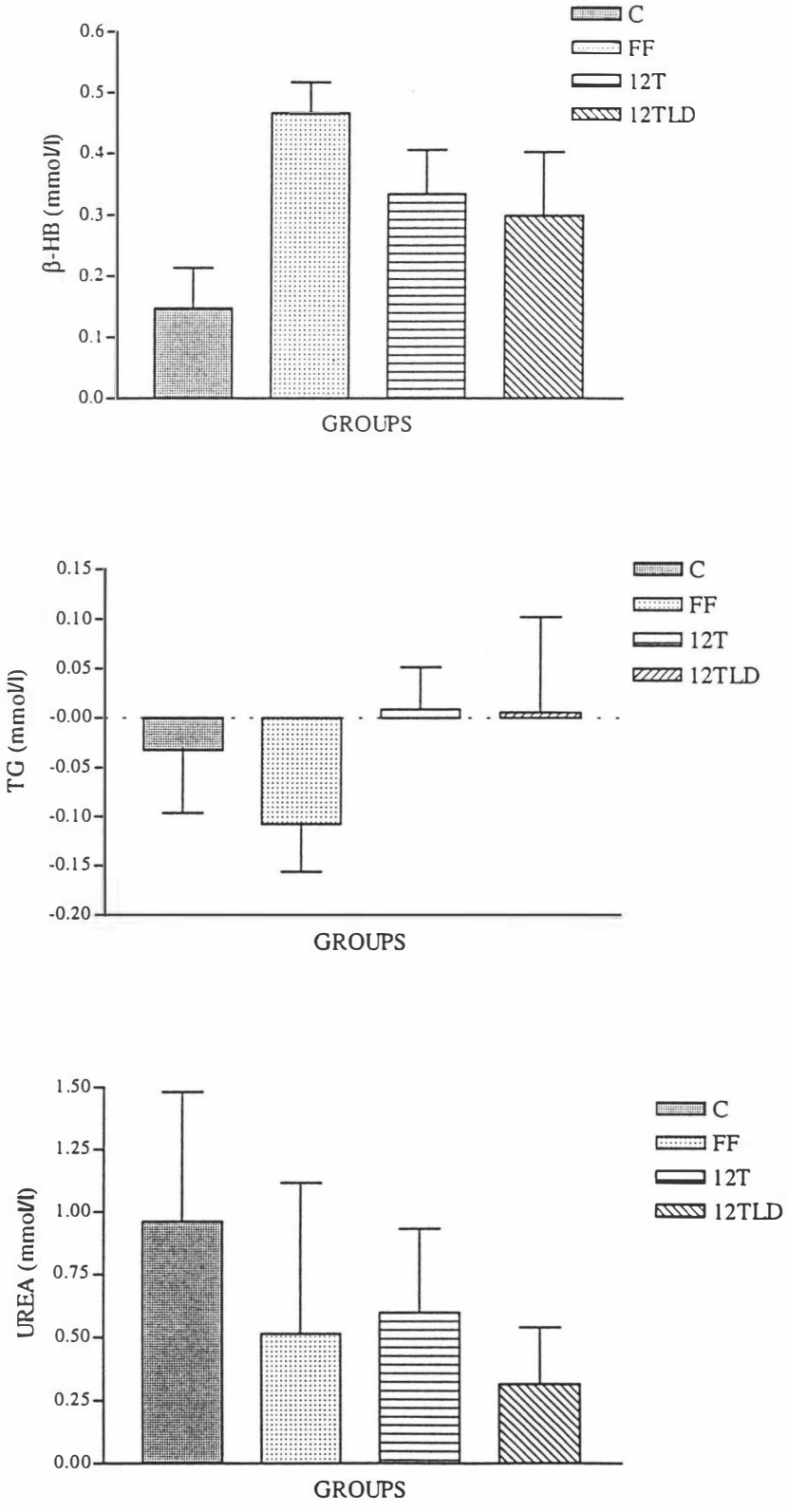


Fig 3.17 Mean changes (0 to 31h) (±SEM) in plasma concentrations of beta-hydroxybutyrate (β-HB), triglycerides (TG), and urea in unfed calves transported for 12 hours at high (0.2m^2/calf) or low (0.4m^2/calf) stocking densities.

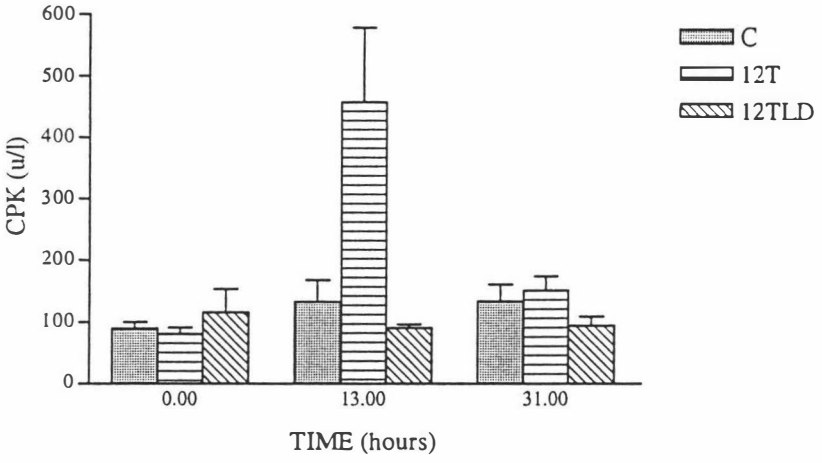


Fig 3.18 Mean plasma concentrations (±SEM) of creatine phosphokinase (CPK) in unfed calves transported for 12 hours at high (0.2m^2/calf) and low (0.4m^2/calf) stocking densities.

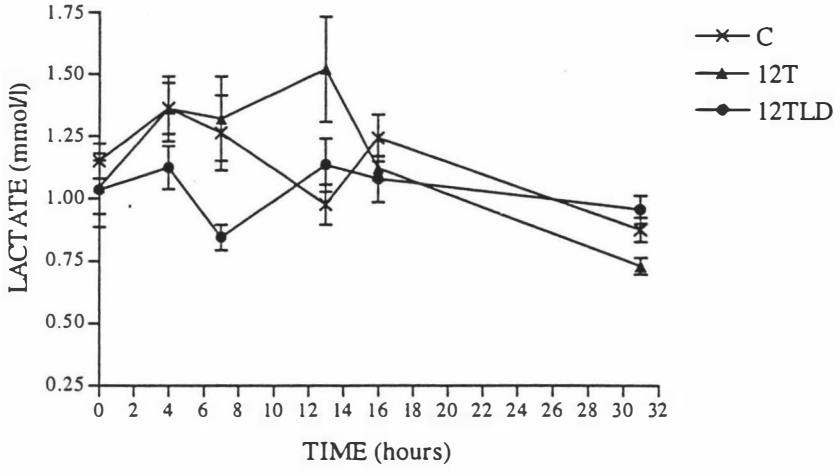


Fig 3.19 Mean plasma concentrations (±SEM) of lactate in unfed calves transported for 12 hours at high (0.2m^2/calf) and low (0.4m^2/calf) stocking densities.

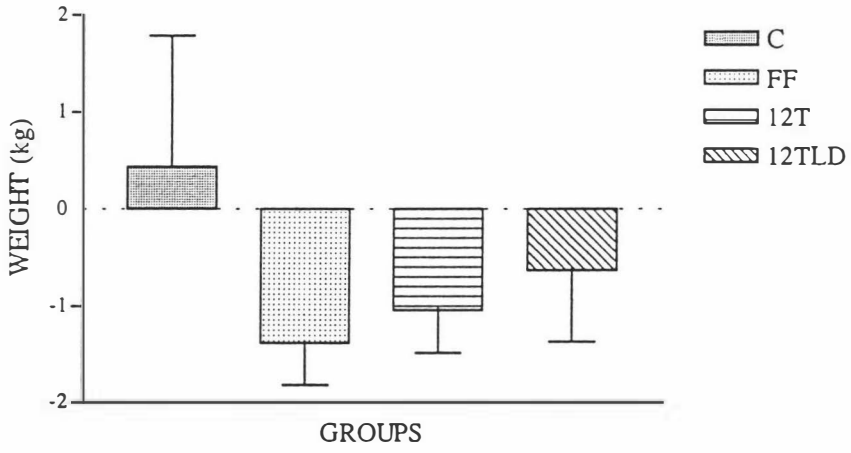


Fig 3.20 Mean change (±SEM) in weight (0 to 31h) in unfed calves transported for 12 hours at high (0.2m^2/calf) and low (0.4m^2/calf) stocking densities.

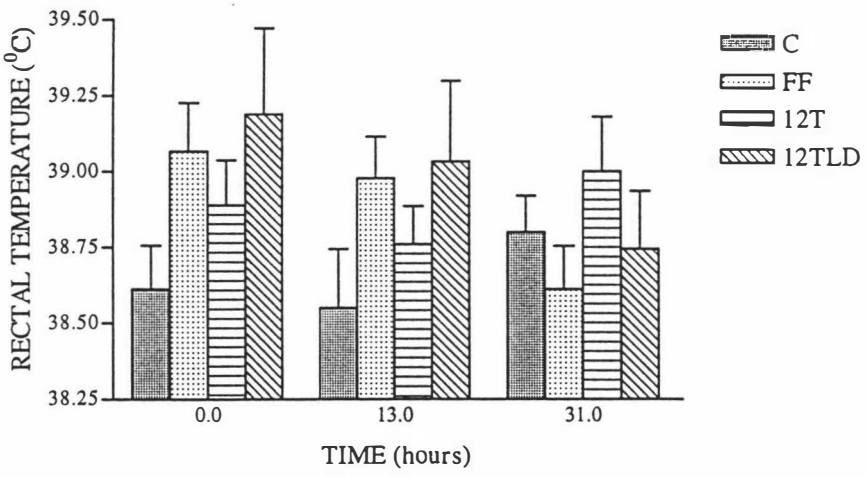
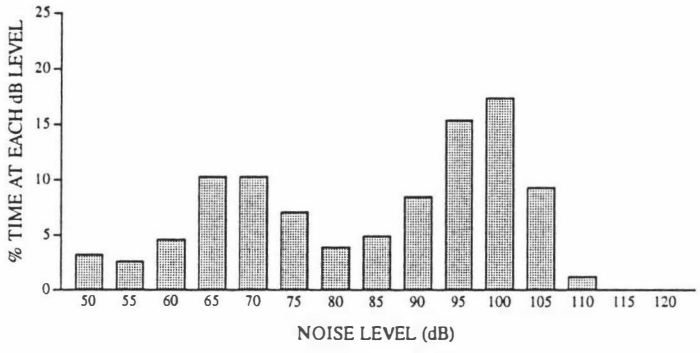
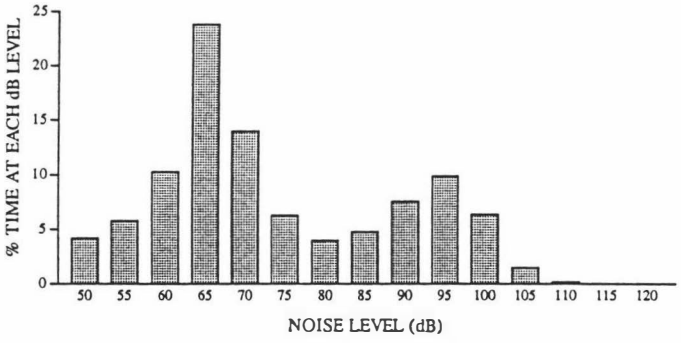


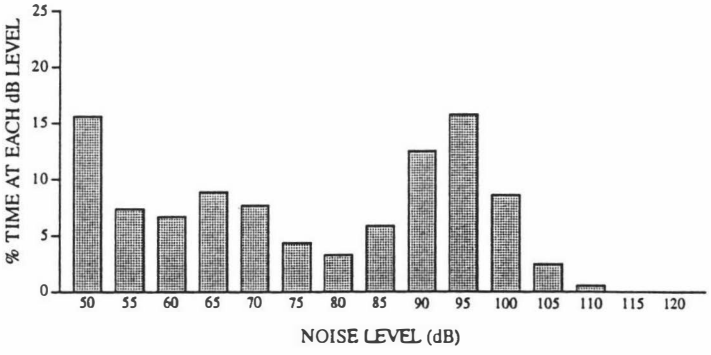
Fig 3.21 Mean rectal temperatures (±SEM) of unfed calves transported for 12 hours at high (0.2m^2/calf) and low (0.4m^2/calf) stocking densities.



a) the first three hours of transport (6.40 am - 10 am)



b) the second three hours of transport (10.07 am - 1.12 pm)



c) the final six hours of transport (1.15 - 7.11pm)

Fig 3.22 Noise levels (dB) in the truck during transport, expressed as the percentage of time spent at different noise levels.

3.5 DISCUSSION

In this study the effects of transport on calves were superimposed on the effects of withholding food from calves (Chapter 2). Data obtained during the experiment supported several main conclusions. Firstly, at the recommended high stocking density 3 hours of transport did not appear to have any added physiological or metabolic effect on unfed calves. Secondly, a glucose-sparing effect apparently occurred in unfed calves transported for 12 hours; and thirdly, there was evidence to suggest some physical exertion or bruising occurred in unfed calves transported for 12 hours. At half the recommended stocking density, transportation at a lower density apparently had few effects metabolically on unfed calves.

Effect of transport duration on unfed calves.

The glucose response of calves in group 3T was similar to that observed in FF calves. Both groups displayed hypoglycemia relative to the present control calves and to normal values for plasma glucose concentrations in calves (Wood *et al.*, 1971; Kinsbergen *et al.*, 1994) after 16 hours. The decline in plasma glucose concentrations continued until it reached a nadir at 25 hours (Fig 3.8). The increases in beta-hydroxybutyrate concentrations of calves in groups FF and 3T suggests that lipids were being utilised to provide fuel for metabolism. The lack of significant change in urea concentrations and the comparison with normal plasma urea concentrations from other young calves (Kinsbergen *et al.*, 1994) suggests there was still an adequate supply of carbohydrate and lipid substrates for metabolism.

Physical exertion in groups C and 3T, as indicated by plasma concentrations of lactate and creatine phosphokinase, was minimal. Concentrations of lactate fluctuated during the 31 hours in C and 3T calves and both groups showed a similar pattern (Fig 3.11). The increase seen in lactate concentrations in C and 3T calves at 0-4 hours and in C at 13-16 hours may be have been a response to feeding. Newborn lambs exhibited an increase in plasma lactate concentrations after feeding following a period of starvation (Mellor, 1987). However this postprandial rise in lactate was not observed in group C following feeding at 25 hours. The concentrations of lactate in C and 3T calves were

slightly lower than those recorded in resting calves from other studies (Lewis *et al.*, 1975; Lindt and Blum, 1994; Warriss *et al.*, 1995) which would suggest minimal physical exertion had occurred.

Concentrations of creatine phosphokinase were similar to those observed in calves and older cattle at rest (Warriss *et al.*, 1995; Knowles *et al.*, 1997). No changes were observed in concentrations of creatine phosphokinase in calves from groups C and 3T (Fig 3.10a). These results illustrate that unfed calves transported for 3 hours and unfed calves that were not transported responded in a similar manner metabolically. Hence, in this study, 3 hours of transport had no detected added effect of exercise or muscle damage on unfed calves.

Whereas the plasma glucose concentrations in FF and 3T calves began to decline after 13 hours, 12T calves maintained higher, fairly constant plasma glucose levels until 19 hours (Fig 3.8). After this time glucose concentrations declined at the same rate as that which occurred earlier in FF and 3T calves. Thus it would appear that the glucose response of unfed calves to food withdrawal was delayed 6 hours by 12 hours of transport. As mentioned previously (Chapter 2), in the initial stages of fasting, plasma glucose is supplied largely by glucose derived from liver glycogen. An alternative source of glucose for muscle tissue is muscle glycogen. In humans mobilisation of muscle glycogen is thought to be the major mechanism for sparing blood glucose during exercise (Dohm *et al.*, 1986). Muscle glycogen is utilised during contraction of skeletal muscle, but it is only of direct use in the muscle tissue itself (Alexander, 1979). Consequently, contracting muscles preferentially utilise muscle glycogen which reduces their need for glucose supplied from the plasma and thereby contributes to the maintenance of higher plasma glucose levels and spares liver glycogen. Liver glycogen alone can meet the carbohydrate requirements of newborn lambs for only about 2 hours, but this period of time may be extended to 10 hours if the lamb is shivering (Alexander, 1979; Mellor and Cockburn, 1986). It is possible that the delayed glucose decrease observed in group 12T was due to muscular exertion which caused mobilisation of muscle glycogen. This produced an apparent glucose-sparing effect which reduced the rate of liver glycogen depletion. Consequently, higher plasma glucose levels were maintained in 12T calves for 19 hours compared with 13 hours in groups FF and 3T.

Explanations for the occurrence of muscular activity in group 12T include the necessity for calves to counteract the movement of the truck whilst standing during transport, or the calves shivered during transport in response to cold exposure, or both.

Calves in the 12T group were stocked at a high density on the truck ($0.2m^2$/calf). At this density the calves were packed together when standing making it difficult for them to lie down. Standing on a moving vehicle for this amount of time involves some degree of physical exertion associated with bracing against the movement of the truck as it rounds corners and changes speed (Tarrant *et al.*, 1992). Thus muscular activity would have been required for the calves to maintain their balance whilst the truck was moving. This was indicated by results obtained from plasma lactate and creatine phosphokinase concentrations in 12T calves. Although lactate concentrations have been reported to reach concentrations of 13 mmol/l after moderate treadmill exercise (Lindt and Blum, 1994), and the present concentrations of less than 1.75 mmol/l indicated a restful state (Lindt and Blum, 1994; Warriss *et al.*, 1995), there was nevertheless some evidence of muscular activity. The present lactate levels were maintained during the 12 hours of transport in contrast to those in C and 3T calves which declined (Fig 3.11). Therefore, although lactate concentrations in 12T calves were not suggestive of significant anaerobic metabolism, they did indicate that some physical activity had occurred in 12T calves.

Longer journeys have been associated with higher creatine phosphokinase levels implying increasing exertion (Warriss *et al.*, 1995). In one study where calves were transported for up to 15 hours the maximum creatine phosphokinase concentration was about 1000u/l (Warriss *et al.*, 1995). Although there was an overall increase in creatine phosphokinase concentrations in 12T calves, the results were variable in individual animals (Fig 3.10b). Not all calves exhibited an increase in creatine phosphokinase concentration and in those that did, the increases had different magnitudes. As increased concentrations of creatine phosphokinase may also result from muscle damage such as bruising, it is difficult to determine the cause of these increased concentrations. Creatine phosphokinase in the blood has been found to increase with increasing severity of bruising (Tarrant, 1990). Therefore the variability seen in creatine phosphokinase concentrations from our calves may be a result of differing degrees of muscle damage.

The other possible cause of muscular activity is shivering. Standing on a moving truck causes air to circulate around the animal which can create a wind chill effect. If the surrounding air temperature is low, the animal may become cold-stressed especially if the calf is wet. Young calves are considerably more sensitive to cold than adult cows, the lower critical temperature (estimated from results obtained by Gonzalez-Jimenez and Blaxter, 1962) for 1-2 week old calves being around 11 ^{0}C. As the mean environmental air temperatures during the time of this study ranged between 7 and 13 ^{0}C, it is likely that some degree of heat production in the form of shivering would have occurred.

Thus the glucose-sparing effect seen in 12T calves may be explained by the muscular exertion which occurred during transport as a result of maintenance of balance and/or shivering. The absence of similar effects in the 3T group suggests that the demand for physical activity was greater during 12 hours than during 3 hours of transport. A similar degree of muscular activity would have occurred in both groups, but 12T calves were required to maintain this level of activity for 4 times as long as 3T calves. Therefore, in this study transport duration apparently did affect the response of unfed calves to transport.

Effect of stocking density during transport on unfed calves.

Calves stocked at a density of 0.4m^2/calf (group 12TLD) displayed a similar glucose response to that seen in the FF and 3T calves (Figs 3.8 and 3.16). Whereas unfed calves transported for 12 hours at a high density exhibited the metabolic changes described above, transportation for 12 hours at a low density apparently had no added effect on unfed calves. It is likely therefore that a similar degree of physical exertion occurred in 12TLD calves as in FF and 3T animals. This may be explained by calves in 12TLD lying down during transportation, which would therefore minimise the physical activity involved in maintenance of balance. As these calves were stocked at a lower density this would have been possible. A lack of significant change in creatine phosphokinase concentrations has been recorded in previous studies in which transported calves spent at least one third of the journey lying down (Kent and Ewbank, 1986a; Knowles *et al.*, 1997). Also lower stocking densities have been associated with lower levels of creatine

phosphokinase and less severe bruising (Tarrant, 1990). Moreover, lying down may also minimise the degree of heat production required during transport, as below thermoneutral environmental temperatures, calves have a lower heat preservation capacity during standing than during lying (Schrama *et al.*, 1993). It is thought that this may be due to a larger exposed surface area, increased peripheral vasodilatation and an increased heat production in muscles close to the surface in standing animals (Schrama *et al.*, 1993). Calves lying down would therefore have a lower critical temperature than calves that are standing, so at the same environmental temperature, the need to shiver in lying calves would probably be less than in those that are standing. Shivering was not noticed in FF calves, and as FF and 12TLD calves produced similar metabolic responses, it may be assumed that the amount of shivering occurring in 12TLD was minimal during transport.

Although it has been stated that calves lying down have a greater capacity to conserve heat than those standing, it is probable that 12T and 12TLD calves had a similar abilities to conserve heat despite the fact that 12T calves were standing. This would result from the higher stocking density in 12T calves which caused them to be packed together and therefore decreased the overall exposed surface area of each animal. Therefore, as observable shivering was minimal both in 12T and 12TLD calves, and by the above reasoning 12T and 12TLD calves probably had similar heat conservation capacities, the main factor causing physical activity in 12T calves would be bracing against the movement of the truck.

It would appear from this and other studies in calves and lambs that when given the opportunity, these young animals lie down when transported (Knowles *et al.*, 1993; Knowles *et al.*, 1997). This is as would be expected due to the decreased physical demand on the animal when lying down. In older animals it has been shown that a high stocking density was associated with a greater stress response, bruise score and number of falls than medium or low densities (Tarrant *et al.*, 1988). However at low densities cattle showed clear preferences with regard to their orientation to the direction of travel and mobility was increased, therefore decreasing the risk of loss of balance and falling (Tarrant *et al.*, 1992).

Other physiological and metabolic effects of transport.

There was little evidence to suggest dehydration occurred in the unfed, transported calves during this study. A similar result was found in fasted 1-3 week old calves during transport in a study by Kent and Ewbank (1986a). In contrast to these results dehydration was detected in other experiments involving the transportation of young calves and older cattle (Warriss *et al.*, 1995; Knowles *et al.*, 1997). The reasons for these differences in hydration state from the different experiments are not apparent, but when dehydration did occur, recovery of hydration state was rapid, therefore suggesting that dehydration was not severe.

The metabolic parameters measured suggested that the demand for energy reserves in 12T and 12TLD calves was not extreme. This was supported by the rectal temperatures recorded for these animals which remained within the normal range, suggesting that the supply of energy reserves was sufficient to maintain heat production. During winter the calf may be exposed to cold, wet and windy conditions during transport which would necessitate increased heat production to maintain body temperature. Consequently there would be a greater demand for available energy reserves. Calves appear to be unable to regulate their body temperature closely when they are transported during the winter (Knowles *et al.*, 1997). Thus, it is likely that in colder weather than occurred here, the response of these unfed calves to transport would have been more marked with regard to utilisation of energy reserves.

In a study by Kent and Ewbank (1983), transported calves lost more weight during the transport period than did their starved control calves. This was attributed to greater defaecation and urination. A direct correlation has been found between weight loss and journey duration, but there is some uncertainty about the proportions of loss attributable to voiding gut contents and to tissue wastage (Leach, 1982). In our study transported calves lost approximately 1 to 1.5 kg during the experiment which was similar to the amount lost in unfed, untransported animals (group FF). No correlation between weight loss and journey time was seen in our calves.

Other transport stressors.

The metabolic parameters used in this experiment indicated that transport began to have measurable effects on unfed calves between 3 and 12 hours after its onset. However it has been suggested for the calf, that the duration of transport was of minor importance when compared to the number of other stressors involved (Sartorelli *et al.*, 1992). Observations from another study in which calves were placed on a transport simulator indicated that the metabolic response to transport was fully expressed at 15-30 minutes and the main stressors related to transport were thought to be vibration and noise (Locatelli *et al.*, 1989). The main source of noise appeared to be rattling of the metal gates, walls, and pen divisions. Exposure of animals to noise may result in auditory, nonauditory or behavioural modifications (Head *et al.*, 1993). Head *et al.*, (1993) reported from the work of other authors that the sound threshold at which a behavioural response in animals was found to occur was 85-90 db, whereas noises that exceeded this threshold caused more pronounced behavioural responses such as freezing and retreating. The present calves were exposed to noise levels equal to, or greater than, the above sound threshold for approximately 50% of the time (Fig 3.22 a,b,c). However, it is difficult to interpret the effect of these noise levels without further information, as noise intensity and duration also affect the response of the animal (Ames and Arehart, 1972).

Results from several studies in both calves and lambs have suggested that the loading/unloading procedures and the initial part of the journey are the most stressful times for the animal when transported (Kent and Ewbank, 1983; Kent and Ewbank, 1986 a,b; Warriss *et al.*, 1995). Moreover, it was found that in cattle aged 3 weeks, 3 months and 6 months, the level of stress caused by loading and unloading increased with age. These authors measured plasma cortisol concentrations to indicate the degree of stress occurring in the animal during transport. Elevated plasma cortisol and glucose concentrations reflect activation of the pituitary adrenal axis (Kenny and Tarrant, 1987). The relatively high initial glucose concentrations in calves from our study may have reflected stress caused by handling as described in chapter 2. If the loading procedure had caused the calves to become stressed we may have expected to see an increase in glucose concentrations after loading. As this was not observed it could be assumed that

loading the calves did not cause a greater degree of stress than that already occurring as a result of prior handling. As the calves appeared to have adapted to handling before 13 hours, a stress response to unloading in the 12T and 12TLD groups would have been more obvious, none apparently occurred (as judged by plasma glucose concentrations). A typical cortisol response involves a rapid increase in cortisol concentration during the first 15 to 30 minutes, while the magnitude of the response and its subsequent recovery time are determined by the severity of the stimulus (Locatelli *et al.* 1989; McMeekan, 1997). It may be that unloading the calves did initiate a cortisol-induced glucose response, but that the stimulus was only sufficient to cause a short-lived or small increase in cortisol. If this was the case, cortisol concentrations would have recovered within an hour and therefore the glucose response would not have been detected with our sampling frequency.

Conclusions.

Results from this study where calves were transported in mild weather conditions during 30 hours without food led to the following conclusions:

- The main factor to cause metabolic responses in transported calves was considered to be mildly increased physical activity resulting from the need to maintain balance during transport.
- When calves were transported for 12 hours at a stocking density of $0.2m^2$/calf, the response to this mild physical activity first became evident after 3 hours of transport.
- No increased physical exertion was detectable in calves transported for 12 hours at a stocking density of 0.4 m^2/calf.
- Minimal dehydration occurred and although energy reserves were utilised this was not extreme.

Under the circumstances described for this experiment, calves appeared to tolerate up to 12 hours transport and a period of 30 hours without food, without detectable metabolic compromise. For this reason, stocking calves at a lower density than the recommended $0.2m^2$/calf would hardly be necessary. In a different situation such as an increased duration of transport or extremes of temperature, calves may not tolerate the period of

transport and food deprivation as well as the animals in this study did, and a lower stocking density may then be beneficial to the animals.

CHAPTER 4: INITIAL PHYSIOLOGICAL RESPONSES OF HAND-REARED DAIRY CALVES AGED 1-2 WEEKS TO REFEEDING AFTER FASTING AND TRANSPORT.

4.1 SUMMARY

In the previous chapters (2 and 3) the effects of pre-slaughter transport and fasting in young dairy calves were examined. Typically animals in this situation do not have access to food once they have left the farm. Feeding the animal during the process of pre-slaughter transport and lairage may benefit both the animal and the meat industry, by simultaneously increasing the welfare state of the animal and the financial return for the industry.

In this chapter the initial physiological response of calves to feeding was examined after 30 hours without food in transported and non-transported calves, and after the first three hours after transport. Groups included C, FF, HF, 3T, 12T and 12TLD as in the previous chapters but as well as the treatments described, calves in these groups were fed at 31 hours. In addition 3TR and 12TR calves were treated in a similar manner to 3T and 12T calves but were fed after transport. PCV and plasma concentrations of total proteins, glucose, triglycerides and urea were measured at 31, 32, 33 and 34 hours in groups C, FF, HF, 3T, 12T and 12TLD. PCV and plasma concentrations of glucose and lactate were measured in groups 3T, 3TR, 12T and 12TR. The results indicate that feeding after 30 hours of fasting is associated with a decrease in glucose clearance, possibly as a result of decreased insulin responsiveness and/or decreased insulin sensitivity. It is thought that the decreased glucose clearance resulted from metabolic overcompensation due to delayed adjustment of hormones and metabolites from the starved state to the fed state. Feeding immediately after 3 or 12 hours of transport restored plasma glucose levels to be within the normal range. It was concluded that feeding young calves following a period of transport and starvation was beneficial to the animal.

4.2 INTRODUCTION

Domestic animals destined for slaughter are generally not fed for financial and practical reasons once they have left the farm Apart from the cost involved in purchasing food and supplying labour, feeding animals prior to slaughter complicates the slaughter process. Feeding animals prior to slaughter increases the chance of ruptured intestines during evisceration and increases the amount of gut contents to be disposed of (Professor N. Gregory, pers. comm.). It has also been reported that pre-slaughter feeding complicates bleeding and taints the carcass (Ingram, 1964).

Withholding food from animals prior to slaughter may also have disadvantages. There is evidence that carcass weight reduction resulting from pre-slaughter starvation was a source of loss to the meat industry (Kirton *et al.*, 1972). Liveweight loss during the initial stages of fasting was determined by the degree of gastrointestinal 'fill' and the level of fill affected the time between the start of fasting and the beginning of carcass weight loss (Kirton *et al.*, 1968). In lambs, carcass weight loss began between 8 and 24 hours after lambs were removed from pasture (Kirton *et al.*, 1971). A loss of 0.25 kg by 24 hours was reported in lambs (Kirton *et al.*, 1971) and 0.73 kg in calves (Kirton and Paterson, 1973). It has been suggested that young milk fed calves suffer greater carcass loss during starvation than grazing lambs or adult cattle as calves have no nutrient reserves in the rumen (Kirton and Paterson, 1973). In contrast to older ruminants, most of the stomach contents were digested in the first 24 hours in calves (Kirton *et al.*, 1968).

In addition to the carcass, by-products obtained from the bobby calf such as the vell (a source of rennin), are important financially. Starvation causes weight reduction in some of these by-products and therefore a decrease in income (Kirton and Paterson, 1973). Ingram (1964) found that feeding up to a short time before slaughter was beneficial. Weight losses that occurred during pre-slaughter starvation were prevented by a meal 6 hours before the animals were killed and a higher carcass yield was obtained. This author also reported that instead of tainting the meat, feeding animals before slaughter improved meat quality. Thus feeding young domestic animals prior to slaughter may be of economic benefit.

In conjunction with possible economic benefits, feeding after starvation and transport improves the welfare status of the animal by replenishing body reserves and re-establishing the hydration state. If an animal is fatigued, feeding restores glycogen in the muscles and liver allowing a normal postmortem pH decline in the meat and reducing weight loss in the liver (Ingram, 1964).

Monitoring the recovery rate of parameters after feeding may be a way of assessing the physiological and metabolic state of the animal during transport and starvation. Knowles *et al.* (1993) found three stages in the recovery of lambs after transport. After the first 24 hours following transport the blood variables associated with short-term stress (PCV, cortisol and glucose) and dehydration had recovered and the levels of lactate had fallen. Ninety-six hours after transport liveweight and levels of beta-hydroxybutyrate, urea, free fatty acids and liver glycogen had stabilised. After 144 hours a fuller recovery had occurred, most of the creatine phosphokinase had been cleared and plasma protein levels had stabilised (Knowles *et al.*, 1993). Feeding the animals after transport and starvation may decrease the time taken for parameters to return to normal levels and may also indicate the extent to which energy reserves were exhausted. The results obtained by measuring the short term responses of calves to feeding can be compared with results from similar studies (Chapters 2 and 3; Knowles *et al.*, 1993; Knowles *et al.*, 1997) to determine the changes in physiological and metabolic state of the calves induced by starvation and transport.

Aims of this study.

To evaluate the initial physiological and metabolic responses of 1-2 week old calves to feeding:

- After 30 hours of food deprivation in transported and non-transported calves.
- After unloading following transport of 3 or 12 hours duration.

4.3 MATERIALS AND METHODS

This research was conducted as one trial which was performed as 2 identical experiments on separate days (21-22/8/95 and 4-5/9/95). The materials and methods for this chapter with regard to animals (2.3.1) and methods (2.3.4) are as described in chapter 2 and for transport (3.3.1) are as described in chapter 3.

4.3.1 Groups

Ten calves (5 per experiment) were allocated to each group such that ages and weights were evenly distributed (Table 4.1). The average weight of the calves on the day before the experiment was 40 kg.

	TREATMENT								
GROUP n=10	fed 50ml/kg at 6.30am feed	fed 25ml/kg at 6.30am feed	fed 50ml/kg every 12h	unfed for 30h	trans. 3h density 0.2m^2/calf	trans. 12h density 0.2m^2 /calf	trans. 12h density 0.4m^2 /calf	fed 50ml/kg after transport	fed 50ml/kg at 31h
C	√	-	√	-	-	-	-	-	√
FF	√	-	-	√	-	-	-	-	√
HF	-	√	-	√	-	-	-	-	√
3T	√	-	-	√	√	-	-	-	√
3TR	√	-	-	-	√	-	-	√	√
12T	√	-	-	√	-	√	-	-	√
12TR	√	-	-	-	-	√	-	√	√
12TLD	√	-	-	√	-	-	√	-	√

Table 4.1 Summary of treatments.

Control (C). Calves were fed 50 ml/kg body weight twice daily (as recommended in the Code) at 12 hourly intervals beginning on the morning of the first experimental day at 6.30 am. They also had ad libitum access to water and calf pellets. Calves were not transported and remained in their pens during the experiment. Calves were fed 50 ml/kg again at 31 hours (fed at 0, 13, 25 and 31h).

Full Feed (FF). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access

to water. Calves were not transported and remained in their pens during the expeiment. They were fed 50 ml/kg again at 31 hours.

Half Feed (HF). Calves were fed 25 ml/kg body weight (half the recommended amount) on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water. Calves were not transported and remained in their pens during the experiments. They were fed 50 ml/kg again at 31 hours.

3 Hours Transport (3T). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 3 hours starting immediately after the second blood sample was taken. During transport the available area was $0.2m^2$/calf. Calves were fed 50 ml/kg again at 31 hours.

3 Hours Transport/Refed (3TR). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Calves were transported by truck for 3 hours immediately after the second blood sample was taken. During transport the available area was $0.2m^2$/calf. Calves were fed 50 ml/kg at 4 hours (after transport) and again at 31 hours and had access to water when not being transported.

12 Hours Transport (12T). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 12 hours immediately after the second blood sample was taken. During transport the available area was $0.2m^2$/calf. Calves were fed 50 ml/kg again at 31 hours.

12 Hours Transport/Refed (12TR). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Calves were transported by truck for 12 hours immediately after the second blood sample was taken. During transport the available area was $0.2m^2$/calf. Calves were fed 50 ml/kg at 13 hours (after transport) and again at 31 hours and had access to water when not being transported.

12 Hours Transport/Low Density (12TLD). Calves were fed 50 ml/kg body weight on the morning of the first experimental day at 6.30 am. Thereafter they were not fed for 30 hours but had access to water when not being transported. Calves were transported by truck for 12 hours immediately after the second blood sample was taken. During transport the available area was $0.4m^2$/calf. Calves were fed 50 ml/kg at 31 hours.

4.3.2 Experimental Procedures

For all animals a 9 ml blood sample was taken at 0 hours (6 am) then calves were fed according to their group. Another blood sample was obtained after this feed at 1 hour (7 am) after which blood samples were taken from all animals every 3 hours for 30 hours. After the 31 hour blood sample, all animals were fed a volume of 50 ml/kg and further blood samples were obtained hourly for the next 3 hours. Blood samples were therefore obtained at 0, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 32, 33 and 34 hours.

Those animals being transported were bled on the truck which returned every 3 hours for this purpose. Following transport the calves were bled before being unloaded and returned to their respective pens. Groups 3TR and 12TR were fed a volume of 50 ml/kg following blood sampling at 4 and 13 hours respectively. In addition to the procedure described above, groups C, 3T and 3TR were blood sampled at 5 and 6 hours and groups C, 12T, and 12TR were sampled at 14 and 15 hours.

All animals were weighed prior to the experiment and again at the end of 31 hours. In groups C, 3T and 3TR rectal temperatures were obtained following blood sampling at 0, 4, 13 and 31 hours. In groups FF, HF, 12T, 12TLD and 12TR rectal temperatures were taken at 0, 13 and 31 hours. A video recording of the behaviour of the calves after transport was made between 11 am - 1 pm, 2.30 pm - 5pm and 8.30 pm - 9 pm on day 1, but the recordings did not provide sufficient detail for analysis. The experimental protocol is summarised in Table 4.2.

4.3.3 Blood and Plasma Measurements

PCV was estimated by the microhaematocrit technique shortly after the blood samples were taken. At Ruakura Animal Health Laboratory, plasma samples were analysed for concentrations of triglycerides, which were determined on an Hitachi 717 Autoanalyser (30^0C) using the enzymatic colorimetric test (modified according to Wahlefeld, 1974); and lactate using the UV method (Noll, 1974). At Massey University plasma samples were analysed on an Hitachi 704 Multi-channel analyser for concentrations of glucose, using the enzymatic colorimetric method (Trinder, 1969) Boehringer Mannheim kit no. 1448668; total plasma protein, using the Biuret method (Weichselbaum, 1946) Boehringer Manneheim kit no. 1553836; and urea, using the kinetic UV test (Neumann and Ziegenhorn, 1977) Boehringer Manneheim kit no. 1489364.

4.3.4 Presentation of Results

PCV and plasma concentrations of total proteins, glucose, triglycerides, urea and lactate were expressed in tables as the mean ± standard error of the mean (SEM) for each group at the sample time. These parameters were also expressed graphically as the mean change ± SEM where the change in concentration was calculated for each calf in the group over 3 hours and the mean of these differences was determined for the group. Plasma glucose concentrations were expressed graphically as the mean ± SEM for each group at each sample time and as the mean incremental change in plasma glucose concentration for each group at each sample time.

Analysis of these data was performed using GraphPad Prism™ version 2.0. When the significance of differences between more than two groups at a particular sample time were required, a one-way analysis of variance (ANOVA) was conducted when data had equal variances, otherwise a Kruskal-Wallis test for non-parametric data was used. ANOVA (Bonferronis) or Kruskal Wallis (Dunns) post-tests were used to establish which differences were significant between the pairs. Where data were presented as mean changes, a one-sample t-test was used to determine whether the changes were significantly different from zero. Areas under the curve were calculated for the graph of

mean plasma glucose concentrations and for the graph of mean incremental changes in plasma glucose concentrations.

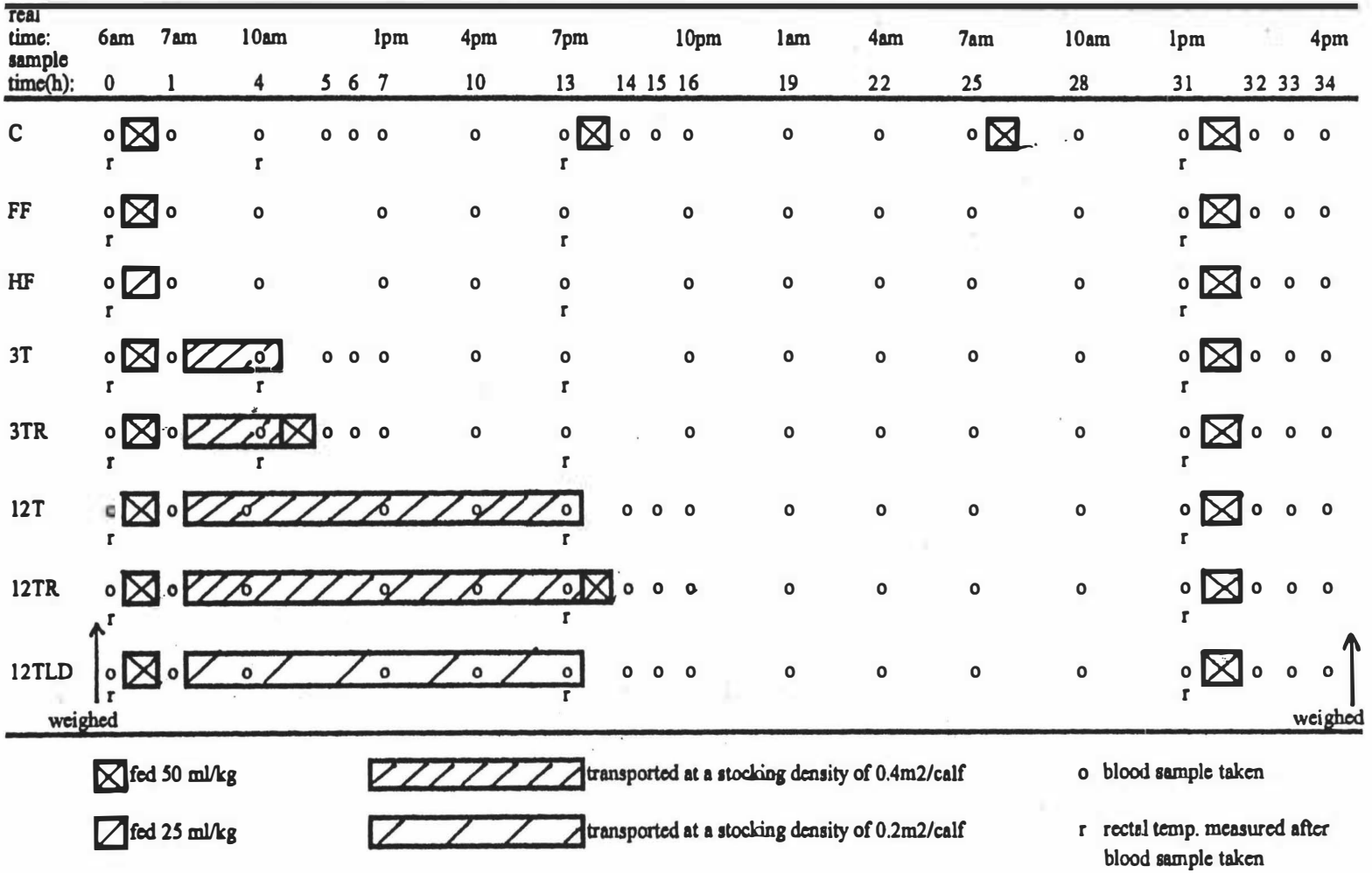


Table 4.2 Summary diagram of experimental protocol

4.4 RESULTS

4.4.1 Refeeding After 30 hours Without Food

Indices of hydration state.

Mean values and changes in PCV and total plasma protein concentrations (Table 4.3) were within normal ranges. No significant differences from zero were observed for the changes in PCV or TPP concentrations (Figs 4.1 and 4.2), so that mean changes in concentration of these parameters after feeding were minimal. All groups responded similarly to refeeding as no significant differences were observed between groups.

Indices of metabolic state.

The mean changes that occurred in glucose concentrations after refeeding were similar in all food-deprived groups (Fig 4.3 a,c) but glucose concentrations in control calves were significantly different to these ($p<0.001$). Plasma glucose concentrations of control calves were within the normal range at 31 hours and remained unchanged after refeeding (Table 4.4). Glucose concentrations in calves in the 5 food-deprived groups were low at 31 hours but returned to control levels after 2 hours (Fig 4.3b). Concentrations continued to increase during the last hour.

Mean plasma triglyceride concentrations in calves at 31 hours ranged from 0.19 - 0.33 mmol/l (Table 4.4). Mean changes that occurred in triglyceride concentrations after feeding were variable (Fig 4.4) but these changes were not significantly different from zero. There were no significant differences in triglyceride concentrations between groups.

Mean plasma urea concentrations had increased at 3 hrs after feeding in all groups (Fig 4.5). This increase was only significantly different from zero in 12TLD calves ($p<0.001$). There were no significant differences between groups.

4.4.2 Refeeding After Transport

Indices of hydration state.

Although the PCVs of calves transported for 3 hours (3T and 3TR) were within the normal ranges, a significant decrease in PCV occurred in these calves during the first three hours after transport (3T: $p<0.05$, 3TR: $p<0.001$). No significant differences occurred between the PCVs of calves that were fed after 3 hours of transport (3TR) and calves that were not fed after 3 hours of transport (3TR) (Fig 4.6). No significant change in PCV of calves transported for 12 hours (12T and 12TR) was observed (Fig 4.6). The PCVs for these calves were also within normal ranges (Table 4.5).

Indices of metabolic state.

Mean increases in plasma glucose concentrations were significantly different from zero in calves transported for 3 hours (3T: $p<0.005$, 3TR: $p<0.05$). These mean increases were similar in 3T and 3TR calves (Fig 4.7). A decrease in mean glucose concentration was observed in 12T calves ($p<0.05$), whereas calves that were fed after 12 hours transport (12TR) produced an increase in glucose concentrations ($p<0.001$) similar to that seen in the calves transported for 3 hours (Fig 4.7) (Table 4.5).

Indices of physical state.

Changes in mean plasma lactate concentration appeared to be a function of whether or not the calves had been fed after transport as opposed to the duration of transport (Fig 4.8). A decrease in lactate concentrations was seen in calves that were not fed after transport (3T and 12T) although this was not significant. In calves that were fed after transport, a similar and significant increase in plasma lactate concentrations was observed (3TR: $p<0.05$, 12TR: $p<0.005$) (Table 4.5).

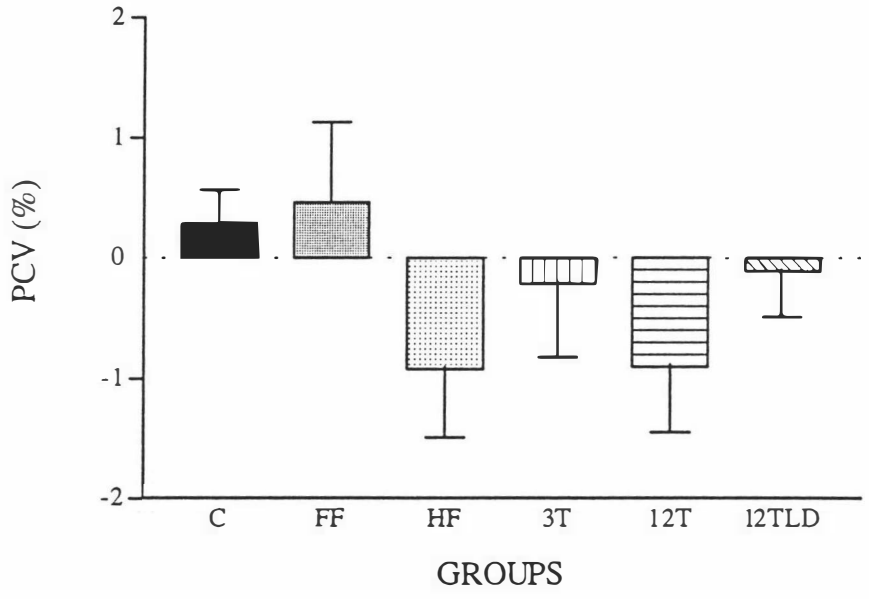


Fig 4.1 Mean change (31 to 34h) in PCV (± SEM) after refeeding at 31h following 30h without food in transported and non-transported calves.

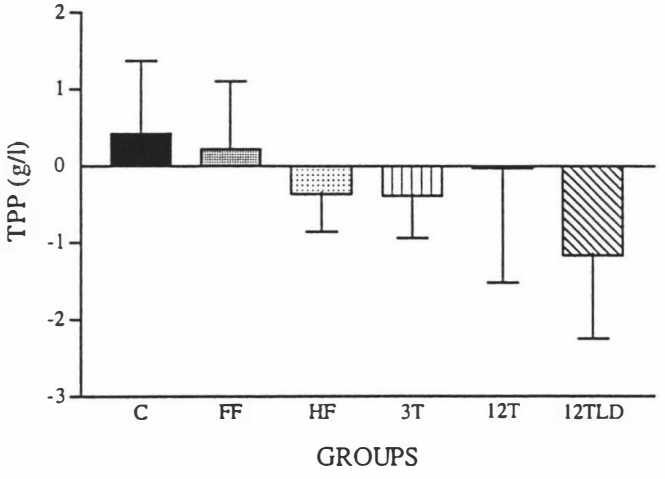


Fig 4.2 Mean change (31to34h) in total plasma protein (TPP) concentration (± SEM) after refeeding at 31h following 30h without food in transported and non-transported calves.

GROUP	PCV (%)		TPP (g/l)	
	31h	34h	31h	34h
C	34 ± 2	32 ± 2	69 ± 4	68 ± 4
FF	35 ± 2	36 ± 2	68 ± 4	68 ± 4
HF	36 ± 1	36 ± 1	68 ± 3	68 ± 4
3T	35 ± 1	35 ± 1	68 ± 4	66 ± 5
12T	34 ± 2	34 ± 2	63 ± 4	63 ± 4
12TLD	34 ± 2	34 ± 2	68 ± 2	67 ± 2

Table 4.3 Mean concentrations ± SEM of hydration parameters after refeeding at 31h and 3h later at 34h in transported and non-transported calves following 30h of food deprivation.

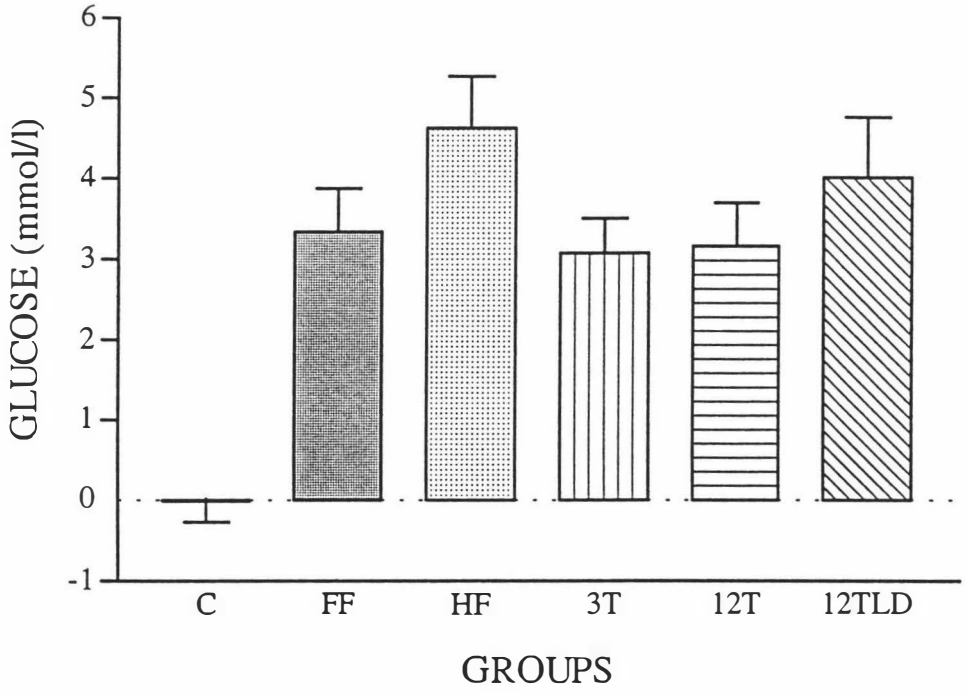


Fig 4.3a Mean change (31 to 34h) in plasma glucose concentrations (±SEM) after refeeding at 31h following 30h without food in transported and non-transported calves.

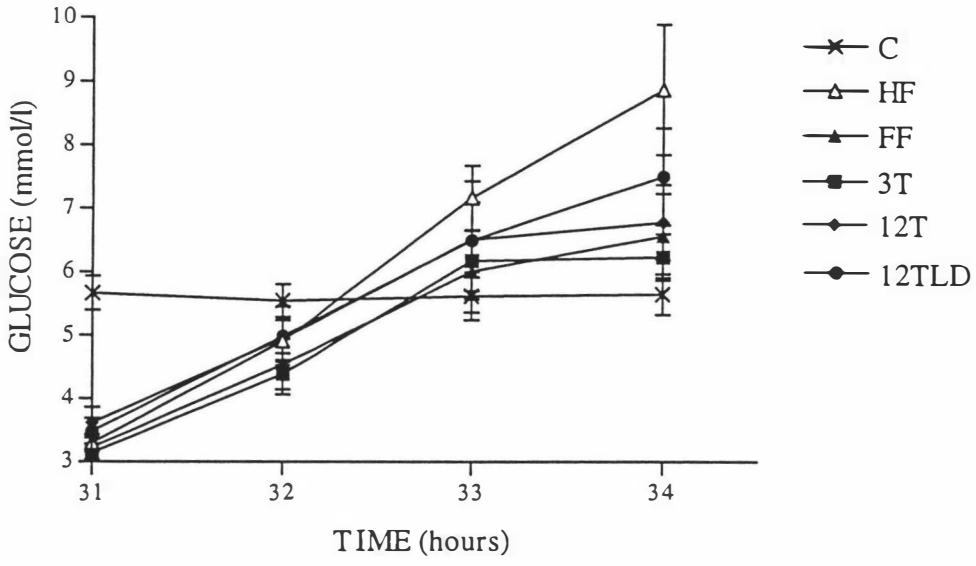


Fig 4.3b Mean plasma glucose concentrations (±SEM) after refeeding following 30h of food deprivation in transported and non-transported calves.

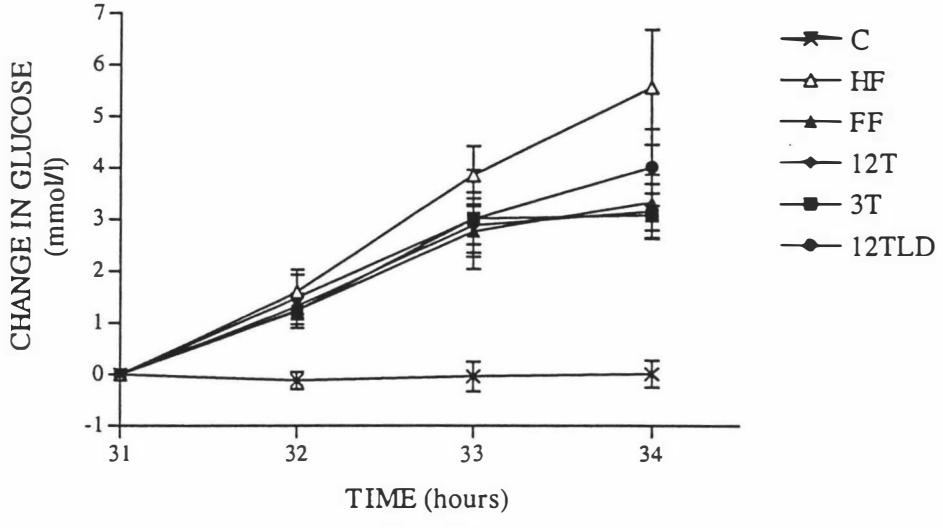


Fig 4.3c Mean incremental changes in plasma glucose concentration (±SEM) after refeeding following 30h without food in transported and non-transported calves.

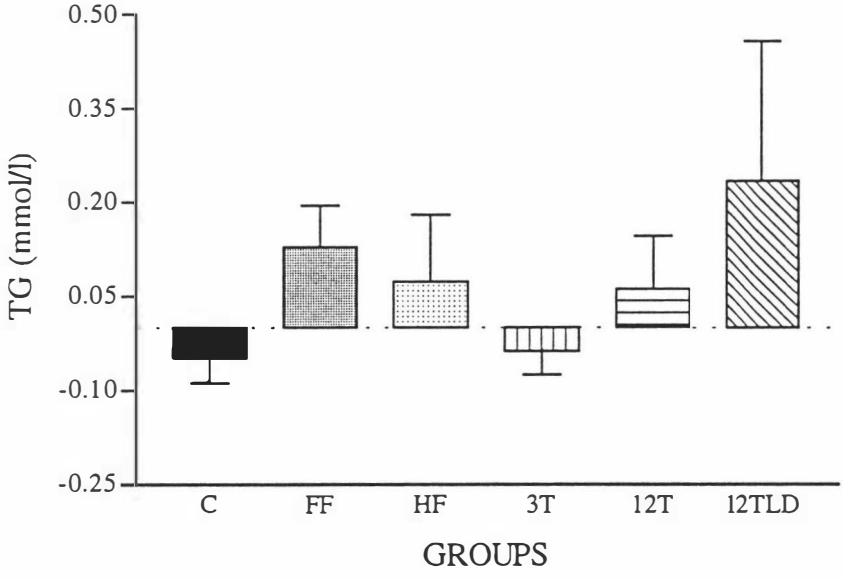


Fig 4.4 Mean change (31 to 34h) in plasma triglycerides (TG) concentrations (±SEM) after refeeding at 31h following 30h without food in transported and non-transported calves.

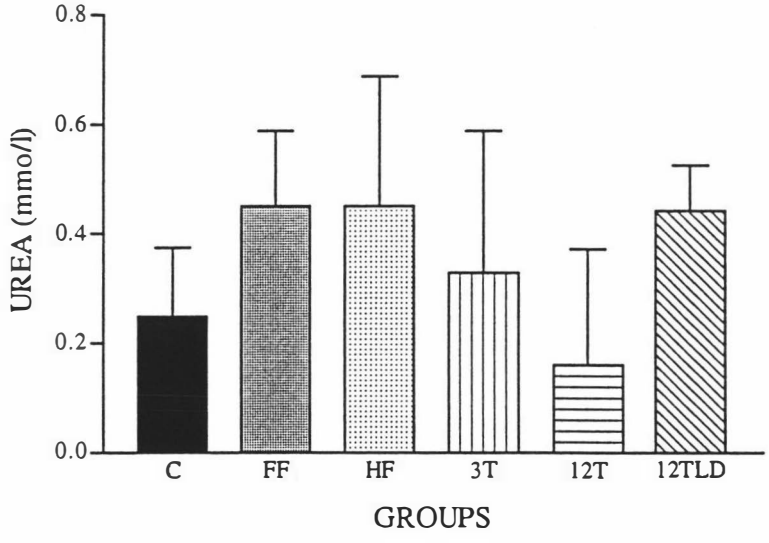


Fig 4.5 Mean change (31 to 34h) in plasma urea concentrations (±SEM) after refeeding at 31h folowing 30h without food in transported and non-transported calves.

GROUP	GLUCOSE (mmol/l)		TG (mmol/l)		UREA (mmol/l)	
	31h	34h	31h	34h	31h	34h
C	5.7 ± 0.3	5.7 ± 0.3	0.31 ± 0.04	0.26 ± 0.03	4.1 ± 0.4	4.3 ± 0.5
FF	3.3 ± 0.2	6.6 ± 0.7	0.19 ± 0.01	0.32 ± 0.07	3.9 ± 0.3	3.9 ± 0.6
HF	3.3 ± 0.1	8.0 ± 0.6	0.20 ± 0.02	0.16 ± 0.04	3.7 ± 0.2	4.1 ± 0.3
3T	3.2 ± 0.1	6.2 ± 0.4	0.21 ± 0.02	0.16 ± 0.04	3.9 ± 0.3	4.2 ± 0.3
12T	3.6 ± 0.2	6.8 ± 0.6	0.25 ± 0.03	0.29 ± 0.09	3.5 ± 0.5	3.6 ± 0.5
12TLD	3.5 ± 0.2	7.5 ± 0.8	0.33 ± 0.06	0.57 ± 0.20	3.3 ± 0.3	3.7 ± 0.3

Table 4.4 Mean concentrations ± SEM of metabolic parameters after refeeding at 31h and 3h later at 34h in transported and non-transported calves following 30h of food deprivation.

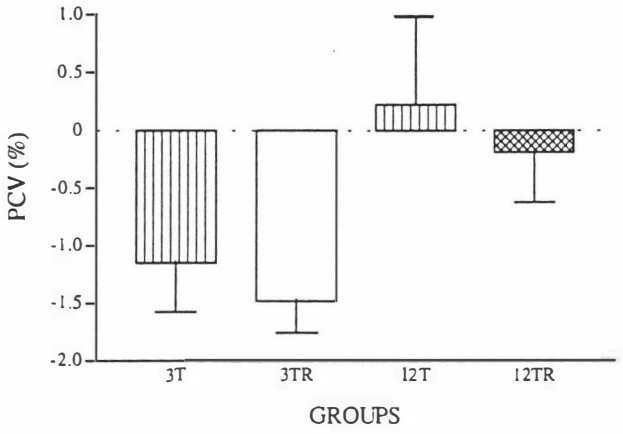


Fig 4.6 Mean change in PCV (± SEM) in calves that were refed after transport (3TR & 12TR) and not refed after transport (3T & 12T). The change was calculated from 4 to 7h in calves transported for 3h and from 13 to 16h in calves that were transported for 12h.

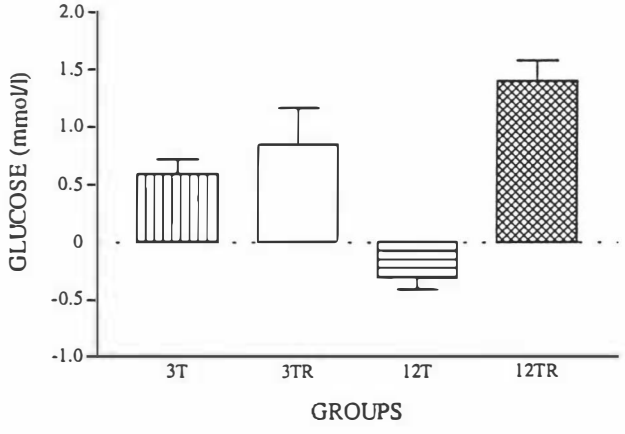


Fig 4.7 Mean change in plasma glucose concentration (±SEM) in calves that were refed after transport (3TR & 12TR) and not refed after transport (3T &12T). The change was calculated from 4 to 7h in calves that were transported for 3h and from 13 to 16h in calves that were transported for 12h.

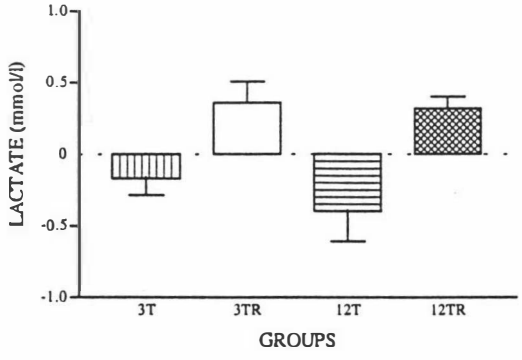


Fig 4.8 Mean change in plasma lactate concentrations (±SEM) in calves that were refed after transport (3TR &12TR) and not refed after transport (3T &12T). The change was calculated from 4 to 7h in calves that were transported for 3h and from 13 to 16h in calves that were transported for 12h.

GROUPS	PCV (%)		GLUCOSE (mmol/l)		LACTATE (mmol/l)	
	4h	7h	4h	7h	4h	7h
3T	35.1 ± 0.9	33.7 ± 1.1	5.2 ± 0.2	5.7 ± 0.2	1.3 ± 0.1	1.1 ± 0.1
3TR	37.5 ± 1.7	36.1 ± 4.8	5.2 ± 0.3	6.2 ± 0.4	1.1 ± 0.1	1.5 ± 0.1
	13h	16h	13h	16h	13h	16h
12T	35.4 ± 2.1	35.0 ± 1.9	5.7 ± 0.3	5.4 ± 0.3	1.5 ± 0.2	1.1 ±0.1
12TR	35.4 ± 1.8	35.5 ± 1.8	5.7 ± 0.2	7.0 ± 0.3	1.1 ± 0.1	1.5 ± 0.1

Table 4.5 Mean concentrations ± SEM of parameters in calves that were refed after transport (3TR &12TR) and not refed after transport (3T &12T).

4.5 DISCUSSION

Refeeding after 30 hours without food.

No significant changes occurred in PCV or total plasma protein concentration after the 3 hours following refeeding. This is to be expected as normal levels of hydration were maintained during the fasting period in both transported and non-transported calves (Chapters 2 and 3).

Prior to refeeding at 31 hours, food-deprived calves (FF, HF, 3T, 12T, 12TLD) had similar plasma glucose concentrations of around 3.5 mmol/l, whereas control calves at 31 hours had concentrations of around 5.7 mmol/l (Figs 2.11, 3.8, 3.16). When the calves were refed, glucose concentrations in food-deprived calves rapidly increased over 3 hours to concentrations greater than those observed in control calves. Although there were no significant differences in glucose concentrations between food-deprived groups 3 hours after refeeding (34 hours), the overshoot in glucose concentrations above control levels was significant in HF and 12TLD calves (Fig 4.3b). This overshoot after refeeding indicates that the rate of entry of glucose into the plasma was greater than its rate of removal.

A greater quantity of lactase synthesised during starvation may contribute to an increased rate of entry of glucose into the plasma. Sucking calves obtain blood glucose from the products of intestinal carbohydrate digestion and hepatic glycogenolysis (Church, 1976). The pre-ruminant animal ingests colostrum and milk of which the digestible carbohydrate is lactose (Webster, 1984). Lactose is broken down in the gut by intestinal lactase to yield glucose and galactose and galactose is further metabolised in the liver to also form glucose (Clarenburg, 1992). Studies in rats have found that starvation increases the activity of intestinal lactase (Leichter *et al.*, 1987). The increase in lactase activity also enhances the absorption of lactose metabolites as indicated by transfer of the constituent monosaccharides into the blood both in vivo and in vitro (Leichter *et al.*, 1987). This finding may be explained by the de novo synthesis of lactase molecules which occurs during starvation in the rat (Nsi-Emvo and Raul, 1984). It has also been shown that the specific activity of lactase increases with the period of

starvation (Yamada *et al.*, 1983). Therefore the 30 hour period of food deprivation imposed on the calves in our study may have stimulated an increase in lactase activity in the intestinal epithelium. Consequently, when the animals were refed, the rate of lactose digestion may have been high, as would be absorption of its products, leading to a rapid increase in plasma glucose concentrations.

The rate of glucose removal from plasma is largely regulated by the action of insulin. When an animal is fed there is an increase in blood glucose concentrations resulting from digestion and absorption of food. Increasing plasma glucose concentrations initiate secretion of insulin from the pancreas. In this situation an animal is said to be *insulin responsive*. Insulin promotes the uptake of glucose by target tissues, in which case an animal is said to be *insulin sensitive* (decreased insulin sensitivity is also called increased insulin resistance). Glucose is then used immediately or stored as endogenous body reserves. This action of insulin modulates plasma glucose concentration, preventing the postprandial rise in glucose concentration from over-shooting normal plasma levels. The sensitivity of insulin action in an animal can be determined by injecting insulin intravenously, either alone or with glucose (which may be administered orally or intravenously), and monitoring the rate at which plasma glucose concentrations decrease (Heard and Henry, 1969). The combined effects of insulin responsiveness and insulin sensitivity contribute to the overall *glucose tolerance* of an animal. Thus it is likely that the rate of glucose removal from plasma is dependant on glucose tolerance. Glucose tolerance is usually measured by determining the rate at which plasma concentrations of glucose decrease following intravenous or oral glucose administration (Heard and Henry, 1969).

It is possible that glucose tolerance is temporarily decreased when an animal makes the transition from the starved to the fed state. During a period of starvation plasma glucose concentrations decrease as was observed in the food-deprived calves from our study. Decreased plasma glucose concentrations cause insulin secretion to be suppressed and although we did not measure insulin in this study, a decrease in basal plasma insulin concentrations during fasting has been reported by other authors (Malaisse *et al.*, 1967). In this situation the animal appears to be adapting metabolically to the starved state. Fasting was also found to cause insulin resistance in rat adipose tissue both in vitro

(Huber *et al.*, 1965; Kasuga *et al.*, 1977) and in vivo (Goldman and Cahill, 1964). The development of insulin resistance in peripheral tissues during fasting may be a mechanism to prioritise the use of available glucose for those tissues and organs that require glucose as their primary and/or sole, energy substrate. When an animal is fed after a period of starvation in which insulin resistance has developed, it appears to take a few hours before the animal becomes metabolically readjusted to the fed state and insulin resistance is reversed. During this time the rate of glucose clearance from plasma may be slow which could explain the overshoot in glucose concentrations in HF and 12TLD calves. The return of plasma glucose concentrations of food-deprived calves in our study to normal levels within 3 to 4 hours of refeeding was consistent with other reports (Rollin *et al.*, 1986; Hostettler-Allen *et al.*, 1994).

Reasons for the development of impaired glucose tolerance in HF and 12TLD calves and not in other food-deprived calves following refeeding are unclear. There appears to be no apparent reason as to why 12TLD calves showed relative glucose intolerance after refeeding, as calves in groups HF, FF, 3T and 12TLD showed similar glucose responses during the 30 hours of food-deprivation. It is possible that the smaller volume of food given to HF calves at the beginning of the experiment resulted in a metabolic difference that was significant enough to cause glucose intolerance relative to that seen in other food-deprived groups. Thus glucose intolerance in HF calves may have resulted from a relatively decreased nutrient availability, but this was not reflected in the plasma concentrations of glucose during the 30 hours of food-deprivation.

Findings by Heard and Henry (1969) imply that the mechanisms for uptake of plasma glucose in the young immature animal were less dependent on, or were relatively insensitive to, the action of insulin, so that these animals had low insulin sensitivity leading to decreased glucose tolerance. It has been suggested that a lack of glucokinase in neonatal animals contributed to a low glucose tolerance (Rollin *et al.*, 1986). Glucokinase is an enzyme in the liver which catalyses the phosphorylation of glucose, thereby enabling glucose to remain in hepatocytes (Clarenburg, 1992). Its equivalent in most other cells is hexokinase. Rollin *et al.* (1986) reported from the work of others that there are low or non-existent levels of glucokinase in the livers of newborn rats, dogs, and camels. Glucokinase first appears in rats at 16 days and reaches adult

activities at 26-28 days (Walker and Holland, 1965). A lack of glucokinase and hexokinase in neonates may affect the ability of the tissues to take up glucose, consequently decreasing insulin sensitivity and therefore glucose tolerance. It is unlikely that a lack of glucokinase contributed to the apparent decrease in glucose tolerance seen after refeeding in HF and 12TLD calves, as all calves in our study were of a similar age.

The changes that occurred in plasma triglyceride concentrations after refeeding were varied but not significant. These results were similar to the triglyceride response observed in calves during the period of food deprivation and transport (Chapters 2 and 3). As indicated in those chapters, given the limited number of samples taken, plasma triglyceride concentrations are not a reliable indicator of lipid mobilisation. Concentrations of beta-hydroxybutyrate provide more information about whether or not lipids are being mobilised. During the food deprivation period, calves appeared to be mobilising lipids as indicated by the increased concentrations of beta-hydroxybutyrate in food-deprived calves relative to control calves (Chapter 2). Following refeeding in food-deprived groups, there was an increase in plasma glucose concentrations which would stimulate insulin secretion. Insulin enhances lipogenesis and inhibits lipolysis in adipose tissue and muscle (Clarenburg, 1992), so that had plasma concentrations of beta-hydroxybutyrate been measured in this experiment, it would be expected that a decrease in beta-hydroxybutyrate concentration would have accompained or followed the postprandial glucose rise.

No significant changes in concentrations of plasma urea were observed following refeeding. No significant change in urea concentrations occurred during the period of food deprivation (Chapters 2 and 3), so that a change in urea concentration following refeeding would not be expected, especially within 3 hours.

Refeeding after transport.

It is not likely that the decrease in PCV between 4 and 7 hours observed in calves transported for 3 hours was a hydration effect as it occurred in both 3T and 3TR calves (Fig 4.6). As explained in chapter 2, it is possible that the calves were stressed by

activities occurring prior to or at the beginning of the experiment which resulted in splenic contraction and high initial PCVs. The subsequent adaptation of the calves to noise and handling, an adaptation that continued during the first 3 hours after refeeding, would therefore explain the decrease in PCV seen in 3T and 3TR calves. Support for this idea comes from the abscence of a PCV change in calves during the first 3 hours after transport for 12 hours, whether or not they were refed (12T and 12TR calves). Measurements of plasma total protein concentrations would have been useful to support this theory - if total plasma protein concentrations remained at normal levels it would have confirmed the absence of dehydration. Nevertheless, refeeding calves immediately after transport had no apparent effect on their hydration state as indicated by similar responses in PCV in 3T and 3TR, and in 12T and 12TR (Fig 4.6).

The increase in plasma glucose concentrations between 4 and 7 hours seen in calves transported for 3 hours (Fig 4.7) was probably due to the fact that they had been fed 4 hours previously. Although there was a slightly larger increase in plasma glucose concentrations from calves fed immediately after transport (3TR), the change in concentration was not significantly different from that seen in calves transported for 3 hours and not refed (3T). It is likely that the effect of refeeding after 3 hours transport was masked by the initial feed. This is reinforced by results obtained from calves transported for 12 hours. After 12 hours glucose concentrations had returned to baseline levels and were beginning to decline in 12T calves (Figs 3.8, 3.16), hence the smaller change in their glucose concentration (Fig 4.7). When calves were fed immediately after 12 hours of transport (12TR), glucose concentrations rapidly increased over the next 3 hours. It would appear from these results that refeeding calves after transport of duration greater than 3 hours helps restore glucose concentrations to normal levels.

An increase in plasma lactate concentrations was observed in calves that were fed after transport regardless of its duration (Fig 4.8), which implies a lactate response to feeding rather than transport. A lactate response to feeding has also been seen in lambs which were fed after 9 hours of food deprivation (Mellor, 1987). The lambs' plasma lactate concentrations, which had previously remained relatively constant, increased during the first 3 hours after feeding and then declined to baseline values 3 hours later (Mellor, 1987).

In a previous study where young calves were transported for 24 hours (Knowles *et al.*, 1997), it was found that feeding the calves during transport did reduce adverse effects to some extent but it was questionable whether it was worth disrupting the journey for the minor benefits of feeding during transport. As the maximum recommended transport duration in New Zealand is 12 hours (AWAC, 1993), it would not be worth interrupting a journey to refeed the animals. Results from our study (Chapter 3) indicate that in mild weather conditions, calves can tolerate up to 12 hours of transport stocked at a density of $0.2m^2$/calf without significant metabolic compromise. However if the weather conditions during transport were more extreme, calves may have a greater energy requirement and could benefit from being fed after transport. If animals were to be fed in the lairage following transport, it would be necessary to ensure that they were capable of drinking. Carcass dehydration in lambs has been observed irrespective of the presence or absence of water and it is thought that lambs may not drink in unusual surroundings (Kirton *et al.*, 1968). It may also be necessary, especially with young calves, for the calf to learn to drink from troughs or buckets before provision of water is worthwhile. Whether or not the practical implications (mainly time and money) of feeding the animals would outweigh the benefits would need to be examined.

Conclusions.

The following conclusions may be drawn from this study.

After transport and 30 hours without food:

- Apart from a glucose response, the initial response to refeeding transported and non-transported calves after 30 hours of food-deprivation indicated no significant change in their hydration or metabolic states.
- Refeeding transported and non-transported food-deprived calves caused plasma glucose levels to return to normal within 3 hours and in 2 groups (HF and 12TLD) caused an overshoot in glucose concentrations from control levels.
- The overshoot in plasma glucose concentrations observed in HF and 12TLD calves after refeeding may have been caused by an increase in the ratio of the 'rate of glucose entry into plasma' to the 'rate of glucose removal from plasma'.

- Increased lactase activity has been reported to occur during periods of starvation. It is postulated that this may cause increased plasma glucose concentrations and in conjunction with a decreased rate of glucose clearances from plasma, result in an overshoot in glucose concentrations in HF and 12TLD calves.
- Insulin resistance, which has been reported to develop during periods of starvation may cause a decreased rate of glucose clearance from the plasma. This represents ametabolic adjustment to starvation, the reversal of which, in HF and 12TLD calves, was delayed.
- In HF calves the overshoot may be explained by the lower volume of milk fed prior to food deprivation causing a relative decrease in nutrient availability. The reason for the overshoot in glucose concentrations in 12TLD calves is not clear.

Immediately after transport

- The effects of feeding after 3 hours of transport were masked by the first feed the calves had been given just before transport began.
- Feeding after 12 hours of transport maintained plasma glucose concentrations within the normal range observed in fed calves (group C).

CHAPTER 5: GENERAL DISCUSSION

5.1 GENERAL CONCLUSIONS

This study was conducted in mild climatic conditions and apart from the period of transportation, calves had access to water at all times. Under these conditions and using the metabolic parameters outlined in Chapter 1, the following conclusions may be drawn from this work.

Effects of feeding and fasting.

- Feeding 50 ml/kg of milk or stored colostrum at 12 hourly intervals is sufficient to maintain acceptably high plasma glucose concentrations in calves aged 1-2 weeks.
- A period of 30 hours without food had apparently minimal adverse effects on the hydration state and metabolic state of 1-2 week old calves. There was no evidence to suggest that calves were significantly dehydrated, and they were able to maintain energy levels during this time without excessive protein catabolism.
- Calves fed 25 ml/kg on the morning of feed withdrawal exhibited a similar metabolic response to those fed 50 ml/kg.

Effects of transport duration and density.

- The metabolic response of food-deprived calves transported for 3 hours at a density of 0.2 m^2/calf were similar to those of non-transported food-deprived calves. Thus, it appears that 3 hours of transport also caused minimal adverse metabolic effects in food-deprived calves.
- The response of food-deprived calves to 12 hours transport at a density of 0.2 m^2/calf was significantly different from that of non-transported food-deprived calves, as transported calves maintained normoglycemia for 6 hours longer than non-transported calves. This difference was thought to be caused by mildly increased physical (muscular) activity occurring in calves transported for 12 hours. That

activity, required to maintain balance in the moving truck, produced a plasma glucose sparing effect which was thought to have resulted from mobilisation of muscle glycogen (Chapter 3).

- The response of food-deprived calves transported for 12 hours at a lower stocking density (0.4m^2/calf) was similar to that of non-transported food-deprived calves. This response suggests that there was minimal physical activity which was attributed to the fact that these calves lay down during transport (Chapter 3).

Effects of refeeding.

- Refeeding transported and non-transported calves after 30 hours without food re-established plasma glucose concentrations at normal levels within 3 hours.
- Plasma glucose concentrations of HF and 12TLD calves overshot normal levels by 3 hours after refeeding. It is likely that this overshoot occurred as a result of an increased ratio of the 'rate of glucose entry into plasma' to 'rate of glucose removal from plasma'. Factors that may have caused an increase in this ratio include increased lactase activity in the gut and the development of insulin resistance, both of which have been found to occur in starved animals (Chapter 4).
- Thus in HF and 12TLD calves, the overshoot in plasma glucose concentrations suggests that the metabolism of these animals had adjusted to the starved state such that when they were fed, there was metabolic overcompensation in the form of insulin resistance to glucose uptake by tissues. In HF calves, this may be explained by the lower volume of milk fed prior to food deprivation. Thus it is possible that these animals would have had less energy substrates available before the starvation period than calves in other groups. The reason for the overshoot in 12TLD calves is unclear (Chapter 4).
- Although feeding immediately after 3 hours of transport did not markedly affect the decline in plasma glucose concentrations, feeding after 12 hours of transport at a density of 0.2 m^2/calf appeared to be beneficial to the calves as indicated by plasma glucose concentrations which returned to normal fed levels.

Summary.

With regard to metabolism, hand-reared dairy calves aged 1-2 weeks appear to tolerate a period of up to 12 hours transport during 30 hours of food deprivation quite well. Thus the recommendations given in the Code regarding nutrition and transport would appear to be adequate for these calves during the process of pre-slaughter transport and lairage. However these experiments were conducted in relatively benign conditions and in different circumstances the calves may not fare as well (see 5.3 Future Research).

5.2 ASSESSMENT OF PARAMETERS

One of the major limiting factors of an experiment are the parameters chosen to measure the animals responses and the way in which these parameters are used. The following discussion outlines what the parameters used in this study were measuring, how this relates to the calf's response to treatment, and the suitability of the parameters for our purposes.

Indices of hydration state.

Packed cell volume (PCV) is defined as 'the percentage of the volume of whole unclotted blood occupied by the erythrocytes' (Blood and Studdert, 1988). Provided no loss of erythrocytes has occurred, changes in PCV either reflects changes in plasma volume (Boyd, 1981) such that an increase in PCV indicates dehydration, or splenic contraction in response to the sympathoadrenomedullary system. Likewise, increases in the concentration of plasma proteins reflect changes in plasma volume provided no loss of protein has occurred. In younger calves, the plasma concentrations of total proteins may be affected by intake of immunoglobulins from the milk (Chapter 2), but these proteins can only be absorbed for up to 48 hours after birth (Stott *et al.*, 1979). As calves in this study were aged from 1 to 2 weeks, TPP concentrations would not be affected by immunoglobulin intake. Splenic contraction, which may be initiated by excitement, pain or catecholamine release, can cause an increase in PCV (Persson, 1967). Thus to provide an indication of the degree of dehydration it is more accurate to

measure the change in both PCV and TPP as this allows for non-hydrational changes in PCV. The slight increase in PCV at the beginning of our experiment may have resulted from splenic contraction, but PCVs soon stabilised around normal values suggesting the calves had become accustomed to the noise of the experiment and to being handled (Chapter 2). It is possible that removing blood during sampling causes a decrease in PCV. The volume of red cells removed during sampling was calculated to be 65 ml which represents a change in PCV of 2%. It is therefore unlikely that blood sampling significantly affected PCV. Concentrations of total plasma proteins also remained normal which supported the conclusion that the animals were not dehydrated. As losses of erythrocytes and protein were not likely to have occurred, measuring changes in PCV and TPP provided a reasonable indication of the hydration state of calves in this experiment.

Indices of metabolic state.

Plasma glucose concentrations were measured as an index of the availability of carbohydrate. Glucose enters the plasma following absorption from the gut, breakdown of glycogen in the liver or hepatic formation of glucose during gluconeogenesis (Kaneko, 1989). Glucose is removed from the plasma in two contrasting situations. The first is when energy is required, thus glucose is taken up by the peripheral tissues and oxidised to provide energy. The second is when there is excess glucose in the circulation such as after a meal, so that glucose is taken up by the liver, muscles and other tissues and is stored as glycogen. Changes in plasma glucose concentrations stimulate secretion of hormones, primarily insulin and glucagon but also catecholamines and glucocorticoids, to control the level of blood glucose and maintain appropriate concentrations (Clarenburg, 1992). Thus the concentration of glucose in the plasma is the net result of an equilibrium between the rates of entry and removal of glucose in the circulation (Kaneko, 1989) and reflects changes in the rates of production and utilisation of glucose no matter where they occur (Clarenburg, 1992). Hence plasma glucose concentrations are a good indicator of overall carbohydrate availability and reflect sensitively the full range of nutritional levels (Mellor, 1987). When using plasma glucose concentrations as an index of metabolic state, stress must be avoided as sympathetic discharge may cause increased plasma glucose concentrations (Guyton,

1991). It is possible that calves in the present study were initially stressed as indicated by elevated PCVs (Chapter 2). Stress effects may be avoided by desensitising calves to the procedures prior to the experiment.

To represent metabolism of lipids, plasma concentrations of triglyceride and beta-hydroxybutyrate were measured. Triglycerides, composed of three fatty acids and one glycerol molecule, are obtained from the diet and are either catabolised in the liver to provide energy for metabolism, or are stored in adipose tissue for later use. To enable transportation of triglycerides in the blood, they must first be incorporated into chylomicrons or lipoproteins (Clarenburg, 1992). If energy is required, triglycerides in the liver are broken down to their component fatty acids and glycerol molecules. These products are then transported in the blood to peripheral tissues where they are oxidised. If there is an insufficient supply of hepatic triglyceride, triglyceride from adipose tissue is mobilised, transported to the liver as fatty acids, where they are oxidised for energy or are redeposited as triglyceride in the liver (Guyton, 1991). Consequently, plasma concentrations of triglyceride only measure the input from the gut to the adipose tissue or liver and they do not indicate of lipid catabolism (Wood, *et al.*, 1971). Moreover, measuring plasma triglycerides in our study was of little value as only initial and final values were obtained. More significant information may have been obtained if triglyceride concentrations had been measured more frequently. Mobilisation or utilisation of lipid would be better indicated by measuring concentrations of glycerol or fatty acids. A large proportion of the initial degradation of fatty acids occurs in the liver, especially when large amounts of lipids are being mobilised for energy (Guyton, 1991). However the liver uses only a small portion of fatty acids for its own intrinsic metabolic processes. The majority of the remaining fatty acids form ketone bodies, predominantly beta-hydroxybutyrate, which are oxidised for energy in peripheral tissues (Guyton, 1991). Thus increased concentrations of plasma beta-hydroxybutyrate are representative of lipid catabolism. Plasma beta-hydroxybutyrate is a less sensitive index of a moderate degree of undernourishment than fatty acid concentration but it is more useful in cases of more severe undernourishment where fatty acid levels are approaching or have reached their maximum (Russel *et al.*, 1967; Mellor, 1987). As lipid metabolism is in a state of dynamic equilibrium, it may be best portrayed by measuring

concentrations of several different lipid components and using these results to provide an overall picture.

Plasma concentrations of urea were measured to represent protein metabolism. Synthesis of urea in the liver provides a nontoxic mechanism for excretion of ammonia during catabolism of amino acids (Kaneko, 1989). After its formation, urea diffuses from the liver cells into the body fluids and is excreted by the kidneys (Guyton, 1991). A certain amount of body proteins are degraded, deaminated and oxidised every day if no protein is ingested. This is known as obligatory protein loss (Guyton, 1991). Increased concentrations of plasma urea may occur if the animal is on a high protein diet, has impaired renal function, or has an increased rate of protein catabolism (Kaneko, 1989). Decreased concentrations may result from a low protein diet or liver failure and may also be observed in neonatal animals. It is not until the quantity of stored fats begins to run out that the amino acids begin to be deaminated and oxidised for energy in significant quantities. In a situation where an adult animal is starved, this does not usually occur for about two weeks. From this point on tissue protein begins to degrade (Guyton, 1991). Protein metabolism is minimal during the first day after birth in unfed newborns (Mellor and Cockburn, 1986), but with increasing age calves are capable of catabolising protein (Chapter 2). As calves in our study were not receiving diets containing high levels of protein, it may be assumed that the diet would not have been a significant factor in causing changes in plasma urea concentrations. It is possible that impaired kidney function could cause changes in urea concentrations, but mean plasma concentrations of urea obtained from calves in the present study were within normal ranges (Chapter 2). Thus if changes had occurred in plasma concentrations of urea in calves from this study, it is most likely that they would have resulted from protein catabolism.

It follows that if the changes in plasma concentrations of glucose, beta-hydroxybutyrate and urea are viewed together and exhibit changes consistent with their known metabolic roles, an accurate general picture of the metabolic status of the animal will be obtained. These parameters were used effectively in that manner in this study.

Indices of physical state.

Plasma concentrations of lactate and creatine phosphokinase were measured to provide some indication of whether or not physical exertion occurred during transportation. Creatine phosphokinase is found in many types of cells but has its highest specific activity in skeletal muscle (Kaneko, 1989). In muscle, this enzyme makes ATP available for use in muscular contraction by the phosphorylation of ADP from creatine phosphate (Kaneko, 1989). Creatine phosphokinase has a plasma half-life of 2-4 hours, so that even marked elevations in creatine phosphokinase may return to normal within 12 to 24 hours. Thus for plasma concentrations of creatine phosphokinase to be useful in representing muscular exercise, concentrations would need to be measured around every 2-4 hours. Increased concentrations of plasma creatine phosphokinase are indicative of muscular contraction or muscle damage. Vigorous exercise or prolonged shipping have been found to cause up to a fourfold increase in plasma creatine phosphokinase without producing histological evidence of muscle damage (Smith, 1996). As only large increases in plasma activity of creatine phosphokinase are clinically indicative of muscle damage, smaller changes in concentration may represent muscular contraction. As increases in creatine phosphokinase concentrations from calves in this study were relatively small, it may be assumed that these changes resulted from muscular contraction.

Lactate is formed from pyruvate during anaerobic glycolysis (Kaneko, 1989). Energy used during strenuous activity is derived from existing ATP in muscle cells, creatine phosphate in the cells, anaerobic energy release and oxidative energy release. Energy derived from ATP and creatine phosphate is only sufficient to maintain maximum muscle contraction for a few seconds (Guyton, 1991). Release of energy by glycolysis provides energy faster than oxidative metabolism. Most of the energy required during strenuous activity that lasts from 5 seconds to 2 minutes is derived from anaerobic glycolysis. Consequently, the glycogen content of muscles during strenuous exercise decreases and lactic acid concentration of the blood increases (Guyton, 1991). Thus plasma concentrations of lactate are a useful indicator of muscular activity in situations of muscular oxygen depletion such as strenuous bouts of exercise or shivering. Lactate concentrations are probably of little value in reflecting mild exercise and were therefore

of little use in this study other than to indicate the absence of strenuous exercise or shivering.

5.3 FUTURE RESEARCH

The ability of the calf to tolerate transport may be significantly influenced by the climatic conditions. Throughout our experiment, temperatures remained between 7 and 13 ^{0}C. Higher temperatures, particularly in conjunction with lack of water may result in significant dehydration. Calves in our study had access to water throughout the duration of the experiment except during transport. Although it is recommended that water be freely available to calves in the lairage, water is usually in troughs and it is possible that the calves do not learn to drink from a trough if they have been accustomed to feeding from teat feeders. In this situation calves may become dehydrated. In colder temperatures and especially with wind and rain, calves require an increased metabolic rate to maintain normal body temperatures. It is relatively difficult for the animals to achieve this during transport as movement is restricted thereby preventing them from generating heat through physical exercise involving locomotion. Thermal balance can be maintained by shivering which generates heat from skeletal muscle (Carstens, 1994) but energy must be mobilised from endogenous stores to fuel this heat production. It would therefore seem worthwhile to conduct similar experiments to those described in this thesis in low air temperatures such as 0-10 ^{0}C combined with wind and rain, and in high air temperatures such as 20-30 ^{0}C with water unavailable.

Although the design of this experiment included one group of calves that were fed half the recommended volume on the morning prior to transport and food-deprivation, we did not account for calves receiving less than the recommended volume for a significant period of time before this as would probably be the case for weaker or less dominant calves. In this situation it is possible that the calves' energy reserves would be significantly depleted before the period of food deprivation so that they would more rapidly be compromised energetically. To determine whether calves were affected by feeding level for an extended period prior to transport and food-withdrawal, calves

could be fed a lower volume than is recommended (25ml/kg twice daily) for up to a week prior to the beginning of the experiment.

There appeared to be a glucose sparing effect in food-deprived calves transported for 12 hours (Chapter 3). This was attributed to muscular activity resulting from shivering and/or muscular activity involved in maintaining balance. Whether or not the calves were visibly shivering could be determined by videoing the animals during transport. To determine whether liver glucose was being spared by the use of muscle glycogen, simultaneous measurements of liver glycogen and muscle glycogen could be obtained over the 30 hour period. This may be achieved by taking biopsy samples. If muscle glycogen use does spare liver glycogen, it is likely that there would be a progressively decreasing ratio of muscle glycogen to liver glycogen in calves transported for 12 hours than in non-transported calves. The ethical cost to animals of any repeated biopsy would have to be weighed against the anticipated benefits that such work would generate before deciding to proceed.

As previously mentioned, the current study was conducted in relatively benign conditions and the calves appeared to tolerate the process of pre-slaughter transport and lairage without significant metabolic compromise. As a comparison with this situation, similar measurements of metabolic parameters could be obtained from calves at the freezing works prior to slaughter. Although the background of these calves in terms of the way they were treated and the conditions they were exposed to would not be known, the information obtained would indicate what happens to the calves in reality. Significant differences between the results obtained from the freezing works situation and those seen in this study may indicate that calves are not being treated according to the recommendations given in the Code, or that more extreme weather conditions are detrimental to the calves, whereas similar results would suggest the recommendations were being applied to good effect.

REFERENCES

Alexander G. (1962) Energy metabolism in the starved new-born lamb. *Australian Journal of Agricultural Research.* **13:** 144-164.

Alexander G. (1979) Cold thermogenesis. *International Review of Physiology: Environmental Physiology III.* **20:** 43-155.

Ames D.R., Arehart L.A. (1972) Physiological response of lambs to auditory stimuli. *Journal of Animal Science.* **34:** 994-998.

A.W.A.C. (1993) Code of Recommendations and Minimum Standards for the Welfare of Bobby Calves. Code of Animal Welfare No. 8. Animal Welfare Advisory Committee. Ministry of Agriculture and Fisheries, Wellington, New Zealand.

A.W.A.C. (1997) Code of Recommendations and Minimum Standards for the Welfare of Bobby Calves. Code of Animal Welfare No. 8 (revised). Animal Welfare Advisory Committee. Ministry of Agriculture and Fisheries, Wellington, New Zealand.

Barnes R.J., Fowden A.L., Silver M., Comline R.S. (1977) Liver glycogen concentrations in the fetal lamb and pig. *Annales de Recherches Veterinaires.* **8:** 374-378.

Battaglia F.C., Meschia G. (1986) An Introduction to Fetal Physiology. New York: Academic Press, Orlando.

Bauchart D., Durand D., Laplaud M., Forgez P., Goulinet S., Chapman J.M. (1989) Plasma lipoproteins and alipoproteins in the preruminant calf, *Bos* spp: density distribution, physiochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis. *Journal of Lipid Research.* **30:** 1499-1514.

Blaxter K.L., Wood W.A. (1951) The nutrition of the young ayrshire calf: 3. The metabolism of the calf during starvation and realimentation. *British Veterinary Journal.* **5:** 29-55.

Blood D.C., Studdert V.P. (1988) Baillere's Comprehensive Veterinary Dictionary. Baillere Tindall, London.

Blood D.C, Radostits O. (1989) Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 7th edition. Bailliere Tindall Limited, London. Pp 14-15.

Boyd J.W. (1981) The relationship between blood hemoglobin concentration, packed cell volume and plasma protein concentration in dehydration. *British Veterinary Journal.* **137:** 166-172.

Braun J.P., Tainturier D., Laugier C., Benard P., Thouvenot J.P., Rico A.G. (1982) Early variations of blood plasma gamma-glutamyl transferase in newborn calves - a test of colostrum intake. *Journal of Dairy Science.* **65:** 2178-2181.

Carstens G.E. (1994) Cold thermoregulation in the newborn calf. *Veterinary Clinics of North America: Food Animal Practice.* **10** (1): 69-105.

Challis J.R.G., Lye S.J. (1986) Parturition. Chp 2. In: Oxford Reviews of Reproductive Biology. **8:** 61-129. Clarendon Press, Oxford.

Church D.C. (1976) Digestive Physiology and Nutrition of Ruminants. Vol 1: 34-35. D.C. Church, Oregon.

Clarenburg R. (1992) Physiology of Domestic Animals. Moseby-Year Book Inc, Missouri.

Cresteil T., Leroux J. (1977) Early postnatal metabolism of amino acids in the rat. *Pediatric Research.* **11:** 720-723.

Daniels L.B., Perkins J.L., Krieder D., Tugwell D., Carpenter D. (1974) Blood glucose and fructose in the newborn ruminant. *Journal of Dairy Science.* **57** (10): 1196-1200.

Dirks K. (1995) *Meterological Society of New Zealand (Inc.) Newsletter.* **63.**

Dohm G.L., Beeker R.T., Israel R.G., Tapscott E.B. (1986) Metabolic responses to exercise after fasting. *Journal of Applied Physiology.* **61** (4): 1363-1368.

Eales F.A., Gilmour J.S., Barlow R.M., Small J. (1982) Causes of hypothermia in 89 lambs. *Veterinary Record.* **110:** 118-120.

Eales F.A., Small J. (1980) Summit metabolism in newborn lambs. *Research in Veterinary Science.* **29:** 211-218.

Edwards A.V. (1970) Carbohydrate metabolism in young animals. In: Physiology of Digestion and Metabolism in the Ruminant. Proceedings of the 3rd International Symposium. Edited by A. Phillipson. Oriel Press, England. Pp 181-198.

Edwards S.A. (1982) Factors affecting the time to first suckling in dairy calves. *Animal Production.* **34:** 339-346.

Evers B. (1989) Hormonal effects on protein turnover. In: Protein Metabolism in Farm Animals - Evaluation, digestion, absorption and metabolism. H.D. Brock, B. Eggum, A. Low, O. Simon, T. Zebrowska. Oxford University Press, London. Chp 10: 367-403.

F.A.W.C. (1997) Report on the welfare of dairy cattle. Farm Animal Welfare Council, Surrey. December.

Fey H. (1971) Immunology of the newborn calf: its relationship to colisepticemia. *Annals. New York Academy of Science.* **176:** 49-63.

Fowden A.L. (1980) Effects of arginine and glucose on the release of insulin in the sheep fetus. *Journal of Endocrinology.* **85:** 121-130.

Fraser A.F. (1979) The nature of cruelty to animals. *Applied Animal Ethology.* **5:** 1-4.

Girard J., Ferre P. (1982) Metabolic and hormonal changes around birth. In: Biochemical Development of the Fetus and Neonate. C. Jones. Amsterdam: Elsevier, pp 517-551

Girard J., Ferre P., Pegorier J., Duee P. (1992) Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. *Physiological Reviews.* **72** (2): 507-839.

Goldman J.K., Cahill G.F. (1964) Insulin effect on glucose-^{14}C incorporation into rat livers in adipose tissue in vivo. *Metabolism, Clinical and Experimental.* **13:** 572-578.

Gonzalez-Jimenez E., Blaxter K.L. (1962) The metabolism and thermal regulation of calves in the first month of life. *British Journal of Nutrition.* **16:** 199-212.

Gruber W. (1978) Inhibition of creatine kinase activity by Ca^{2+} and reversing effect of ethylenediaminetetraacetate. *Clinical Chemistry.* **24:** 177-178.

Guyton A.C. (1991) Textbook of Medical Physiology, 8th edition. W.B Saunders, Philadelphia. Chp 69: 75-78, 82.

Head H.H., Kull R.L., Campos M.S., Bachman K.L., Wilcox C.J., Cline L.L., Hayen M.J. (1993) Milk yield, milk composition and behaviour of Holstein cows in response to jet aircraft noise before milking. *Journal of Dairy Science.* **76:** 1558-1567.

Heard C.R.L., Henry P.A.J. (1969) Glucose tolerance and insulin sensitivity. *Clinical Science.* **37:** 37-44.

Hostettler-Allen R.L., Tappy L., Blum, J.W. (1994) Insulin resistance, hypoglycemia, and glucosuria in intensively milk-fed calves. *Journal of Animal Science.* **72:** 160-173.

Huber A.M. Gershoff S.N., Antoniades H.N. (1965) The effect of fasting and refeeding on isolated rat adipose tissue activity in the presence of a crystalline and "bound" insulin. *Metabolism.* **14:** 619-624.

Ingram M. (1964) Feeding meat animals before slaughter. *Veterinary Record.* **76** (46): 1305-1310.

Kaneko J.J. (1989) Clinical Biochemistry of Domestic Animals, 4th edition. Academic Press Inc, California.

Kasari T.R. (1994) Physiologic mechanisms of adaptation in the fetal calf at birth. *Veterinary Clinics of America: Food Animal Practice.* **10** (1): 127-136.

Kasuga M., Akanuma Y., Iwamoto Y., Kosaka K. (1977) Effects of fasting and refeeding of insulin receptors and glucose metabolism in rat adipocytes. *Endocrinology.* **100** (5): 1384-1390.

Katunguka-Rwaskishaya E., Larkin, Kelly W.R. (1985) Some haematological and blood biochemical components in milk-fed calves. *Irish Veterinary Journal.* **39:** 138-142.

Kenny F.J., Tarrant P.V. (1987) The reaction of young bulls to short-haul road transport. *Applied Animal Behaviour Science.* **17:** 209-227.

Kent J.E., Ewbank R. (1983) The effect of road transportation on the blood constituents and behaviour of calves. I. Six months old. *British Veterinary Journal.* **139:** 228-235.

Kent J.E., Ewbank R. (1986a) The effect of road transportation on the blood constituents and behaviour of calves. II. One to three weeks old. *British Veterinary Journal.* **142:** 131-139.

Kent J.E., Ewbank R. (1986b) The effect of road transportation on the blood constituents and behaviour of calves. III. Three months old. *British Veterinary Journal.* **142:** 326-335.

Kinsbergen M., Sallmann H.-P., Blum J.W. (1994) Metabolic, endocrine and haematological changes in 1-week-old calves after milk intake, in response to fasting and during total parenteral nutrition. *Journal of Veterinary Medicine A.* **41:** 268-282.

Kirton A.H., Moss R., Taylor A.G. (1971) Weight losses from milk and weaned lambs in mid Canterbury resulting from different length of starvation before slaughter. *New Zealand Journal of Agricultural Research.* **14:** 149.

Kirton A.H., Paterson D.J. (1973) Effects of starvation before slaughter on some body components of bobby calves. *New Zealand Journal of Experimental Agriculture.* **1:** 115-119.

Kirton A.H., Paterson D.J., Duganzich D.M. (1972) Effect of pre-slaughter starvation in cattle. *Journal of Animal Science.* **34** (4): 555-559.

Kirton A.H., Quatermain A.R., Uljee A.E., Carter W.A., Pickering F.S. (1968) Effect of 1 and 2 days ante-mortem fasting on live weight and carcass losses in lambs. *New Zealand Journal of Agricultural Research.* **11:** 891-902.

Klein S, Sakurai Y, Romijn J, Carroll R. (1993) Progressive alterations in lipid and glucose metabolism during short-term fasting in adult young men. *Endocrinol. Metabolism.* **28:** E801-E806.

Knowles T.G., Wariss P.D., Brown S.N., Edwards J.E., Watkins P.E., Phillips A.J. (1997) Effects on calves less than one month old of feeding or not feeding them during road transport of up to 24 hours. *Veterinary Record.* **140:** 116-124.

Knowles T.G., Wariss P.D., Brown S.N., Kestin S.C. (1994) Long distance transport of export lambs. *Veterinary Record.* **134:** 107-110.

Knowles T.G., Wariss P.D., Brown S.N., Kestin S.C., Rhind S.M., Edwards J.E., Anil M.H., Dolan S.K (1993) Long distance transport of lambs and the time needed for subsequent recovery. *Veterinary Record.* **133:** 286-293.

Leach T.M. (1982) Physiology of the transport of cattle. In - Current Topics in Veterinary Medicine and Animal Science, Vol. 18: Transport of animals intended for breeding, production and slaughter. R. Moss (editor). Martinus Nijhoff Publishers, Boston. Pp57-72.

Leichter J., Goda T., Koldovsky O. (1987) Dependency of lactose absorption on lactase activity in starved rats. *Canadian Journal of Physiology and Pharmacology.* **65** (11): 2287-2290.

Lewis H.B., Rhodes D.C. (1978) Effects of intramuscular injection on serum creatine phosphokinase (CPK) values in dogs. *Veterinary Clinical Pathology.* **7** (2): 11-12.

Lewis L.D., Phillips R.W., Elliot C.D. (1975) Changes in plasma glucose and lactate concentrations and enzyme activites in the neonatal calf with diarrhea. *American Journal of Veterinary Research.* **36** (4): 413-417.

Li P. (1985) β-hydroxybutyrate. *Clinical Chemistry Newsletter.* **11** (2): 13.

Lindt F., Blum J.W. (1994) Growth performance, hematological traits, meat variables, and effects of treadmill and transport stress in veal calves supplied different amounts of iron. *Journal of Veterinary Medicine.* **41:** 333-342.

Locatelli A., Sartorelli P., Agnes F., Bondiolotti G.P., Picotti G.B. (1989) Adrenal response in the calf to repeated simulated transport. *British Veterinary Journal.* **145:** 517-522.

MacKenzie D.D.S. (1984) Immunoglobulin transfer. Dairy Cattle Society of the New Zealand Veterinary Association. Proccedings of the Seminar held at Massey University, 30 November - 1 December. Pp 9-27.

McMeekan C. (1997) Evaluation of strategies for alleviating dehorning distress in calves. MSc thesis. Massey University.

Malaisse W., Malaisse-Lagae F., Wright P. (1967) Effect of fasting upon insulin secretion in the rat. *American Journal of Physiology.* **213:** 843-848.

Martinez A., Padbury J., Humme J., Evans C., Shames L. (1990) Plasma catecholamines and their physiologic thresholds during the first ten days of life in sheep. *Journal of Developmental Physiology.* **13:** 141-146.

Mayor F., Cuezva J.M. (1985) Hormonal and metabolic changes in the perinatal period. *Biology of the Neonate.* **48:** 185-196.

Mellor D.J. (1987) Feeding pregnant ewes and newborn lambs during experiment. In: Animal Models in Fetal Medicine. Ed. P. Nathanielsz. Perinatology Press. Chp 3: 55-92.

Mellor D.J. (1990) Meeting the colostrum needs of newborn lambs. *In Practice.* **12** (6) 239-244.

Mellor D.J. (1992) Nutrition and gastrointestinal development in the newborn. *Proceedings of the Nutrition Society of New Zealand.* **17:** 25-37.

Mellor D.J., Cockburn F. (1986) A comparison of energy metabolism in the newborn infant, piglet and lamb. Review article. *Quarterly Journal of Experimental Physiology.* **71:** 361-379.

Mellor D.J., Reid C.S.W. (1993) Concepts of animal well-being and predicting the impact of procedured on experimental animals. In: Improving the Well-being of Animals in the Research Environment. Proceedings of the ANZCCART conference, Sydney, October. Pp 3-18.

Naylor J.M., Kronfeld D.S., Bech-Neielsen S., Bartholomew R.C. (1977) Plasma total protein measurement for prediction of disease and mortality in calves. *Journal of the American Veterinary Medical Association.* **171** (7): 635-628.

Neumann U., Ziegenhorn J. (1977) Kinetic enzymatic method for determination of urea in serum with the LKB 8600 Reaction Rate Analyzer. *Scandanavian Journal of Clinical and Laboratory Investigation.* **37:** supplement 147, abstract 97.

Noll F. (1974) L- (+)- Lactate. Determination with LDH, GPT and NAD. In: Methods of Enzymatic Analysis, 2nd edition. H.U. Bergmeyer, editor. Academic Press Inc, NY. P 1475.

Nsi-Emvo E., Raul F. (1984) Stimulation of lactase synthesis induced by starvation in the jejunum of adult rat. *Enzyme.* **31** (1): 45-49.

Oltner R., Berglund B. (1982) Blood levels of haemoglobin, leukocytes, glucose, urea, creatinine, calcium, magnesium and inorganic phosphorous in dairy calves from birth to 12 weeks of age. *Swedish Journal of Agricultural Research.* **12:** 23-28.

Owens S.W., Sartin J.J., Kemppainen R.J., Cummins K.A., Bartol F.F., Bowman M.A. (1986) Developmental alterations in the regulation of glucagon and insulin secretion in Holstein calves. *American Journal of Veterinary Research.* **47** (2): 263-269.

Perino L., Sutherland R., Woollen N. (1993) Serum gamma-glutamyl transferase activity and protein concentration at birth and after suckling in calves with adequate and inadequate passive transfer of immunoglobulin G. *American Journal of Veterinary Research.* **54** (1): 56-59.

Persijn J.P., Van der Slik W. (1976) A new method for the determination of gamma-glutamyltransferase in serum. *Journal of Clinical Chemistry and Clinical Biochemistry.* **14:** 421-427.

Persson S. (1967) II. Studies of variations in plasma volume, total blood volume and total hemoglobin in horses. *Acta Veterinaria Scandanavica Supplement.* (**19**-suppl.): 84-121.

Rollin R.E., Fettman M.J., Phillips R.W. (1986) Age-related changes in carbohydrate tolerance in healthy neonatal calves. *American Journal of Veterinary Research.* **47** (7): 1583-1585.

Ronge H., Blum J.W. (1988) Somatomedin C and other hormones in dairy cows arounf parturition, in newborn calves and in milk. *Journal of Animal Physiology and Animal Nutrition.* **60:** 168-176.

Russel A.J.F., Doney J.M., Reid R.C. (1967) The use of biochemical parameters in controlling nutrient stores in pregnant ewes, and the effect of undernourishment during pregnancy on lamb birth-weight. *Journal of Agricultural Science.* **68:** 351-358.

Sartorelli P., Dominoni S., Agnes F. (1992) Influence of duration of simulated transport on plasma stress markers in the calf. *Journal of Veterinary Medicine A.* **39:** 401-403

Schrama J.W., Arieli A., Brandsma H., Luiting P., Verstegen M.W.A. (1993) Thermal requirements of young calves during standing and lying. *Journal of Animal Science.* **71:** 3285-3292.

Shannon A.D., Lascelles A.K. (1966) Changes in the concentration of lipids and some other constituents in the blood plasma of calves from birth to 6 months of age. *Australian Journal of Biological Science.* **19:** 831-839.

Shelley H.J. (1960) Blood sugars and tissue carbohydrate in fetal and infant lambs and rhesus monkeys. *Journal of Physiology.* **153:** 527-552.

Shelley H. (1961) Glycogen reserves and their changes at birth. *British Medical Bulletin.* **17:** 137-143.

Siegmund O. (1973) The Merck Veterinary Manual, 4th edition. Merck & Co. Inc, USA. Pp 1389-1393.

Siddons R.C., Smith R.H., Henschel M.J., Hill W.B., Porter J.W.G. (1969) Carbohydrate utilisation in the pre-ruminant calf. *British Journal of Nutrition.* **23:** 333-341.

Sisson S., Grossman J. (1953) The Anatomy of Domestic Animals, 4th edition, revised. W.B. Saunders Company, Philadelphia. Pp 451-453.

Slater J.S., Mellor D.J. (1981) Within-day variations in the composition of maternal and fetal plasma from catheterised ewes fed once daily or at hourly intervals during late pregnancy. *Research in Veterinary Science.* **31:** 224-230.

Smith B. (1996) Large Animal Internal Medicine. Diseases of horses, cattle, sheep and goats, 2nd edition. Mosby-Year Bk Inc, St Louis. Chp 22: 441-469.

Stott G.H., Marx D., Menefee B.E., Nightengale G. (1979) Colostral immunoglobulin transfer in calves. I. Period of Absorption. *Journal of Dairy Science.* **62:** 1632-1638.

Tarrant P.V. (1990) Transportation of cattle by road. *Applied Animal Behaviour Science.* **28:** 153-170.

Tarrant V., Grandin T. (1993) Cattle transport. In: Livestock Handling and Transport,. CAB International, England. Pp 109-126

Tarrant P.V., Kenny F., Harrington D. (1988) The effect of stocking density during 4 hour transport to slaughter on behaviour, blood constituents and carcass bruising in Friesian steers. *Meat Science.* **24:** 209-222.

Tarrant P.V., Kenny F.J., Harrington D., Murphy M. (1992) Long distance transportation of steers to slaughter: effect of stocking density on physiology, behaviour and carcass quality. *Livestock Production Science.* **30:** 223-238.

Thickett B., Mitchell D., Hallows B. (1986) Calf physiology and nutrition. In: Calf Rearing. Farming Press Limited, Ipswich. Chp 3: 55-75.

Thompson J.C., Pauli J.V. (1981) Colostral transfer of gamma glutamyl transpeptidase in calves. *New Zealand Veterinary Journal.* **29:** 223-226.

Trinder P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry.* **6** (Pt 1-2): 24-27.

Ventorp M., Michanek P. (1991) Cow-calf behaviour in relation to first suckling. *Research in Veterinary Science.* **51:** 6-10.

Vermorel M., Dardillat C., Vernet J., Saido, Demigne C. (1983) Energy metabolism and thermoregulation in the newborn calf. *Annales de Recherches Veterinaires.* **14:** 382-389.

Wahlefeld A.W. (1974) In: Methods of Enzymatic Analysis, 2nd English edition. H. Bergmeyer (editor). Academic Press Inc, NY. P 1831.

Warriss P.D., Brown S.N., Knowles T.G., Kestin S.C., Edwards J.E., Dolan S.K., Phillips A.J. (1995) Effects on cattle of transport by road for up to 15 hours. *Veterinary Record.* **136:** 319-323.

Webster J. (1984) Calf husbandry, health and welfare. Granada Publishing Limited, London. Chap 1, 2, 4, 6 and 7.

Weichselbaum T.E. (1946) An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *American Journal of Clinical Pathology.* **10:** 40-49.

White D., Baxter M. (1994) Hormones and Metabolic Control, 2nd edition. Edward Arnold, Great Britain.

Wood A.S., Bayley H.S., MacLeod G.K. (1971) Imposing a weekly fast on calves receiving a milk replacer diet once and twice per day: blood glucose and plasma lipid patterns. *Journal of Dairy Science.* **54** (4): 509-514.

Yamada K., Goda T., Bustamante S., Koldovsky O. (1983) Different effect of starvation on activity of sucrase and lactase in the rat jejunoileum. *American Journal of Physiology.* **244:** G449-G455.