A STUDY OF PHYSIOLOGICAL DIFFERENCES BETWEEN LOW AND HIGH BREEDING INDEX FRIESIAN HEIFERS

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ABSTRACT

Friesian heifers from two genetic lines divergently selected for milk production were compared in their metabolic physiology and endocrinology in three experiments. Studies were conducted on the heifers, which were matched for age and bodyweight, in order to identify metabolic differences which might be used as genetic markers for lactational performance.

In the first experiment diurnal variation in plasma metabolite and hormone concentrations and responses to metabolic challenges of glucose, insulin, glucagon, and adrenaline, were measured in 6 high breeding index (HBI) and 6 low breeding index (LBI) heifers aged 6 to 8 months and fed 75% or 125% maintenance energy requirement (MER).

Basal plasma concentrations of creatinine, GH and NEFA were not influenced by selection line. Plasma insulin concentrations after feeding were greater in the LBI than in the HBI heifers. Relative to the concentrations which existed at the time of feeding, the elevation in plasma glucose concentration was greater in the HBI than in the LBI heifers from 7 to 9 hours after feeding. Elevation in plasma urea concentration on feeding was greater in HBI than in LBI heifers. Urea concentrations then declined more rapidly in the selected animals during the postprandial period such that concentrations were lower in HBI than in LBI heifers from 11 till 23 hours after feeding. Responses to metabolic challenge were generally not different between the lines and there were no line x allowance interactions except in the NEFA response to adrenaline where HBI heifers responded more than LBI heifers at 75% MER but not at 125% MER.

When compared with heifers fed 125% MER, those fed 75% MER exhibited: increased plasma creatinine concentrations; a smaller increment in plasma urea concentration after feeding; greater plasma NEFA levels in the post-prandial period; lower insulin concentrations during a 24 hour sampling period; decreased insulin release and glucose removal after glucose administration; greater plasma NEFA concentrations and reduced glucose clearance after insulin injection; enhanced glycogenolytic responses to glucagon and adrenaline;

andincreased lipolytic responses to glucagon and adrenaline.

In the second experiment, 8 HBI and 8 LBI Friesian heifers aged 6 months were treated with progesterone by Controlled Internal Drug Release (CIDR) devices and fed 70% MER. Initially, basal plasma metabolite and hormone concentrations were measured in samples collected during a 6 hour intensive sampling period. In the following period, the line x dose interactions of intravenous glucose (0, 75, 150 and 300 mg/kg lwt) and insulin (0, 0.1, 1, and 10 ug/kg lwt) on metabolic responses were evaluated in a split-plot design carried out over a period of 8 days.

Basal plasma urea and creatinine concentrations were marginally greater ($P<0.10$) in the LBI heifers than in the HBI heifers but no differences were found between the two lines in plasma concentrations of GH, insulin, glucagon, glucose or NEFA. No significant line differences were found in the number of secretion spikes or the magnitude of the spikes for basal GH or insulin. Glucagon concentrations were measured using a specific double antibody radioimmunoassay developed as part of this programme.

There were marked dose effects of both glucose and insulin challenges on concentrations of insulin, glucose and NEFA. In addition, the HBI heifers released more insulin than the LBI heifers after the glucose challenge in a manner independent of glucose dose. Moreover, volume of plasma glucose distribution (Vd), or the distribution coefficient (Δ) was smaller, and glucose disappearance rate greater (in terms of elimination rate constant (k) or the half-life ($t_{1/2}$) of the injected glucose), in the HBI than in the LBI heifers.

Insulin challenge resulted in slightly higher plasma insulin concentrations in the HBI heifers than in the LBI heifers. No significant interactions of line x dose in plasma metabolites and hormone concentrations were observed after either glucose or insulin challenges.

The third experiment compared 8 HBI and 8 LBI yearling heifers, fed 140% MER and receiving progesterone treatment, with respect to: diurnal patterns of plasma concentrations of metabolites and hormones; volume of body fluid distribution; ingestive behaviour in terms of rate of eating; responses of lipolysis and glycogenolysis to adrenaline challenge at various times after feeding and fasting; metabolic responses to fasting and refeeding; and pancreatic insulin release and glucose disappearance after glucose challenges administered before and after the withdrawal of progesterone-impregnated CIDRs.

Diurnal plasma concentrations of glucose, were greater, but plasma urea and creatinine levels were lower, in HBI than in LBI heifers. Plasma glucagon levels at the onset of feeding/refeeding were only briefly greater in HBI heifers than in LBI heifers. The volumes of urea distribution, plasma distribution (as measured by Evans blue (T1824) distribution), and the extracellular fluid distribution (as measured by thiocyanate (NaSCN) distribution) were similar between the HBI and LBI heifers. In general, rate of eating was similar between the lines over the experiments except it was greater in the LBI than in the HBI heifers on the first day of measurement. In addition, the eating rate fell substantially in the LBI but not in HBI heifers 28 hours after the withdrawal of progesterone-CIDRs.

Lipolytic response to adrenaline was minimal 7 hours after feeding, and maximal after 72 hours of fasting, whereas the reverse was true for glycogenolytic responses. There were significant line x time of challenge interactions in pre-challenge plasma NEFA concentrations, HBI heifers fasted for 72 hours exhibiting greater elevation in plasma NEFA concentration. Time of challenge relative to feeding/fasting did not, however, influence the magnitude of selection line effects on lipolytic or glycogenolytic responses.

Basal plasma insulin concentration and pancreatic insulin release after glucose challenges were greater in HBI than the LBI heifers, irrespective of the presence or absence of progesterone-impregnated CIDRs. Although basal plasma glucose concentration was greater in the HBI than in the LBI heifers, glucose disappearance was similar between

the two lines following glucose challenge in this experiment. There was a significant line x progesterone presence/withdrawal interaction in the pre-challenge plasma glucose concentrations. Plasma glucose concentrations were greater in the HBI than in the LBI heifers 46 hours after the removal of progesterone CIDRs but not prior to removal of the CIDRs.

These results demonstrated that genetic variation exists in nitrogen, lipid, glucose and insulin metabolism between the HBI and the LBI heifers. Appropriate experimental conditions such as different feeding regimens, use of metabolic challenges and control of oestrous activity, alone or in combination, were useful means of maximising these genetic differences. While these metabolic characteristics have the potential to become markers for dairy merit, their genetic relationships with milk production should be confirmed in further studies and these traits should also be evaluated in progeny tested bulls before their wide use in dairy cattle breeding.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
A_{kp}	Alkaline phosphatase
AMI	Amylases
ANOVA	analyses of variance
BHBA	β-hydroxybutyrate
C_0	Concentration at time=0 (after challenge)
CCK	Cholecystokinin
CIDR	Progesterone-impregnated controlled internal drug releaser
CNS	Central nervous system
cpm	Counts per minute
C.V.	Coefficient of variation
DM	Dry matter
DNAFP	DNA Finger Print
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GIP	Gastric inhibitory peptide
GnRH	Gonadotrophin releasing hormone
GP_γ	Guinea pig gamma globulin
h^2	Heritability
IGF(s)	Insulin-like growth factor(s)
HBI	High breeding index
k	Fractional removal rate of injected metabolite
LH	Luteinizing hormone
LBI	Low breeding index
LWT	Live weight
MANOVA	Repeated-measures analyses (multivariate analyses of variance)
MER	Maintenance energy requirement
MHC	Major histocompatability complex
MJ	Megajoules
NaSCN	Sodium thiocyanate
NEFA	Non-esterified fatty acids
ng	Nanogram
PCV	Packed (red) cell volume
PL	Placental lactogen
Prl	Prolactin
pg	Picogram
R	Repeatability
RFLP	Restriction fragment length polymorphism
r_g	Genetic correlation
RIA	Radioimmunoassay
S.D.	Standard deviation
s.e.	Standard error of the mean
SGOT	Serum glutamic oxaloacetic transamines
$t_{1/2}$	Half-life
T1824	Evans blue dye
T_3	Triiodothyronine
T_4	Thyroxine
Tf	Transferrin
TRH	Thyrotropin-releasing hormone
µg	Microgram
Vd	Volume of fluid distribution
VIP	Vasoinhibitory peptide.
VMH	Ventromedial hypothalamus
Δ	Fluid distribution coefficient

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CHAPTER ONE: INTRODUCTION

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I. Dairy cattle breeding and the role of genetic markers

1. Progeny testing and its limitations

The fundamental aim of dairy cattle breeding programmes is to estimate the genetic merit of animals in a population and select the superior individuals for breeding the next generation, so increasing milk production and productive efficiency. Current progeny testing schemes for estimating the breeding values of superior bulls are based on the theory of quantitative genetics. In those schemes, young bulls are mated by artificial insemination (AI) to a large number of cows in a range of environments (e.g. different dairy farms throughout the country). The milk production of the daughters is measured and used to assess the genetic merit of the bulls. Although the genes of the identified superior bulls can then be disseminated widely by AI, the rate of genetic improvement is still relatively slow and progeny testing is expensive. This is mainly because a large number of daughters is required to calculate breeding values of the bulls, and the results are normally not available until the bulls are 5 to 6 years old (i.e. the generation interval is very long). In addition, the genetic merit of breeding heifers is not estimated accurately by the current breeding schemes. Simulated gene flows indicate that accurate selection of the dams of the female replacements is required for maximum genetic gain (McDaniel and Dantine 1983; Woolliams and Smith 1988). Further, the correlation between the first lactation (normally only this information is used for calculating the breeding value of the bull) and the lifetime performance of the heifers is not unity (Powell et al. 1981; Powell and Norman 1988). All of these factors, but particularly the long generation interval, conspire to limit the rate of genetic gain in current dairy breeding programmes.

2. Potential uses of genetic markers in dairying

The development of genetic markers (i.e. metabolic characteristics of an animal which may be used to predict genetic merit) could have a number of advantages for the dairy industry,

particularly if the use of the markers was inexpensive and applicable to both bulls and heifers. These potential benefits include: early identification of both bulls and heifers of superior genetic merit, allowing shorter generation intervals; increased chance that exceptional individuals would be identified because a larger number of cattle, and thus a larger genetic pool, could be screened; matings where both the heifers and bulls had been selected for superior genetic merit; reduction of breeding costs if the use of genetic markers was able to replace the progeny test; early culling of inferior bulls and heifers; and more accurate comparison of the genetic value of animals from different herds or different countries. The combination of these could lead to annual rates of genetic improvement substantially greater than the present 1- 1.5 % (Blake et al. 1986; Strandlberg and Danell 1988).

II. Approaches to identifying genetic markers

There are two main approaches which can be taken in the search for genetic markers. One relies on comparisons between animals from selection lines and the other on comparisons between bulls which have been progeny tested and whose genetic merit is accurately known.

1. Selection lines

The selection of superior bulls and their wide use in AI programmes in the past three decades has facilitated the development of lines of dairy cattle differing in genetic merit for milk production. Such lines are now available in several countries including England, the United States of America, Denmark and New Zealand. These lines are maintained by selecting heifers of low or high genetic merit (based on average parental merit) and mating them to bulls of appropriate genetic merit as assessed by progeny testing. Lines differing in mean breeding values by 20 to 30% have been established by these procedures.

In general, selection for milk yield, either in special lines or in the population as a whole, has resulted in the production of genetically superior cows with the following characteristics (Bryant and Trigg 1981; Davey et al. 1983; Barnes et al. 1985; Bauman et

al. 1985; Grainger et al, 1985; Peel and Bauman 1987; Holmes et al. 1987; Holmes 1988):

a) increased yield of milk and milk solids;
b) similar milk solids content, or small increases in milk fat concentration (in NZ) or slightly reduced concentration of solids (in other countries);
c) increased milk flow and milking time;
d) slightly lighter weights in Friesians, but heavier weights in Jerseys;
e) increased feed intake per unit of live weight;
f) no difference in ability to digest or metabolise gross dietary energy;
g) more efficient conversion of feed into milk, i.e. a greater portion of dietary energy being converted to milk;
h) lose more or gain less LW during lactation;
i) no difference in fasting heat-production or in maintenance energy requirements during the non-lactating period;
j) greater udder volume (l/cow, or $l/kg^{.75}$), but similar milk secretion per unit of udder volume in Jerseys. In Friesians, no difference exists in udder volume but superior cows have greater milk secretion per unit of udder volume;
k) Substantial differences in partitioning of absorbed nutrients to milk secretion vs tissue deposition.

The major advantage of studying selection lines is the substantial differences in average genetic merit which exist between the lines, even though the genetic values of individuals within a line are unknown. Therefore the physiological differences associated with the genetic selection are maximised between the lines and can be identified more easily. The identification of these physiological differences, in the selection line cows or their calves, may be used as a method of identifying potential genetic markers.

However, the use of the selection lines for identifying genetic markers also has the following restrictions: a) only limited gene pools are used to create these lines so that results may not be

representative of the whole population; b) genetic drift may occur in the small population of the lines; and c) the estimated genetic merit (eg via use of ancestry records) of a line may overestimate or underestimate the actual genetic merit particularly if the population is small.

2. Progeny tested bulls

Another approach to identifying genetic markers is to compare the physiological characteristics of young bulls whose genetic merit is accurately determined by progeny test. This method has two major advantages over use of selection lines: a) there should be no environmental covariation between marker levels and milk yield as the effect has been corrected in progeny testing by having daughters of the bulls represented in a range of farms in different environments; b) the genetic variation for milk production of the bulls is estimated within the range from which further genetic improvement is to be made (whereas the difference in selection lines may not represent the future direction of selection pressure because the low lines, in particular, are not of similar breeding values to the bulls which we wish to select using markers). However, the use of progeny testing is expensive and has the disadvantage of a long interval between the time when markers can be measured (normally when bulls are 12-15 months of age) and when their genetic merit can be evaluated (after the progeny test results become available when bulls are 4-5 years of age). Alternatively, older bulls, which have already been progeny tested, can be used to identify the genetic markers. This method relies on the assumption (as yet unproven) that there are strong correlations between the marker values when measured in young bulls and in the same bulls when they are 5 years of age or older. The logistical problems of handling mature bulls are also much greater.

III. Types of genetic markers

Considerable attention has been given, in the past, to conformational or type traits as predictors of genetic merit (e.g. in heifer selection). Though genetic correlations may exist between these traits and milk yield the results have been inconsistent (Gowen 1933;

Touchberry 1951; Blackmore et al. 1958; Norman et al. 1981; Sieber et al. 1988), and genetic improvement via this route has been slow. It is even reported that cows selected for conformation traits produced less milk than those selected directly for increased milk yield (Voelker and Ludens 1982).

Another approach to indirect selection is to identify biochemical characteristics which are linked with the genes determining milk production and could be used as genetic markers for predicting dairy merit. Over the last 40 years much work has been done to study the relationship between milk production and the polymorphisms present in various bovine proteins (reviewed by Kiddy 1979; Gahne 1982). More recently polymorphisms in DNA and the possible linkage with milk production have received much attention. Moreover, genetic variation in metabolic traits such as blood concentrations of metabolites and hormones has been identified in dairy cattle (Kitchenham et al. 1975; Stark et al. 1978; Peterson et al. 1982), especially between genetically divergent lines of dairy cattle (Tilakaratne et al. 1980; Flux et al. 1984; Barnes et al. 1985). Thus a number of potential genetic markers of lactational performance exist.

1. Polymorphisms

1) Protein polymorphisms

Protein polymorphism is due to differences in one or more amino acids in the protein molecule, and is controlled by single or multiple alleles with simple inheritance. There are numerous reports studying the relationships between the polymorphisms of various serum and milk proteins and milk yield in dairy cattle. For example, the possible relationship between polymorphisms in red blood cell antigens and milk production has been examined extensively in different populations of dairy cattle of varying size and genetic diversity. However, only the B group has been consistently associated with changes in percentage of milk fat (Neimann-Sorensen and Robertson 1961). These relationship are not sufficiently strong to have any practical value for selecting superior animals.

Effects of polymorphisms in other bovine proteins and enzymes, such as the major histocompatibility complex (MHC), transferrin (Tf), amylases (AmI) and alkaline phosphatase (A_{kp}) on milk traits have also been examined by many workers (see reviews: Kiddy 1979; Gahne 1982). Despite the high incidence of polymorphisms in these proteins and enzymes in cattle, consistent associations between particular polymorphisms and milk production have not been established. An exception is in milk protein polymorphism which has been found to be related to milk yield, milk composition and processing properties of the milk (Mclean et al. 1984; Gibson 1987).

Selection for polymorphisms with simple inheritance, in theory, has limited value in dairy cattle breeding unless major genes regulating milk production are discovered. This is because even if the initial genetic correlation between the marker traits and milk production is strong, selection will soon fix these alleles and reduce the genetic correlation to zero.

2) DNA Polymorphisms

Polymorphisms of proteins essentially reflect polymorphisms in the genomic DNA. These mutations can occur as single nucleotide subsitutions, or they can involve the gain, loss or rearrangement of nucleotides in segments of up to a few thousands nucleotides. Recent developments in the analysis of DNA have revealed a large amount of variation in the composition of genetic material within species including cattle (Kashi et al. 1986; Fries et al. 1989).

Restriction Fragment Length Polymorphism (RFLP): Hybridization patterns of cloned and labelled DNA sequences ("probes") with RFLP provide a direct measure of variation in the genomic DNA. They are detected by separating, by gel electrophoresis, fragments of (RFLP) DNA derived from the digestion of genomic DNA with restriction endonucleases (Montgomery et al. 1988). Changes in the length of RFLPs can result from an insertion or a deletion of a piece of DNA between the enzyme cutting sites, but the majority of RFLPs revealed so far appear to be single point mutations.

The RFLP has potential application in dairy breeding as a means of identifying the major genes for milk production or the linkage of marker loci with the major genes. It may be that genetic selection has favoured active regulatory variants of genes for hormones such as GH and prolactin, which are required to support mammogenesis and lactogenesis (Cowie et al. 1980). Hallerman et al. (1987) recently studied the RFLPs in Holstein-Friesian cattle at the GH and prolactin loci, and found considerable variation in the GH locus that may be caused by genomic insertion/deletion, while few RFLPs were present at the prolactin loci. Their relationship with milk yield has not, however, been examined.

DNA Fingerprint (DNAFP): DNAFP is a technique to measure the highly variable regions present in several short sequences of the DNA molecule defined as "minisatellites". The minisatellite length variation is detected using restriction enzymes that do not cut the repeat unit, resulting in a set of stably inherited markers that are highly polymorphic. The multiple banding patterns so produced contrast with the single band of the RFLPs. The inheritance of variation in DNAFP is also in a Mendelian manner. The mechanism of genetic variation controlling quantitative traits has been attributed to minor variation at regulatory sites via their influence upon structural genes (Robertson 1985). Thus studies of variation in DNAFP and RFLPs may lead to an understanding of the genetic mechanisms controlling milk production, and allow the manipulation of quantitive traits at the genomic level (Beckman and Soller 1987; Fries et al. 1989).

2. Metabolic characteristics

Evidence for genetic variation in physiological traits has been found in dairy cattle in recent years (Hart et al. 1978; Stark et al. 1978; Gibson et al. 1987). The significance and potential use of these traits as genetic markers has been emphasized by several recent reviews (Gorski 1979; Land 1981; Pollak et al. 1984; Sejrsen and Lovendahl 1986; Woolliams and Smith 1988; McCutcheon and Mackenzie 1989).

It is known that milk production is influenced by polygenic mechanisms and that variation in yield between cows can be attributed partially to differences in the complement of additive genes present. These additive genes may involve the regulation of a few or many of the physiological pathways essential to milk production by modifying the amount or activity of enzymes and hormones. The interactions of these metabolites, enzymes and hormones may be more important than the absolute amounts themselves. Genetic control of milk yield is polygenic, and a number of loci are likely involved. These genes may be expressed in the female at different stage of mammogenesis, lactogenesis or galactopoiesis, and would be of great potential use as genetic markers, in bulls and heifers, if expressed before puberty.

Genetic correlations between milk production and polygenic metabolic traits may be causal when the metabolite is a precursor, product, or (to a lesser extent) by-product of milk synthesis. This represents a correlation between intermediates and the end products of the same physiological reaction. Thus, any metabolic characteristics correlated with genetic merit (as assessed by ongoing or potential production) may be useful predictors of genetic merit for quantitative traits.

The usefulness of physiological traits as genetic markers for milk production will, however, depend upon several parameters of the traits including their heritability (h^2), genetic correlation with milk production (r_g) and repeatability (R) (Rowlands and Manston 1976). Heritability (h^2) is the genetic variation expressed as a proportion of phenotypic variation, while r_g measures the relationship between the marker traits and the breeding value. Repeatability is the correlation between repeated records on an individual animal. The response in genetic improvement by using marker traits will be valuable only if these parameters h^2, r_g and R are sufficiently large.

Relatively high values have been reported for some of these parameters in the limited studies available and these results encourage the search for metabolic markers. Stark et al. (1978) found significant genetic correlations between the breeding values (based on progeny test) of Friesian bulls and blood concentrations of urea

(r_g=0.24), inorganic phosphate (r_g=-0.24), and potassium (r_g=-0.25) (all P<0.05). Peterson et al. (1982) reported that the heritabilities for plasma levels/activities of alkaline phosphatase, creatinine and amylase are moderately high and that genetic correlations between milk yield and alkaline phosphatase and creatinine are large (Table 1.1). These results were confirmed in several other studies (Tilakaratne et al. 1980; Rowlands et al. 1983, Table 1.2; Ma et al. 1985). The repeatabilities of plasma insulin, GH, T_3 and T_4 concentrations were

Table 1.1 Heritabilities (h^2)of serum components and genetic correlations (r_g) with 305 day milk production.

	h^2	r_g		
		milk	fat	protein
Urea	0.17	0.46	0.53	0.33
Uric acid	0.14	0.39	0.48	0.40
Total protein	0.20	0.15	0.06	0.06
Albumin	0.19	0.33	0.23	0.33
Alkaline phosphatase	0.52	-0.94	-0.91	-0.89
SGOT	0.17	0.19	0.20	0.12
Creatinine	0.44	-0.60	-0.60	-0.68
Potassium	0.23	0.57	0.16	0.45
Amylase	0.43	0.21	0.10	0.02

(Peterson et al. 1982)

Table 1.2. Heritabilities (h^2), repeatabilities (R), and genetic correlations (r_g) of serum components with growth rate in British Friesian bull calves

	h^2	R	r_g
Glucose	0.41	0.47	0.30
Cholesterol	0.53	0.56	0.06
Urea	0.29	0.30	0.71
Albumin	0.38	0.64	-0.29
Globulin	0.65	0.64	-0.12
Haemoglobin	0.55	0.59	-0.46
Inorganic phosphate	0.49	0.51	0.38
Calcium	0.16	0.41	-0.27
Magnesium	0.33	0.53	0.43
Sodium	-	0.14	-
Potassium	0.82	0.39	-0.38
Copper	0.44	0.65	-0.10
Growth rate	0.35	-	-

(Rowlands et al. 1983)

estimated in young calves of high and low genetic merit by Land et al. (1983). They concluded that whereas the concentration of insulin in a single sample is a poor indicator of the insulin status of an animal,

the mean of several samples is a good indicator of the animal's insulin status over time, at least over the interval from 6 to 16 weeks of age (the range of ages examined in that study). By contrast, the concentration of thyroid hormones in a single sample is indicative of the animal's status at that time, but the status apparently changes with time i.e. there is little variation within a day but considerable changes in the ranking of animals over a period of weeks.

It is necessary to evaluate potential markers in the population in question, at an age appropriate to the breeding programme and under conditions similar to those which could be applied in practice. This is because these parameters of the traits can vary among different populations and within a population due to differences in genetic components, in permanent or temporary environmental components, or in any combination of these components (Rowlands 1983). Therefore, it is possible that a marker which is effective in one population may not be so reliable in another population e.g. North American vs New Zealand herds. Also the repeatability of the markers may vary with age, and since there are substantial advantages in selecting at or before puberty, it would be sensible to evaluate potential markers in young stock.

IV. Metabolic differences between divergent dairy lines selected for milk production

Differences in plasma concentrations of metabolites and hormones between high and low genetic merit dairy cattle of various ages have been reported recently by a number of research groups. Differences have been found in basal levels, in responses to feeding/fasting and in responses to metabolic challenges.

1. Basal plasma concentrations of hormone and metabolites

1) Hormones

i) **GH:** That GH is galactopoietic in lactating cows has long been known (reviewed by: Cowie et al. 1980; McCutcheon and Bauman 1985;

Peel and Bauman 1987; Hart 1981, 1988). Therefore it is not too surprising to find increased GH concentrations in the plasma of high genetic merit cows under various experimental conditions and among different populations (Barnes et al. 1985; Kazmer et al. 1986; Bonczek et al. 1988; Lukes et al. 1988). The differences in plasma GH levels of lactating cows are, however, not always significant (Flux et al. 1984).

The effect of genetic selection on plasma GH level has also been studied in young calves. Basal plasma GH levels were greater in high breeding index (HBI) than in low breeding index (LBI) calves fed milk in one trial when the calves were 11 to 18 days old but similar between the lines in a second trial when the calves used were 10 days older (Xing et al. 1988). Land et al. (1983) also reported that basal plasma GH levels were similar in high and low genetic merit calves at 2-3 months of age. In another study, basal plasma GH concentrations were similar in HBI and LBI bull calves at 8 months of age but were greater in the HBI line during fasting (Mackenzie et al. 1988).

The greater GH level in the high genetic merit line may be due to a greater GH secretion in this group. Greater GH release was observed in high genetic merit cows after TRH challenge (Kazmer et al. 1986), and in HBI calves after injection of arginine on one occasion (Xing et al. 1988) but not in another study when the calves were 10 days older than those used in the previous trial. The difference in plasma GH concentration between the high and low genetic merit cows during lactation may, however, reflect a larger energy deficit due to greater milk secretion in the high genetic merit line, rather than a genetically determined difference in GH secretion. It is known that energy deficit increases plasma GH concentration in lactating cows (Hart et al. 1978; Flux et al. 1984). Although a clear relationship between genetic merit and plasma GH levels has not been established, the data of Mackenzie et al. (1988) suggest control of energy balance may be effective in enhancing differences in plasma GH concentration between the lines.

ii) **Insulin:** The metabolic role of insulin in ruminants is similar to that in monogastric animals (Bassett 1975, 1981; Bines and

Hart 1982; McDowell 1983; Brockman and Laarveld 1986; Weekes 1986) Insulin is required for mammogenesis and for the maintenance of established lactation in various species (Erb 1977; Cowie et al. 1980).

The effect of genetic merit for milk production on plasma insulin concentration has been studied in cattle. Flux et al. (1984) reported that plasma insulin concentrations were greater in HBI than in LBI cows when the cows were fed 70% of ad libitum intakes in mid-lactation/early pregnancy. Barnes et al. (1985) also found that plasma insulin concentrations were higher in high genetic merit heifers than in low genetic merit heifers before and after feeding and after insulin challenge (6 mU/kg). On the other hand, there is one report that plasma insulin concentrations were lower in high genetic merit cows when blood samples were collected via jugular venipuncture on days 42 and 161 postpartum (Bonczek et al. 1988). Using similar venipuncture sampling methods Osmond et al. (1981) found no relationship between plasma insulin concentrations of Friesian bulls and their breeding values based on a progeny test.

In newborn calves, basal plasma insulin concentration was greater in the HBI line than in the LBI line after overnight fasting and milk feeding in one trial but not in another (Xing et al. 1988). Plasma insulin concentration was also relatively lower in low genetic merit calves at 2 to 3 months of age in another study (Sinnett-Smith et al. 1987). In 6 month old bull calves, basal plasma insulin levels were slightly greater in the HBI line than in the LBI line during normal feeding, and the difference became significant during fasting (Mackenzie et al. 1988).

The greater basal plasma insulin concentration in the HBI line may arise from several mechanisms, including: a) Increased sensitivity of the pancreas to releasing stimuli, as was demonstrated by the greater insulin release in HBI than in LBI animals when a glucose challenge was given to young bulls (Mackenzie et al. 1988) and an arginine challenge was given to milkfed calves (Xing et al. 1988); b) Higher plasma levels of metabolites which stimulate insulin secretion, as indicated by the hyperinsulinaemia and hyperglycaemia/euglycaemia which often

coexist in the Massey University HBI line (Flux et al. 1984; Mackenzie et al. 1988; Xing et al. 1988); c) A lower rate of insulin removal from the circulation, as hyperinsulinaemia was prolonged in the high genetic merit cattle following insulin challenge (Barnes et al. 1985); d) A smaller insulin distribution volume which would also favour greater insulin concentrations given a fixed rate of entry.

iii) **Glucagon:** Though not well studied, the metabolic role of glucagon in ruminants is believed to be similar to that in monogastrics (Bassett 1981; McDowell 1983; Brockman and Laarveld 1986). Glucagon increases blood glucose concentration by stimulating glycogenolysis and promoting gluconeogenesis, stimulates the rate of protein degradation and stimulates hepatic uptake of amino acids for gluconeogenesis (Riis 1983). Glucagon may have a permissive effect on lipolysis by antagonising the anabolic effect of insulin.

In view of the role of glucagon in glucose metabolism, Flux et al. (1984) postulated that differences in plasma glucagon concentration may have existed between the HBI and LBI cows and been responsible for the glucose differences observed between the two lines. So far, however, only one study has examined plasma glucagon levels in high and low genetic merit dairy cattle (Barnes et al. 1985). The results of that study showed that plasma glucagon level was significantly higher in low genetic merit heifers before and after feeding, after insulin challenge, at 6 and 12 months of age. The data indicate that glucagon concentration should be investigated further as a potential marker of genetic merit.

iv) **Prolactin** (Prl): Eley et al. (1981a and 1981b) reported that basal plasma prolactin concentrations were higher in low than in high genetic merit Jersey cows prepartum. Barnes et al. (1985) showed that plasma prolactin concentrations were lower in high genetic merit animals after insulin administration in Holstein-Friesian heifers of 6, 12, 18, and 24 months of age. Several reports also showed that basal plasma prolactin concentrations were relatively but not significantly greater in low than in high genetic merit cows (Barnes et al. 1985; Kazmer et al. 1986; Bonczek et al. 1988). The greater prolactin

concentration in the low genetic merit than in the high genetic merit cattle may be responsible for the greater body weight in the low line (Barnes et al. 1985). The anabolic effect of prolactin in ruminants is also indicated by the observation that plasma prolactin concentration increased post-prandially but decreased during periods of feed restriction (McAtee and Trenkle 1971; Serjsen et al. 1983; Kazmer et al. 1986).

v) Placental lactogen: Placental lactogen (PL), a hormone similar to GH and Prl in structure and sharing many of their biological activities, is secreted from the placenta. It augments insulin secretion, promotes protein conservation and NEFA release from adipose tissue, and decreases the peripheral utilisation of glucose (Metcalfe et al. 1988). Decreases in blood glucose or fasting can cause an increase in plasma PL levels in humans (Cowie et al. 1980). There was one report that plasma placental lactogen concentration was greater in high yielding breeds and in high producing cows within the high yielding breed (Bolander et al. 1976), but this has not been substantiated.

vi) Insulin-like growth factors (IGFs I and II): IGF-I stimulates milk production in bovine mammary tissue in vitro (Baumrucker 1986), and a recent study by Davis et al. (1988) suggested IGF-II may be involved in the GH-mediated galactopoietic effect. Mackenzie et al. (1988) showed that IGF-I concentrations were similar in HBI and LBI bull calves at 8 months old. However Ahlborn-Breier et al. (1987) found a positive relationship between IGF-I levels and the genetic merit of progeny tested Friesian bulls. They concluded that genetic variation in IGF-I and its relationship with genetic merit for milk yield may be masked in some years by the effects of varying nutritional management.

vii) Thyroid hormones: The thyroid hormones, thyroxine (T4) and triiodothyronine (T3), stimulate the basal metabolic rate via the metabolism of carbohydrate, lipids and proteins, by increasing the activities of specific enzymes that contribute to oxyen consumption. They also stimulate blood concentrations of other hormones such as GH and Prl.

Joakimsen et al. (1971) and subsequently Sorensen et al. (1981), showed that thyroxine degradation rate in dairy bulls and the milk yield of their daughters were correlated. However, a report by Osmond et al. (1981) showed a poor correlation between the plasma concentrations of T_3 and T_4 in a single plasma sample from dairy bulls and the bulls' genetic merit based on a progeny test.

In a comparison of dairy cattle from two selection lines, Land et al. (1983) showed that plasma concentrations of T4 and T3 were greater in high genetic merit calves than in low genetic merit calves. Conversely Sejrsen et al. (1984) found that plasma T_4 levels after feeding were greater in LBI heifers at 3.5 months of age. A negative correlation between the estimated breeding value and plasma concentrations of T3 and T4 was apparent in calves at 3.5 and 7 months of age.

viii) Cortisol: Glucocorticoids affect the metabolism of carbohydrate, lipid and protein by increasing the synthesis of a number of key enzymes in the glucogenic pathway within hepatocytes. The action of cortisol is anabolic in liver but catabolic in muscle and adipose tissue, where it inhibits glucose uptake and promotes proteolysis and lipolysis (Riis 1983). The released amino acids become substrates for gluconeogenesis in the liver or kidney. Cortisol maintains levels of lipolytic enzymes that are activated through the actions of the catecholamines. Glucocorticoids are also required for lactogenesis and mammogenesis in ruminants (Erb 1977; Cowie et al. 1980).

Flux et al. (1984) speculated that the difference in plasma glucose between their lines may have been a result of differences in glucocorticoid metabolism. When measured in milk fed calves, however, plasma cortisol levels were similar in the HBI and LBI lines (Xing et al. 1988).

ix) Progesterone, Oestrogen and Luteinising Hormone (LH): Eley et al. (1981a and 1981b) reported that plasma progesterone concentrations were higher in Jersey cows of high genetic merit prior to parturition, whereas plasma oestrogen (oestrone) and LH levels were greater in the

low genetic merit cows. They suggested that the differences in maternal plasma concentrations of progesterone, oestrone and LH might be affected directly or indirectly by the fetus or placenta.

2) Metabolites

i) **Glucose:** Glucose is a very important energy source for normal functions of the CNS and muscle activity (including smooth muscle of the alimentary tract). Glucose is also required as a precursor/substrate for the synthesis of lactose and milk fat and for fetal growth (Bergman 1983a, 1983b). Gluconeogenesis in liver and kidney is the major source of glucose production in cattle (Bergman 1983b). Availability of plasma glucose and glucose uptake by mammary gland are the major determinants of rate of milk secretion (Bines and Hart 1982; Bauman and Elliot 1983; McDowell et al. 1988).

Flux et al. (1984) observed that plasma glucose level was lower in LBI cows fed 70% of appetite than in HBI cows fed the same level or cows of either line fed ad libitum. Plasma glucose concentration also tended to be higher in high than in low genetic merit lactating heifers before and after feeding and after a low dose of insulin challenge (6 mU/kg) (Barnes et al. 1985). Gibson et al. (1987) reported that plasma glucose level prior to parturition was greater in high than in low genetic merit cows but a greater fall in plasma glucose concentration occurred in the high genetic merit group in the period around calving.

Basal plasma glucose levels after overnight fasting were lower in LBI than in HBI calves fed indoors, and the differences were diminished after milk feeding (Xing et al. 1988). However, no selection line differences in plasma glucose concentration were found in younger (5 to 8 days of age) calves under field conditions (milk was fed but calves had access to grass). Greater plasma glucose concentrations were also found in 8 month old bull calves of high genetic merit during a re-feeding period after a short-term fast, but not in the normal feeding period (Mackenzie et al. 1988). Basal plasma glucose levels were greater, but not significantly so, in high genetic merit than in low genetic merit calves fed a diet of concentrates plus hay

(Tilakaratne et al. 1980; Sejrsen et al. 1984). Furthermore, positive correlations between plasma glucose levels and estimated breeding values were found in ruminant calves (Sejrsen et al. 1984) and in milk fed calves (Xing 1985). However, there were also reports that basal plasma glucose levels were similar between high and low genetic merit calves (Bridges et al. 1987; Sinnett-Smith et al. 1987).

The reasons for the glucose differences between the lines, where they occur, are not known. Flux et al. (1984) suggested that variation in plasma glucocorticoid or glucagon levels between the lines could be responsible for the glucose differences in lactating cows. It has also been speculated that the HBI calves have a greater glucose concentration because they are relatively resistant to the glucoregulatory effect of insulin (Xing et al. 1988).

ii) **NEFA and glycerol:** Unless ruminants are fed a high fat diet, NEFA and glycerol in their blood originate largely (more than 90%) from lipolysis in the adipose tissue (Giesecke 1983; Leat 1983). Released NEFA are mainly bound to albumin to be transported from adipose tissue to liver, muscle, mammary gland and other organs in need of energy substrates. The ability to mobilise adipose tissue reserves during lactation is an important factor affecting milk production. Cows of high genetic merit produce more milk fat than those of low genetic merit and lose more body weight early in lactation. Both of these effects are believed to reflect greater mobilisation of fatty acids from adipose tissue (Davey et al. 1983; Barnes et al. 1985; Holmes 1988).

Although Flux et al. (1984) found that plasma NEFA concentrations were similar between HBI and LBI cows, Gibson et al. (1987) found that plasma NEFA concentrations were generally greater in high genetic merit cows during the first weeks after parturition. Similarly, Barnes et al. (1985) observed that NEFA concentrations were greater in lactating cows of high genetic merit. In an in vitro study, McNamara and Hillers (1986a) found that the basal rate of glycerol release from adipose tissue was greater in high genetic merit cows in late pregnancy and in lactation. In addition, lipogenesis in the adipocytes was lower in

high genetic merit than in low genetic merit cows (McNamara and Hillers 1986b). Unfortunately it is not clear whether these differences reflect basal differences in metabolism of adipose tissue between the lines or whether they have been caused by differences in energy balance.

When measured in young animals, basal plasma NEFA concentrations were similar between the high and low genetic merit calves in several studies (Tilakaratne et al. 1980; Sejrsen et al. 1984; Bridges et al. 1987; Sinnett-Smith et al. 1987). A report by Barnes et al. (1985), however, showed that plasma NEFA concentrations were greater in high genetic merit heifers at 6 months of age but not when they were 12 and 18 months old. Though basal plasma NEFA concentrations were often similar between the high and low genetic merit lines, greater elevation of plasma NEFA was observed after acute fasting in calves (Tilakaratne et al. 1980) and after challenges of catecholamines in heifers of high genetic merit (McNamara and Hillers 1986).

iii) **Ketone bodies:** BHBA and other ketone bodies were high in high genetic merit cows around calving (Gibson et al. 1987) and in lactating cows fed restricted energy intake (Flux et al. 1984). However they were similar in fully fed lactating cows of divergent genetic merit (Flux et al. (1984) and in young calves of high and low genetic merit (Tilakaratne et al. 1980; Sinnett-Smith et al. 1987).

iv) **Urea:** A major source of plasma urea in cattle after feeding is from the degradation of dietary nitrogenous compounds. The excess ammonia that escapes capture by rumen microbial cells is passively absorbed through the rumen epithelium and converted into urea by the liver. A part of the urea is returned to rumen, by simple diffusion from blood or secretion into saliva. Another source of plasma urea is the catabolism of amino acids from body protein, a process influenced by a number of hormonal and physiological factors (Riis 1983).

Stark et al. (1978) and subsequently Peterson et al. (1982) showed that blood urea level was genetically correlated with milk production in dairy cows. Greater plasma urea concentrations in high than in low genetic merit cows were also reported during the period

before and after feeding, and after insulin challenge (Barnes et al. 1985).

Basal plasma urea concentration tended to be similar between the high and low genetic merit calves at 3 to 7 months of age when the animals were sampled under conditions of good feeding (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). However, the rise in plasma urea was greater in low genetic merit calves when the calves were fasted for several days. In contrast, Barnes et al. (1985) reported that plasma urea concentrations measured before and after feeding were greater in high than in low genetic merit heifers at 6 months of age. Similarly, Mackenzie et al. (1988) reported a greater rise in plasma urea concentration on re-feeding in HBI bulls than in LBI bulls (6 months old) after 3 days of fasting.

The mechanisms responsible for the differences in plasma urea concentration between the selection and control lines are still poorly understood. Tilakaratne et al. (1980) proposed that a decreased rate of body protein degradation might be responsible for the lower plasma urea concentration in high genetic merit calves during fasting. Barnes et al. (1985) attributed the greater urea level in the high genetic merit cattle to a greater rate of dietary protein degradation. Neither of these hypotheses has been examined experimentally.

v) **Blood proteins:** Plasma proteins can be readily divided into 3 fractions- albumin, globulin and fibrinogen- which account for 2-3% of total body proteins. Average concentrations of these 3 fractions are 3.5, 4 and 0.5 g/dl, giving a total of 8 g protein per 100 g plasma (Rowlands 1980). Fibrinogen is a single protein which is converted to fibrin when blood clots. The albumin fraction is also homogenous and because of its relatively high concentration and small molecular weight (68,000 MW) it makes a major contribution to the colloid osmotic pressure of blood. Albumin also acts as a carrier in the transport of various metabolites and hormones such as NEFA, Ca^{++}, T_3 and, T_4 in the blood. The globulin fraction is extremely heterogenous, containing a large number of proteins with different functions. The major component consists of the immunoglobulins which carry the antibody activity of the plasma. In lactating cows, plasma proteins decrease at the peak of

lactation, probably due to a greater body protein turnover (Rowlands 1980; Riis 1983).

Gibson et al. (1987) reported that high genetic merit lactating heifers had higher plasma globulin levels than low genetic merit heifers immediately after, and about 60 days after, parturition. Plasma immunoglobulin levels also tended to be greater in milk fed calves of high genetic merit (Xing 1985). In ruminant calves, total plasma protein and plasma globulin were greater in high genetic merit calves during feeding or fasting (Tilakaratne et al. 1980).

vi) Haematocrit: Eley et al. (1981a) reported that the haematocrit was greater in high genetic merit Jersey heifers. However haematocrit was similar in milk fed calves of the HBI and LBI lines (Xing 1985).

vii) Hormone sensitive lipase activity: McNamara (1989) and Smith and McNamara (1988) reported that the activity of hormone sensitive lipase was greater in the adipocytes from cows of a selected line than in those from the control line. However, the possibility that these differences were confounded with differences in energy balance cannot be discounted.

2. Energy balance and fasting/refeeding

1) Energy balance

It is well established that plane of nutrition can affect the blood concentrations of metabolites and hormones in animals. Underfeeding or fasting normally results in decreased plasma concentrations of insulin, glucagon, prolactin, thyroid hormones, urea, glucose and protein, but increased concentration of GH, NEFA and ketone bodies (Hove and Blom 1973; Trenkle 1978, 1981; Bassett 1975, 1981; Athanasiou and Phillips 1978; Baird et al. 1979; Kertz et al. 1982; Flux et al. 1984; Johnsson et al. 1985; Blum et al. 1982; Frohli and Blum 1988b). These changes are often accompanied by reductions in energy expenditure and thermogenesis, development of peripheral resistance to insulin, deterioration of glucose tolerance and decreased

clearance of catecholamines (Olefsky et al. 1973; Danforth 1985; Frohli and Blum 1988a, 1988c). High levels of (energy) intake and overfeeding often result in increased body weight and produce metabolic effects opposite to those of underfeeding and fasting. For instance nutritionally-induced obesity in heifers and sheep is associated with hyperglycaemia and hyperinsulinaenmia (McCann and Reimers 1985a, 1986; McCann et al. 1987).

The effect of level of energy intake on the expression of genetic variation in blood profiles has been investigated in few studies in lactating cows and heifers (Flux et al. 1984; McNamara and Hillers 1986a and 1986b). In the study of Flux et al. (1984), lactating cows of HBI and LBI lines were fed freshly cut pasture at either 70% or 100% of appetite level. The results showed that: a) Plasma glucose concentration was significantly lower in the under fed LBI group than in other groups ($P<0.05$); b) GH concentrations were higher in the cows on restricted intake ($P<0.05$) and in HBI cows but the differences between LBI and HBI cows were not significant. c) Plasma insulin levels were consistently higher for the HBI cows on restricted intake than for those in other groups, among which differences were not significant; d) Plasma concentration of B-hydroxybutyrate was significantly higher in the cows on restricted intake ($P<0.01$) and the concentration fell immediately after feeding.

McNamara and Hillers (1986a, 1986b) examined lipogenesis and lipolysis in the adipose tissue of high and low genetic merit lactating heifers, under two nutritional regimens. Animals of each genetic group were allotted to ad libitum diets of 40% hay and 60% concentrate or of 60% hay and 40% concentrate from 0 to 140 days of lactation. Results of that study showed that lipogenesis rates at 15, 30, 60, and 180 days postpartum in heifers fed low energy diets were 107, 66, 21, and 166% of those in heifers fed high energy diets. Rates of lipogenesis in high genetic merit heifers were 40, 10, 45, and 66% of those in low genetic merit heifers at 15, 30, 60, and 180 day postpartum. Basal glycerol release in high genetic merit cows was 64, 17, 40, 23, 20, and 42% greater than in low genetic merit cows at -30, -15, 30, 60, 180, and 349 days relative to parturition. The lipolytic response to catecholamines increased prior to parturition, and increased further in

early lactation, then remained elevated during lactation and into the dry period. However, this response was unaffected by feeding diets with different energy content. These results showed that lipogenesis in bovine adipose tissue decreased according to decreased energy intake and to increased milk fat secretion resulting from genetic selection, whereas rates of lipolysis were affected only by genetic selection and not by nutritional levels.

In another study, Smith and McNamara (1988) studied the cellularity of, and activity of hormone sensitive lipase in, the adipose tissue of high and low genetic merit lactating heifers. Animals of each genetic group were allotted to treatments of either 36:64 or 71:29 barley concentrate:alfalfa hay diet fed ad libitum from 0 to 140 day of lactation. The results showed that mean adipocyte diameter (MCD), adipocyte number per gram (NPG), and hormone sensitive lipase activity were greater in high genetic merit cows during early lactation. Changes in NPG were enhanced by dietary energy restriction.

Besides the influence of energy balance, plasma profiles of hormones and metabolites can also be affected by the level of protein intake, or the ratio of energy:protein in the diet. It is known that high ratios of protein:energy intake will result in increased plasma urea levels in lactating cows whereas this increase can be inhibited by adding concentrates to the diet (Rowlands 1980; Oltner and Wiktorsson 1983). Borger et al. (1973) reported that steers on isocaloric diets containing 11.0 or 12.5% protein had significantly higher insulin levels than steers on an isocaloric diet containing 9.5% protein. Additionally, the correlation between the amount of protein digested in the intestines and plasma insulin concentration was positive, whereas the correlation between the digested protein and GH was negative, in sheep (Bassett et al. 1971).

2) Fasting/Refeeding

Acute fasting has been used to widen metabolic differences between the high and low genetic merit calves. Several studies showed that plasma urea levels were greater in the low genetic merit than in the high genetic merit calves after 2 to 4 days fasting (Tilakaratne et al.

1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). However, Mackenzie et al. (1988) reported that urea levels were similar during a 3 day fast but greater in the HBI bull calves than in the LBI group during a refeeding period after the fast.

Greater elevations in plasma NEFA concentration after acute fasting was found in high genetic merit calves in one study, (Tilakaratne et al. 1980) but not in others (Sejrsen et al. 1984; Sinnett-Smith et al. 1987; Mackenzie et al. 1988). In bull calves plasma insulin and GH concentrations were significantly greater in the HBI line than in the LBI line during a 72 hour fasting period (Mackenzie et al. 1988). Plasma insulin and glucose were greater in HBI bull calves during the refeeding period of that study.

3. Metabolic challenges

Various metabolic challenges have been used to elicit metabolic differences between high and low genetic merit lines. The tests are based on the assumption that the ability of the metabolic process to respond to the challenge may be related to the genetic merit of the animal for milk production. Metabolic challenges offer a number of advantages including: An ability to detect homeorhetic changes in response to homeostatic signals, even when no changes in basal hormone/metabolite concentrations are apparent (Sechen et al. 1989); measurement of responses which involve specific tissue or organs (e.g. pancreatic sensitivity to a glucose challenge); and the opportunity to simultaneously measure related metabolic responses (e.g. tissue sensitivity to insulin concurrent with pancreatic sensitivity to glucose in the glucose challenge, Bergman et al. 1985).

1) **Glucose:** Intravenous glucose challenge (175 mg/kg) caused greater insulin release in HBI than in LBI bull calves (Mackenzie et al. 1988).

2) **Arginine:** Arginine challenge (40 mg/kg) to milk fed calves caused greater elevation in plasma GH concentration in HBI calves on one occasion but not on another when the calves used were older (by 10 days) and had had more access to grass prior to the experiment (Xing et

al. 1988). Insulin responses to the arginine challenge were similar between the lines when the calves were fed, but were greater in the HBI calves during fasting (Xing et al. 1988). Using a similar arginine challenge regimen, Mackenzie et al. (1988) did not observe any metabolic differences between the HBI and LBI bull calves at 6 month of age.

3) **Propionate:** Injection of sodium propionate (0.5 mmol/kg) into the circulation caused a substantial rises in plasma concentrations of glucose, but decreased plasma NEFA and urea, in both high and low genetic merit calves. Moreover, the pre-challenge differences in plasma NEFA between the high and low lines were reduced after the infusion (Tilakaratne et al. 1980). No differences in metabolic responses between the lines after propionate injection were reported by Sinnett-Smith et al. (1987).

4) **Insulin:** Barnes et al. (1985) showed that insulin challenge (6 mU/kg) resulted in increased plasma concentrations of glucose, urea, and insulin. In addition, the urea and insulin concentrations following the challenge were greater in the high genetic merit heifers when they were 6 and 24 months old. Using a high dose of insulin (265 mU/kg) Mackenzie et al. (1988) showed that insulin challenge resulted in a greater decrease in plasma glucose levels in the HBI bull calves.

5) **Glucagon:** Mackenzie et al. (1988) reported that glycogenolytic response to glucagon challenge (0.1 µg/kg) was smaller in HBI bull calves compared with LBI bull calves.

6) **Adrenaline:** Greater glycogenolytic, but similar lipolytic, responses were observed in HBI heifers vs LBI heifers at 6 months of age following adrenaline challenges over a range of doses (0.25, 0.5 and 1.0 µg/kg), and following a high dose (1.0 µg/kg) of noradrenaline (Bridges et al. 1987). McNamara and Hillers (1986), using an *in vitro* method, observed greater lipolytic responses to catecholamines (10^{-4} M) in the adipose tissue of lactating cows of high genetic merit. In the latter study, responses to noradrenaline were 15, 20, 18, and 15% greater in high genetic merit than in low genetic merit cows, and responses to adrenaline were 12, 20, 14, and 50% greater in high

genetic than in low genetic merit cows, at 30, 60, 180, and 349 days postpartum. These results suggested that the sensitivity of the adipose tissue to lipolytic signals was enhanced in the high genetic merit line.

7) Growth hormone releasing-factor (GHRF): Massri et al. (1985) injected GHRF (0.1 uU/kg, hpGRF) into 8 high and 8 low genetic merit heifer calves aged 6 to 7 months. After the challenge, plasma GH increased by more than 10 fold and peak plasma GH was reached within 20 min in both high and low lines. However no differences in plasma GH levels were found between the two lines.

8) Thyrotropin releasing hormone (TRH): Kazmer et al. (1986) compared the metabolic responses to TRH challenge (0.33 µg/kg) in 50 Holstein cows of different genetic merit, and found that plasma GH before and after the challenge was greater in high genetic merit cows. Eley et al. (1981b) reported that TRH challenge (100 µg/animal) resulted in a greater prolactin release in Jersey cows of low genetic merit on day 10 postpartum.

9) Gonadotropin releasing hormone (GnRH): Eley et al. (1981b) could not find any metabolic differences in responses to GnRH (100 µg/animal) challenge between high and low genetic merit Jersey cows on day 10 postpartum. It should be noted that all animals in that study received the same dose of challenge (100 µg) rather than being challenged according to their body weight.

10) Growth hormone (GH): Acute GH (NIH-GB-B9; 0.98 µ/mg) challenge (0.1 mg/kg) in calves did not result in differences in plasma concentrations of glucose or urea between high and low genetic merit calves (Land et al. 1983). This is in contrast to the increased plasma NEFA and glucose levels, but decreased plasma urea, which are sometimes observed after chronic GH treatment in ruminants (Davis et al. 1969; McDowell et al. 1988; Eisemann et al. 1989; Sechen et al. 1989). Moreover the use of an acute GH challenge appears to be an illogical approach because it is the homeorhetic (chronic) actions of GH, rather then any acute mechanisms, which are central to its regulation of metabolism (Bauman and McCutcheon 1986).

4. Summary of the metabolic differences between the high and low genetic lines

The effects of selection line on metabolic profiles in dairy cattle so far reported are summarised in Table 1.3.

Table 1.3 Summary of the reported differences in plasma metabolite and hormone concentrations between high and low genetic merit cattle within breed
(+: value is greater in HBI cattle),
(-: value is smaller in HBI cattle)

	Cows	Heifers	Bulls	Calves (prepubertal)
GH	+	+	+	+
Prolactin (Prl)	-	-		
Placental lactogen	+			
Insulin	- +	+	+	+
Glucagon	-	-		-
T3	+			+
T4				+
Progesterone	+			
Oestrogen	-			
LH	-			
IGF-I			+	
Urea	- +	+	+	- +
Creatinine	-			
Globulin				+
Immunoglobulin				+
Plasma protein				+
Glucose	+	+	+	+
NEFA	+	+		+
BHBA	+			
Ketones	+			
Potasium	-			
Inorganic phosphate (Pi)	-			
Haematocrit (PCV)	+			
Alkaline phosphatase	-			
Hormone sensitive lipase	+			
Mammary α-lactalbumin synthesis to Prl treatment		+		
Lipogenesis	-			
Arginine-hyperinsulinmia				+
Arginine-hyper-GH-release				+
Insulin-hypoglycaemia			+	
Catecholamines-hyperglycaemia		+		
Catecholamines-hyperlipidaemia	+			
Glucagon-hyperglycaemia				-
Heart-rate at feeding				+

Ahlborn-Breier et al. 1987; Barnes et al. 1985; Bonczek et al. 1988; Bridges et al. 1987; Eley et al. 1981a, 1981b; Flux et al. 1984; Gibson et al. 1987; Harrison et al. 1988; Kazmer et al. 1986; Kazmer et al. 1989; Land et al. 1983; Lukes et al. 1988 McNamara 1989; McNamara and Hillers 1986a, 1986b; Mackenzie et al. 1988; Massri et al. 1985; Sejrsen et al. 1984; Sinnett-Smith et al. 1987; Smith and McNamara 1988; Tilakaratne et al. 1980; Vega et al. 1988; Xing 1985; Xing et al. 1988; Kitchenham et al. 1975; Stark et al. 1978; Peterson et al. 1982; Ma 1985.

There is increasing information supporting the view that genetic variation, caused by selection for milk production, exists in metabolic traits of dairy cattle. This variation has been observed primarily in plasma concentrations of GH, prolactin, insulin, thyroid hormones,

glucose, NEFA, protein and urea. In addition, metabolic challenges such as injection of glucose, catecholamines, insulin, glucagon and TRH, and fasting/refeeding have been effective in magnifying some of the differences which occur between the lines, Moreover, genetic variation in physiological traits is detected not only in mature cows but also in young calves, both male and female.

V. Purpose and scope of the investigation

Although the effects of genetic selection for milk production on metabolic profiles in blood have been demonstrated recently in dairy cattle, the results so far reported are based upon limited observations and thus are not conclusive. For instance, inconsistent or sometimes conflicting results exist with respect to the effect of genetic merit on plasma concentrations of GH, insulin, glucose, NEFA, and urea. A greater elevation in plasma NEFA concentration after fasting in the high genetic merit calves was observed in one study (Tilakaratne et al. 1980) but not in others (Sejrsen et al. 1984; Sinnett-Smith et al. 1987; Mackenzie et al. 1988). Both greater and lower plasma urea concentrations have been found in high genetic merit lines (greater: Barnes et al. 1985; Mackenzie et al. 1988; lower: Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). The reasons for these discrepancies have not been elucidated. Besides the possible effect of difference in the genetic composition of the populations in different studies, variation in experimental conditions (non-genetic effects) can also have a marked influence on the results. Of the non-genetic effects, differences in level of feed intake, type of feed, feeding regimen, sampling regimen, and dose of challenges may have contributed to these discrepancies. However, so far few studies have examined the effects of these factors on metabolic characteristics of the high and low genetic merit lines.

The selection of heifers is a major weak point of traditional breeding programmes. If genetic markers could be developed for identifying heifers of superior genetic merit, then the rate of genetic improvement would be increased substantially. Heifers may also serve as a model for bulls since there are no clear examples in the literature of genetic merit x sex interactions in metabolic

characteristics. Thus markers identified in heifers could potentially be incorporated into schemes for selecting bulls either for progeny testing or for alternative programmes.

Therefore, Friesian heifers from the two selection lines at Massey University were used in these experiments. The two lines, selected with assistance from the Livestock Improvement Division of the New Zealand Dairy Board, differ by 20% in terms of estimated breeding index and previous studies have identified metabolic differences between the lines (Flux et al. 1984; Bridges et al. 1987; Mackenzie et al. 1988; Xing et al. 1988). In particular, differences detected in these studies have been most apparent when non-genetic factors, such as age, live weight, feed intake and feeding management are controlled. In the present investigation, emphasis was placed on controlling these factors so that, within experiments, animals in each genetic group were matched as closely as possible. Furthermore, the possible effect of stage of the oestrous cycle on metabolism was controlled and evaluated by giving progesterone to the heifers during the last two experiments (Chapters 4 and 5) after the indication of a possible influence of oestrous cycle on metabolism from the first trial (Chapter 3).

Plasma concentrations of GH, insulin, glucagon, glucose, NEFA, urea, and creatinine were compared between the HBI and LBI heifers in this study, not only because they are important participants in the overall metabolism of the animal, but also because genetic variation in these parameters has been detected or implied by the results of earlier studies at Massey University and elsewhere. Interrelationships may exist among these hormones and metabolites. For example it has been hypothesised that differences in plasma glucagon levels may be one of the regulatory factors responsible for the glucose difference between the lines (Flux et al. 1984), but such differences have not been examined in the Massey University lines.

Therefore the objectives of this study were to:

1. Develop a radio-immunoassay capable of measuring the plasma levels of glucagon in cattle.

2. Scan the differences in basal plasma concentrations of metabolites and hormones between the two lines of heifers (HBI vs LBI) in relation to time of the day and onset of feeding.

3. Measure the differences in metabolic responses between the two lines of heifers to various metabolic challenges including glucose, insulin, glucagon and adrenaline injections, and fasting/refeeding.

4. Assess the effect of energy balance on the differences in plasma concentrations of metabolites and hormones and in the response to metabolic challenges in LBI and HBI heifers.

5. Test interactions between dose of glucose and insulin challenges and genetic merit on response to these challenges.

6. Evaluate interactions between genetic merit and time relative to feeding on metabolic response to adrenaline challenge

7. Verify the possibility of interactions between stage of the oestrous cycle and genetic merit on metabolic response to glucose challenge

CHAPTER TWO: DEVELOPMENT AND APPLICATION OF A DOUBLE ANTIBODY RADIOIMMUNOASSAY FOR BOVINE PLASMA GLUCAGON

CHAPTER TWO: DEVELOPMENT AND APPLICATION OF A DOUBLE ANTIBODY RADIOIMMUNOASSAY FOR BOVINE PLASMA GLUCAGON

I. INTRODUCTION

Glucagon is a small polypeptide hormone containing 29 amino acids and secreted from the pancreatic A-cells. It plays an important role in maintaining blood glucose concentrations in animals, by stimulating hepatic glycogenolysis and gluconeogenesis (thus antagonising the hypoglycaemic effect of insulin), and favouring lipid release and utilisation. The catabolic effects of glucagon on muscle and liver protein, as well as the effect of stimulating hepatic uptake of the released amino acids, are thought to be part of the mechanism by which glucagon stimulates gluconeogenesis. The central role occupied by glucagon in ruminant metabolism may be highlighted by the fact that virtually all glucose in mature animals is derived through gluconeogenesis, a process influenced to a large extent by glucagon. Therefore, differences in energy and nitrogen metabolism between the HBI and LBI cattle could be accounted for, in part, by differences in blood glucagon metabolism between these two groups of cattle (Chapter I).

Despite its importance in the maintenance of homeostasis, glucagon has not been widely studied in all species, including ruminants. This is mainly due to the greater difficulty in developing a reliable radioimmunoassay (RIA) for glucagon than for other hormones. The major problem is associated with the production of antibodies to glucagon. Immunisation against glucagon often results in the production of two major types of glucagon antibodies in the serum. One type of antibody has weak cross-reactivity with other polypeptides and is directed against the COOH terminal of the glucagon molecule. This type of antibody is known as "COOH terminal specific" or "pancreatic glucagon specific". Hormones recognised by this type of antibody are often referred to as IRG (immunoreactive glucagons). The other type of antibody, now known to be directed against the NH_2 terminal and the central region of the glucagon molecule, is often referred as "NH_2 terminal specific" or "non-specific glucagon antibody". It is now established that antibodies which recognise the NH_2 terminal end of

glucagon have affinity for various non-glucagon polypeptide hormones, especially hormones secreted from the intestinal tract, such as the glucagon-like peptides (gut glucagons). This is because of the homogeneity in their structures with glucagon secreted from the pancreatic A cells. Polypeptides which react mainly with NH_2 terminal specific antibody are often referred to as glucagon-like immunoreactivity (GLI). Therefore, the production of antibodies specific to glucagon is an essential element in the development of a valid RIA for glucagon.

Besides the difficulty of producing glucagon antibodies, the assay procedure for glucagon is more restricted due to the fact that endogenous glucagon in plasma is unstable and can be easily damaged during incubation of the assay. Furthermore, it is not always possible to produce ^{125}I-labelled glucagon with high specificity and stability for use in the RIA due to damage caused by introducing the ^{125}I into the glucagon molecule or inadequate purification of the ^{125}I-labelled glucagon. Thus the establishment of a glucagon radioimmunoassay is dependent upon overcoming these difficulties. This Chapter reports on the procedures which were used to develop a pancreatic-specific glucagon RIA for use in study of the low and high breeding index cattle.

II. Materials and methods

1. Source of hormones

Bovine glucagon (Sigma G4250, Lot No. 65F-0674) was used as the antigen for raising glucagon antisera and for the reference standards. Glucagon (Eli Lilly Company, Indianapolis, Indiana, USA, Lot No. 258-25J-120) was iodinated with ^{125}I as the radioactive trace in the assay. Insulin (Sigma No. I-5500, Lot No. 55F-0536) and glucagon-like peptide I (Sigma No G-3265, Lot No. 36F08221) were used as reference materials for testing the specificity of the antisera raised against glucagon.

2. Generation of glucagon antisera and antisera to guinea pig gamma globulin

1) Antigen preparation: Glucagon was conjugated to bovine serum albumin (BSA) by the glutaraldehyde method (Frohman et al. 1970). Briefly, 4 mg glucagon and 12 mg BSA (Sigma, Cat. No. A-8022, Fraction V) were dissolved in 1.6 ml of 0.2 M borate buffer (Williams and Chase, 1968) at pH 10 and 0.8 ml of 0.022 M glutaraldehyde solution was added dropwise with constant stirring. The coupling reaction was allowed to proceed for 24 hours at room temperature during which time a deep yellow colour developed. The glucagon-BSA complex was dialysed against 1200 ml (500 volumes) of 0.1 M phosphate buffer, pH 7.0 for 48 h with 2 changes of the buffer, after which the complex (4mg/2.88ml) was stored frozen at -12°C until required.

Conjugated glucagon was diluted with saline to 0.5 or 0.25 mg/ml for two different immunisation methods (I and II respectively). The diluted solution (0.5 ml) was emulsified with 0.5 ml Freund's complete adjuvant or with incomplete adjuvant (Difco, Laboratories, Detroit, Michigan, USA) for each guinea pig at each injection.

2) Animals: Eight young healthy female guinea pigs were divided randomly into 2 groups for the production of glucagon antisera by two different immunisation methods, as proposed by Professor D.S. Flux (method I) and Dr. K.M. Moriarty (method II), respectively.

3) Immunization procedures: In both methods, the guinea pigs were injected at monthly intervals on 4 occasions before a booster injection was given and the animals bled. For method I, 250 µg glucagon was emulsified with Freund's complete adjuvant for the first 3 injections and injected intradermally at 6-8 sites on the back of the guinea pigs. For the fourth injection, glucagon was emulsified with Freunds' incomplete adjuvant and the injection was given subcutaneously (as mild ulcer development on the sites of previously injected skin made further intradermal injections inappropriate).

For method II, 125 µg glucagon was emulsified with Freund's complete adjuvant for the first injection, and incomplete adjuvant was used for the 3 remaining injections. Injections were given subcutaneously at multiple sites on the neck, inside the thigh and along the back of the animal.

A booster injection of 100 µg of free glucagon in sterile physiological saline was given subcutaneously to all animals 5 months after the first injection. Ten days later the guinea pigs were bled by cardiac puncture. Blood was collected into heparinised centrifuge tubes and placed on ice. Plasma was harvested after centrifugation (2500g, 4°C), diluted at 1:100 with assay buffer (without trasylol), aliquoted into small vials (0.3 ml) and stored at -12°C.

4) Production of second antibody: Guinea pig gamma globulin (GPγ) was prepared from normal guinea pig plasma by precipitation with $(NH_4)_2SO_4$ (Bohn 1978). Larger molecules in the plasma were first precipitated and removed with 18% $(NH_4)_2SO_4$ and then the concentration of $(NH_4)_2SO_4$ was increased to 33%. The resulting precipitate was washed twice in 40% $(NH_4)_2SO_4$, dialysed against 0.1 M NH_4HNO_3 and lyophilised. The GPγ was injected into sheep, using the same procedure as method II for glucagon antibody production described above (Professor D.S. Flux, personal communication).

The GPγ produced by the salting out method was also added to the assay tubes to increase the volume of the precipitate produced on the addition of the second antibody.

3. Preparation of reference standards.

Assay buffers for preparing glucagon stock solution and reference standards, as well as for glucagon assays, were prepared as follows. A mixture of 90% 0.01 M PBS phosphate buffer + 10% 0.1 M EDTA-PBS buffer was made up to contain 1.6 mg/ml BSA and 200 kallikrein inhibitor units (KIU)/ml of Aprotinin (Sigma No A-6279, Lot 47F-8020), at pH 7.5. Glucagon stock solution was prepared by dissolving 1 µg glucagon in 100 µl 0.01 N NaOH and further diluted with assay buffer to give a solution of 10 µg/ml glucagon. Glucagon standards (0.05, 0.1, 0.2, 0.4, 0.8,

1.6, 3.2, 6.4, and 12.8 ng/ml) were prepared by serial dilution of the stock solution with assay buffer. The standards were aliquoted and stored frozen at -20°C for up to 6 months until used in the assay.

4. Glucagon iodination and purification of the ^{125}I-labelled glucagon

The iodination of glucagon was based on the chloramine-T method of Greenwood et al. (1963), and the iodinated glucagon was purified according to Jorgensen and Larsen (1972).

A 1.1x15 cm column of QAE-Sephadex A-25 (Pharmacia AB Laboratory Separation Division, Uppsala, Sweden, Lot No. LD 00481) in 0.08 M Tris, 0.02 N HCl, 0.08 M NaCl (pH 8.6) buffer was prepared. The column was equilibrated with the same buffer containing, in addition, 1% human serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia CSL, B676 AD-4461), and 50 KIU/ml of Aprotinin, at a rate of 10 ml/h.

For iodination, approximately 10 µg glucagon was dissolved in 0.01 N NaOH to give a final concentration of 0.4 µg/ul. Twenty five µl of freshly prepared glucagon solution was added to a siliconised vial with a tapered bottom and mixed with 5 µl (0.5 mCi) ^{125}I and 25 µl 0.4 M phosphate buffer, pH 7.2 using a siliconised pipette. Five µl of 0.4% Chloramine-T (this and all the following reagents were made up in 0.04 M phosphate buffer, pH 7.4) was added to the vial rapidly and again mixed. Ten seconds later the reaction was stopped by adding in succession 20 µl of 0.24% $Na_2S_2O_5$, 50 µl of 1% KI, 5 µl of 2M TRIS and 1 ml of column eluent buffer. A sample (10 ul) of this mixture was collected to measure the total radioactivity in the reaction vial and for subsequently calculating the recovery of ^{125}I from the column. The remaining contents of the vial were transferred to the top of the anion exchange column with a disposable pipette and flow rate through the column adjusted to 10 ml/hr. Fractions (1 ml) of the eluent were collected into LP3 plastic assay tubes and 10 µl from each tube was counted for plotting the elution profile using a gamma counter (LKB Wallac, 1261 Multigamma, Wallac OY, Finland). Two examples of the elution pattern are shown in Figure 2.1. The fractions (29-35) corresponding to the major part of the main peak, which accounted for

approximately 40% of the total counts applied to the column, were collected and stored at -20°C until use. A high proportion of the radioactivity applied remained on the column as described by Jorgenson and Larson (1972).

5. Radioimmunoassay method

The procedure for the glucagon RIA was as follows. Glucagon standards or glucagon in unknown samples (100 ul) were added, in triplicate, to 3-ml plastic assay tubes (LP3, Luckhams Co., England) and mixed with 100 µl of the glucagon antibody (diluted at 1:12,000 and 1:16,000 for GP2 and GP3 respectively). Three control tubes without standards (i.e. 100 µl assay buffer instead of standard/sample) were added as 100% or Bo tubes and three control tubes without antiserum (i.e. 100 µl assay buffer plus 100 µl GPγ instead of glucagon antisera) were added as non-specific binding tubes within each assay. After 24 hours incubation at room temperature, 50 µl ^{125}I-glucagon (approx. 5000 cpm) was added to the tubes and into two blank tubes (as total counts), and incubated at room temperature for a further 24 hours. Then, the second antibody (50 µl of sheep anti-guinea pig gamma globulin antiserum at 1:40 dilution) was added. Following 72 hour incubation at 4°C, the glucagon-antibody complex was precipitated using a centrifuge at 2500 g and 4°C for 30 minutes. The supernatant was removed from the tube by aspiration. The assay tubes containing the standards or samples were counted in a gamma counter for 60 seconds. The value of Bo varied from 30 to 45% of total counts, and the value of non-specific binding from 3 to 5% of total counts for most of the assays.

III. RESULTS

1. Validation of the radioimmunoassay system

1) Characterization of the antisera

i) Titre: The titre measures the maximum binding capacity (i.e. the total number of binding sites) of the antiserum and determines the dilution at which the antiserum can be used for a RIA. Normally, the

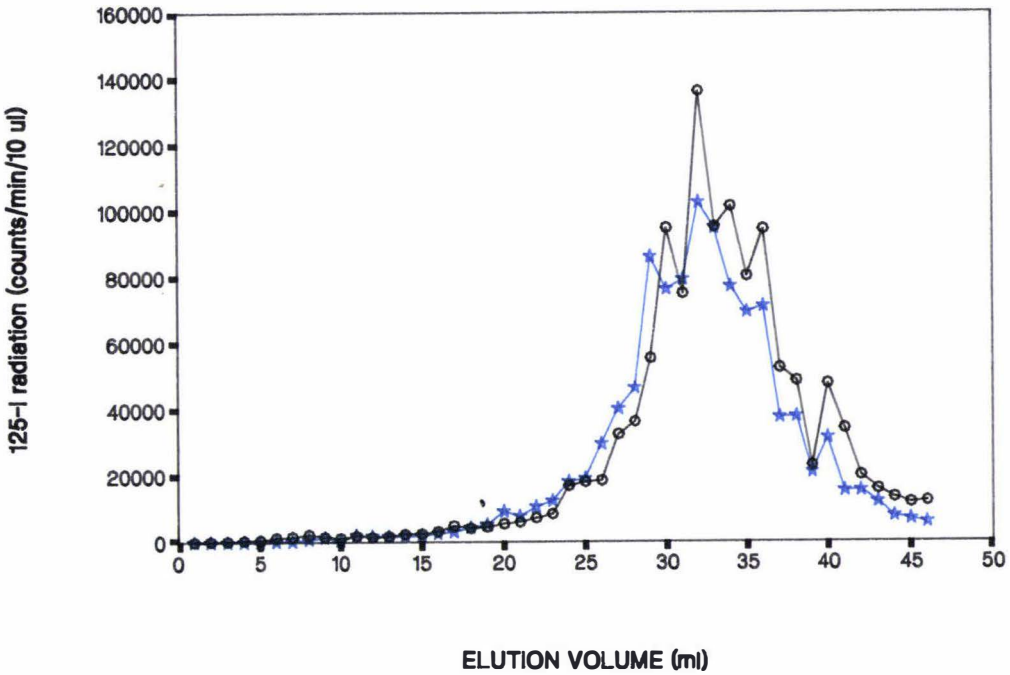
titre of an antiserum can be assessed as the dilution factor at which 50% of the ^{125}I-glucagon is bound in the absence of any unlabelled hormone in the assay tube. In the present study, the antisera were diluted at 1:4,000, 1:8,000, 1:12,000, 1:16,000 and 1:24,000 and incubated with 50 µl ^{125}I-glucagon (5000 cpm) for 24 hours at room temperature. The separation of antibody-bound from free ^{125}I-glucagon was achieved with the addition of a sheep anti-guinea pig gamma globulin antiserum and incubation at 4°C for 72 hours before centrifugation. The binding results are shown in Figure 2.2. Only two guinea pigs, GP2 and GP3 (both immunised by method I) developed antisera with relatively high titres. An antiserum with a high titre is desirable for a RIA in that only a small amount of antiserum is required to carry out a large number of assays. Thus further characterisation was carried out only with these two antisera.

ii) Affinity: The antiserum affinity or equilibrium constant Ka is a measure of the attraction between the antibody and the antigen, in this case glucagon. It determines how far the reaction: [antibody] + [glucagon] = [antibody-glucagon], can be pushed towards the right hand side of the equation. Antibodies with a higher affinity yield RIA standard curves with increased assay sensitivity. The affinities for GP2 and GP3 antisera were calculated from the Scatchard plot (Segal, 1975) using the standard curves and resulted in the values 2.56×10^{10} L/mol and 3.1×10^{10} L/mol, respectively.

iii) Sensitivity: The sensitivity of the assay was estimated as the smallest amount of unlabelled glucagon which could be distinguished statistically ($P<0.01$) from a zero sample in triplicate determination of the standard curves. The sensitivity of an assay using the antiserum of GP2 was approximately 150-200 pg/ml when the antiserum was diluted at 1:12,000, and for GP_3 was approximately 50-100 pg/ml when used at 16,000 dilution.

iv) Specificity: The specificity of an antiserum is indicated by the degree to which the antiserum binds only the desired antigen. In this study, the specificity of antisera from GP2 and GP3 was tested by incubating ^{125}I-glucagon with the antisera in the presence of glucagon-like peptide I or insulin at concentrations of 3.125, 6.25,

Figure 2.1 The elution pattern of ^{125}I-labelled bovine glucagon from QAE-Sephadex A 25 equilibrated with 0.08 M TRIS, 0.02 N HCl, 0.08 M NaCl, 1% human albumin and 50 KIU/ml trasylol (pH 8.5), and eluted at 10 ml/hour (example 1, *-*, and example 2, o-o).



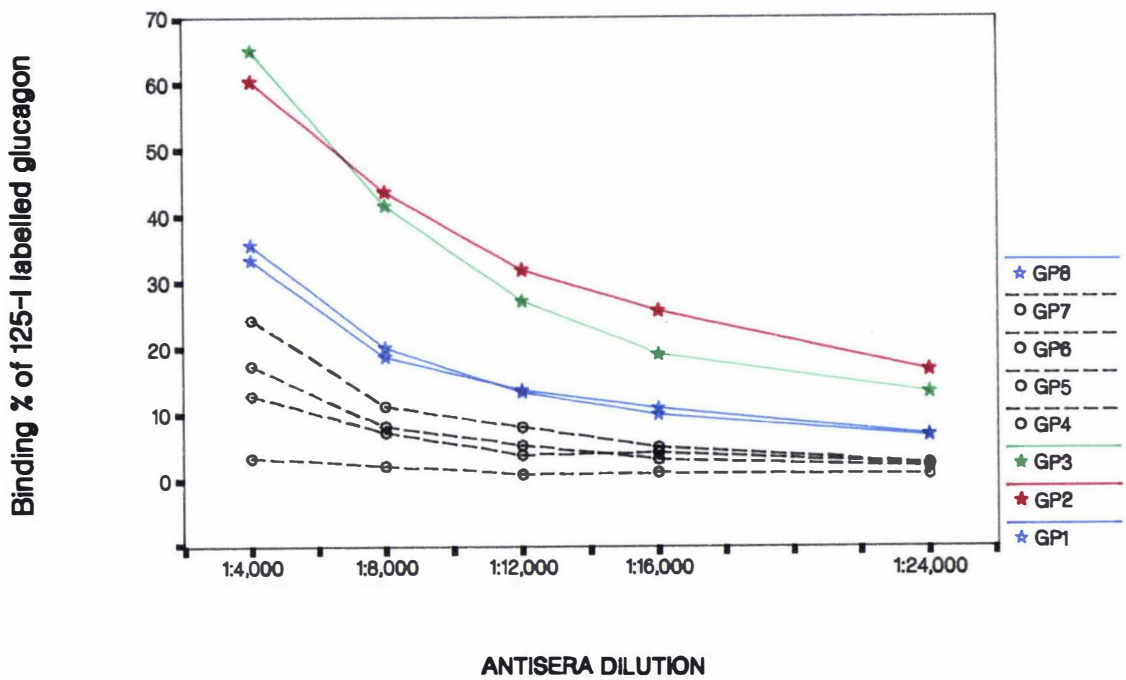


Figure 2.2 Evaluation of the titres of glucagon antisera elicited in 8 young female guinea pigs after 5 months of monthly injections of glucagon-bovine serum albumin of : 0.25 mg/animal at multiple intradermal sites (method I, solid lines); 0.125 mg/animal at multiple subcutaneous sites (method II, broken lines). Booster injections of 100 ug of free glucagon in sterile saline were given to the guinea pigs 10 days before collecting the blood samples.

12.5, 25, 50, 100, 200, 400 and 800 ng/ml. The relative potencies of these two hormones in competing with pancreatic glucagon for glucagon antibody were evaluated using the ratio of displacement of the ^{125}I-glucagon caused by pancreatic glucagon and by glucagon-like peptide I and insulin. At concentrations up to 800 ng/ml, neither glucagon-like peptide I nor insulin exhibited significant displacement of the ^{125}I-labelled glucagon (Figure 2.3) from the antisera from either GP2 or GP3. Thus the cross reactivities with insulin and glucagon-like peptide I were less than 0.5% and 0.1% for the antisera of GP2 and GP3 respectively.

v) Precision: Precision is a measure of the variation observed between repeated determinations on the same sample. Intra-assay precision was determined by calculating the mean coefficient of variation between triplicate determinations of 50 randomly-chosen pools and unknown samples. The inter-assay coefficient of variation was calculated from 6 quality control pools (with plasma glucagon concentrations spiked from 2 to 8 ng/ml) that were assayed in triplicate in 6 different assays. The pools were made up with plasma collected from either fed or fasted animals including 6 young calves, 5 heifers and one bull. The intra- and inter-assay coefficients of variation (C.V.) for assays using GP2 antisera were 11.5% and 18% respectively. The intra-assay C.V. for the assay using GP3 antisera was 10% (no inter- assay C.V. available for GP3 as only one assay was conducted using GP3 antisera).

2) Parallelism: Parallelism is a measure of the extent to which hormone in the unknowns is able to displace labelled antigen from the first antibody, compared with the standards. The presence of parallelism is an essential requirement for a valid radioimmunoassay. A lack of parallelism can indicate that: natural hormone in the samples differs from the hormone used in the standard curves in molecular structure or immunoreactivity; the binding between the hormone in the samples and the antibody is interfered with (promoted or inhibited) by other factors in the plasma; and excessive degradation of the radio-labelled antigen during incubation.

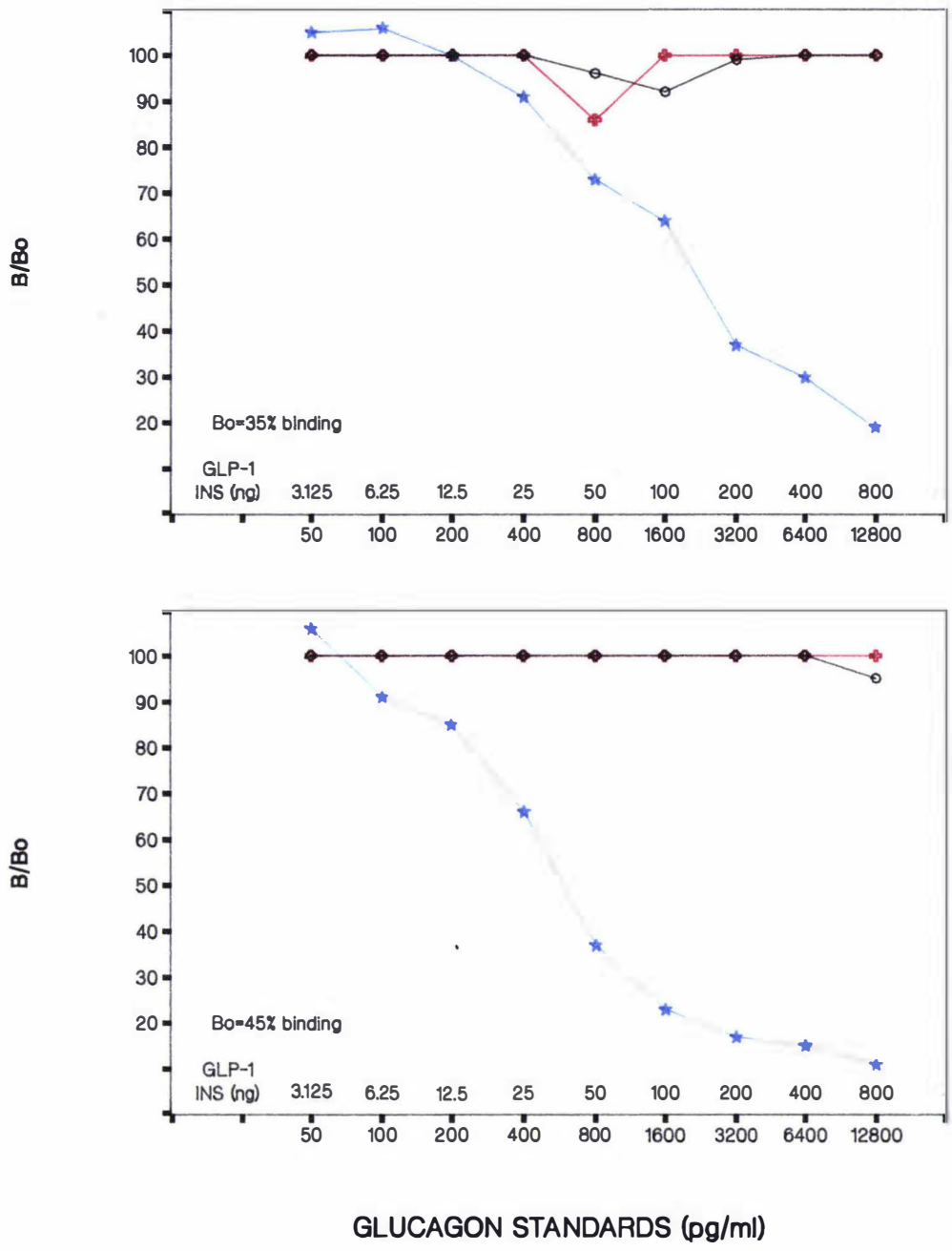


Figure 2.3 Displacement of ^{125}I-glucagon from the antisera of guinea pig 2 (GP2) (upper panel) and GP3 (lower panel) by glucagon standards (*--*), and by glucagon-like peptide I (o--o) and bovine insulin (+--+) at concentrations of 3.125 to 800 ng/ml

The parallelism of the glucagon RIA was assessed using the GP2 and GP3 antisera at a 1:16,000 dilution in an assay in which 25, 50, 100 and 200 ul samples of the the 6 pool samples were assayed in triplicate. The lack of parallelism was recognised if there were consistant deviations (for instance at 20% or greater) between the results of different dilutions. The results in Figure 2.4 shows that there were parallel changes between the curves of glucagon standards and the diluted samples for both antisera.

2. Plasma glucagon in heifers

Plasma samples were taken from 32 progesterone-treated heifers in two experiments (for detail see Chapters 4 and 5). In the first experiment, 6 samples were collected hourly from heifers fed 75% maintenance energy requirement (MER) but which had not been fed for more than 17 hours before the sampling. In the second experiment, plasma samples were taken from 16 heifers fed 125% MER and on two occasions: 1) 38 samples taken over a 29 hour period during which time the heifers had been fed twice; 2) 12 samples taken during a period of 73 hours involving fasting and subsequent refeeding. Samples from the first experiment were assayed using the antiserum of GP3, while samples from the second experiment were assayed using the antiserum of GP2. The changes in mean plasma glucagon concentrations from these two experiments are shown in Figure 2.5.

There was considerable variation in plasma glucagon concentration within and between the heifers in the two experiments (range: 0.15 to 6 ng/ml). Feeding and refeeding resulted in rapid increases in plasma glucagon concentration, which returned to pre-feeding levels soon after feeding (Figure 2.5, middle and lower panels). Moreover, mean plasma glucagon concentrations of the heifers were lower ($P<0.05$) during the fasting period (Figure 2.5 lower-panel) than during the normal fed period (Figure 2.5 middle-panel) (0.687 vs 1.161 ng/ml). These results are, in general, consistent with the reported effects of feeding and fasting on plasma glucagon concentration (Marliss et al. 1970; Bassett 1972; Ostaszewski and Barej 1979; de Jong 1981).

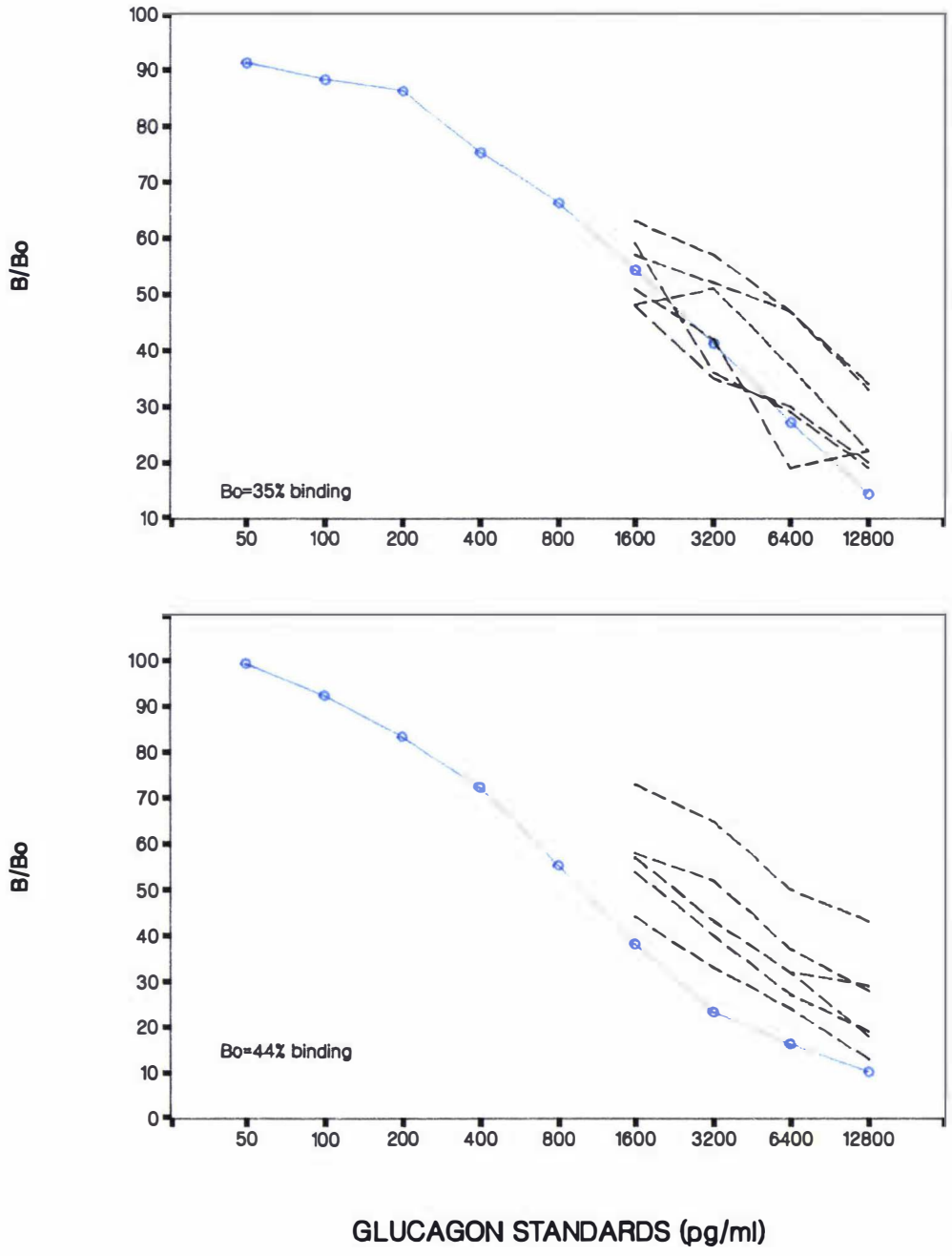


Figure 2.4 Evidence of parallelism: Standard curves of glucagon were produced using the glucagon antisera of GP2 (upper panel) and GP3 (lower panel) at 1:16,000 dilution, while the broken lines represent the displacement of ^{125}I-glucagon when bovine plasma samples were added at 4 dilutions (see: parallelism)

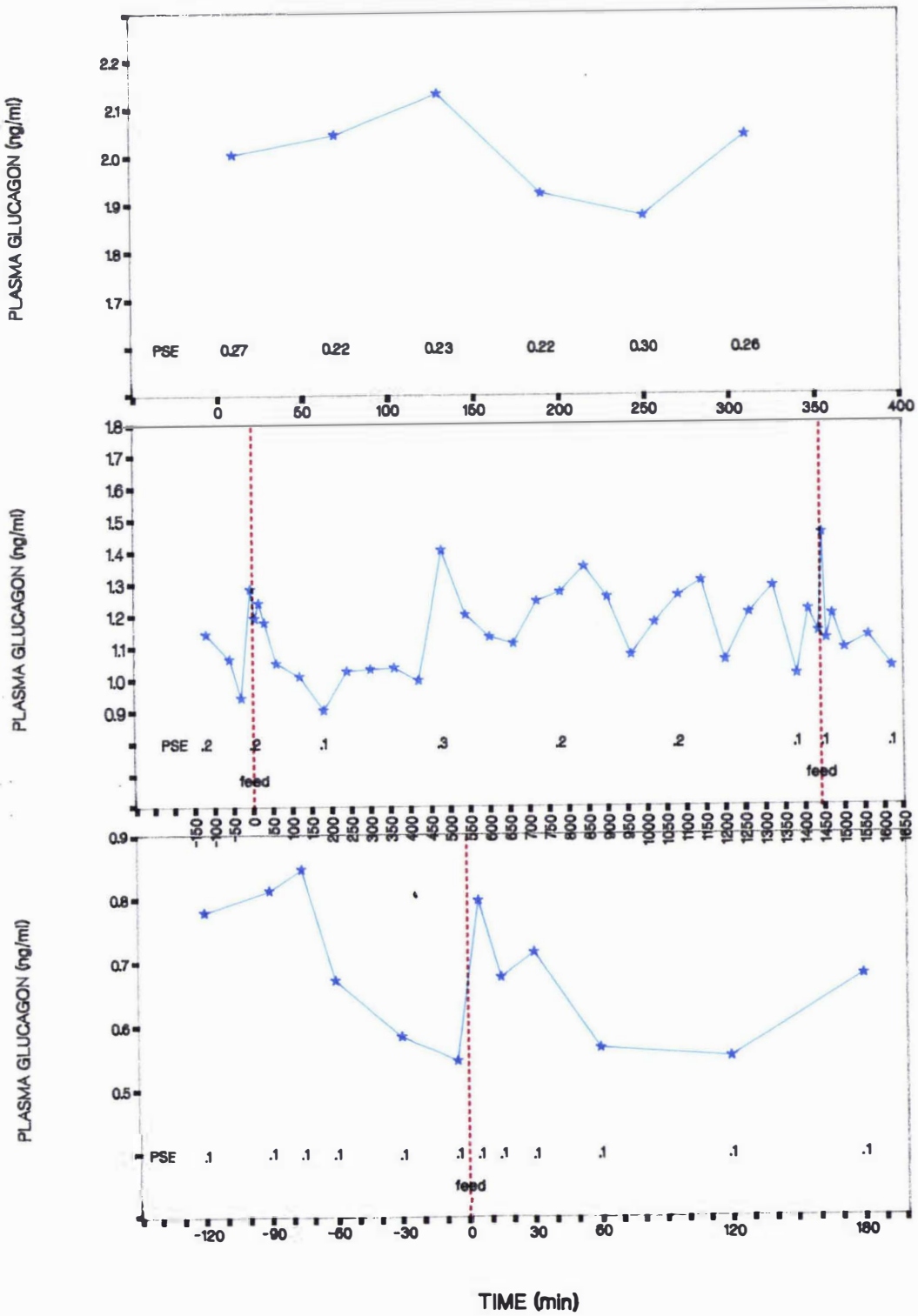


Figure 2.5 Plasma glucagon concentrations in progesterone-treated heifers: (1) samples collected hourly for 6 hours from 16 heifers following 17 hours fasting (upper panel); (2) samples collected hourly for 29 hours from 16 heifers fed at times 0 and 1440 minutes (mid-panel), and over a 70 hour fasting-refeeding period (lower-panel). Feedings commenced at times indicated by the vertical reference line.

IV DISCUSSION

Glucagon is a small polypeptide and thus a poor antigen. The coupling of the glucagon to some larger protein, such as bovine serum albumin, is essential for generating antisera of high sensitivity. Apart from this, the coupling procedure may also increase the chance of producing antisera specific to pancreatic glucagon. Procedures favouring the coupling between the NH_2 terminal of glucagon and the carrier protein will leave a free COOH terminal, thus giving more likelihood of a glucagon-specific antibody being produced. Several successful coupling procedures have been developed for the glucagon molecule (Ensinck 1983), and they may favour the binding of the carrier protein to different parts of the glucagon molecule as these methods differ in coupling reagents and carrier proteins. The exact mechanism controlling the coupling procedure is, however, not clear but is thought to be influenced by reaction conditions such as pH, temperature, and concentration of buffer.

The lack of cross-reactivity with glucagon-like peptide I and insulin by the glucagon antisera produced in GP2 and GP3 suggests that these two antisera are specific to pancreatic glucagon and directed only against the COOH terminal end of the molecule. Glucagon-like peptide I (glicentin) has substantial structural homology at the NH_2 end with pancreatic glucagon. It is thus one of the most likely polypeptides from the gut to cross-react with non-specific glucagon antisera. The cross-reactivity of other gut hormones such as cholecystokinin (CCK), gastric inhibitory peptide (GIP) and vasoactive intestinal peptide (VIP) with the glucagon antisera of GP2 and GP3 was not examined in this study. While they could be potential candidates for a weak cross-reactivity with glucagon antisera, their importance is likely to be minimal given the very weak cross-reactivity of GP2 and GP3 antisera with glucagon-like peptide I (Schenck 1977; McEvoy et al. 1977).

The use of guinea pigs to produce glucagon antisera was successful in this study. The substantial differences in the titre of the antisera between the two immunisation methods (Figure 2.1) show that method I induced a significantly higher response than method II. In

method I, the adjuvant-glucagon mix was injected intradermally which probably resulted in a lower rate of release than from the subcutaneously injected depot. This would likely have prolonged stimulation of the immune system. It has been suggested that various routes of delivery may be ranked in decreasing order of effectiveness in stimulating immune response to a poor antigen as follows: lymph nodes, the knee joints, intradermal, intramuscular, subcutaneous and intravenous (Hurn and Landon, 1971). The continued use of Freund's complete adjuvant in method I may also have resulted in a more prolonged sensitization of the immune system. The difference between methods I and II in the antigen dosage delivered (0.25 mg vs 0.125 mg) would probably have had minimal effect as it is generally accepted that antisera response is relatively independent of immunogen dosage over a wide range of doses, once a certain minimum quantity is reached. This study was, however, not designed to establish such a minimal requirement.

Antibodies with a high sensitivity will show a marked decline in tracer binding for only small changes in hormone concentration in the standard curve. In this study, the sensitivities of the GP**3** and GP**2** (50 and 200 pg/ml respectively) were relatively higher than others reported in the literature (2-5 pg/ml, Holst and Aasted 1974). This may be a reflection of differences in binding affinity between the antisera produced in the present study and those produced by others (Holst and Aasted 1974). In practice the sensitivity of an assay can be improved to certain extent by: decreasing the amount of the antibody added to the assay tubes (Skelley et al. 1973); adding less or using better quality of radio-labelled antigen in the assay tube (Skelley et al. 1973); increasing incubation time of the first antibody with the unlabelled antigen; and delayed addition of tracer (Skelley et al. 1973). In this study, dilutions of the GP2 and GP3 antisera at 1:12K, and 1:16k resulted in assay sensitivities which permitted almost all of the unknown samples to lie within the linear portion of the standard curve. It may be noted that the GP3 was more suitable for detecting low plasma glucagon levels than GP2 as the sensitivity of GP3 (50 pg/ml) was lower than that of GP2 (200 pg/ml).

The glucagon levels obtained in the progesterone-treated heifers using the above assay method were comparable with that in 6 month old heifers (Barnes et al. 1985), but were relatively high compared to the results of others (cows: about 100-500 pg/ml, Satin et al 1988; Sechen et al. 1989) under similar conditions. It is not known if this was due to a stimulatory effect of progesterone on plasma glucagon levels in the heifers. Plasma glucagon levels are higher in females with a high circulating progesterone, such as during pregnancy (Daniel et al. 1974; Luycke et al. 1975; Hornnes and Kuhl 1980; Hornnes 1985). In addition, loss of the potency of stored glucagon standards, which is inevitable even in the presence of trasylol (Heding 1971), and the degradation of ^{125}I-glucagon in plasma during incubation can also give higher than normal results (Eisentraut et al. 1968). Alternatively, the difference in absolute levels of immunoreactive glucagon between this and other studies could reflect the use of different standards. This could be checked by comparing the standards used in this laboratory with those used in other laboratories.

Nevertheless, the method for glucagon assay described here fulfils the criteria of a RIA in respect of high sensitivity and specificity for measuring glucagon in heifers. The intra- and inter-assay coefficients of variation lie within acceptable levels. The parallelism between the curves of glucagon standards and the diluted samples has further proved validity of the assay. Finally, responses in plasma glucagon to the effects of feeding and fasting are consistent with the results of other studies.

CHAPTER THREE: DIURNAL VARIATION IN PLASMA METABOLITE AND HORMONE CONCENTRATIONS AND RESPONSE TO METABOLIC CHALLENGES IN HIGH BREEDING INDEX AND LOW BREEDING INDEX FRIESIAN HEIFERS FED AT TWO ALLOWANCES

CHAPTER THREE: DIURNAL VARIATION IN PLASMA METABOLITE AND HORMONE CONCENTRATIONS AND RESPONSE TO METABOLIC CHALLENGES IN HIGH BREEDING INDEX AND LOW BREEDING INDEX FRIESIAN HEIFERS FED AT TWO ALLOWANCES

ABSTRACT

Diurnal variation in blood metabolite and hormone concentrations and their responses to acute intravenous injections of glucose, insulin, glucagon and adrenaline were examined in twelve 6-8 month old Friesian heifers born to either high breeding index (HBI) or low breeding index (LBI) parents. Heifers were fed at below- (75%) and above- (125%) their maintenance energy requirement (MER) in a switchback trial (single reversal) to test the effects of selection line and its interaction with energy balance.

When compared with heifers fed 125% MER, those fed 75% MER exhibited: increased plasma creatinine concentrations; a smaller increment in plasma urea concentration after feeding; greater plasma NEFA levels in the post-prandial period; lower insulin concentrations during a 24 hour period; decreased insulin release and glucose removal after glucose administration; greater plasma NEFA concentrations and reduced glucose clearance after insulin injection; enhanced glycogenolytic responses to glucagon and adrenaline; and increased lipolytic responses to glucagon and adrenaline.

Basal concentrations of insulin, creatinine, GH and NEFA were not influenced by selection line. Relative to the concentrations which existed at the time of feeding, HBI heifers showed a significantly greater rise in plasma glucose 7-9 hours after feeding. Plasma urea concentration also rose more rapidly in HBI heifers but then declined more rapidly in the postprandial period such that concentrations were lower in HBI than in LBI heifers from 11 till 23 hours after feeding. Responses to metabolic challenge were generally not different between the lines and there were no line x allowance interactions except in the NEFA response to adrenaline where HBI heifers responded more than LBI heifers at 75% MER but not at 125% MER.

It is concluded that the feeding regime used in this experiment was effective in causing substantial variation in blood hormone/metabolite concentrations and in responses to metabolic challenges. However, there was no evidence of marked allowance x selection line interactions. Differences between the lines were limited to plasma urea and glucose concentrations and were dependent upon the sampling time relative to feeding.

I. INTRODUCTION

The study of the high breeding index (HBI) and low breeding index (LBI) selection lines of Friesian cattle at Massey University has shown that differences in plasma hormone and metabolite concentrations are apparent in the heifer and bull calves, as well as in the mature cows. These differences are primarily seen in aspects of glucose and insulin metabolism (Flux et al. 1984; Bridges et al. 1987; Mackenzie et al. 1988; Xing et al. 1988). However, the differences have not always been consistent between years and between different groups of animals. One possible reason for this is the presence of interactions between the selection lines and the environmental conditions to which the animals are exposed. Thus, for example, Flux et al. (1984) found that differences between the HBI and LBI lactating cows were most apparent when they were underfed.

The objective of this experiment, therefore, was to determine the effects of energy status on basal hormone and metabolite concentrations and on the responses of these hormones and metabolites to metabolic challenges in heifer calves from the two selection lines.

II. MATERIALS AND METHODS

1. Animals

Twelve 7- to 8-month old Friesian heifers, 6 born to parents of the high breeding index (HBI) line and 6 born to parents of the low breeding index (LBI) line, were used in the experiment. They were raised on the Dairy Cattle Research Unit at Massey University and originated from the same lines as those used in the earlier studies

(Flux et al. 1984). The estimated mean BI based on ancestry information was 132±3 (Mean ± SD) for the HBI group and 109±2 for the LBI group. Live weights (lwt) at the start of the experiment were 180 ± 28 kg and 173 ± 23 kg for HBI and LBI heifers, respectively.

2. Experimental design

The experiment was designed as a switchback trial involving a 2X2 factorial arrangement of selection line (HBI vs LBI) and energy allowance (75% vs 125% of maintenance energy requirement). The 12 heifers were divided into two blocks, A and B, with 3 HBI and 3 LBI animals in each block. Block A received the 75% maintenance energy requirement (MER) treatment followed by the 125% MER treatment while in Block B the sequence of feeding levels was reversed. Basal plasma concentrations of hormones and metabolites and their responses to acute glucose, insulin, glucagon and adrenaline injections were measured in each of the periods.

Prior to each experimental period, the heifers had been grazing ad libitum on mixed ryegrass/white clover pasture. At the start of the period they were housed in tie-stalls for a 15-day feeding adjustment. Heifers were fed mixed pasture hay (predominantly perennial ryegrass/white clover) to appropriate levels in each period. Maintenance was calculated as 0.55 MJ ME/$kg^{.75}$ (Holmes et al. 1984) and the hay was assumed to contain 8.6 MJ ME/kg DM at 85% DM (Holmes et al. 1984). Hay was offered once daily at 1600h and heifers had free access to clean water.

On day 16 of each period, jugular cannulae were inserted under local anaesthesia. The following day, blood sampling for basal plasma hormone and metabolite concentrations commenced at 1600h (i.e. coincident with feeding) and continued at hourly intervals for 24 hours. On day 19 glucose (0.4 g/ml prepared for intravenous infusion by National Dairy Association, Palmerston North, New Zealand) was injected (0.3 g/kg lwt) at 0930h and blood samples collected at -30, -15, -5, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 27, 32, 42, 52, 62, 72, 82, 92, 102, 122, 142, 162, and 182 minutes from the time of injection (t=0). Challenges of insulin (10 µg/kg lwt, Sigma Cat No. 5500, 26.4

IU/mg), glucagon (0.175 µg/kg lwt, Sigma Cat No. G-4250) and adrenaline (1 µg/kg lwt, Travenol Laboratories, NZ. Ltd., Auckland,NZ) were administered on days 20, 21 and 22, respectively. Insulin and glucagon were dissolved in sterile physiological saline (National Dairy Association, Palmerston North, New Zealand) containing 30 mg/ml bovine serum albumin (Sigma, Cat No. A-8022, Fraction V) while adrenaline was dissolved in sterile saline and protected from light prior to administration by enclosing vials and syringes in aluminium foil. Each of the challenges was administered at 1000, 1200 or 1400 h in 3 sub-groups of 4 heifers (balanced for BI and energy allowance) to facilitate blood sampling. Blood samples were collected at -15, -10, -5, 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes from the time of the challenges (t=0). Blood (6 ml) was collected into centrifuge tubes containing 100 ul of 35% (w/v) sodium citrate as the anticoagulant and was centrifuged immediately at 2500 g and 4°C. The plasma was then harvested and stored frozen at -20°C until analysed.

At the end of period I, the heifers were returned to grazing for 10 days and offered pasture ad libitum. They were then housed for a second adjustment period (with the level of energy intake reversed for each animal), recannulated and subjected to the same procedures as in period I. The first challenge given in period II was again glucose but the sequence of other challenges was reversed (i.e. adrenaline, glucagon then insulin) to minimise any biases due to order of challenge.

3. Blood analyses

Plasma glucose concentration was determined on a Yellow Springs Instrument Model Y-27 Industrial Analyser. Plasma non-esterified fatty acid (NEFA) concentrations were determined colourimetrically using NEFA assay kits (Wako Pure Chemical Industries Ltd, Japan). The assay was modified as described by McCutcheon and Bauman (1986). Urea and creatinine were analysed by the autoanalyser methods of Marsh et al. (1965) and Chasson et al. (1961), respectively. Intra- and inter-assay coefficients of variation for glucose, urea, creatinine and NEFA were 2.4 and 3.5, 1.3 and 0.4, 1.9 and 1.0, and 2.6 and 3.8% respectively. Double-antibody radioimmunoassay (RIA) methods for

plasma insulin and growth hormone (GH) were as described by Flux et al. (1984). Bovine insulin (Sigma Chemical Co. No I-5500, Lot No:55F-0536, 23.4 IU/mg) was used for iodination and as the reference standard in insulin RIA. Bovine GH, (USDA-bGH-I-1, Lot No: AFP 6500, 3.2 IU/mg) and (USDA-bGH-B1, Lot No: AFP-5200, 1.9 IU/mg), were used for iodination and as reference standards respectively in the GH assay. The intra- and inter-assay coefficients of variation for insulin RIA were 8.2 and 12.4%, and for GH RIA, 8.6 and 13.2%, respectively.

4. Statistical analysis

The model for statistical analysis was based on the split-plot analysis of Gill and Hafs (1971):

$$Y_{ijkl} = U + L_i + S_j + LS_{ij} + H_{ijk} + P_k + LP_{ik} + SP_{jk} + LSP_{ijk} + e_{ijkl}$$

where U = overall mean
Y_{ijkl} = the observed value
L_i = effect of the ith selection Line (i=1, 2)
S_j = effect of the jth Sequence (j=1, 2)
H_{ijk} = between-Heifer error term
P_k = effect of kth Period (k=1, 2)
e_{ijkl} = within-heifer error term

and where interactions are as shown. Because of the single reversal nature of the experiment, the sequence x period interaction is equivalent to the MER effect, while the line x sequence x period interaction tests the line x MER effect.

Challenge and basal data were analysed by applying the above model at each sampling time. Where differences existed between the two selection lines prior to challenge (i.e. in baseline concentrations), data were corrected by subtracting the mean prechallenge concentrations of the hormone or metabolite from observed concentrations at each of the post-challenge samples. A corresponding correction was used for the diurnal plasma hormone/metabolite concentrations using the first sample as an approximation of the baseline value. Analysis was then

performed as described above on baseline-corrected data. The analyses were performed using the statistical package 'REG' (Gilmour 1985).

III. RESULTS

1. Body weight changes: The live weight change of the heifers over the experimental periods was not affected by selection line. The effect of energy allowance on live weight change was significant ($P<0.001$), with heifers offered 125% MER gaining 4.2 $\pm$ 1.7 (S.E.) kg and heifers offered 75% MER losing 10.2 $\pm$ 1.1 kg body weight.

2. Basal hormone and metabolite concentrations: Diurnal changes in plasma concentrations of hormones and metabolites for each of the four line x MER groups are shown in Figures 3.1-3.4.

Glucose concentration fell in all animals for the first 1 to 2 hours after feeding and then increased steadily, reaching peak concentrations after 9 to 15 hours (Figure 3.1). There were no apparent differences between the selection lines in glucose concentration and effects of energy allowance were only occasionally significant. Glucose data were further analysed by subtracting the first observation from the subsequent values to test the treatment effect on plasma glucose concentrations relative to those which existed at the time of feeding. The results showed a greater ($P<0.05$) increment of plasma glucose concentration in the HBI heifers than in the LBI heifers from 7 to 9 hours after feeding. The glucose concentration increment was also greater ($P<0.05$) in the heifers fed 75% MER than in those fed 125% MER at intervals from 3 to 15 hours after feeding (Figure 3.1).

Plasma insulin concentration was greater ($P<0.05$) in LBI than in HBI heifers at 2, 4 and 7 hours after feeding (Figure 3.2). Thereafter, the selection line difference became nonsignificant but there was a significant line x allowance interaction ($P<0.05$) on occasions during the interval 6 to 21 h post feeding. This was mainly due to the high insulin levels in the LBI heifers offered the 125% MER. Allowance exerted a significant effect on insulin concentration from 6 to 10 and from 13 to 22 hours after feeding, with animals fed at 125%

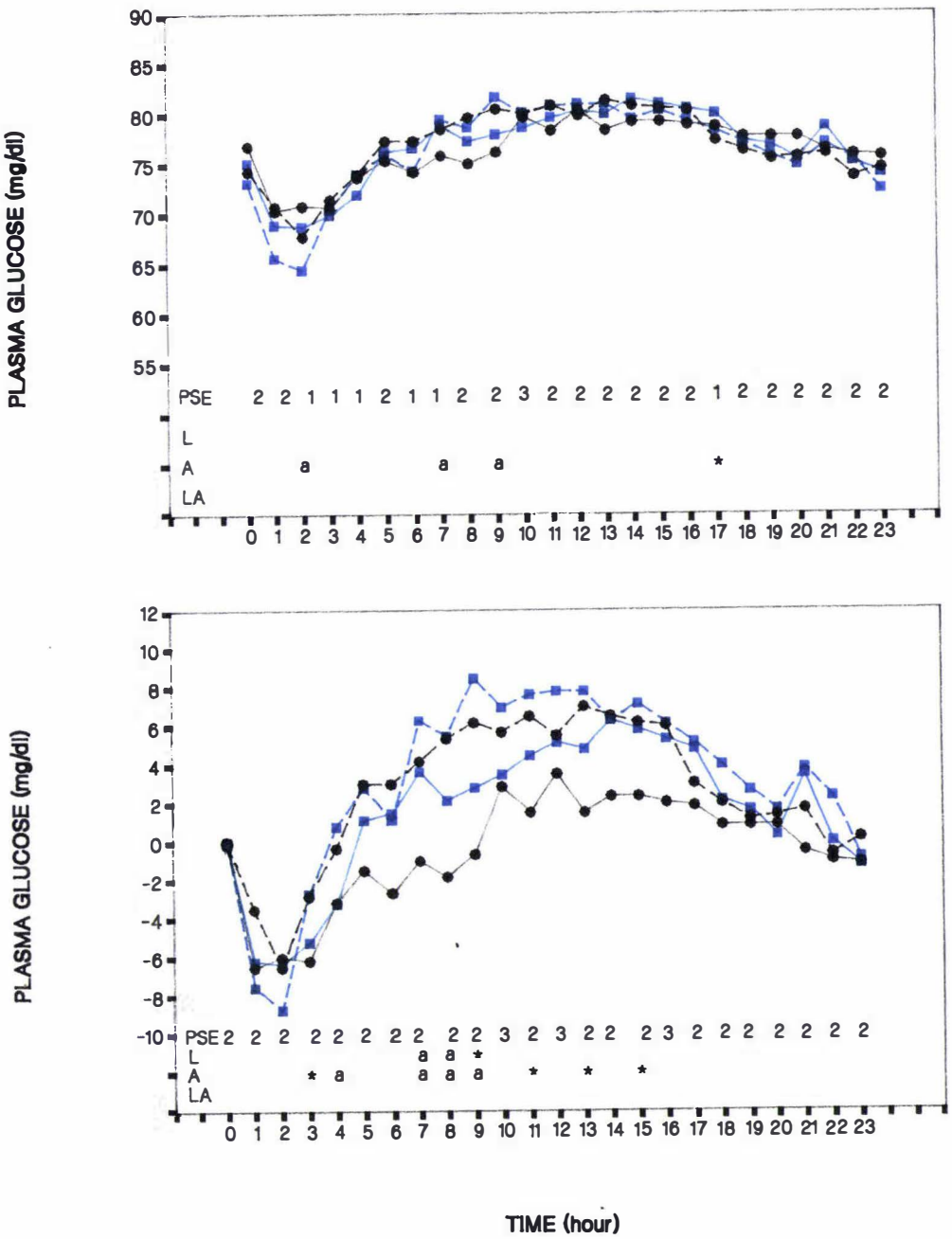


Figure 3.1 Basal plasma concentrations of glucose uncorrected (upper panel) and corrected for pre-feeding glucose concentration (lower panel) in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line)) and 125% (solid line) maintenance energy requirement. Heifers were offered hay at time 0 (1600h). (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

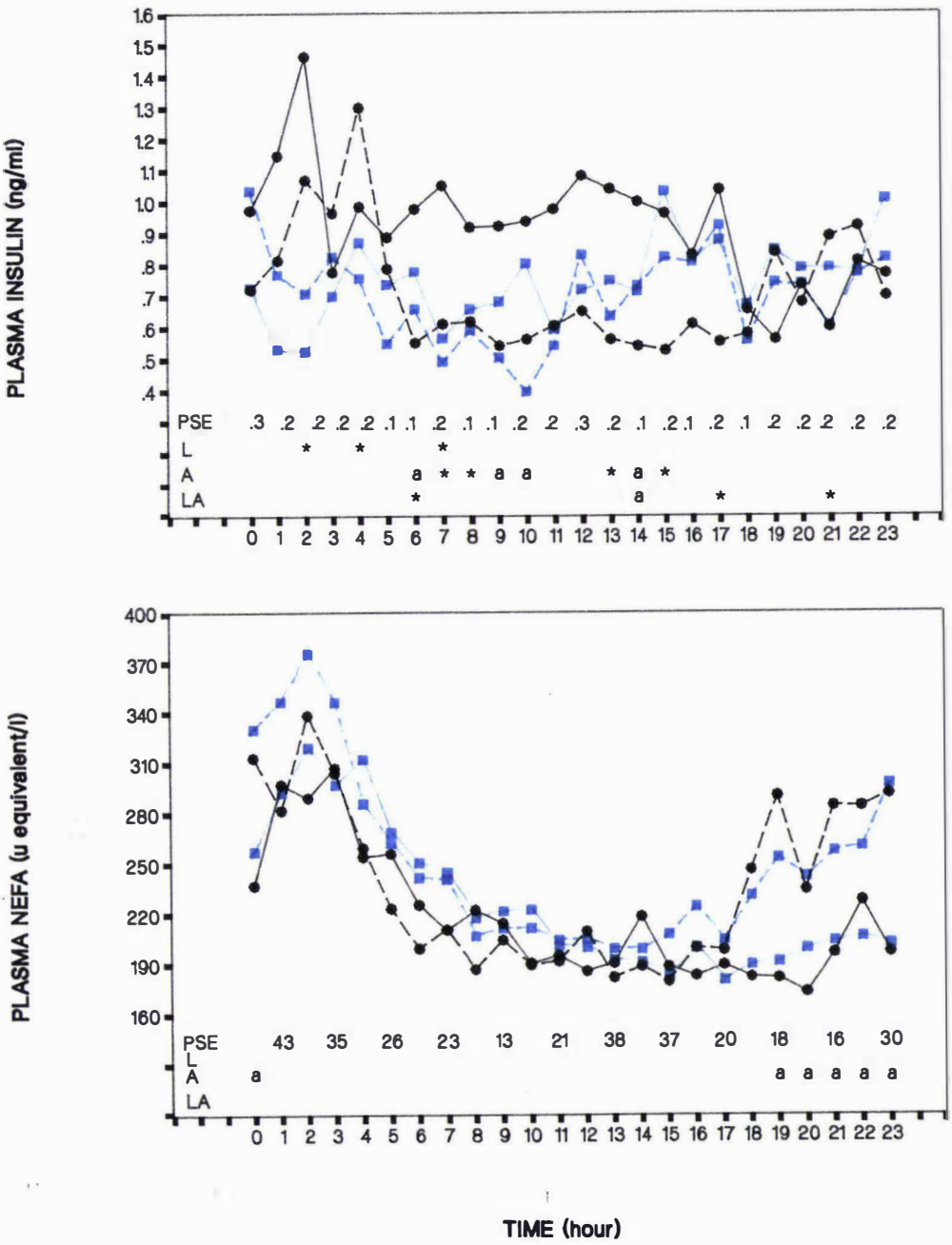


Figure 3.2 Basal plasma concentrations of insulin (upper panel) and NEFA (lower panel) in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. Heifers were offered hay at time 0 (1600h). (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

MER having higher insulin levels ($P<0.05$) than those fed 75% MER.

The diurnal pattern of plasma NEFA concentration was the reverse of that for glucose (Figure 3.2). NEFA concentrations were high immediately after feeding but declined thereafter. Significantly higher NEFA levels were observed in the heifers fed 75% MER than in heifers fed 125% MER immediately before feeding (i.e. at time zero) and during the period 19 to 23 hours after feeding ($P<0.05$). No effects of selection line on NEFA concentrations were observed.

Feeding resulted in a marked change in plasma urea concentrations with the peak occurring 4 to 7 hours after feeding and a nadir between 16 and 20 hours. Plasma urea concentrations were relatively higher in the HBI heifers than in the LBI heifers during the first 5 hours after feeding, and this difference was significant ($P<0.05$) from 1 to 2 hours after feeding when the data were corrected by subtracting the concentration at zero time. As the animals entered a postabsorptive state, the urea concentration decreased more rapidly in the HBI heifers. As a result, the HBI heifers had a significantly lower ($P<0.05$) plasma urea level, relative to that at feeding, from 11 hours after feeding till the end of sampling (Figure 3.3). Plasma urea was also significantly higher in the heifers fed 125% MER compared with those fed 75% MER from the start of sampling until 17 hours after feeding ($P<0.05$). However, this was primarily due to the difference which already existed at the time of feeding. Relative to this initial level, heifers on 75% MER had greater plasma urea concentrations from 16 to 23 hours after feeding (Figure 3.3) coincident with their greater NEFA levels.

Plasma creatinine increased substantially in the 75% MER heifers following feeding (Figure 3.4). There was no selection line effect on plasma creatinine over the 24 hour period and no line x allowance interaction even though HBI heifers fed at 125% MER showed consistently low creatinine levels. Heifers fed 75% MER generally maintained higher creatinine concentrations than those in the 125% MER group but the difference was significant ($P<0.05$) only during the first 7 hours after feeding and from 15 to 23 hr after feeding.

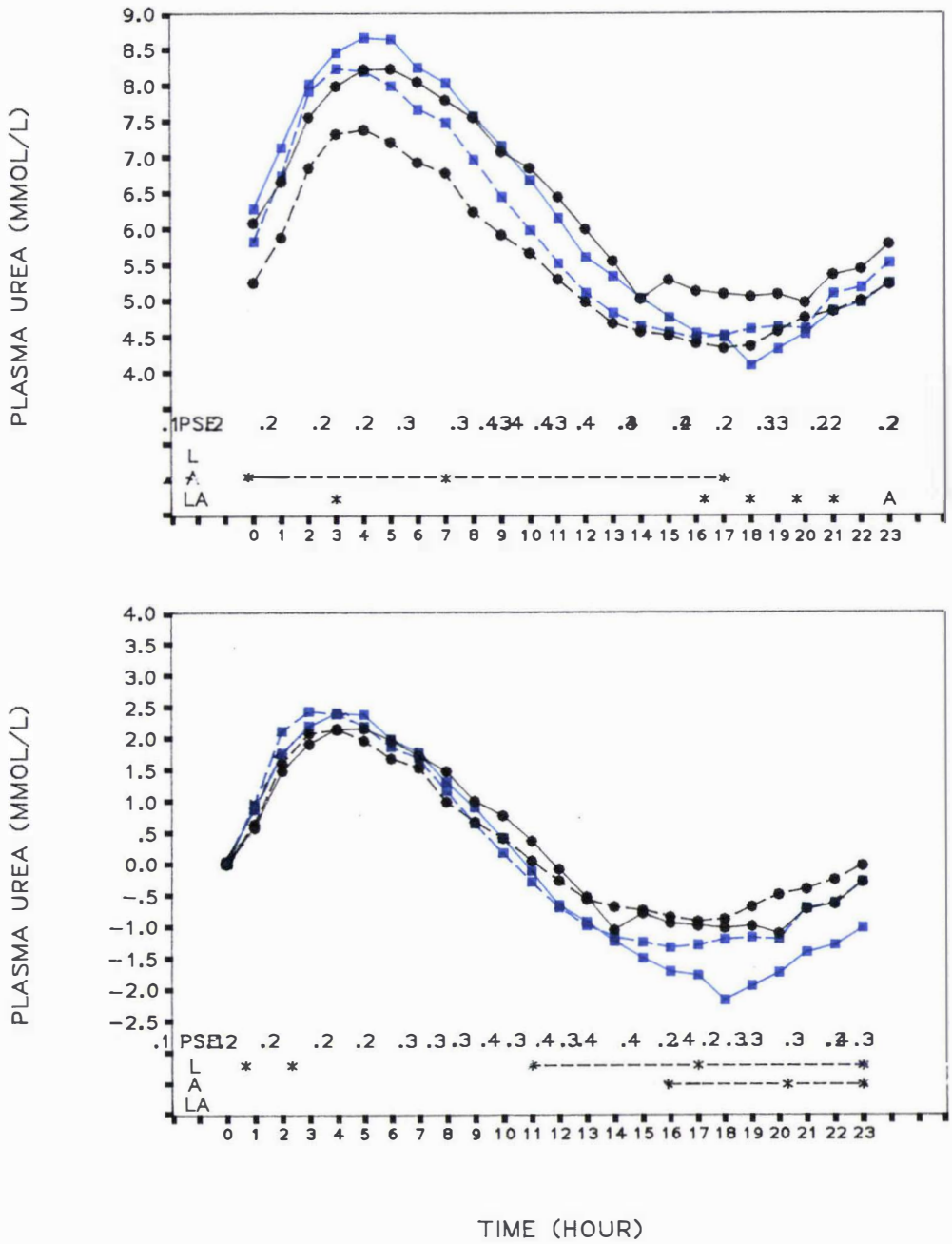


Figure 3.3 Basal plasma concentrations of urea uncorrected (upper panel) and corrected for pre-feeding urea concentration (lower panel) in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. Heifers were offered hay at time 0 (1600h). (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

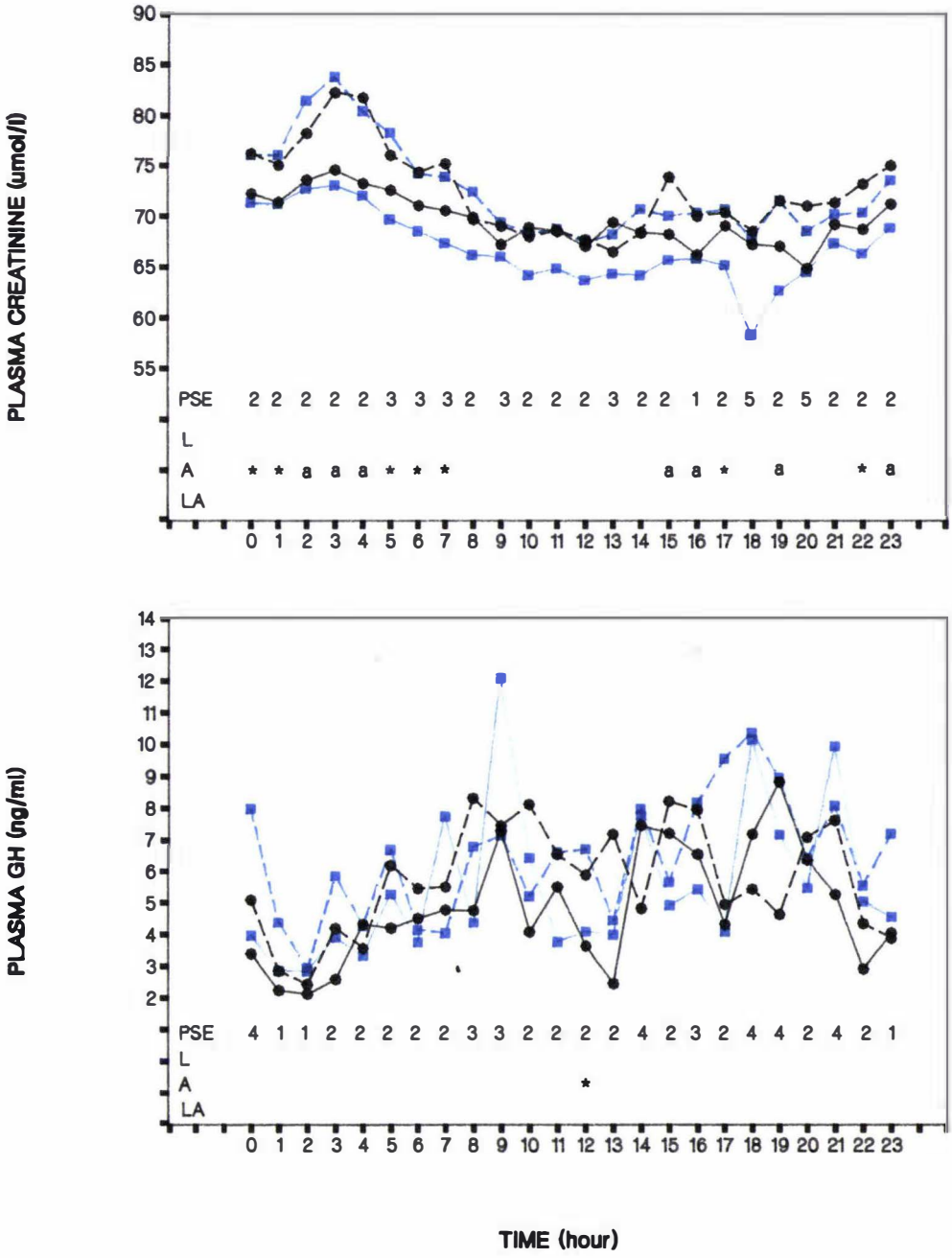


Figure 3.4 Basal plasma concentrations of creatinine (upper panel) and GH (lower panel) in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. Heifers were offered hay at time 0 (1600h). (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

There was no effect of selection line or allowance on plasma GH concentrations (Figure 3.4).

3. Challenges

Metabolic responses to the intravenous administration of glucose, insulin, glucagon and adrenaline for each of the four experimental groups are shown in Figures 3.5-3.8.

1) Glucose Challenge:

Glucose: A peak plasma glucose concentration of about 300 mg/dl was achieved in all heifers 2 minutes after glucose infusion (Figure 3.5). Glucose concentration then declined rapidly during the following 60 minutes but it was not not until 120 minutes after challenge that basal concentrations were reached. There was no significant effect of selection line on plasma glucose during the challenge but the effect of allowance was significant ($P<0.05$) from 6 to 10, and from 19 to 122 minutes after challenge, with the 75% MER group maintaining a higher level of blood glucose.

There was an immediate elevation of plasma insulin in all the heifers following the glucose challenge. LBI heifers appeared to have a greater insulin response than HBI heifers, but the difference was significant ($P<0.05$) only at 42 minutes after challenge. Heifers fed at 125% MER showed a higher level of insulin than heifers fed at 75% MER ($P<0.05$) from 2 to 6, 19 to 27 and at 82 minutes following the challenge.

2) Insulin challenge

Administration of insulin resulted in a steady decline in plasma glucose concentrations in all groups of heifers (Figure 3.6). This trend was sustained until the end of sampling at 60 minutes post-challenge. No significant differences due to selection line or allowance were found. However, when corrected for pre-challenge values, plasma glucose concentrations were significantly lower in heifers fed 125% MER than in heifers fed 75% MER at 2.5 and 5 minutes

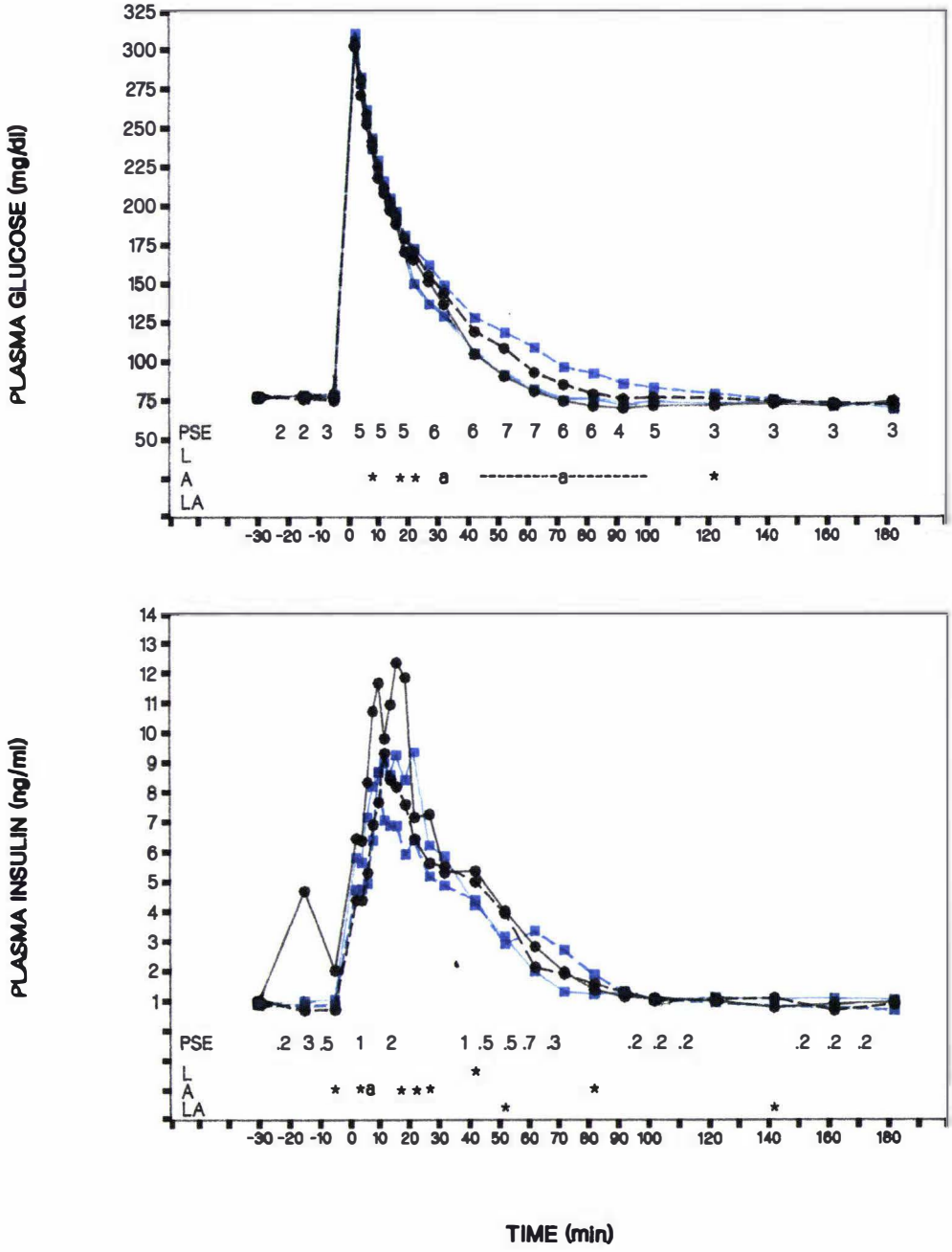


Figure 3.5 Plasma concentrations of glucose (upper panel) and insulin (lower panel) in response to glucose challenge (0.3 g/kg lwt) in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

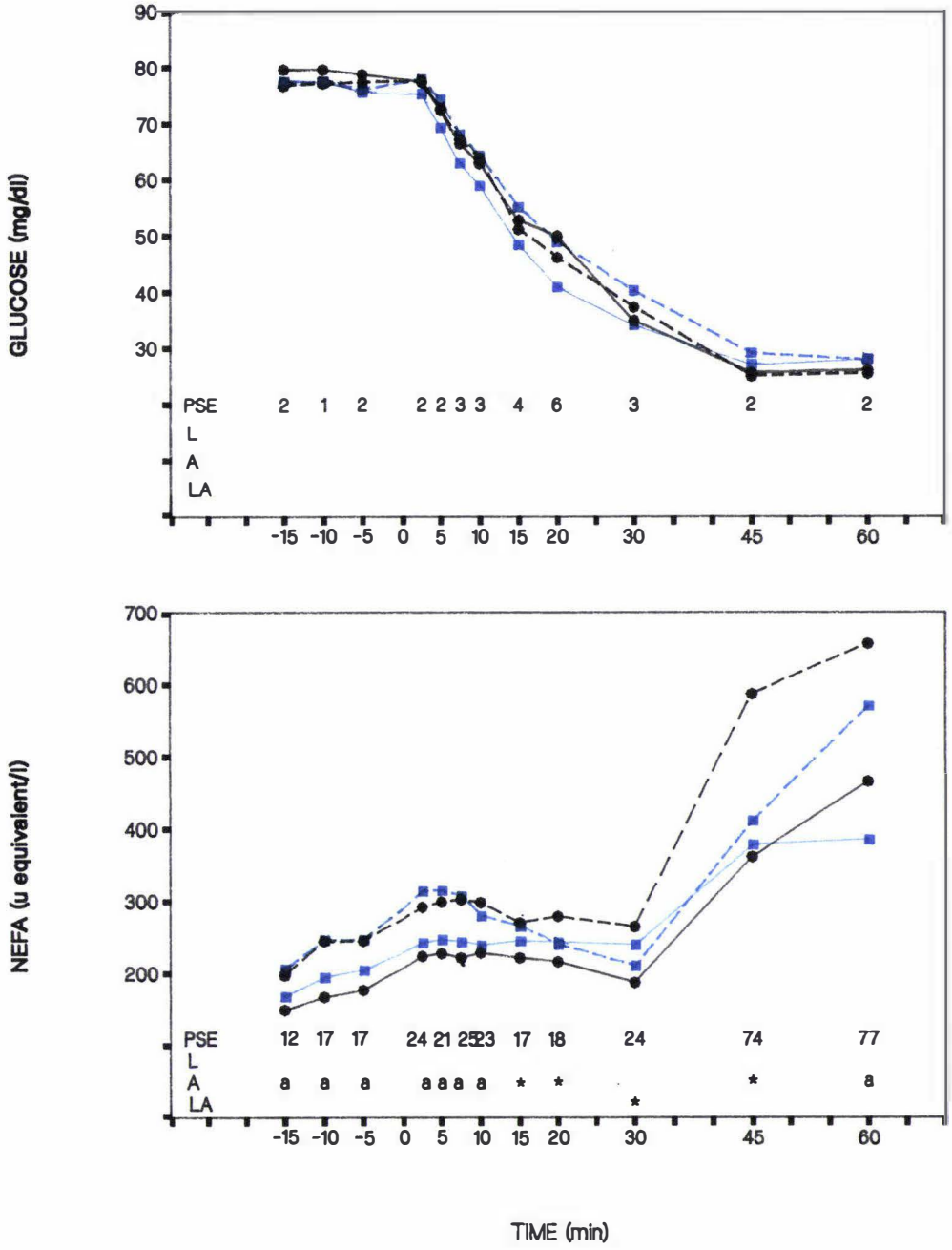


Figure 3.6 Plasma concentrations of glucose (upper panel) and NEFA (lower panel) in response to insulin challenge (10 µg/kg lwt) administered at time 0 in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

after challenge ($P<0.05$).

There was a biphasic response of plasma NEFA concentration to insulin challenge (Figure 3.6). The first minor increase in plasma NEFA occurred soon after insulin infusion and was followed by a transient decline in plasma NEFA. The major increase in plasma NEFA occurred 45 minutes after insulin injection. No differences due to selection line were observed. The difference between the two allowance groups was significant before and following the insulin challenge, with the heifers fed at 75% MER maintaining higher plasma NEFA concentrations ($P<0.01$). The post-challenge difference was due to the carry-over from pre-challenge differences as indicated by nonsignificant allowance effects in baseline-corrected data.

3) Glucagon challenge

The glucagon challenge resulted in a rapid elevation of plasma glucose concentrations in the heifers from about 80 mg/dl to about 105 mg/dl (Figure 3.7). Glucose then declined and regained the pre-challenge concentrations at about 45 minutes after the challenge (Figure 3.7). There were no differences due to selection line. Basal glucose was higher ($P<0.05$) in the heifers fed 125% MER than in heifers fed 75% MER prior to glucagon challenge (80.5 vs. 76.5 mg/dl). However, the situation reversed following the challenge and the baseline-corrected data showed that the elevation in plasma glucose was significantly greater in the 75% MER than in 125% MER group following glucagon challenge ($P<0.05$).

There was a small increase in plasma NEFA concentration in response to glucagon injection (Figure 3.7). No significant differences were found between HBI and LBI heifers in plasma NEFA concentration before or after the challenge. Heifers fed 75% MER showed significantly ($P<0.05$) higher NEFA concentrations following the challenge than those fed 125% MER from 2.5 to 20 minutes after the injection. However, this difference was nonsignificant after adjustment for pre-challenge values.

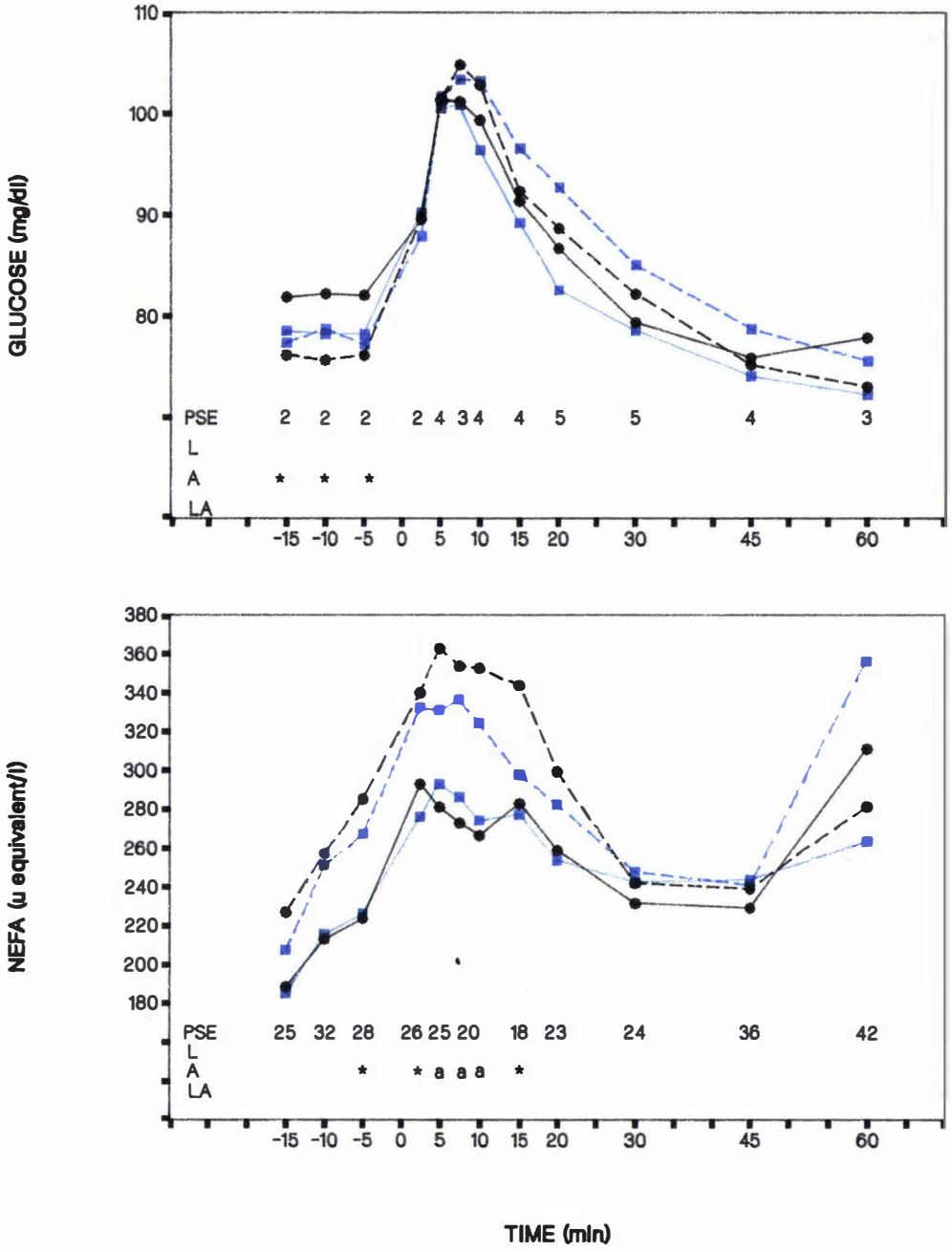


Figure 3.7 Plasma concentrations of glucose (upper panel) and NEFA (lower panel) in response to glucagon challenge (0.175 µg/kg lwt) administered at time 0 in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

4) Adrenaline challenge:

Plasma glucose increased in all heifers in response to adrenaline injection (Figure 3.8). The glucose concentration prior to and after the challenge was slightly lower in HBI heifers than in LBI heifers and that difference was significant ($P<0.05$) at 2.5 mins when data were corrected for pre-challenge levels. Heifers fed 75% MER generally maintained lower pre-challenge but higher post-challenge plasma glucose concentrations than heifers fed 125% MER and the post-challenge difference was significant ($P<0.05$) at 7.5 and from 15 to 30 minutes (after baseline correction).

Plasma NEFA was significantly higher in HBI than in LBI heifers at -10, 2.5, and 7.5 min from adrenaline challenge, but the difference observed in the post-challenge samples become non-significant after baseline correction. Basal NEFA was significantly higher ($P<0.05$) in 75% MER than in 125% MER heifers and the difference was further enhanced ($P<0.01$) following the adrenaline injection. The analysis using baseline-corrected data showed a significant line x allowance interaction from 7.5 to 45 minutes after adrenaline injection ($P<0.05$), with HBI heifers fed 75% MER exhibiting a greater NEFA response than the other groups.

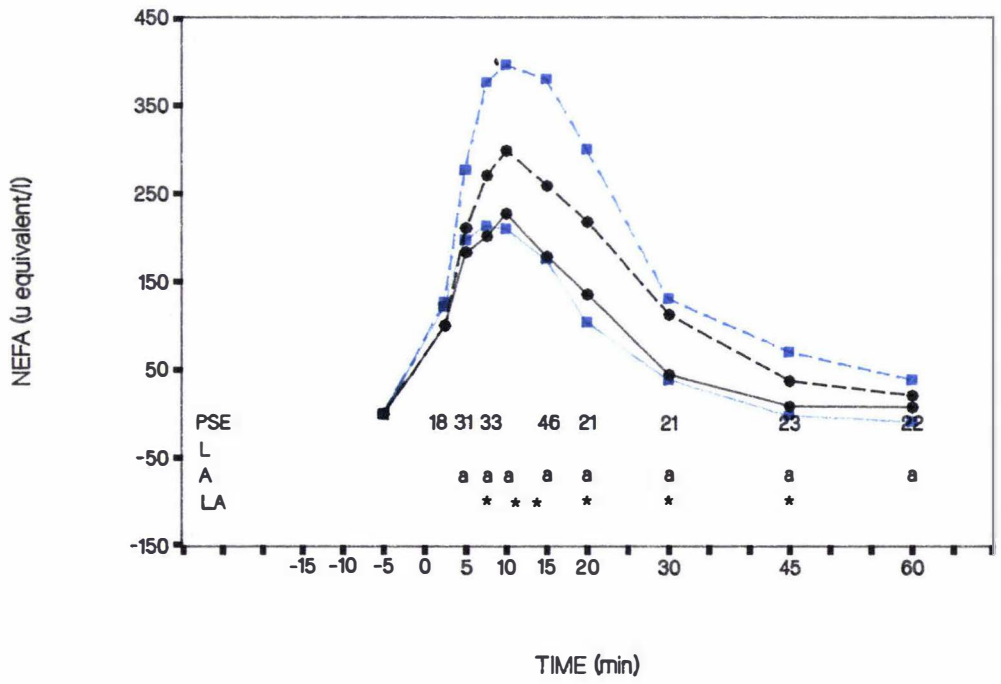


Figure 3.8 Plasma concentrations of NEFA, corrected for pre-challenge concentration, in response to adrenaline challenge (1 µg/kg lwt) administered at time 0 in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=$P<0.05$, a=$P<0.01$)

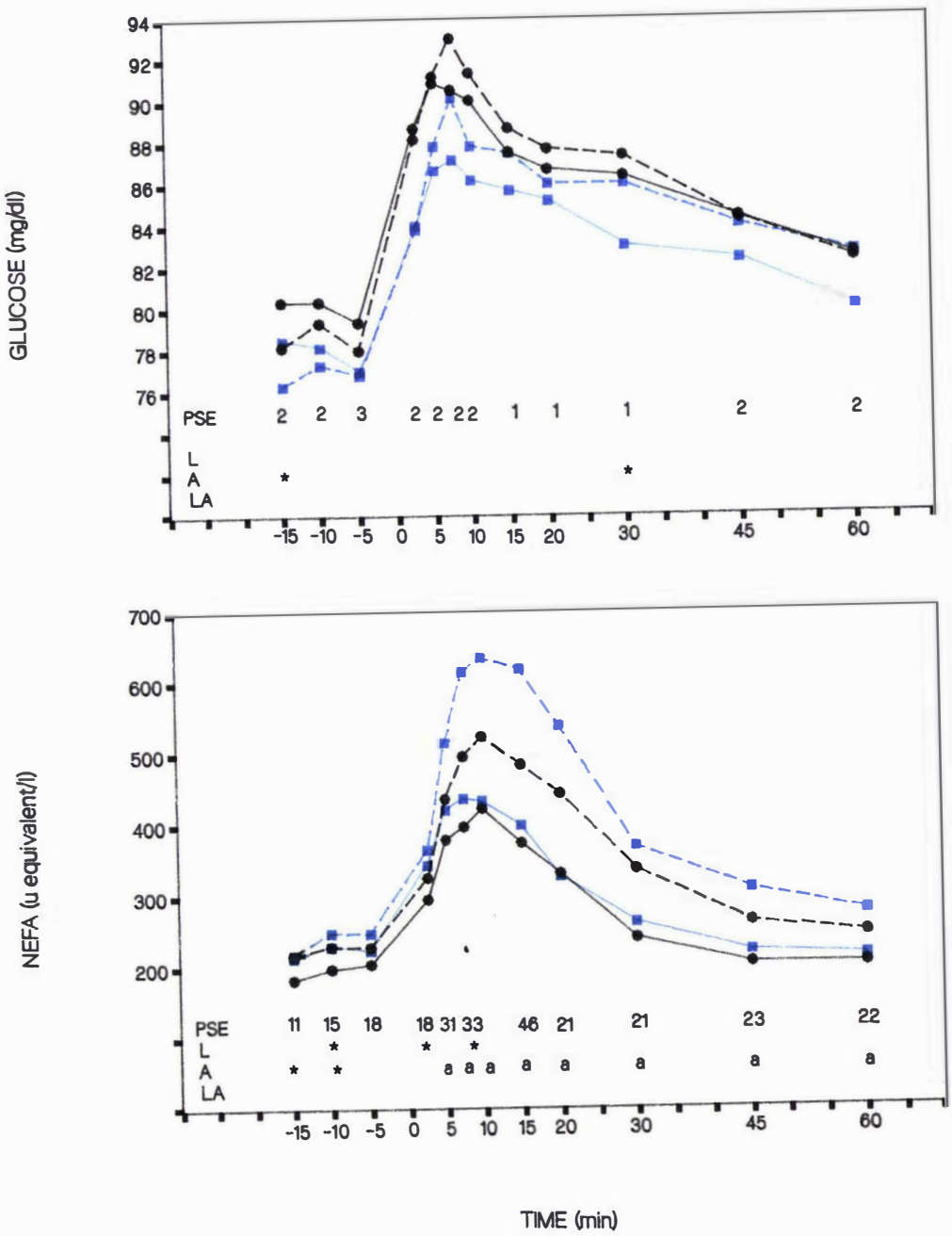


Figure 3.8 Plasma concentrations of glucose (upper panel) and NEFA (lower panel) in response to adrenaline challenge (1 µg/kg lwt) administered at time 0 in 6 high breeding index (square) and 6 low breeding index (circle) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. (L=selection line effect, A=allowance effect, LA=line x allowance interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

IV. DISCUSSION

The objective of this experiment was to assess the effect of energy balance on the diurnal variation in plasma concentrations of metabolites and hormones, and on their responses to metabolic challenges, with specific reference to possible differences between the HBI and LBI heifers. Considerable diurnal variation was observed in most of the blood metabolites (Figures 3.1-3.4) which should be taken into account when interpreting blood metabolite profiles in heifers. The general pattern of diurnal variation and the responses to the metabolic challenges were, however, consistent with previous reports in the literature (Vasilatos and Wangsness 1981; Van Soest 1982; Flux et al. 1984; Bridges et al. 1987; Mackenzie et al. 1988).

Energy allowances were chosen to produce marked differences in metabolic state. In this respect they were successful. Relative to those fed 125% MER, heifers fed 75% MER had lower basal concentrations of insulin (Figure 3.2) and urea (Figure 3.3) but higher concentrations of NEFA (Figure 3.2) and creatinine (Figure 3.4). This is consistent with the 75% MER group having depressed insulin secretion, reduced entry of urea from the gut, and altered basal lipolysis and proteolysis. Following the glucose challenge, there was reduced release of insulin into blood in the 75% MER group (Figure 3.5), an effect which would have been at least partly responsible for the reduced rate of glucose clearance from the circulation. Heifers fed 75% MER also appeared to be less sensitive to the glucose-clearing effects of insulin, as indicated by their higher glucose concentrations following the insulin challenge (Figure 3.6).

Heifers fed 75% MER also exhibited different responses from those fed 125% MER in glycogenolytic and lipolytic responses to the glucagon and adrenaline challenges (Figures 3.7 and 3.8). The elevation in plasma glucose (baseline-corrected) was greater in 75% MER heifers after the challenges, presumably reflecting greater glycogen breakdown. However, whereas the adrenaline challenge resulted in significantly greater NEFA concentrations in the 75% MER heifers, the same was not true for the glucagon challenge (baseline-corrected).

Despite the fact that the selection lines were compared at differing energy balances there were no apparent differences in basal plasma concentrations of GH, NEFA or creatinine between LBI and HBI heifers. The greater plasma glucose concentration (baseline corrected, $P<0.05$) in the HBI heifers than in the LBI heifers after feeding was sustained only briefly from 7 to 9 hours after feeding when maximum fermentation from hay would be expected to occur in the rumen (Van Soest 1982). This difference in glucose concentration between the HBI and LBI heifers may have reflected either a more rapid entry of gluconeogenic products from the alimentary tract or a reduced basal glucose utilisation by the peripheral tissues in the HBI group. Greater basal plasma glucose concentrations were also observed in milk-fed calves (Xing et al. 1988) and lactating cows (Flux et al. 1984) of high genetic merit in comparison with animals of low genetic merit.

Plasma insulin concentration tended to be higher in LBI heifers immediately after feeding, and the line x MER interaction was significant at intervals during the postprandial period, primarily because the LBI group offered 125% MER maintained higher insulin than the other groups. These results are in contrast with earlier studies in which higher basal plasma insulin levels were observed in HBI milkfed calves (Xing et al. 1988) and lactating cows (Flux et al. 1984) than in the respective LBI groups.

Relative to the levels at the time of feeding, the increases in plasma urea concentration were significantly greater in the HBI heifers than in the LBI heifers in the first few hours immediately after feeding (Figure 3.3). Plasma urea concentration then declined more rapidly in the HBI heifers after peak concentrations were attained (5 hours after feeding). Greater plasma urea concentrations following feeding have also been reported in lactating cows (Barnes et al. 1985) and fasted bull calves (Mackenzie et al. 1988) of high genetic merit. Conversely, lower plasma urea concentrations in calves of high genetic merit were reported (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). In the latter case, however, the urea differences were detected only when the calves were fasted for a period but not during *ad lib* feeding (Tilakaratne et al. 1980). The

mechanisms responsible for the observed patterns of plasma urea concentration are yet to be elucidated. One possibility is that differences exist in the digestive function between the two lines i.e. that dietary nitrogen was absorbed more quickly by the HBI heifers after feeding, and the lower plasma urea levels in the postprandial period were due to the relatively more emptied rumen. Alternatively, more urea may have been recycled back to the rumen during the postprandial period in this group. Another possibility is that differences in urea distribution space existed between the HBI and LBI groups, with LBI heifers maintaining a greater urea space relative to the HBI heifers. Thus, the higher plasma urea level in the HBI group at the time of feeding may have been due to its distribution into a relatively smaller space. A smaller urea space would also have favoured the more rapid disappearence of urea in the postprandial period since a greater proportion of the space would be cleared by the kidney per unit time.

No differences were observed between the HBI and LBI heifers in plasma glucose clearance following insulin or glucose challenges, or in release of pancreatic insulin in response to exogenous glucose. Thus the results demonstrate that the pancreatic B-cell sensitivity to acute stimulation with glucose and the response of peripheral tissues to endogenous insulin were apparently similar for the two lines under the present experimental conditions. An earlier report showed that insulin response to arginine challenge was greater in milkfed HBI calves (Xing et al. 1988). It may be that arginine is a more effective stimulus than glucose for highlighting differences in pancreatic sensitivity between the two lines of heifers. It may be equally possible that the dose of insulin used in this study was too high and evoked maximum peripheral tissue responses, rather than a physiological response, to insulin. Another possibility is that the pancreatic responses to glucose and glucoregulatory effect of insulin in the animals were affected by physiological state. For example, the stage of the oestrous cycle, which was not controlled in the heifers used in the present study, can affect glucose and insulin metabolism in cattle (McCann and Reimers 1986).

Selection line did not influence the glycogenolytic response of

the heifers to adrenaline or glucagon challenges. However, HBI calves offered 75% MER had greater lipolytic responses, as measured by NEFA concentration in the plasma, to adrenaline than the other three groups. This lends further support to the notion that high genetic merit cattle have an ability to mobilise more body energy reserves at times of various nutritional challenges such as during fasting (Tilakaratne et al. 1980) and during peak lactation (Davey et al. 1983). The mechanisms responsible for the increased lipolytic response to adrenaline are still unknown. A recent study by Smith and McNamara (1988) showed that activity of hormone sensitive lipase was greater in high genetic merit cows although this comparision was confounded with differences in energy balance. Similarly, high yielding cows treated with somatotropin (GH) showed both increased blood NEFA concentration and enhanced lipolytic response to adrenaline (Sechen et al. 1989). However, differences in GH concentrations were not detected between the two lines in this study although a more frequent sampling regime would be required to establish the point unequivocally. It is also of interest to compare the present results with those of Bridges et al. (1987) who observed a greater plasma glucose response, but not lipolytic response, to adrenaline in HBI heifers. The discrepancy in the results between the two studies might have arisen because of differences in the feeding regime. Feeding was once daily in the present study whereas, in that of Bridges et al. (1987), the heifers were fed more frequently and challenged while they still had access to feed. Studies by Blum et al. (1982) and Frohli and Blum (1988b) showed that NEFA response to adrenaline in dairy cattle was minimal following feeding but increased markedly during fasting. Thus responses of LBI and HBI cattle to the lipolytic and glycogenolytic effects of adrenaline might be influenced by time from feeding as well as by chronic effects of energy allowance.

In summary, the divergent energy balances employed in the present experiment, alone or in combination with metabolic challenges, produced substantial differences in many plasma parameters. There was evidence for a line x allowance interaction in NEFA response to adrenaline, the line differences being greatest at 75% MER. Differences were also apparent between the lines in the changes in plasma glucose and urea levels which occurred after feeding. These differences were not

affected by energy allowance and were most apparent when 'baseline' concentrations (those at the time of feeding) were corrected for. Clearly, such baseline (prefeeding) values need to be more clearly defined in future studies by improving the sampling regime.

CHAPTER FOUR: METABOLIC DIFFERENCES BETWEEN HIGH BREEDING INDEX AND LOW BREEDING INDEX HEIFERS TREATED WITH PROGESTERONE-CIDRS: BASAL METABOLITE AND HORMONE CONCENTRATIONS AND DOSE EFFECTS OF GLUCOSE AND INSULIN CHALLENGES ON METABOLIC RESPONSES

CHAPTER FOUR: METABOLIC DIFFERENCES BETWEEN HIGH BREEDING INDEX AND LOW BREEDING INDEX HEIFERS TREATED WITH PROGESTERONE-CIDRS: BASAL METABOLITE AND HORMONE CONCENTRATIONS AND DOSE EFFECTS OF GLUCOSE AND INSULIN CHALLENGES ON METABOLIC RESPONSES

ABSTRACT

The effects of genetic selection for milk production on metabolic physiology were examined in 8 HBI and 8 LBI Friesian heifers treated with progesterone in a two-period experiment. In the first period, the basal plasma concentrations of GH, insulin, glucagon, glucose, NEFA, urea and creatinine were measured over a 6 hr intensive sampling period (at 10 min intervals). In the second period, the dose effects of intravenous glucose (0, 75, 150 and 300 mg/kg lwt) and insulin (0, 0.1, 1, and 10 µg/kg lwt) challenges on metabolic responses were tested in a split-plot design carried out over a period of 8 days.

Basal plasma urea and creatinine concentrations were marginally greater ($P<0.10$) in the LBI heifers than in the HBI heifers but no differences were found between the two lines in plasma concentrations of GH, insulin, glucagon, glucose or NEFA. No significant line differences were found in the number of secretion spikes or the magnitude of the spikes for basal plasma GH or insulin during the 6 h period.

The effects of dose of either glucose or insulin on plasma concentrations of insulin, glucose and NEFA were significant. There were significant differences between the two lines in insulin secretion in response to glucose challenges. The HBI heifers released more insulin than the LBI heifers in the initial period (0-12 min following the challenge), and this difference was independent of glucose dose. Lower plasma glucose concentrations in the HBI than in the LBI heifers after glucose challenges were apparently due to the secondary effect of the greater hyperinsulinaemia in the former group. Analysis of the disappearance of glucose from the plasma showed that the glucose elimination rate constant (k) and glucose concentration at time zero (C_0) were greater in the HBI heifers than in the LBI heifers, whereas the volume of glucose distribution (Vd), the distribution coefficient

(Δ) and the half-life ($t_{1/2}$) of the injected glucose were greater for the LBI than for the HBI heifers.

Plasma insulin concentrations after insulin challenge were slightly higher in the HBI heifers than in the LBI heifers, whereas plasma glucose and NEFA level did not differ between the lines after the challenge. No significant interactions of line x dose in plasma metabolites and hormone concentrations were observed after either glucose or insulin challenges.

These results showed that while the basal plasma concentrations of metabolites and hormones were not significantly influenced by genetic selection for milk production, the pancreatic insulin secretion and glucose disappearance in response to glucose challenge were significantly greater in HBI than in LBI heifers treated with progesterone.

I. INTRODUCTION

The physiological responses of two lines of Friesian heifers, one of high genetic merit and the other of low genetic merit, to two levels of feeding and to various metabolic challenges were investigated in the previous study (Chapter 3). Some of the results for insulin metabolism in that study were at variance with earlier reports on lactating cows (Flux et al. 1984), new born calves (Xing et al. 1988) and 6 month old bull calves (Mackenzie et al. 1988). For example, plasma insulin concentrations were greater in the high breeding index (HBI) groups than in the low breeding index (LBI) groups in the three earlier studies and the response of pancreatic insulin secretion to arginine infusion was higher in the HBI newborn calves than in the LBI calves. In contrast, in the experiment described in Chapter 3, plasma insulin concentrations were similar in heifers from the two lines irrespective of the conditions applied (such as metabolic challenges and variation in feed allowance), which were intended to accentuate the differences between the lines. One possible explanation is that insulin metabolism is influenced by the physiological state of the animal. The earlier studies were carried out on pregnant cows (Flux et al. 1984) or pre-pubertal calves (Xing et al. 1988) whereas some of the heifers in

Chapter 3 had reached puberty and stage of the oestrous cycle may affect insulin and glucose metabolism in cattle (McCann and Reimers 1986). Furthermore, only single doses of glucose or insulin were given (Chapter 3) and the possibility of a line x dose interaction exists, i.e. the magnitude of line differences may be dependent upon the dose of metabolite or hormone administered.

Another inconsistency between studies, with regard to the selection line effect, is in respect to plasma GH concentration. There are reports that plasma GH concentrations are higher in dairy cattle of high genetic merit (Kazmer et al. 1983; Barnes et al 1985; Xing et al. 1988), but the differences were not always significant in the Massey University selection lines (Flux et al. 1984; Chapter 3). These latter results may be due, in part, to insufficient frequency of sampling to detect differences between the lines.

Therefore, the first objective of this study was to determine the selection line effect on the basal concentrations of plasma metabolites and hormones, especially GH and insulin, by applying a frequent sampling regime. The second objective was to evaluate the effect of doses of glucose or insulin used in the challenge experiments on the metabolic responses of the heifers from the two lines. For this study, possible effects of the stage of the oestrous cycle were controlled by treating all heifers with progesterone.

II. MATERIALS AND METHODS

1. Animals

Sixteen 6-month old Friesian heifers, 8 born to parents of high breeding index (HBI) and 8 born to parents of low breeding index (LBI), were used in this experiment. They originated from the same lines as those used in earlier studies (Davey et al. 1983; Flux et al. 1984; Chapter 3) and were raised on the Dairy Cattle Research Unit at Massey University. The estimated mean BI based on ancestry information was 135 $\pm$ 2 (Mean $\pm$ S.D.) for the HBI group and 111 $\pm$ 2 for the LBI group. Live weight at the start of the experiment was 132 $\pm$ 7 kg and 137 $\pm$ 14 kg for HBI and LBI heifers respectively.

2. Experimental procedures

Prior to the experiment, the heifers had been grazing ad libitum on mixed ryegrass/white clover pasture with access to hay supplements (due to the fact that pasture growth had been affected by a dry season). The heifers were then housed indoors for 16 days to adjust to the changes in conditions, including feeding. Heifers were fed mixed pasture hay (predominantly perennial ryegrass/white clover) at 75% maintenance energy requirement (MER). Maintenance was calculated as 0.55 MJ ME/$kg^{.75}$ (Holmes and Wilson 1984) and the hay was assumed to contain 8.6 MJ ME/kg DM at 85% DM (Holmes and Wilson 1984). Hay was offered once daily at 1600h throughout the experiment and the heifers had free access to clean water.

Oestrous cycling of the heifers was controlled by inserting intravaginally a progesterone-impregnated controlled internal drug releaser (CIDR, Type B, EAZI-breed, AHI Plastic Moulding Co., Hamilton, New Zealand) on day 11 of the adjustment period. Jugular cannulae were inserted into the heifers under local anaesthesia on day 17, one day before the intensive blood sampling started.

The experiment was conducted in two periods. In period I, a 6 hour intensive blood sampling regime was designed to measure the profiles of GH secretion as well as other metabolite and hormone concentrations. Blood samples were collected into pre-chilled centrifuge tubes, containing 100 ul of 35% (w/v) sodium citrate, at 10 minute intervals for 6 hours, beginning at 1000 h. Plasma was harvested and aliquoted into vials after centrifugation. Aprotinin (Sigma No. A-6279 Lot No. 47F-8020) was added to 1 ml aliquots of the plasma samples, collected at 10, 70, 130, 190 and 270 and 330 minutes relative to the start of sampling, at 1000 KIU/ml equivalent to trasylol, to inhibit proteolytic activity for the glucagon assay. Plasma samples were stored at -20°C before being analysed. Animals were allowed one day's rest before being sampled in period II.

Period II involved administration of intravenous glucose and insulin challenges. Heifers were divided into 2 blocks (balanced for BI) with block 1 receiving glucose followed by insulin and block 2 receiving the reverse sequence. Within each block, heifers received glucose or insulin challenges in a 4 x 4 Latin Square arrangement (replicated in BI). Four glucose doses (0, 75, 150 and 300 mg/kg) and 4 insulin doses (0, 0.1, 1 and 10 µg/kg) were administered, one on each day. Thus the combination of challenges (4 glucose followed by 4 insulin or vice versa) took a total of 8 days.

Insulin and glucose were prepared for the challenges as follows. Insulin (Sigma Chemical Company, St Louis, Mo., USA, Cat. No. I-5500, 23.4 IU/mg) was dissolved in 3% bovine serum albumin (Sigma Cat. No. A-8022, Fraction V) in physiological saline to the appropriate concentrations and volumes (7-10 ml/animal) and administered as an intravenous injection over 10-20 seconds. Glucose (0.4g/ml, prepared for intravenous infusion by National Dairy Association, Palmerston North, New Zealand) was diluted with saline and infused (50 to 100 ml/animal) into the heifers within 2 minutes. Zero dose involved injections of sterile saline. The challenges were conducted in two batches, at 1000 h or 1200 h on each day, and blood samples were collected at -15, -10, -5, 4, 8, 12, 16, 20, 30, 40, 50, 60, 75, 90, and 120 minutes relative to glucose/insulin injections. Samples were collected into pre-chilled centrifuge tubes, containing 100 ul of 35% (m/v) sodium citrate, and processed as described above.

3. Blood analyses

Urea and creatinine concentrations in plasma were analysed by the autoanalyser methods of Marsh et al. (1965) and Chasson et al. (1961), respectively. Glucose was determined enzymatically by the method of Rosevear et al. (1969). Plasma non-esterified fatty acid (NEFA) concentrations were determined colourimetrically using NEFA assay kits (Wako Pure Chemical Industries Ltd, Japan) based on the modified procedure of McCutcheon and Bauman (1986). The intra- and inter-assay coefficients of variation for glucose, urea, creatinine and NEFA were 1.4, 3.8; 1.3, 1.9; 0.4, 1.0; and 2.6, 3.8% respectively. Plasma insulin and GH were assayed using the double-antibody

radioimmunoassays previously described by Flux et al. (1984). Bovine crystalline insulin (Sigma No. I-5500, Lot No. 55F-0536, 23.4 IU/mg) was used for iodination and as the reference standard in the insulin RIA. Bovine GH, (USDA-bGH-I-1, Lot No. AFP 6500, 3.2 IU/mg) and (USDA-bGH-B1, Lot No. AFP-5200. 1.9IU/mg), were used for iodination and as reference standards respectively in the GH assay. The intra- and inter-assay coefficients of variation for insulin assay were 8.2% and 12.4% and for GH were 8.6% and 13.2%, respectively.

The insulin assays were initially conducted in such a manner that samples from 2 heifers from each selection line at all the 4 doses were included in each assay. The results of the assays showed that insulin values in samples from the high and low dose challenges samples were often beyond the maximum (12.8 ng/ml) and minimum (0.05 ng/ml) detection limits of the assay.

Plasma insulin concentration in samples taken after injections of medium (1 µg/kg) and high doses (10 µg/kg) of insulin, that were greater than the highest insulin standard (12.8 ng/ml), were then reassayed after serial dilution with 3.5% bovine serum albumin (BSA, fraction V, Sigma) in 0.01 M phosphate-buffered saline (PBS). When the samples were diluted at ratios up to 1:4, they exhibited parallel displacement of ^{125}I insulin standards. However, lack of parallelism occurred when the samples after high-dose insulin injection were diluted at more than 1:4 (i.e. at 1:8 and above) dilution factor (Appendix I). Thus, only the insulin in samples after the medium-dose insulin challenge could be satisfactorily assayed.

Plasma glucagon concentration was determined by the double antibody radioimmunoassay method described in Chapter 2. Bovine glucagon (Sigma No. G4250, Lot No. 65F-0674) was used as the antigen for raising glucagon antisera and as the reference standards. A different source of bovine Glucagon (Eli Lilly company, Indianapolis, Indiana, USA, Lot No. 258-253-120) was used for iodination. The glucagon antiserum was raised in a guinea pig by an immunization method similar to that of Frohman et al. (1970). When used at 1:16,000 dilution, this antiserum had an assay binding of 35% (in absence of unlabelled glucagon). The assay sensitivity was from 50 to 100 pg/ml

with a binding affinity of 3.1×10^{10} L/mol. The assay exhibited less than 1% cross-reaction with both glucagon-like peptide I (Sigma No. G-3265 Lot No. 36F08221) and bovine insulin (Sigma No. I-5500, Lot No. 55F-0536). The second antibody to guinea pig gamma globulin was raised in sheep and used at 1:40 dilution to give an excess of antibody. The iodination of glucagon was based on the Chloramine-T method (Greenwood et al. 1963) and the iodinated glucagon was purified by anion exchange chromatography (Jorgensen and Larsen 1972). The standards used contained 50, 100, 200, 400, 800, 1600, 3200, 6400 and 12800 pg/ml. The intra-assay C.V. was 10.5%. and all the samples were analysed within one assay.

4. Statistical Analysis

1) Six hour sampling

i) Basal plasma concentrations: Differences between the HBI and LBI heifers in plasma concentrations of urea, creatinine, glucose, NEFA, GH, insulin and glucagon from the intensive blood sampling period were initially analysed by ANOVA methods, with selection line as the only factor being examined. Repeated measures-analysis (MANOVA) was then used to further analyse these data to test the overall effect of selection line over the sampling period.

ii) Quantitative analysis of pulsatile GH and insulin secretion observed in the 6 hour period Apart from the estimation of basal concentrations, the patterns of GH and insulin secretion in the HBI and LBI heifers were compared in the following aspects according to the method of Santen and Bardin (1973). 1) The coefficients of variation in plasma GH and insulin concentrations over the 6 hour period. 2) The number of secretion spikes over the sampling period. A spike was identified if there was a 30% or greater increase above the preceding nadir in hormone concentration which was followed by a decline in concentration. (A 30% increase in concentration was more than three times the intra-assay coefficients of variation for both GH and insulin). 3) The magnitude of the GH and insulin spikes as the proportional and absolute increments above the preceding nadir. These parameters were then analysed by ANOVA to test the selection line

effect.

2) Analysis of the data from glucose and insulin challenges

i) Analysis of plasma concentrations of hormones/metabolites

The model for statistical analysis of the data after glucose and insulin challenges was based on the split-plot analysis of Gill and Hafs (1971):

$$Y_{ijklm} = U + L_i + B_j + LB_{ij} + S_k + LS_{ik} + BS_{jk} + LBS_{ijk} + P_l + LP_{il} + BP_{jl} + LBP_{ijl} + D_m + LD_{im} + BD_{jm} + LBD_{ijm} + SP_{kl} + LSP_{ikl} + BSP_{jkl} + e_{ijkl}$$

where Y_{ijklm} = the observed value

U = overall mean

L_i = effect of the ith selection Line (i=1, 2)

B_j = effect of the jth Block (j=1, 2)

S_k = effect of the kth Sequence (k=1...4)

P_l = effect of the lth Period (day) (l=1...4)

D_m = effect of the mth Dose (m=1...4)

LBS_{ijk} = the line x block x sequence interaction which, in the split-plot model, is the appropriate error term for testing the effects of line, block, sequence and their first order interactions.

e_{ijkl} = error term ($LBSP_{ijkl}$) used for testing the effects of period, dose and their interactions.

and interactions between the main effects are as indicated.

Challenge data from the four doses of glucose/saline or insulin/saline were initially analysed by applying the above model at each sampling time. Where differences existed between the two selection lines prior to challenge (i.e. in baseline concentrations), data were corrected by subtracting the mean prechallenge concentrations of the hormone or metabolite from observed concentrations at each of the post-challenge samples. Analyses (on the baseline-corrected data) were then performed as described above. The analyses were performed using the statistical package 'REG' (Gilmour 1985).

ii) Insulin and glucose disappearance curves after insulin and glucose injections

Insulin and glucose disappearance curves were constructed for data arising from intravenous injections of insulin (medium dose only) and glucose respectively. For insulin data after insulin injection, only those observations from 4 to 40 minutes post-challenge were used to estimate the insulin kinetics, as plasma insulin concentration in most heifers had already returned to basal concentrations by 40 minutes following the injection. The data for curve fitting were corrected by subtracting the means of the three pre-injection observations and were analysed by a non-linear, least-squares Gauss-Newton iterative method using a computer package (BMDP85). Initially, both single- and two-compartment models were fitted for these experimental data. As the single-compartment model (1) gave the best fit for the insulin data (after medium dose of insulin injection only), it was used to derive the kinetics of insulin disappearance (Ritschel 1986; Hart et al. 1980). The plasma glucose patterns after glucose challenge showed apparent oscillations in the post-challenge period especially after plasma glucose returned to its basal levels, and the oscillations were not accounted for by the single-compartment model (1). Therefore a modified single-compartment model (2), which includes a component of a triangle function $\cos(p3 \cdot t)$, that best describes the oscillations, was used to fit the glucose data after the glucose challenges (H.Varela-Alvarez, per. comm.).

$$Y = C_0 \cdot e^{-k \cdot t} \quad ----(1)$$

$$Y = p1 + p2 \cdot e^{-k \cdot t} + \cos(p3 \cdot t) \quad ----(2)$$

C_0 = p1 + p2 + 1

$t_{1/2}$ = 0.693/k

Vd = Dose injected/C_0

Δ = Vd/lwt

C = $\Delta \cdot k$

S = M · C

where for the glucose data

Y = plasma concentration (mg/dl) at time t (min)

t = time from challenge (min)

C_0 = hypothetical plasma glucose concentration (mg/dl) at t= 0, obtained by back-extrapolation of the monoexponential decay line

k = elimination rate constant (%/min)

p1= constant value (mg/dl)

p2= coefficient for the exponential component (mg/dl)

p3= an index of fluctuation in plasma glucose concentration (±%)

$t_{1/2}$ = half-life of injected glucose (min)

Vd= volume of distribution (dl, but converted to l in Table 4.3a by dividing by 10)

Δ = distribution coefficients for injected glucose (l/kg lwt) and for the insulin data, in addition to the corresponding values above,

C = metabolic clearance rate of insulin (ml/min/kg)

S = basal plasma insulin secretion rate (ng/min/kg)

M = mean of 3 pre-challenge insulin concentrations (ng/ml)

iii) Calculation of insulin and glucose response areas after glucose injection

The acute (0 to 12 minutes) and the total (0 to 120 minutes) insulin and glucose response areas above the base-line after glucose challenge were calculated using the trapezoidal method (Ritschel 1986). Initially, the maximum acute insulin secretion rate (Vmax) (0-12 min)

in response to glucose challenge and the acute glucose stimulus areas (Km) resulting 50% of Vmax were estimated using Eadie-Scatchard plots (Segal 1975). However, as the doses of glucose applied in this study were far too few for generating these parameters, the calculated Vmax and Km are excluded from further discussion.

Glucose parameters (K, $t_{1/2}$, Vd, Δ) derived for each of the 3 doses of glucose were analysed using the split-plot analysis method as described above. The insulin kinetics (K, $t_{1/2}$, Vd and Δ) derived after the injection of the medium-dose insulin (1 µg/kg lwt) were analysed by ANOVA to test the selection line effect.

III. RESULTS

1. Six hour sampling period:

The profiles of the mean plasma hormone and metabolite concentrations of the HBI and LBI heifers over the 6 hour intensive sampling period are shown in Figures 4.1-4.2.

Mean plasma GH and insulin concentrations were slightly greater in HBI heifers than in the LBI heifers (Figure 4.1 and Table 4.1) but the differences were not significant ($P>0.05$). No differences in plasma glucagon concentrations were found between the two lines.

There was no difference in plasma glucose concentration between the HBI and LBI groups even though glucose concentration appeared to be higher in the HBI than in the LBI heifers during the last 2 hours of sampling (Figure 4.1). Plasma NEFA concentration in the heifers showed a gradual increase over the 6 hour period (Figure 4.2). No significant line effect on plasma NEFA level was observed, but mean plasma NEFA concentrations were generally higher in the HBI heifers than in the LBI heifers.

Plasma urea concentrations showed a gradual increase similar to that of plasma NEFA over the sampling time and urea levels were consistently lower ($P<0.1$) in the HBI heifers than in the LBI heifers (Figure 4.2). These differences were significant ($P<0.05$) at 60, 90,

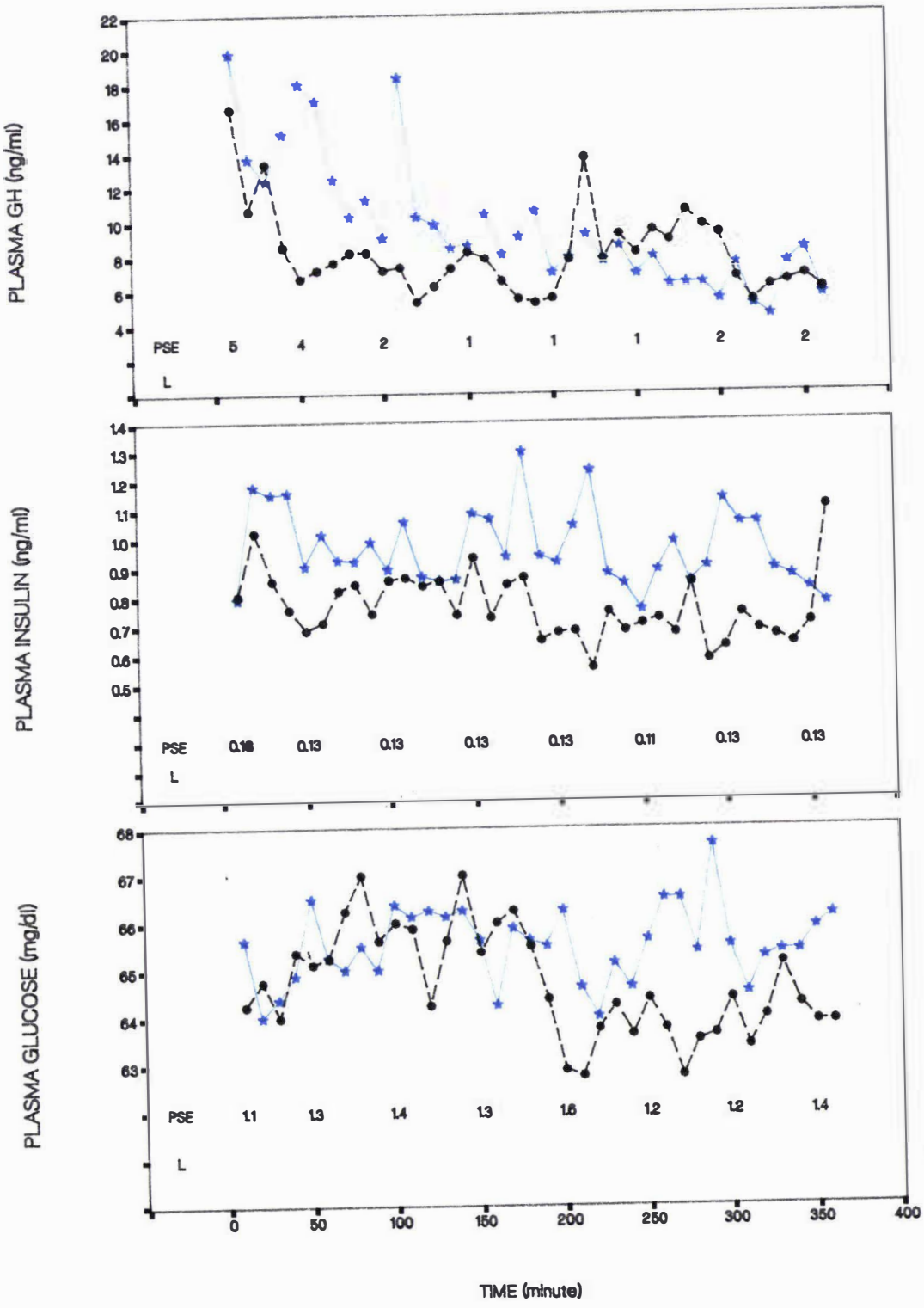


Figure 4.1 Basal plasma concentrations of GH (upper panel) insulin (middle panel) and glucose (lower panel) in 8 high breeding index (*---*) and 8 low breeding index(o---o) heifers treated with progesterone CIDRS. Heifers had last been fed 18 hours before time 0 (1000h) and remained unfed during the sampling period. (L=selection line effect, PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

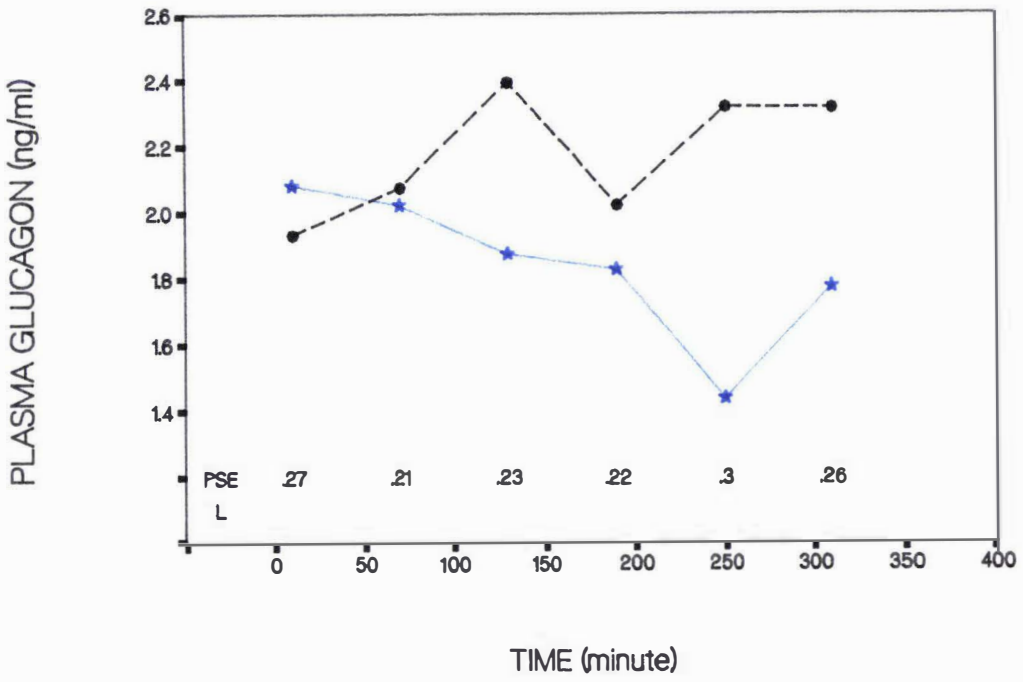


Figure 4.1 Basal plasma glucagon concentrations in 8 high breeding index (*---*) and 8 low breeding index (o---o) heifers treated with progesterone CIDRS. Heifers had last been fed 18 hours before time 0 (1000h) and remained unfed during the sampling period. (L=selection line effect. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

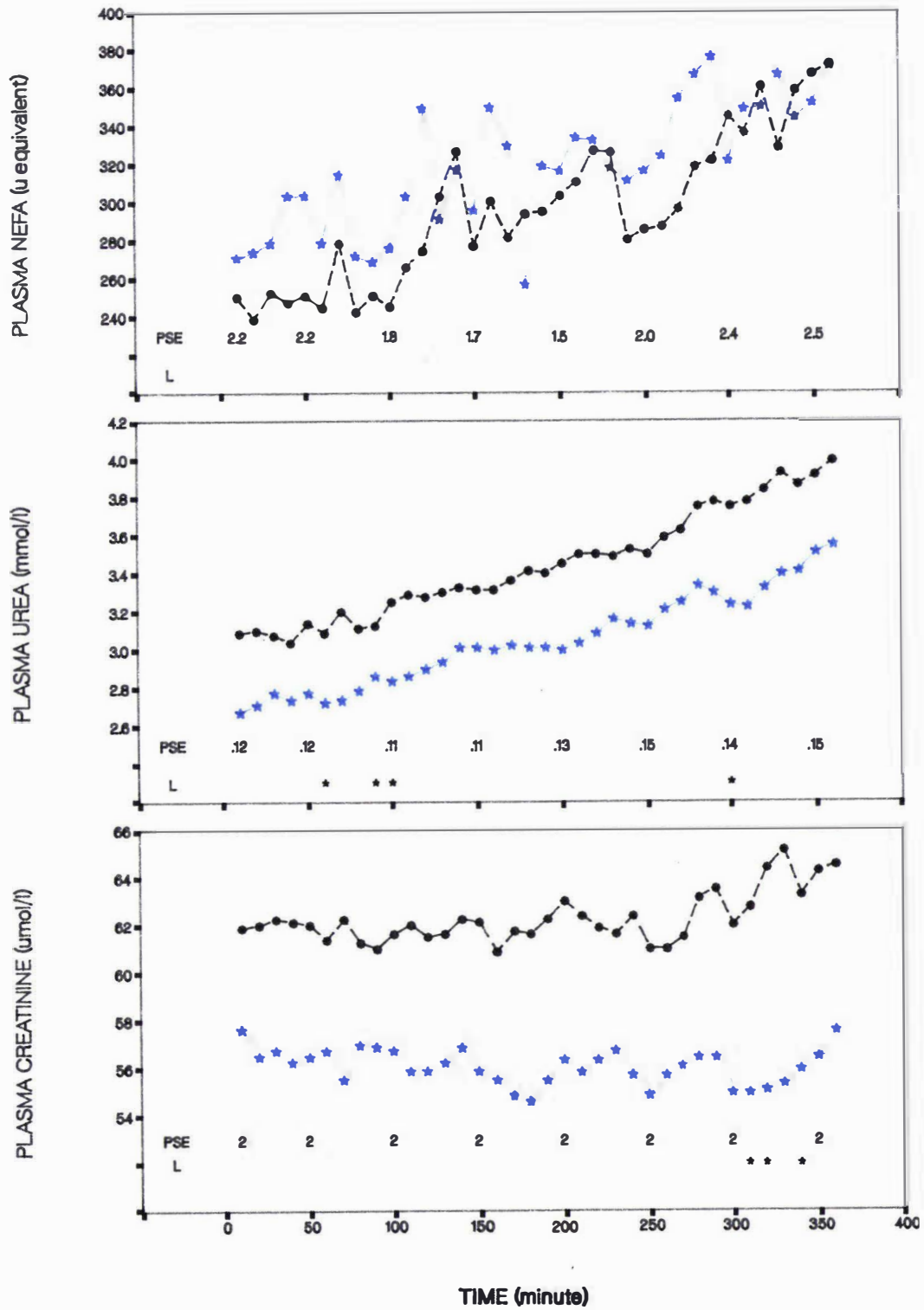


Figure 4.2 Basal plasma concentrations of NEFA (upper panel) urea (middle panel) and creatinine (lower panel) in 8 high breeding index (*---*) and 8 low breeding index(o---o) heifers treated with progesterone CIDRs. Heifers had last been fed 18 hours before time 0 (1000h) and remained unfed during the sampling period. (L=selection line effect. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

100, and 300 minutes relative to the start of sampling. The LBI heifers maintained greater plasma creatinine concentrations than HBI heifers ($P<0.1$) throughout the 6 hour period, and the differences were significant ($P<0.05$) at 310, 320 and 340 minutes relative to the start of sampling (Figure 4.2).

Table 4.1 Means and standard errors (SE) for the integrated plasma hormone and metabolite concentrations collected at 10 minute intervals over a 6 hour period in 8 HBI and 8 LBI heifers.

	HBI heifer	LBI heifer
GH (min·ng/ml)		
Mean	562.45	467.23
S.E.	144.51	79.42
Insulin (min·ng/ml)		
Mean	56.46	44.10
S.E.	13.54	9.48
Glucagon (min·ng/ml)		
Mean	9.08	10.93
S.E.	1.33	1.69
Glucose (min·mg/dl)		
Mean	3820.77	3773.16
S.E.	90.22	100.92
NEFA (min·umol/l)		
Mean	18591.00	17250.50
S.E.	1240.70	1751.36
Urea (min·mmol/l)		
Mean	326.94	363.03
S.E.	13.42	14.99
Creatinine		
Mean (min·umol/l)	1776.66	2006.87
S.E.	96.03	87.54

2. Characteristics of GH and insulin secretion

The mean values for the parameters of basal GH and insulin secretion for the HBI and LBI heifers are presented in Table 4.2, and the patterns of GH and insulin concentration over the 6 hour period for each individual heifer are shown in Appendices 2 and 3. No significant selection line effects ($P>0.05$) were found for these parameters. The absolute increase in GH and insulin spikes was slightly higher for the HBI group than for the LBI group, whereas the percentage increment of GH spikes (spike height/preceding nadir) was greater for the LBI group than for the HBI group ($P<0.10$).

Table 4.2 Characteristics of GH and insulin secretion determined from the analysis of their concentrations in plasma samples collected at 10 minute intervals for 6 hours from the HBI and LBI heifers.

	HBI		LBI	
	GH	Insulin	GH	Insulin
No. of heifers	8	8	8	8
Mean concentration (ng/ml) ± (SE)	9.73 0.62	0.96 0.05	8.10 0.45	0.76 0.03
Coefficients of Variation (%) ± (SE)	0.60 0.06	0.43 0.037	0.58 0.09	0.42 0.044
No. of Spikes/heifer ± (SE)	7.1 1.7	7.8 1.0	6.6 1.7	7.8 1.7
Magnitude of Spikes (ng/ml) ± (SE)	8.5 1.4	0.55 0.06	7.9 1.0	0.46 0.04
Percentage increase above previous nadir ± (SE)	183 28	120 11	265 39	128 13

3. Challenges

1) Glucose challenge: Plasma insulin and glucose concentrations and their acute (0-12 minutes) and total (0-120 minutes) response areas after injections of the three doses of glucose and one of saline are shown in Figures 4.3-4.5. Plasma insulin and glucose concentrations in HBI and LBI heifers were not affected by the injections of saline but increased significantly ($P<0.05$) in all heifers after each glucose injection.

Plasma glucose concentrations were slightly higher from 4 to 12 minutes postchallenge in the HBI heifers than in the LBI heifers and then decreased more quickly to a lower level ($P<0.1$) from 30 to 120 minutes after the glucose injections (Figure 4.3). The significant line x dose interactions ($P<0.05$) in plasma glucose concentration at 50, 90 and 120 minutes after glucose challenges reflected a higher concentration of glucose in the HBI line than the LBI line following saline injection whereas the order was reversed following the glucose injections. The magnitude of the between-line differences in glucose concentration increased with increasing doses of glucose.

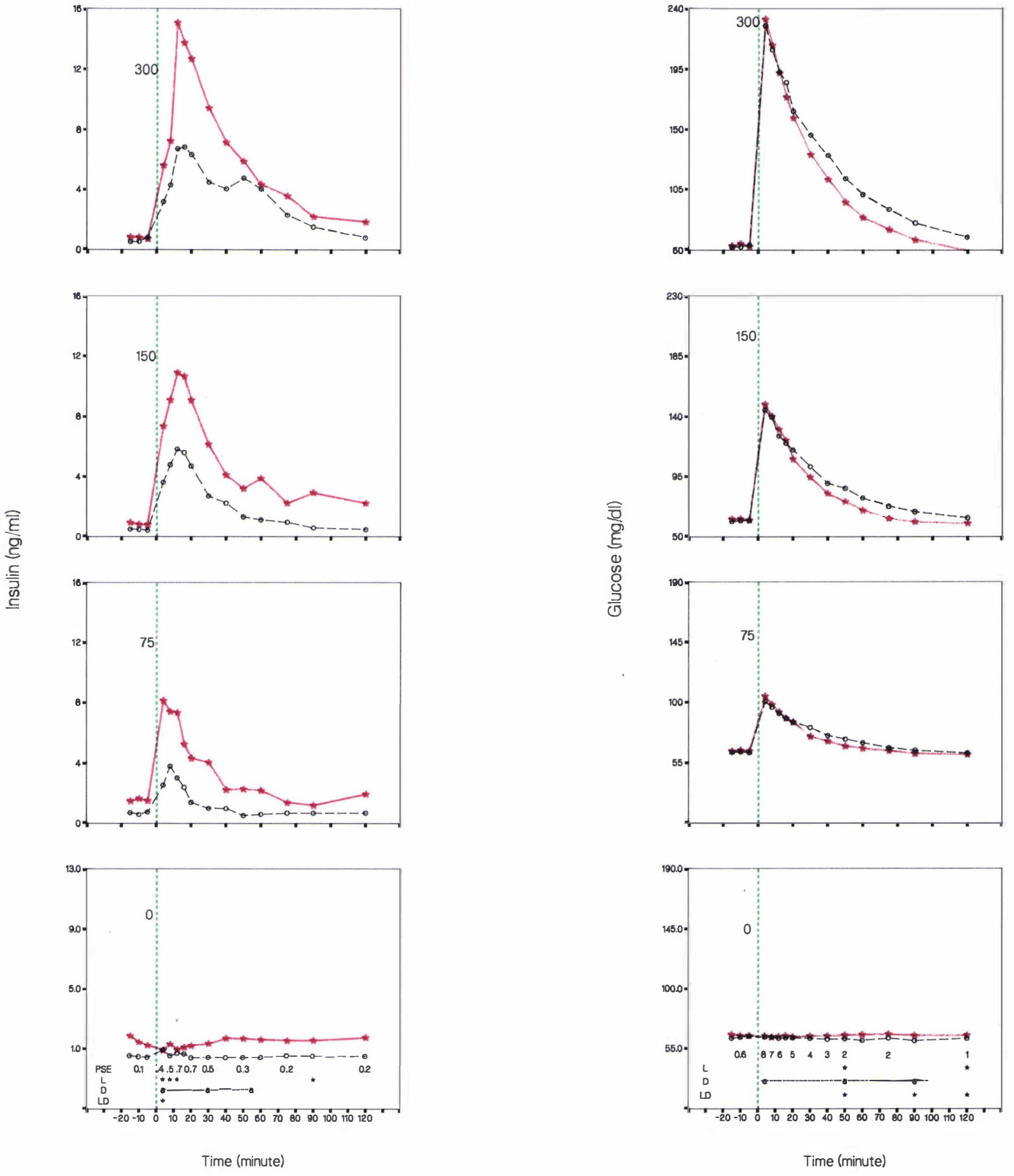


Figure 4.3 Plasma concentrations of insulin (left panels) and glucose (right panels) in response to the challenges of 0 (saline), 75, 150, and 300, mg/kg of glucose administered at time 0 in 8 high breeding index (*---*) and 8 low breeding index (o---o) heifers treated with progesterone CIDRs. (L=selection line effect, D= dose effect, LD= line x dose interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

The analysis of glucose kinetics after glucose injections revealed that the glucose concentration at time zero, C_0 (estimated by back extrapolation) and the glucose elimination rate constant, k, were greater (both $P<0.05$) for the HBI group than for the LBI group (Tables 4.3a and 4.3b). The fluctuation index of glucose concentration following glucose injection was also greater in the HBI group than in the LBI group ($P<0.10$), whereas the volume of glucose distribution, Vd, the distribution coefficients, Δ, and the half-life, $t_{1/2}$, of glucose injected were greater ($P<0.05$) in LBI heifers than in the HBI heifers.

The elimination rate constant, k, and the half-life, $t_{1/2}$, of injected glucose were not affected by the glucose dosage (Table 4.3a-b), but the distribution volume, Vd, and the distribution coefficient, Δ, were greater following the smaller doses of glucose ($P<0.05$) whereas the glucose concentration at time zero, C_0, increased with increasing dose.

The maximum insulin concentrations were observed from 8 to 12 minutes following each glucose challenge. Pre-challenge insulin levels were marginally higher ($P<0.10$) in HBI than in LBI heifers, and the post-challenge difference was significant ($P<0.05$) from 4 to 12 and at 90 minutes following the glucose challenges (Figure 4.3). The peak insulin concentration of HBI heifers was approximately 100% higher than that of the LBI heifers for each dose of glucose, and the acute and total insulin response areas were greater (both $P<0.05$, Figure 4.4) in the HBI heifers than in the LBI heifers.

Furthermore, the analysis of the data, based on the few doses of glucose challenge, showed that the HBI heifers had greater Vmax than did the LBI heifers (101 ± 21 vs 39 ± 14 ng/ml/min, $P<0.05$), whereas the Km were similar in the two lines (152 ± 140 vs 62 ± 386 mg/dl/min, $P>0.05$).

Table 4.3a Means and standard errors (SE) for various parameters describing glucose disappearance after glucose injections of 75, 150 and 300 mg/kg lwt in 8 HBI and 8 LBI heifers.

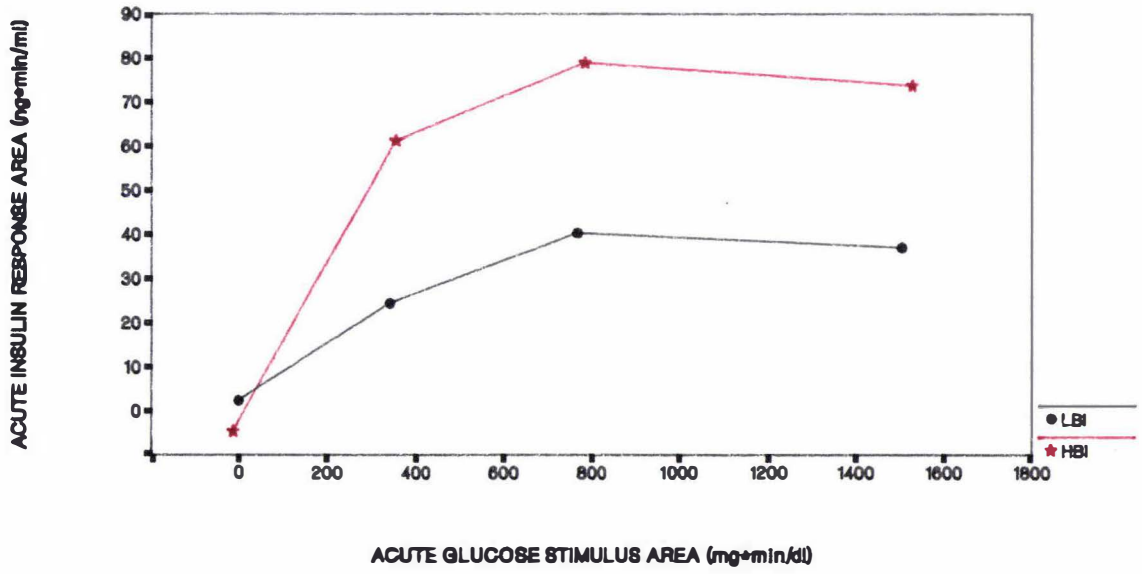
	HBI			LBI		
	75mg/kg	150mg/kg	300mg/kg	75mg/kg	150mg/kg	300mg/kg
Concentration of Glucose at t=0						
Mean (mg/dl)	48.94	102.65	195.73	41.72	93.67	181.33
S.E.	1.89	2.40	3.32	2.10	3.20	6.52
Elimination Rate constant k						
Mean (%/min)	0.042	0.035	0.033	0.026	0.027	0.026
S.E.	0.061	0.003	0.002	0.004	0.003	0.001
Fluctuation index (p3)						
Mean	-0.18	0.12	0.86	0.01	0.09	0.04
S.E.	0.10	0.11	0.05	0.04	0.12	0.42
Distribution Volume Vd						
Mean (l)	20.12	19.08	20.00	24.66	21.81	22.55
S.E.	0.64	0.39	0.41	0.84	0.54	0.63
Distribution Coefficient Δ						
Mean (l/kg)	0.155	0.147	0.154	0.183	0.162	0.167
S.E.	0.006	0.003	0.002	0.010	0.005	0.006
Half-life $t_{1/2}$						
Mean (minute)	18.62	21.46	21.55	30.26	27.13	27.12
S.E.	2.39	2.46	1.58	4.17	2.77	1.08

Table 4.3b Significance of selection line, and dose of glucose injected and line x dose effects on parameters describing glucose disappearance curves from the plasma following glucose injections of 0, 75, 150 and 300 mg/kg lwt in 8 HBI and 8 LBI heifers.

	Line	Dose	LinexDose
Elimination rate constant k (mg/dl/min)	*		
Fluctuation index p3	+	*	*
Concentration of glucose at t=0 (mg/dl)	*	**	
Volume distribution Vd. (l)	***	*	
Distribution coefficent D. (l/kg)	**	*	
Half-life t1/2 (min)	*		

+: P<0.10; *: P<0.05; **: P<0.01

Figure 4.4 (Upper panel) Relationship between the acute (0-12 min) insulin response area and the acute (0-12 min) glucose stimulus area above pre-challenge levels in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers. From left to right the glucose stimulus areas correspond to glucose doses of 0 (saline), 75, 150 and 300 mg/kg lwt.



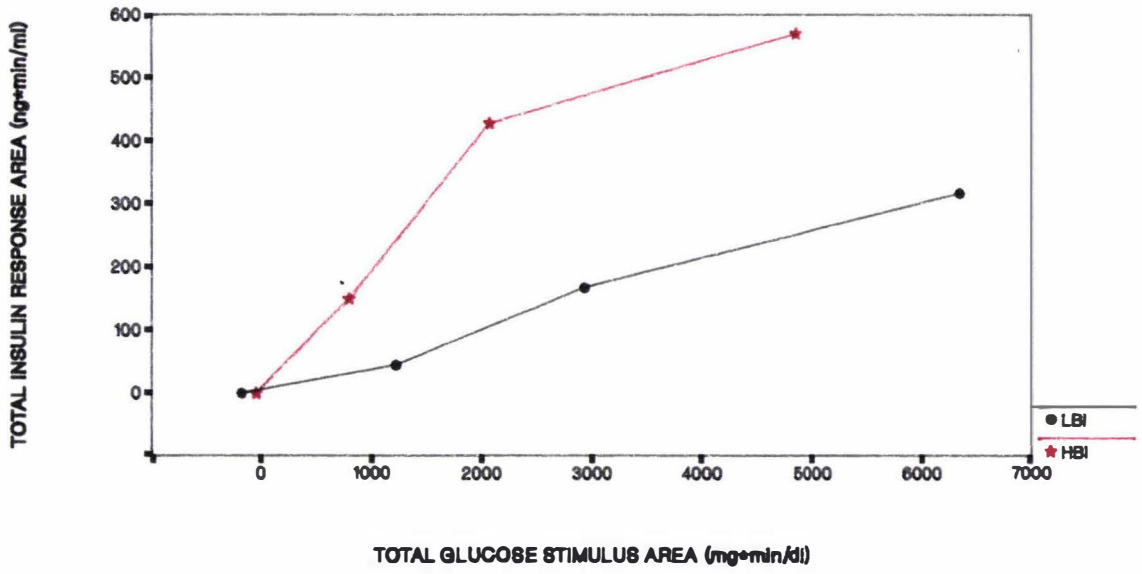


Figure 4.4 (Lower panel) Relationship between the total (0-120 min) insulin response area and the total (0-120 min) glucose stimulus area above pre-challenge levels in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers. From left to right the glucose stimulus areas correspond to glucose doses of 0, 75, 150 and 300 mg/kg lwt.

2) Insulin challenge: Plasma insulin, glucose and NEFA concentrations after insulin injections are shown in Figures 4.5-4.6.

Pre-challenge plasma insulin concentration was slightly higher in the HBI heifers than in the LBI heifers and insulin challenge (1 μg/ml) increased the difference to a significant level ($P<0.05$) at 16, 40, 60 and 75 minutes after the challenge. The analysis of insulin disappearance (Table 4.4) showed that insulin elimination rate constant, k, and metabolic clearance rate, C, were greater in the LBI heifers than in HBI heifers (by 14% and 11% respectively), whereas the half life, $t_{1/2}$, of the injected insulin and basal insulin production/secretion rates, S, were greater in the HBI group than in the LBI group (by 26% and 64% respectively). However, none of these differences was significant ($P>0.05$).

Saline injection did not affect plasma glucose concentrations, whereas the decrease in plasma glucose level was insulin dose-dependent ($P<0.05$, Figure 4.6) from 4 to 120 minutes after the insulin challenges. No significant effects of selection line or line x dose interactions on plasma glucose concentration were observed after the insulin challenges.

Table 4.4 Means and standard errors for various insulin parameters describing the insulin disappearance curves after insulin injection of 1 μg/kg lwt in 8 HBI and 8 LBI heifers.

	HBI	LBI
Basal plasma insulin (ng/ml)	0.65 ± 0.14	0.40 ± 0.08
Elimination rate constant k (fraction/minute):	0.111 ± 0.014	0.127 ± 0.010
Total distribution space (l):	8.1 ± 0.61	7.9 ± 0.62
Distribution coefficient (ml/kg):	61 ± 4	58 ± 4
Half-life (minutes):	7.2 ± 1.2	5.7 ± 0.5
Metabolic clearance rate (ml/min/kg):	6.4 ± 0.6	7.1 ± 0.4
Basal secretion rate (ng/kg/min):	4.55 ± 1.4	2.76 ± 0.5

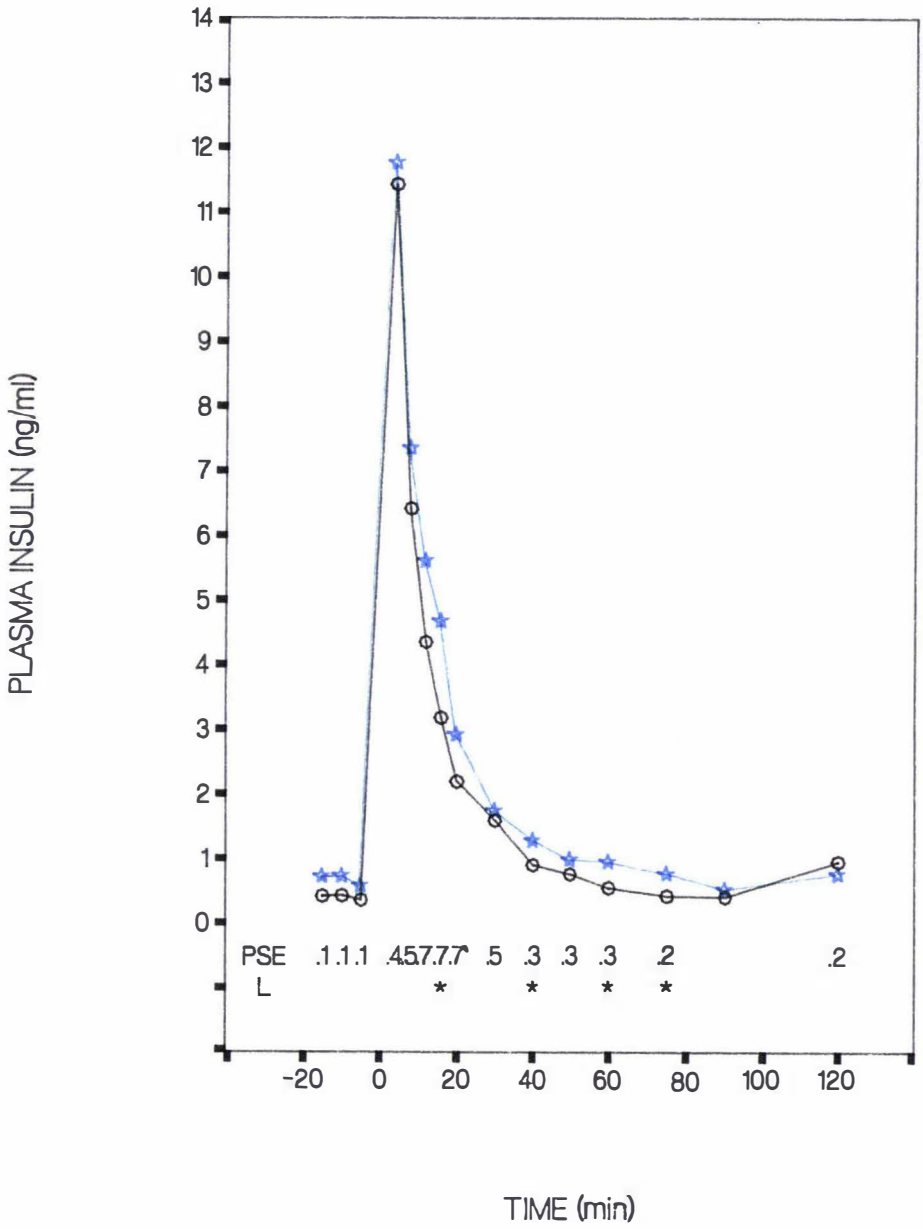


Figure 4.5 Plasma concentrations of insulin after the injection of insulin (1 µg/kg) at time 0 in 8 high breeding index (*---*) and 8 low breeding index (o---o) heifers treated with progesterone CIDRs. (L=selection line effect, PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

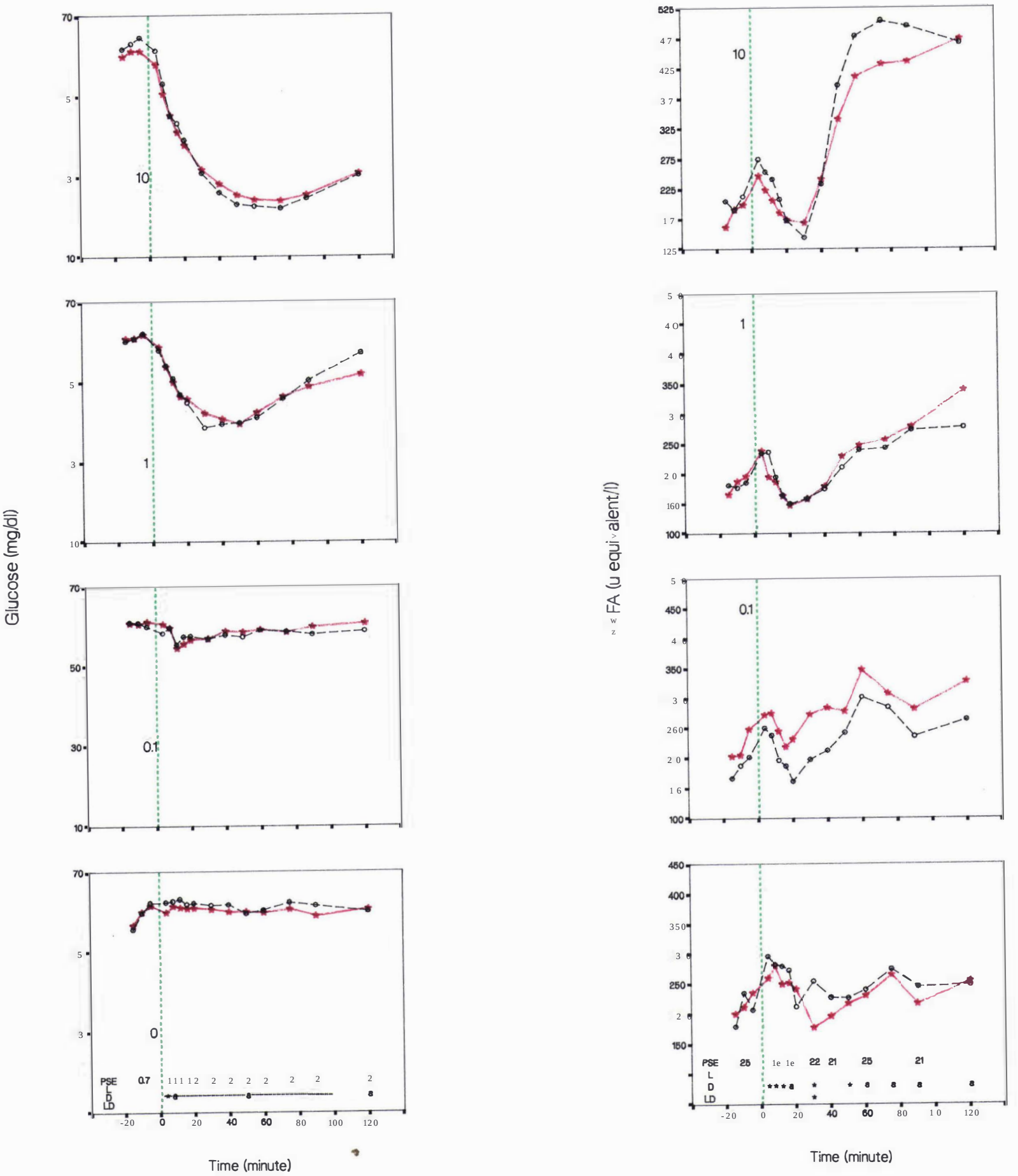


Figure 4.6 Plasma concentrations of glucose (left panels) and NEFA (right panels) in response to the challenges of 0 (saline), 0.1, 1, and 10 µg/kg doses of insulin administered at time 0 in 8 high breeding index (*---*) and 8 low breeding index(o---o) heifers treated with progesterone CIDRs. (L=selection line effect, D= dose effect, LD= line x dose interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

The response to the insulin challenges, as measured by the plasma concentration of NEFA, appeared to be biphasic for each dose, including the saline control. The initial rise in plasma NEFA was rapid and followed by a decline in concentration in all heifers. Plasma NEFA then started to increase again 40 minutes after the challenge. The dose effects of insulin on plasma NEFA were significant ($P<0.05$) at 30 and from 50 to 120 minutes after the challenge, mainly due to the very high NEFA concentrations developed in the heifers which received the high dose of insulin. Plasma NEFA concentrations were not affected by selection line. The significant line x dose interaction ($P<0.05$) in plasma NEFA at 30 minutes after insulin challenge may have occurred by chance as there was no consistent relationship between dose of insulin and magnitude of the line difference.

IV. DISCUSSION

This experiment examined the changes in basal concentrations of plasma metabolites and hormones over a 6 hour intensive sampling period and the responses, to various doses of glucose and insulin, of the HBI and LBI heifers treated with progesterone.

The galactopoietic effect in lactating cows of bovine GH either derived from the pituitary gland or by means of genetic engineering is well known (see review by McCutcheon and Bauman 1984). Therefore it is of interest that higher concentrations of GH were reported in cattle of high genetic merit than in cattle of low genetic merit (Barnes et al 1985; Kazmer et al. 1986; Lukes et al. 1988; Mackenzie et al. 1988; Xing et al. 1988). This GH difference, however, was not always persistent in young calves (Xing et al. 1988; Mackenzie et al 1988) nor was it significant in heifers (Chapter 3), lactating cows (Flux et al. 1984) from the two lines at Massey University. A possible reason for the failure to detect differences between the two Massey University lines was that the sampling regime for measuring GH concentration was inadequate for detecting a pulsatile pattern of release. The results presented in Figure 4.1 and Table 4.2 indicate, however, that even with a sampling regimen at 10-minute intervals, the difference in GH concentration between the HBI and LBI heifers was not significant. It is still possible, although unlikely, that differences in GH

concentration between the lines were missed because the intensive sampling period was too short (i.e. 6 hours).

The high GH concentration in lactating cows of high genetic merit (Barnes et al. 1985; Kazmer et al. 1986; Lukes et al. 1988) may reflect a greater energy deficit caused by increased milk production rather than being the effector of increased milk production. Energy deficit such as that brought about by fasting and undernutrition is effective in elevating blood GH concentration in many species including ruminants (Hart 1981; McDowell 1983). Although the selection line differences in plasma GH concentration have not been demonstrated in the heifers of the present study even with the adoption of different energy balance (Chapter 3), it is still possible that the line effect on plasma GH is confined to a particular range of physiological status, as short-term fasting induced a greater elevation in plasma GH level in HBI bull calves (Mackenzie et al. 1988). An alternative to measuring GH concentration is to measure IGF-1 concentration since it is less variable in blood and its production is stimulated by GH. However, since plasma concentrations of IGF-1 were not different between the two lines of young bulls (Mackenzie et al. 1988) this also supports the view that GH secretion is similar in the two lines.

The lower ($P<0.10$) plasma urea concentrations, during the post-absorptive period, in the HBI than in the LBI heifers were consistent with earlier results (Chapter 3). Lower plasma urea concentrations in high genetic merit calves were also observed when the animals were fasted for a few days (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). Such a difference in plasma urea concentration was attributed by Tilakaratne et al. (1980) to a lower degradation rate of body protein to supply energy in the high genetic merit cattle during energy deficit. However, the observation that the size and the sign of the differences in plasma urea concentrations varied over the day (Chapter 3) led to the alternative hypotheses that there may be differences between the two lines in urea pool size or in ingestive/digestive function. These hypotheses are addressed in Chapter 5.

Negative genetic correlations between plasma creatinine and milk yield have been previously reported for lactating cows (Peterson et al. 1982; Ma et al. 1985). The effect of genetic selection for milk fat yield on plasma creatinine concentration was apparent in this study. Unlike plasma urea which is influenced to a large extent by dietary nitrogen intake, plasma creatinine in animals fed a creatinine-free diet (such as in this experiment) originates from endogenous protein degradation (Waterlow 1969). Thus, the creatinine difference between the two lines may provide further evidence supporting the concept that body protein degradation in the post-absorptive period occurs to a lesser extent in the high than in the low genetic merit cattle (Tilakaratne et al. 1980). However, differences in plasma creatinine concentration may have also reflected a difference in body protein mass or a difference in rate of kidney excretion (Waterlow 1969) between the two lines. Variation in extracellular creatinine distribution may also be a plausible mechanism for the observed difference.

Greater plasma insulin concentrations in selected than in control dairy cattle have been previously observed (cows: Flux et al. 1984; calves: Xing et al. 1988; bulls: Mackenzie et al. 1988). However, plasma insulin concentrations were found to be lower in the HBI heifers than in LBI heifers after feeding (Chapter 3). The latter result may be due to a possible effect of stage of the oestrous cycle as demonstrated by McCann and Reimers (1986). In the present study, the possible effects of oestrous cycling were controlled by progesterone treatment. Basal plasma insulin concentrations were slightly greater in the HBI than in the LBI heifers, and the differences were increased following each of the glucose challenges ($P<0.01$, Figure 4.3). This result confirms the suggestion that differences in plasma insulin metabolism between the two lines are genetically determined (Flux et al.1984; Mackenzie et al. 1988; Xing et al. 1988). In that the acute release of insulin following glucose injection is a valid measure of pancreatic insulin secretion (McCann et al. 1987), and since the volumes of insulin distribution, estimated from insulin disappearance, were similar between the two lines (Table 4.4), the greater insulin level in the HBI heifers after glucose challenge was apparently due to a more active secretion/production of insulin by the pancreas in this group.

It is well established that hyperinsulinaemia causes "down regulation" of insulin receptors, primarily due to increased receptor degradation or, in some cases, due to decreased insulin binding. There is also evidence that the down regulation may involve post-receptor events (Kolterman et al. 1981; Olefsky and Ciaraldi 1981). Insulin resistance associated with hyperinsulinaemia has been demonstrated in obese heifers and sheep (McCann and Reimers 1985b, 1986; McCann et al. 1987). Therefore, the hyperinsulinaemia after glucose challenge in the HBI heifers may have been associated with a state of insulin resistance, thus requiring a greater amount of insulin to clear the same glucose load. The higher plasma insulin concentration, but similar glucose concentration, in HBI heifers compared with LBI heifers after the insulin injection (Figure 4.5) supports this notion. Greater plasma insulin concentrations following insulin injections were also observed in the high genetic merit cattle by Barnes et al. (1985).

The lower plasma glucose concentration and greater rate of glucose disappearance from blood (k) in the HBI than in the LBI heifers following glucose challenges (Figure 4.3 and Table 4.3) may have reflected an increased glucose dispersion into the target tissues, primarily liver, muscle and adipose tissue (Bergman 1983)[b] in this group. The enhanced hyperinsulinaemia would have had a stimulatory effect on glucose disappearance in the HBI heifers. It is interesting to note that glucose distribution space (Vd) was smaller in the HBI heifers than in the LBI heifers (Table 4.3). Smaller distribution could have favoured the initial glucose elimination from the circulation (presumably into urine) in the HBI heifers, especially in those receiving high doses.

The greater degree of fluctuation in plasma glucose concentration (p3) in the HBI heifers after glucose challenge may be the resultant effect of hyperinsulinaemia in this group. Fluctuations in blood metabolite concentrations, especially glucose, are known to be greater at feeding when insulin release is stimulated (Chapters 3 and 5), due to the feedback regulatory mechanisms between insulin and the absorbed nutrients. Oscillations of basal plasma glucose in man and dog have been found to be the effect of pulses of hepatic glucose output (Anderson et al. 1967; Lang et al. 1981), which may be a more

efficient stimulus for insulin release. Alternatively, the greater fluctuations in plasma glucose concentration may also reflect the entrainment, by the sustained hyperinsulinmia, of glucose utilization in the HBI heifers.

The apparently smaller glucose distribution volume in the heifers receiving the largest glucose dose may be a reflection of an increased elimination rate of glucose via the kidney rather than distribution into target tissues. This rapid loss of glucose to urine can increase the value of elimination rate, k, and the glucose concentration at time zero, C_0, thus leading to underestimates of glucose distribution. It is not clear if the differences in glucose distribution volume between the two selection lines were also affected in a similar manner.

In summary, this experiment showed greater basal concentrations of plasma urea and creatinine in the LBI heifers than in the HBI heifers, whereas the differences were not significant for other metabolites and hormones. Significant differences between the lines were also observed in pancreatic insulin secretion in response to glucose challenge, irrespective of the glucose dose. Glucose disappearance rates after glucose challenge were also significantly affected by selection line in that the distribution space was smaller and removal of glucose from blood was more rapid in the HBI group. No significant differences were observed between the lines in the responses of plasma glucose and NEFA concentrations to the insulin challenge. Thus, control of oestrous activity by progesterone treatment may have allowed the expression of differences between the HBI and the LBI heifers in pancreatic insulin release, glucose distribution and glucose removal in response to glucose challenges in a dose-independent manner.

CHAPTER FIVE: PHYSIOLOGICAL DIFFERENCES BETWEEN HIGH BREEDING INDEX AND LOW BREEDING INDEX FRIESIAN HEIFERS TREATED WITH PROGESTERONE: DIURNAL PATTERN OF PLASMA METABOLITE AND HORMONE CONCENTRATIONS, RESPONSES TO METABOLIC CHALLENGES, SIZE OF BODY FLUID COMPARTMENTS, AND RATES OF EATING

CHAPTER FIVE: Physiological differences between high breeding index and low breeding index Friesian heifers treated with progesterone: Diurnal patterns of plasma metabolite and hormone concentrations, responses to metabolic challenges, size of body fluid compartments, and rates of eating

ABSTRACT

In this experiment, various physiological parameters of 8 HBI and 8 LBI yearling heifers, treated with progesterone-CIDRs, were compared including: a) diurnal patterns of plasma concentrations of metabolites and hormones; b) volume of body fluid compartments; c) ingestive behaviour in terms of rate of eating; d) lipolytic and glycogenolytic responses to adrenaline challenge at various times after feeding and fasting; e) metabolic responses to fasting and refeeding; and f) pancreatic insulin release and glucose disappearance after glucose challenge before and 46 h after the withdrawal of progesterone-impregnated CIDRs.

Significant effects of selection line were observed on diurnal plasma concentrations of glucose, urea, and creatinine, with HBI heifers maintaining greater glucose but lower urea and creatinine levels. Plasma glucagon concentrations at the onset of feeding/refeeding were significantly greater in HBI heifers than in LBI heifers. The distribution volumes of urea (total body water), Evans blue (T1824) (plasma volume) and thiocyanate (extracellular fluid volume) were similar between the lines. Rate of eating (feed eaten/unit time) was significantly greater in the LBI heifers than in the HBI heifers on the first day of measurement, but was only marginally greater at subsequent occasions. In addition, the rate of eating fell significantly in the LBI but not in HBI heifers 28 hours after the withdrawal of progesterone-CIDRs.

The magnitude of lipolytic and glycogenolytic responses to adrenaline challenge was significantly influenced by the timing of the challenge relative to feeding and fasting. Lipolytic response to adrenaline was minimal 7 hours after feeding, and maximal after 72 hours of fasting, whereas the reverse was true for glycogenolytic

responses. There were significant line x time interactions in basal plasma NEFA concentration. HBI heifers exhibited greater elevation of NEFA levels with increasing time after feeding.

Fasting caused a significant fall in plasma concentrations of glucose, urea, insulin and glucagon, and a rise in plasma NEFA and creatinine concentrations. The subsequent refeeding induced significant rises in haematocrit, plasma concentrations of urea, creatinine and glucagon, but decreases in plasma NEFA and insulin concentrations. These responses were not affected by selection line except that glucagon concentration was briefly higher in the HBI heifers upon feeding.

Pre-challenge plasma glucose and insulin concentrations, and pancreatic insulin release after glucose challenge, were greater in HBI heifers than in LBI heifers, irrespective of the presence or absence of progesterone-impregnated CIDRs. Plasma glucose concentrations and glucose disappearance rates were similar between the two lines following glucose challenge. A significant line x progesterone presence/withdrawal interaction existed in pre-challenge plasma glucose concentrations, with HBI heifers exhibiting greater plasma glucose levels after the removal of progesterone-CIDR than LBI heifers. Glucose levels were not influenced by selection line prior to CIDR withdrawal.

This experiment demonstrated physiological differences between the HBI and LBI lines. Appropriate manipulation of the feeding regimen and control of oestrous activity by progesterone may be useful methods of maximising the expression of genetic variation in these traits.

I. INTRODUCTION

The previous investigations into physiological differences between the high breeding index (HBI) and low breeding index (LBI) heifers showed that significant differences existed in diurnal plasma urea concentrations, lipolytic response to intravenous adrenaline, and pancreatic insulin secretion in response to a glucose challenge (Chapters 3 and 4). The mechanisms responsible for these differences

and the physiological conditions required for their expression were, however, still not fully understood.

As discussed in Chapter 3, differences between the lines in urea concentration during the day could be attributed to, besides possible differences in endogenous protein metabolism, differences in rates of ingestion/digestion of dietary protein, distribution of urea in body compartments or rates of urinary excretion.

The differential response to adrenaline challenge required further study because differences in glycogenolysis, rather than lipolysis (Chapter 3), were observed between the same two lines of heifers after adrenaline challenges in an earlier report (Bridges et al. 1987). This discrepancy may have been caused by a difference in timing of the adrenaline challenges relative to feeding in the two studies. In the experiment described in Chapter 3 feeding was once daily and the adrenaline challenge was given after the heifers were fasted for 17 hours, whereas in the study of Bridges et al. (1987), the heifers were fed more frequently and challenged while they still had access to feed. A study carried out by Blum et al. (1982) showed that feeding depresses, and fasting enhances, the lipolytic response to adrenaline injection in cattle. Thus the possibility exists of an interaction between metabolic state (i.e. time relative to feeding) and effects of selection line on lipolytic or glycogenolytic responses to the adrenaline challenge.

Insulin release in response to glucose was similar in the two lines when oestrous cycles were not controlled (Chapter 3), but was greater in HBI than in LBI heifers when they were treated with progesterone to prevent cycling (Chapter 4). A study by McCann and Reimers (1986) demonstrated that basal insulin concentration in heifers varied at different stage of the oestrous cycle. Thus, it was of interest to know whether the effect of selection line on insulin release in response to glucose is affected by stage of oestrous cycle in these heifers.

The objectives of this experiment were therefore to examine variation between the two selection lines of heifers in the following aspects: 1) ingestive behaviour in terms of eating rate; 2) diurnal changes in blood metabolite and hormone concentrations, with particular reference to the effects of feeding; 3) volume of the distribution spaces for urea and other markers of body fluid compartments; 4) effect of feeding and fasting on lipolytic/glycogenolytic responses to adrenaline; and 5) pancreatic insulin release in response to glucose challenges during progesterone treatment and at subsequent oestrus.

II. MATERIALS AND METHODS

1. Animals and Management

Sixteen 12 month old Friesian heifers, 8 born to parents of high breeding index (HBI) and 8 born to parents of low breeding index (LBI), were used in this experiment. They originated from the same lines as those used in earlier studies (Chapters 3 and 4) and were raised on the Dairy Cattle Research Unit at Massey University. The estimated mean breeding indices (BI) based on ancestry information were 135 $\pm$ 4 (Mean $\pm$ S.D.) for the HBI group and 111 $\pm$ 1 for the LBI group. Mean liveweights were 205 $\pm$ 17 kg and 217 $\pm$ 11 kg for HBI and LBI heifers respectively.

Prior to the experiment, the heifers had been grazing *ad libitum* on mixed ryegrass/white clover pasture. To facilitate adaptation to indoor feeding, the heifers were deprived of feed for 24 hours before being housed indoors for an 11-day adjustment period. Heifers were fed lucerne chaff at 140% maintenance energy requirement (MER). Maintenance was calculated as 0.55 MJ ME/$kg^{.75}$ (Holmes and Wilson 1984) and the lucerne chaff was assumed to contain 9.5 MJ ME/kg DM at 85% DM (Holmes and Wilson. 1984). Crude protein content of the feed was 19.7% on a DM basis. On alternate days, 5 g of a mineral supplement (59% sodium chloride, 37% sodium sulphate, 4% sodium molybdate) was fed with the lucerne chaff to counteract possible copper toxicity. Fresh water was available *ad libitum*. Feed was offered once daily at 1200h for the first 20 days of the experiment, at 1300h on day 21 and at 1600h from day 22 till the end of the experiment (see later details).

Each heifer was inserted with an intravaginal progestrone releasing device (CIDR^{tm}B, EAZI-breed, AHI Plastic Moulding Co., Hamilton,N.Z) on day 8 to increase progesterone level and thus prevent oestrous cycling of the heifers. The retention rate of the CIDRs was 100% until the withdrawal on day 26. Jugular cannulae were inserted into the heifers under local anaesthesia on day 11.

2. Recording of eating patterns

The rate of eating by the heifers was recorded on days 1, 2, 3, 4, 6, 8, 9, 13, 25, and 26 of the experiment. To facilitate feeding and to minimise a possible effect of feeding anticipation, the feed allowance was preweighed into individual bags one day ahead and kept at a separate site until the start of feeding. Feed intake was measured by recording the weights of the individual feed bins at fixed intervals after feeding on an electronic balance (sensitivity: 1 gram). The bins were weighed in the order of feeding (alternating between heifers from the two lines). On days 1, 2, 8 and 13 bins were weighed every 20 minutes for at least 100 min after feeding, and on days 3, 4, 9, 25, and 26 bins were weighed every 40 minutes after feeding for different time periods as shown in Appendices 4-5. The process of weighing a bin and returning it to the heifer took less than 40 seconds.

3. Experimental procedures

The experiment was divided into 4 periods:

Period I: On day 12 (i.e. one day after cannulation), a 29 hour blood sampling regime commenced at 1000h and 38 samples were collected from each animal at the following times relative to the feeding at 1200h (t=0): -120, -60, -30, -5, 5, 15, 30, 60 min; then hourly until 1380 min (i.e. 1100h on day 13); then at 1410, 1435, 1445, 1455, 1470, 1500, 1560 and 1620 (i.e. -30, -5, 5, 15, 30, 60, 120, and 180) minutes relative to the commencement of feeding at 1200h on day 13. Heifers were allowed one day of rest before the commencement of the experiment in period II.

Period II: On day 15, intravenous injections of a mixed solution of urea, Evans blue dye (T1824) and sodium thiocyanate (NaSCN) at doses of 60, 1, and 20 mg/kg lwt respectively (in approximately 45-60 ml/heifer) were given to the heifers to estimate the volumes of the body fluid compartments in which they distribute. Blood samples were collected at -60, -40, -20, 4, 8, 12, 16, 20, 30, 45, 60, 90, 150, 210, 270 and 330 minutes relative to the time of challenge (1000 h). This was followed by two days rest for the animals before the start of period III.

Period III: Adrenaline challenges of 1 µg/kg lwt were given to the heifers as single pulse injections (in about 10 ml volume) on 4 occasions. The times of injection were: at 1000h (2 hours before feeding, i.e. 22 hours after the last feed) and 1900h (7 hours after feeding) on day 18; at 1000h on day 19 (22 hours after feeding); and at 1000h on day 21 (after 70 hours fasting, the daily allowance of feed having been withheld on days 19 and 20). Blood samples were collected at -15, -10, -5, 4, 8, 12, 16, 20, 30 and 45 minutes relative to the time of adrenaline injections.

Samples were also collected at 41, 46, 51 and 56 hours after feeding on day 18 (i.e. during the fasting period). The heifers were then refed following adrenaline challenges on day 21. To prevent digestive problems, half their allowances were offered at 1300h and the other half at 1700h. Blood samples were collected at -30 -5, 5, 15, 30, 60, 90, 120 and 180 minutes relative to the 1300h refeeding (started after 73 hours fasting). Some heifers did not consume all their feed allowance and most had mild diarrhoea one day after the refeeding. They recovered, however, over the following 3 days before the start of period IV.

Period IV: Intravenous glucose challenges of 150 mg/kg lwt were administered to the heifers within 2 minutes on two occasions. The first was at 1000h on day 25, (i.e. after 17 days of CIDR treatment). CIDRs were then removed from the heifers in the expectation that they would be in oestrus 40-50 hours later (Duirs et al. 1988). The second challenge was given at 1000h on day 27 (46 hours after withdrawal of the CIDR). Blood samples were collected at -30, -15, -5, 4, 8, 12, 16,

20, 24,28, 32, 36, 40, 50, 60, 70, 80, 90, 105 and 120 minutes relative to the glucose injections. The cannula of one LBI heifer blocked on day 25 and the animal had a slightly raised temperature and therefore was removed from the remaining experiments.

Preparation of the challenges: Four grams (g) T1824 (Merck Company, Cat. No. 3169, Lot No. 7476365), 240 g urea (BDH Chemicals Ltd, Analar Grade) and 80 g NaSCN (BDH, Lot No. 13478) were dissolved in physiological saline and made up to 1,000 ml before being filtered through a 2.0 um Millipore filter and aliquoted into appropriately labelled 60 ml syringes. The solution was prepared the night before the challenge and stored at 4°C until used. The adrenaline challenge was constituted by diluting adrenaline stock solution (lmg/ml, David Bull laboratories, Victoria, Australia) to 20 µg/ml with sterile saline and protected from light prior to administration by enclosing vials and syringes in aluminium foil. The glucose challenge was administered as a 0.4g/ml glucose solution (prepared for intravenous infusion by National Dairy Association, P.N., N.Z.).

Blood samples: The blood samples were collected into pre-chilled centrifuge tubes containing, as the anticoagulants, 100 ul of 35% (w/v) sodium citrate (or 1% heparin if the sample was to be assayed for progesterone or for NaSCN/Urea/T1824). Plasma was harvested after centrifugation and stored at -20°C before analysis. In the case of blood samples to be assayed for glucagon concentration, aprotinin (Sigma No. A-6279 Lot No. 47F-8020) was added to the harvested plasma at a rate equivalent to 1000 KIU/ml trasylol, to inhibit proteolytic activity.

4. Blood analyses

Plasma T1824 was determined colourimetrically (Consolazio et al. 1963, detailed procedure in Appendix 6). The standard curve was constituted by diluting the stock solution (4 mg/ml, a proportion of which had been used for the challenge) with physiological saline over a range of 4, 8, 12, and 20 µg/ml. All samples were assayed in duplicate within one assay and the intra-assay coefficient of variation (CV) was less than 1.5%. No blood samples showed haemolysis when the presence

of haemoglobin was checked by eluting the sample through a column of anionic-detergent shredded tissue paper. Plasma NaSCN was also assayed colourimetrically (Consolazio et al. 1963, detailed procedure in Appendix 7). All the samples were determined in duplicate within one assay and the intra-assay CV was less than 3% in each case.

Haematocrit was measured on blood samples collected at 150 and 210 minutes after urea/T1824/NaSCN challenge, and on samples collected at -30, -5, 5, 30, 60 and 120 minutes relative to the refeeding after 70 hours fasting, using microhaematocrit tubes, centrifuge and reader (International micro capillary reader, International Equipment Company, Boston 35, Massachusetts) following the manufacturer's instructions.

Plasma urea (Neumann and Ziegenhorn 1977; Sarre 1959; Mackoy and Mackoy 1927) and creatinine (Bartels et al. 1972; Schirmeister et al. 1964) concentrations were analysed at the Palmerston North Public Hospital using an autoanalyser (BM/Hitachi System 737, Boehringer Mannheim GmbH, Diagnostica). Plasma glucose was determined by an enzymatic colourimetric method (Trinder, 1969; Hoffmeister and Junge 1970) on a Peridochrom glucose autoanalyser (Boehringer Mannheim GmbH, Diagnostica) at Palmerston North Public Hospital. Plasma non-esterified fatty acid (NEFA) concentrations were determined colourimetrically using NEFA assay kits (Wako Pure Chemical Industries Ltd, Japan) based on the modified procedure of McCutcheon and Bauman (1986). The intra- and inter-assay coefficients of variation for urea, creatinine, glucose and NEFA were (respectively): 3.1 and 4.2%; 1.5 and 3.6%; 1.2 and 4.9%; and 2.6 and 3.8%.

Plasma insulin and GH were assayed using the double-antibody radioimmunoassays previously described (Flux et al. 1984). Bovine insulin (Sigma No. I-5500, Lot No. 55F-0536, 23.4 IU/mg) was used for iodination and as the reference standard in the insulin RIA. Bovine GH, (USDA-bGH-I-1, Lot No. AFP 6500, 3.2 IU/mg) and (USDA-bGH-B1, Lot: AFP-5200. 1.9IU/mg), were used for iodination and as reference standards respectively in the GH assay. The intra- and inter-assay coefficients of variation for GH assay were 8.6 and 13.2%, and for insulin were 8.2 and 12.4% respectively.

Plasma glucagon concentration was determined by a double-antibody radioimmunoassay method as described in Chapter 2. Bovine glucagon (Sigma No. G4250, Lot No. 65F-0674) was used for the reference standards and (Eli Lilly company, Indianapolis, Indiana, USA, lot No. 258-253-120) for iodination. The intra- and inter-assay C.V. were 13% and 18% respectively.

Plasma progesterone concentration was analysed by the radioimmunoassay method of Kirkwood et al. (1984) with minor modification. Briefly, 0.5 ml of plasma was extracted with 5 ml toluene:hexane (1:2 v/v). The plasma was frozen for 1 hour and solvent was decanted into clean tubes, dried under air and redissolved in 500 ul ethanol. Duplicate 100 ul samples of ethanol extracts were dispensed into plastic tubes and dried under air, as were duplicate 100 ul samples of standard ethanolic solutions of progesterone (P-1030: Sigma, St Louis, Missouri, U.S.A.) with a range of 6.25, 1.25, 2.5, 5, 10 and 20 ng/ml. A mixture containing: antiserum (courtesy of Dr J. T. France) at a final dilution of 1:18,000 (Tungsubutra and France, 1978); [1,2,6,7$^{3}H^{8}$] progesterone (TRK 413, Amersham, Bucks, U.K.) at 20,000 cpm/100ul; and phosphate-buffered saline containing 0.02 M EDTA and 0.1% gelatin (PBS-EG) in the ratio of 1:1:4 (by volume) was added (600 ul) to each tube and vortexed. After overnight incubation at 4°C, 600 ul of 2.5% (w/v) charcoal (Norit A; A.H.Thomas Co., Philadelphia, U.S.A.) suspension in PBS-EG was added to the tubes, vortexed and then incubated at 4°C for 10 minutes. Tubes were then centrifuged at 3000 g for 10 min at 4°C. The supernatant was decanted into scintillation vials and 5 ml toluene-triton scintillation fluid added before counting for 2 minutes in a Beckman LS 7500 scintillation counter. The assay sensitivity was 0.05 ng/ml. For the four assays performed, the intra- and inter-assay coefficients of variation were 6.3 and 13.7% respectively.

5. Statistical analysis

1) Plasma concentrations of hormones and metabolites:

Differences in plasma metabolite and hormone concentrations between the HBI and LBI heifers for the 29 hour observation period, and

those of fasting/refeeding periods were analysed by ANOVA and MANOVA (repeated measures-analysis) with selection line as the only main effect to be tested. The data from the challenges of adrenaline and glucose were analysed at each sampling time by MANOVA to test the effect of selection line, time and their interaction. The time effect in this model tests for the effects of experimental period (i.e. time relative to feeding for the adrenaline challenge or relative to CIDR withdrawal for the glucose challenge). The line x time interaction tests the hypothesis of equal selection line effects across experimental periods.

Where differences existed between the two selection lines prior to challenge (i.e. in baseline concentrations), data were corrected by subtracting the mean prechallenge concentrations of the hormone or metabolite from observed concentrations at each of the post-challenge samples. MANOVA was then applied to baseline-corrected data as described previously.

2) Disappearance curves for urea, Evans blue (T1824), thiocyanate (NaSCN), and glucose

The urea, T1824, NaSCN, and glucose disappearance curves were constructed for data arising from intravenous injections of urea, T1824, NaSCN, and glucose respectively. The data for curve fitting were corrected by subtracting the means of the three pre-injection observations and were analysed by a non-linear, least-squares Gauss-Newton iterative method using a computer programme package (BMDP85).

i) The data for urea (mmol/l), T1824 (ng/ml), and NaSCN (µg/ml) were fitted into a two-compartment model and the kinetics were calculated according to Ritschel (1986):

$$Y = B \cdot e^{-\beta \cdot t} + A \cdot e^{-\alpha \cdot t} \quad \text{----(1)}$$

where

Y: plasma concentration of urea, T1824 or NaSCN at time t (minute)

B: intercept of back extrapolated monoexponetial elimination

slope with Y axis

A: intercept of distribution slope α with Y axis

β: overall elimination (slow disposition) slope (rate constant)

α: distribution (fast disposition) slope

$C_0 = A + B$

Vd c = dose(injected)/C_0

Vd ss= $(k_{12}+k_{21})$*Vd c/k_{21}

Δ = Vd c/lwt

$K_{12} = A \cdot B \cdot (\beta-\alpha)^2 / C_0 \cdot (A \cdot \beta + B \cdot \alpha)$

$K_{21} = (A \cdot \beta + B \cdot \alpha)/C_0$

$K_{13} = C_0/(A/\alpha + B/\beta)$

$t_{1/2}\alpha = 0.693/\alpha$

$t_{1/2}\beta = 0.693/\beta$

These terms are defined as follows:

C_0: concentration at time=0 estimated by back extrapolation

Vd c: volume of distribution

Vd ss: volume of distribution at steady state

Δ: distribution coefficient

k_{12}: distribution rate constant for transfer from central to peripheral compartment

k_{21}: distribution rate constant for transfer from peripheral to central compartment

k_{13}: elimination rate constant from central compartment

$t_{1/2}\alpha$: α phase half-life

$t_{1/2}\beta$: β phase half-life (regarded as elimination half-life)

ii). The data for glucose after glucose injection was fitted into a modified single-compartment model:

$$Y = p1 + p2 \cdot e^{-k \cdot t} + \cos(p3 \cdot t) \quad ---2)$$

$C_0 = p1 + p2 + 1$

$t_{1/2} = 0.693/k$

Vd = Dose injected/C_0

Δ = Vd/lwt.

These terms are defined as follows:

Y: plasma concentration at time t (minute)

C_0: hypothetical plasma glucose concentration at time 0, obtained by back-extrapolation of monoexponential decline line

k: elimination rate constant (%/min)

p1: constant value (mg/dl)

p2: coefficient of the exponential function (mg/dl)

p3: an index of fluctuation in plasma glucose concentration (±%)

The kinetics for urea, T1824 and NaSCN were analysed by ANOVA to test the selection line effect. Glucose parameters after glucose injection were analysed using the MANOVA to test the effects of selection line, time (relative to CIDR withdrawal) and the line x time interaction. The analyses were performed using the 'SAS' statistical package (1985).

III. RESULTS

1. Plasma metabolite and hormone concentrations during the 29 hour period:

Diurnal changes in mean plasma concentrations of hormones and metabolites for the HBI and LBI heifers are shown in Figures 5.1-5.2.

There was a very rapid but short-lived increase in plasma glucose concentration at the time of feeding and then glucose level fell in all animals for the first 2 hours after feeding (Figure 5.1). Thereafter glucose level increased steadily, reaching peak concentrations at 8 to 9 hours relative to the start of feeding. Glucose concentration was generally greater in the HBI heifers and was significantly ($P<0.05$) greater at -30, -5, 5, 15, 120, 840, and 1320 minutes relative to the time of feeding on the first day, and at 60 and 120 minutes relative to the time of feeding on the second day.

Feeding resulted in a marked increase in plasma urea concentrations with the peak occurring 3 to 5 hours after feeding and being followed by a nadir occurring between 14 and 15 hours after

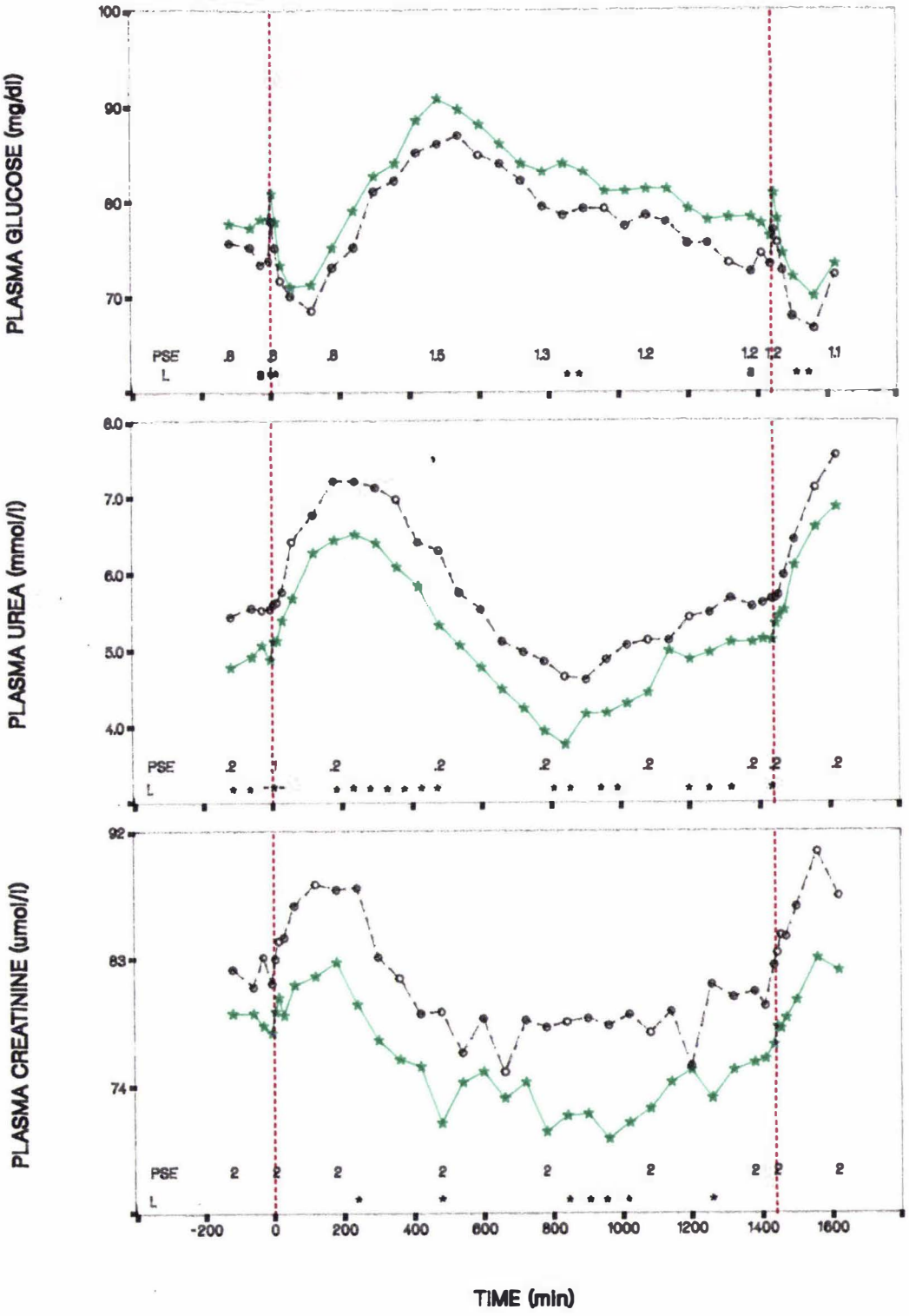


Figure 5.1 Basal plasma concentrations of glucose (upper panel) urea (middle panel) and creatinine (lower panel) over a 29 hour period in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers treated with progesterone-impregnated CIDRs. Heifers were offered lucerne chaff at time 0 and 1440 (both = 1200h) as indicated by the dotted lines. (L=selection line effect. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

feeding (Figure 5.1). Plasma urea concentrations were greater ($P<0.05$) in the LBI heifers than in the HBI heifers at most sampling times during the 29 hour period.

Plasma creatinine increased substantially in all of the heifers during the first 4 hours following feeding (Figure 5.1) and then declined. Though creatinine level was relatively lower in the HBI group than in the LBI group throughout the 29 hour period, it was significant ($P<0.05$) only at 240, 480, 840, 900, 960, 1020, and 1260 minutes relative to feeding.

The highest NEFA concentrations were observed 5 minutes after feeding, but then declined rapidly and were maintained at low values for 800 to 900 minutes after feeding (Figure 5.2). Thereafter, plasma NEFA concentration increased gradually with time. There was no line effect on plasma NEFA concentration, except at the first sample (at -120 minutes) when the concentration was greater ($P<0.05$) for the HBI group than for the LBI group.

Feeding resulted in a significantly greater response ($P<0.05$) in plasma glucagon concentration in the HBI than in the LBI heifers at 30, and 15 minutes relative to the onset of first and second feedings respectively. However this difference was very brief, and thereafter glucagon in both lines was reduced for 6-7 hours after feeding. During the postprandial period plasma glucagon concentration fluctuated in both HBI and LBI heifers and there were no differences between the lines.

2. Urea/T1824/NaSCN injection

Plasma concentrations of urea, T1824, NaSCN and creatinine after the injections of urea, T1824 and NaSCN are shown in Figure 5.3.

Pre-challenge plasma urea concentration was slightly greater ($P>0.05$) in the LBI heifers than in the HBI heifers and a similar difference was maintained following the injection of urea/T1824/NaSCN. Similarly, there were no selection line effects on plasma concentrations of T1824 and NaSCN after the injection, although the

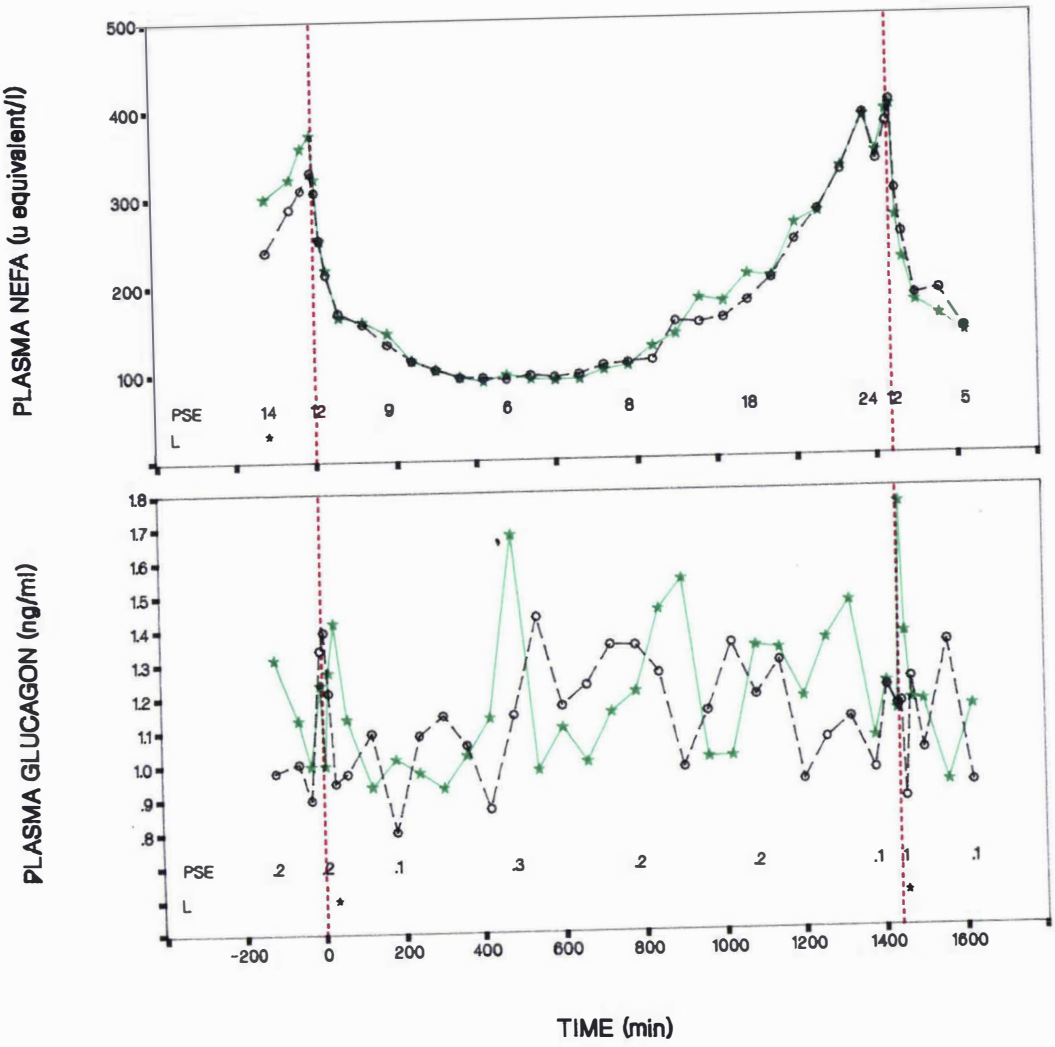


Figure 5.2 Basal plasma concentrations of NEFA(upper panel) and glucagon (lower panel) over a 29 hour period in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers treated with progesterone-impregnated CIDRs. Heifers were offered lucerne chaff at time 0 and 1440 (both = 1200h) as indicated by the dotted lines. (L=selection line effect. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

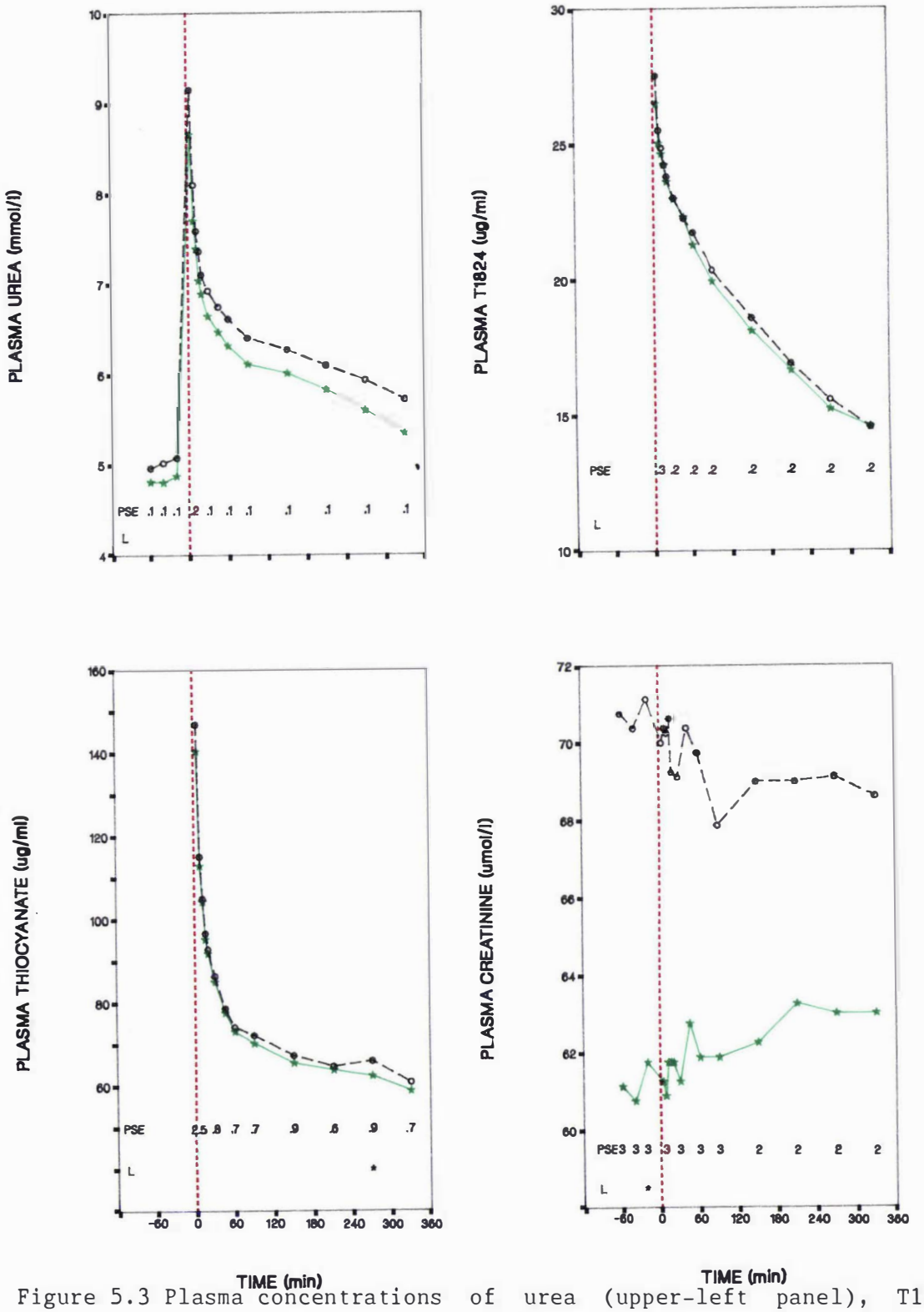


Figure 5.3 Plasma concentrations of urea (upper-left panel), T1824 (upper-right panel), NaSCN (lower-left panel) and creatinine (lower-right panel) in response to the challenges of urea (60 mg/kg lwt), T1824 (1 mg/kg lwt) and NaSCN (20 mg/kg lwt) in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers treated with progesterone-impregnated CIDRs. (L=selection line effect. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

concentrations were slightly higher in the LBI heifers than in the HBI heifers. The only significant difference ($P<0.05$) was for NaSCN at 270 min from challenge. Plasma creatinine concentration was consistently greater ($P<0.10$) in the LBI heifers than in the HBI heifers prior to and following the challenges.

The analysis of the kinetics of urea, T1824 and NaSCN disappearance using the two-compartment model (Table 5.1) showed that selection line had no effect on any of the parameters for urea, T1824 or NaSCN, nor on the volumes of plasma, body water or extracellular fluid as measured by T1824, urea and NaSCN respectively.

3. Adrenaline challenge at different times from feeding

Metabolic responses to the adrenaline challenge administered at -2, 7, 22, and 70 hours from feeding are shown in Figure 5.4.

Basal plasma NEFA concentration and its response to adrenaline challenge were significantly ($P<0.01$) affected by the timing of the challenges relative to feeding, with the greatest basal levels and lipolytic responses being observed in the heifers after 70 hours of fasting. Heifers exhibited the lowest basal plasma NEFA concentration and smallest NEFA response to adrenaline 7 hours after feeding. No differences were observed in plasma NEFA concentration and its response to adrenaline between the two periods at -2 vs 22 hours from feeding, these responses being intermediate between those at 7 and 70 hours after feeding. This is to be expected given that the -2 hour challenge occurred +22 hours after the previous feeding.

There were significant line x time of challenge interactions in pre-challenge plasma NEFA concentrations. Thus HBI heifers exhibited greater NEFA levels relative to those of LBI heifers with increasing time from feeding.

Table 5.1 Kinetics of distribution and disappearance (mean ± SE) of urea, T1824 and NaSCN in 8 HBI and 8 LBI heifers following injections of urea (60 mg/kg lwt), T1824 (1 mg/kg lwt), and NaSCN (20 mg/kg lwt).

	HBI	LBI
Concentration at t=0		
Urea (mmol/l)	5.2 ± 0.29	6.2 ± 0.50
T1824 (μg/ml)	27.3 ± 1.20	30.7 ± 3.30
NaSCN (μg/ml)	167.1 ± 6.90	181.8 ± 5.40
Distribution space (t=0)		
Urea (l)	40.28 ± 1.82	36.32 ± 2.60
T1824 (l)	7.62 ± 0.45	7.44 ± 0.53
NaSCN (l)	24.80 ± 1.00	23.95 ± 0.70
Distribution space (t=12)		
Urea (l)	80.68 ± 3.24	88.58 ± 8.68
T1824 (l)	9.15 ± 0.31	9.52 ± 0.22
NaSCN (l)	39.30 ± 0.99	41.45 ± 1.58
Distribution space (steady state)		
Urea	40.42 ± 1.94	36.43 ± 2.59
T1824	7.63 ± 0.41	7.44 ± 0.54
NaSCN	51.23 ± 1.40	52.83 ± 0.80
Distribution coefficient (l/kg, t=12)		
Urea	0.393 ± 0.012	0.408 ± 0.037
T1824	0.045 ± 0.009	0.044 ± 0.001
NaSCN	0.19 ± 0.002	0.19 ± 0.005
Distribution coefficient (l/kg, steady state)		
Urea	0.198 ± 0.011	0.168 ± 0.012
T1824	0.037 ± 0.002	0.034 ± 0.002
NaSCN	0.250 ± 0.004	0.244 ± 0.003
K_{12}		
Urea	0.003810 ± .000903	0.004415 ± .001451
T1824	0.000317 ± .000169	0.000321 ± .000139
NaSCN	0.000058 ± .000007	0.000075 ± .000012
K_{21}		
Urea	1.18 ± 0.05	1.13 ± 0.06
T1824	3.98 ± 0.68	4.83 ± 1.25
NaSCN	41.12 ± 1.56	44.58 ± 0.94
K_{13} (elim. const.)		
Urea	0.091 ± 0.0011	0.094 ± 0.0012
T1824	0.0020 ± 0.0004	0.0020 ± 0.0003
NaSCN	0.0020 ± 0.0002	0.0020 ± 0.0001
A		
Urea	3.29 ± 0.29	4.27 ± 0.48
T1824	5.16 ± 1.03	6.93 ± 2.62
NaSCN	88.56 ± 6.96	101.32 ± 5.64
B		
Urea	1.89 ± 0.06	1.94 ± 0.07
T1824	22.14 ± 0.63	23.73 ± 0.72
NaSCN	78.53 ± 1.30	80.45 ± 0.92
α		
Urea	0.138 ± 0.014	0.161 ± 0.030
T1824	0.073 ± 0.026	0.060 ± 0.014
NaSCN	0.099 ± 0.007	0.116 ± 0.009
β		
Urea	0.00357 ± .000508	0.00317 ±0.00042
T1824	0.00173 ± .000426	0.00333 ±0.00319
NaSCN	0.00094 ± .000090	0.00091 ±0.00005

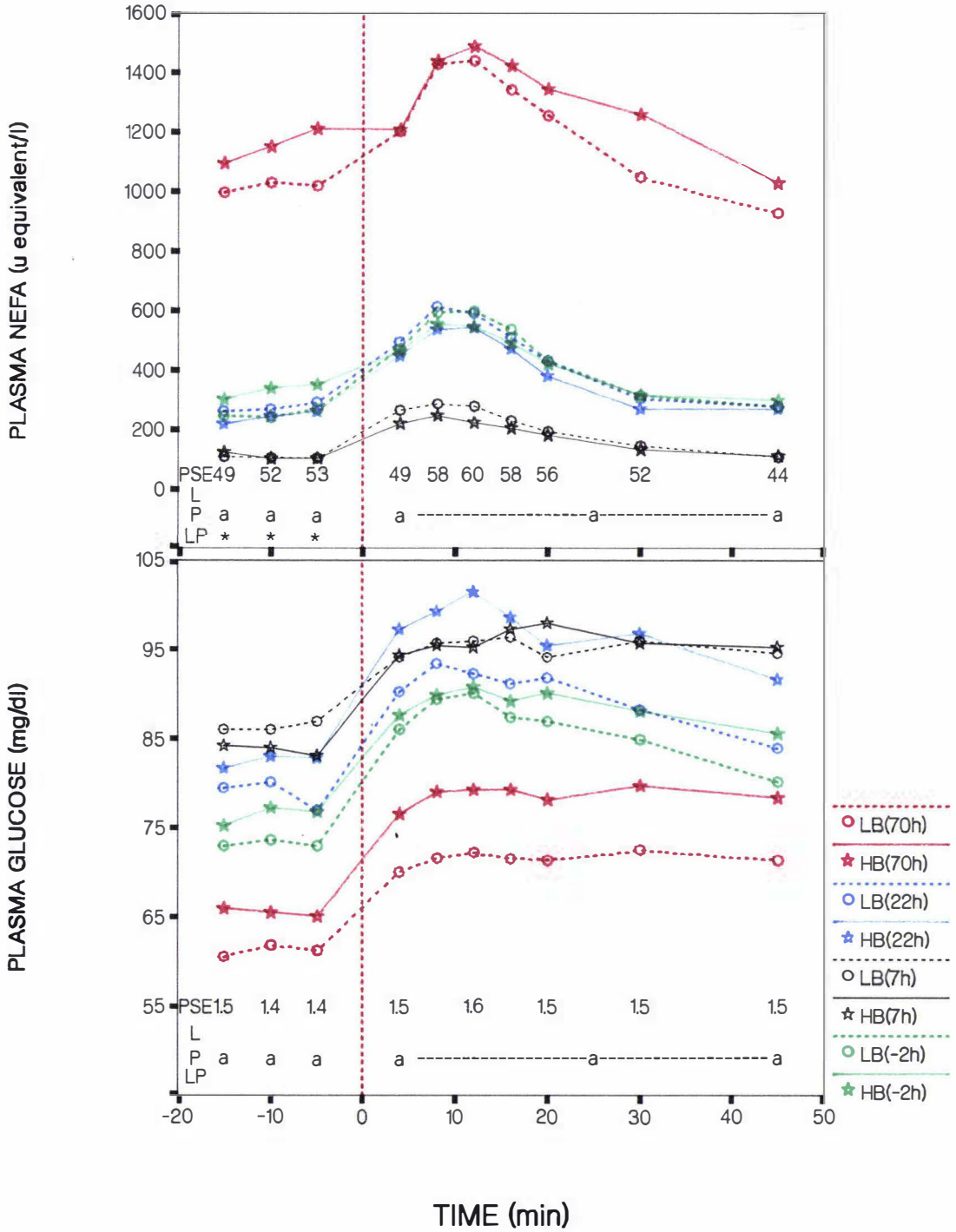


Figure 5.4 Plasma concentrations of NEFA (upper panel) and glucose (lower panel) in response to adrenaline challenge (1 µg/kg lwt) administered at -2 hr (green line), 7 hr (black line), 22 hr (blue line), and 70 hr (red line) relative to the time of feeding in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers treated with progesterone-impregnated CIDRs. (L=selection line effect, P= period effect, LP = line x period interaction. PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

The effect of timing of challenge on plasma glucose concentration and its response to adrenaline was significant ($P<0.05$) from -15 to 45 minutes relative to the challenge, and was the opposite of that on plasma NEFA concentration (Figure 5.4). Basal plasma glucose concentration and the glycogenolytic response to adrenaline were lowest in heifers fasted for 70 hours. Though plasma glucose concentration and the response to adrenaline were relatively greater in the HBI heifers than in the LBI heifers 22 and 70 hours after feeding, the effect of selection line on plasma glucose was not significant ($P>0.05$).

4. Fasting and refeeding

Changes in mean plasma concentrations of hormones and metabolites in the HBI and LBI heifers at 41, 46, 51 and 56 hours of the fasting, and during the refeeding period, are shown in Figure 5.5.

Plasma glucose concentration decreased during the fasting period in all heifers and the lowest values occurred at 46 to 56 hours of fasting. The HBI heifers exhibited greater plasma glucose levels than the LBI heifers all through the fasting period but the difference was significant ($P<0.05$) only after 32 hours of fasting. Refeeding caused a small but rapid rise in plasma glucose concentration which then declined after 5 minutes. No selection line effect on glucose concentration was observed in the refeeding period.

Plasma urea concentrations decreased during the fasting period, with the HBI heifers maintaining slightly lower ($P>0.05$) levels than the LBI heifers. Refeeding resulted in an immediate increment in plasma urea level in all of the heifers. The rise in plasma urea concentration was greater ($P<0.05$) in the HBI heifers than in the LBI heifers 5 and 15 minutes after feeding when the data were corrected for the concentrations 5 minutes before feeding.

Plasma creatinine concentrations increased by about 9% during the period from 32 to 46 hours of fasting and were relatively stable between 46 to 56 hours of fasting for both the HBI and LBI heifers. Refeeding was followed by an increase in creatinine levels. HBI

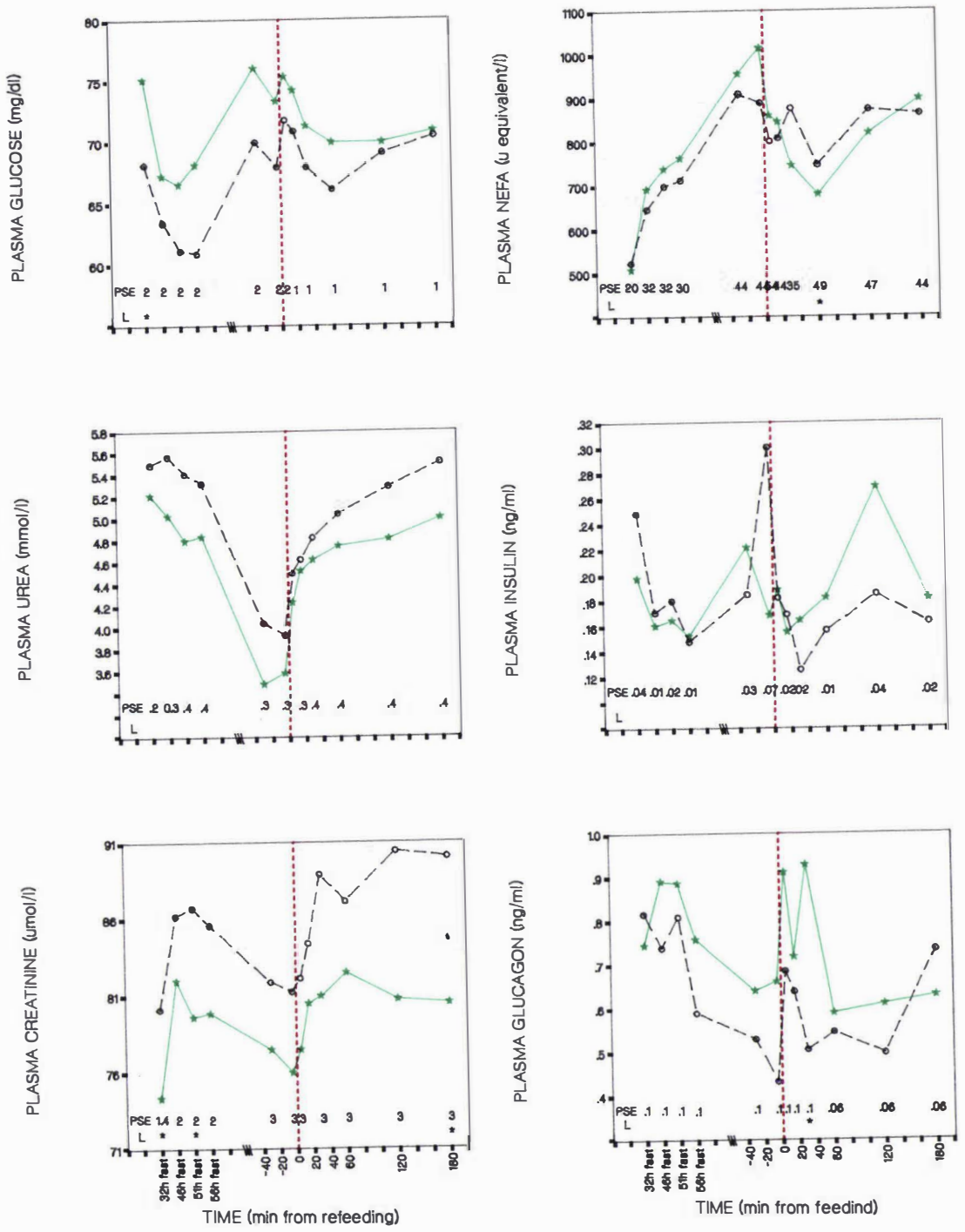


Figure 5.5 Plasma concentrations of glucose (upper-left panel), urea (middle-left panel), creatinine (lower-left panel), NEFA (upper-right panel), insulin (middle-right panel) and glucagon (lower-right panel) over the period of fasting and refeeding (indicated by dotted line) in 8 high breeding index (*--*) and low breeding index (o--o) heifers treated with progesterone-impregnated CIDRs. Heifers were offered lucerne chaff at time 0 (1300h). L=selection line effect. PSE=pooled standard error of . . 05, a=P<0.01)

heifers maintained lower creatinine concentrations throughout the periods of fasting and refeeding and the differences were significant ($P<0.05$) at 41 and 56 hours of fasting.

Plasma NEFA concentrations increased steadily over the fasting period and the highest values were recorded just before refeeding. Refeeding initially resulted in a decline in plasma NEFA concentration but a subsequent rise was observed 2 hours after feeding. Plasma NEFA concentration in the HBI group was higher during the fasting period, and lower during the refeeding period, than in the LBI group. The difference was significant ($P<0.05$) only at 30 minutes after feeding.

Plasma insulin concentration decreased to its lowest level after 56 hours of fasting in the heifers and resumed a higher level just before the refeeding, probably as a result of hyperglycaemia caused by the adrenaline challenge. Refeeding caused a decline in plasma insulin concentration during the first 30 minutes and then a small increase. No selection line effect on plasma insulin was observed during the fasting and refeeding periods.

Plasma glucagon concentration was higher in the HBI heifers than in the LBI heifers during the fasting and refeeding period, and it was significant ($P<0.05$) 30 minutes after refeeding. Refeeding stimulated an immediate glucagon release in all of the heifers.

5. Glucose challenges before and after withdrawal of CIDR

Plasma insulin and glucose concentrations following the glucose challenges administered at 2 hours before and 46 hours after withdrawal of the CIDR are shown in Figure 5.6.

There was an immediate elevation of plasma insulin in all the heifers following the glucose challenge. The response of insulin to glucose was not significantly affected by the presence of CIDR, though peak insulin was slightly greater before CIDR withdrawal than after CIDR withdrawal. Selection line had a significant ($P<0.05$) effect on basal insulin concentration and on pancreatic insulin secretion in response to glucose challenge at both challenge times, with the HBI

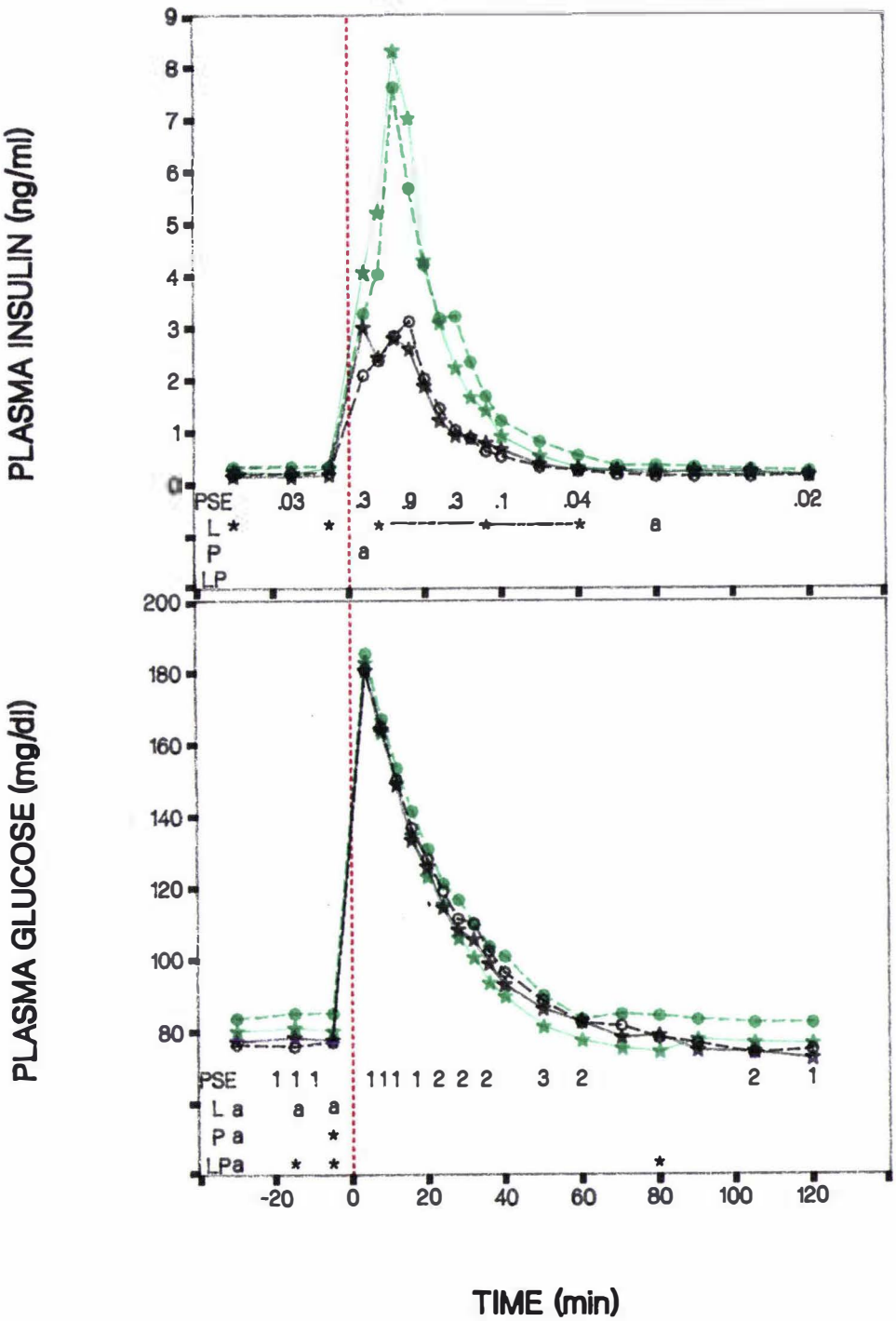


Figure 5.6 Plasma concentrations of insulin (upper panel) and glucose (lower panel) in response to glucose challenge (150 mg/kg lwt) administered at -2 hr (solid line), and 46 hr (broken line relative to the withdrawal of progesterone-impregnated CIDR in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers. (L=selection line effect, P= period effect, LP = line x period interaction. PSE=pooled standard error of the mean. *=P<0.05 P<0.01)

heifers having greater plasma insulin concentrations than the LBI heifers. The difference was significant ($P<0.05$) at -30, -5, from 4 to 60, and at 80 minutes after the glucose challenge for the analysis of combined data. No consistant selection line x time interactions were apparent (i.e. effects of period relative to CIDR withdrawal were additive with those of line). The reduced insulin release at 4 minutes after glucose challenge in the heifers after the CIDR withdrawal may be due to a chance effect.

Table 5.2 Glucose kinetics measured at 2 hours before and 46 hours after CIDR withdrawal in HBI and LBI heifers that received glucose challenges (150 mg/kg lwt)

	HBI		LBI	
	-2 hour[a]	+46 hour[a]	-2 hour[a]	+46hour[a]
Concentration at t=0				
Mean (mg/dl)	129.09	122.75	124.78	124.45
S.E.	3.71	3.53	2.11	3.59
Elim. rate constant k				
Mean (fraction/min)	0.0506	0.0463	0.0453	0.0421
S.E.	0.0039	0.0033	0.0034	0.0041
Fluctuation index p3				
Mean	-0.060	-0.028	-0.088	0.020
S.E.	0.015	0.032	0.045	0.033
Vd				
Mean (l)	24.05	25.22	26.00	26.19
S.E.	1.14	0.92	0.73	1.13
Distribution coef.				
Mean (l/kg)	0.117	0.123	0.120	0.121
S.E.	0.004	0.004	0.002	0.003
Half-life ($t_{1/2}$)				
Mean (min)	14.26	15.58	15.94	17.40
S.E.	1.08	1.18	1.36	1.79

a: Relative to time of CIDR withdrawal

A peak plasma glucose concentration of about 180 mg/dl was achieved in all heifers two minutes after glucose infusion (Figure 5.6). Glucose concentrations then declined to the pre-challenge values at about 60 minutes after the challenge. Pre-challenge plasma glucose concentrations were significantly higher ($P<0.01$) in the HBI heifers than in the LBI heifers. There were, however, no significant effects of selection line on plasma glucose level following the challenge. The effect of the presence of CIDR on plasma glucose was significant only at 30 and 5 minutes before glucose challenge, with a greater glucose value observed after withdrawal of CIDR. Significant ($P<0.05$) interactions between selection line and the presence of CIDR on plasma glucose concentration were also observed at 30, 10 and 5 minutes before the challenge (i.e. the line x period interaction was significant at these times). Analysis of glucose disappearance showed no differences

in glucose elimination rate or in the space of glucose distribution between the lines (Table 5.2). Nor were these parameters affected by the presence vs absence of CIDRs in the heifers.

6. Changes in rate of eating

The rate of eating increased as the experiment progressed. For instance it took about 180 and 120 minutes for the heifers to finish their allowance on days 1 and 8, respectively (Figure 5.7). The LBI heifers maintained a relatively greater rate of eating than the HBI heifers, in terms of amount or percentage of allowance consumed, over the most of the experiment. These differences were, however, only significant ($P<0.05$) from 40 to 120 minutes on day 1 of the observations (Figure 5.7). Though the rate of eating was not affected by the withdrawal of the CIDRs in the HBI heifers it was reduced (by approximately 20%) in the LBI heifers when the intake was measured at 80 minutes (Figure 5.8).

7. Changes in plasma progesterone concentration

Plasma progesterone concentration gradually decreased over the 17 day period after the insertion of the CIDRs (Figure 5.9). A further decrease occurred 46 hour after the withdrawal of the CIDRs, followed by a rise 8 days later. There was no significant effect of line on plasma progesterone concentrations, even though it was slightly higher in the HBI heifers shortly after CIDR insertion and 8 days after CIDR withdrawal. Of the 16 animals in the study, 2 LBI heifers exibited low plasma progesterone levels (less than 1 ng/ml) 8 days following the withdrawal of CIDRs, suggesting that these two heifers had failed to ovulate and form a functional corpus luteum.

8. Changes in blood haematocrit

Haematocrits measured at 150 and 210 minutes after urea/T1824/NaSCN challenge and at -30, -5, 5, 30, 120 minutes relative to the start of refeeding are shown in Figure 5.10. There was a significant rise (10%) in haematocrit in the heifers within 5 minutes of feeding. However, no effects of selection line were observed on the

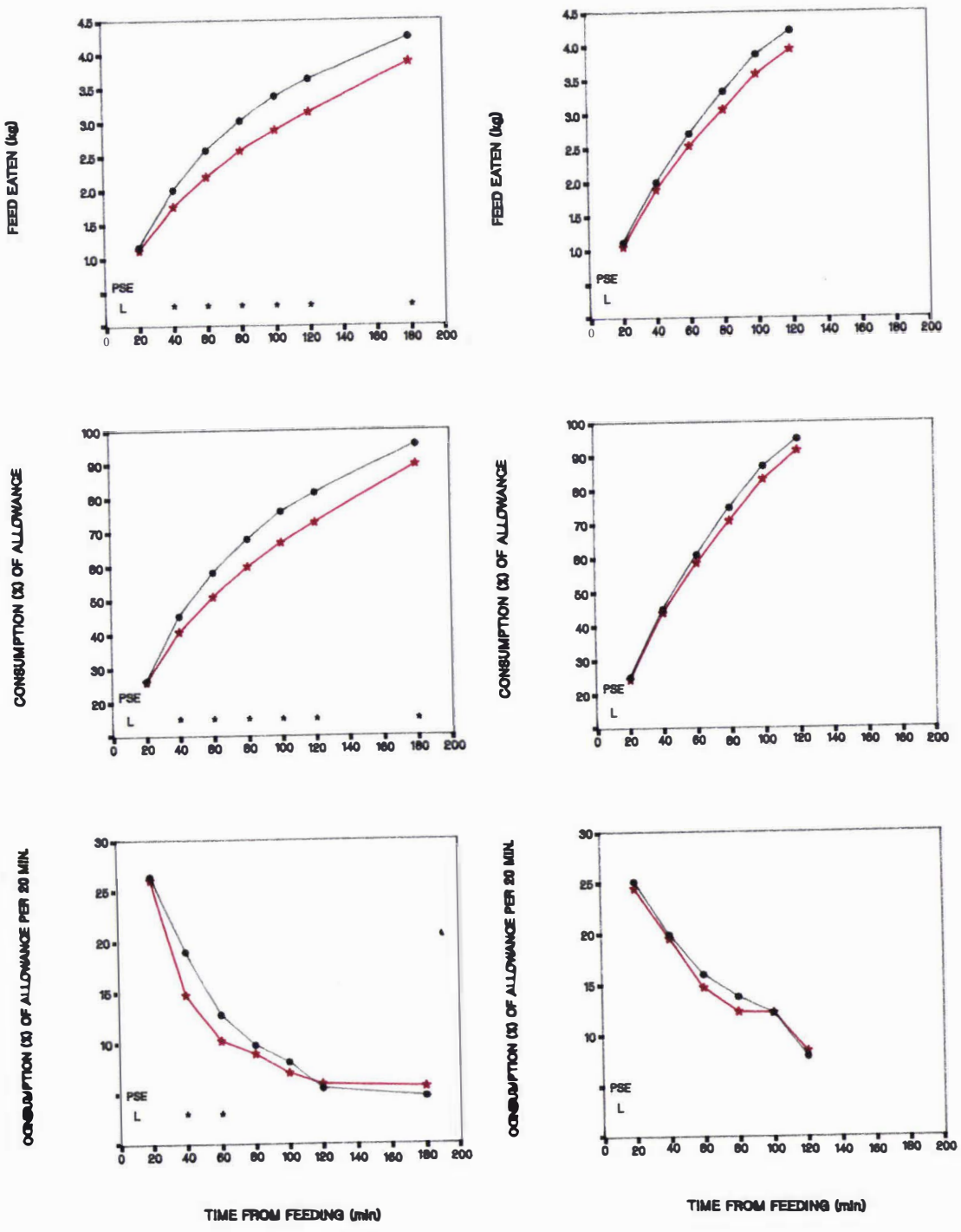


Figure 5.7 The rate of eating in terms of amount (upper panel), or percentage (middle panel) of allowance consumed per unit time, or amount of allowance consumed during successive time intervals of 20 minutes (lower panel) on day 1 (left panels) and day 8 (right panels) of the experiment in 8 high breeding index (*--*) and 8 low breeding index (o--o) heifers (L=selection line effect, PSE=pooled standard error of the mean. *=P<0.05, a=P<0.01)

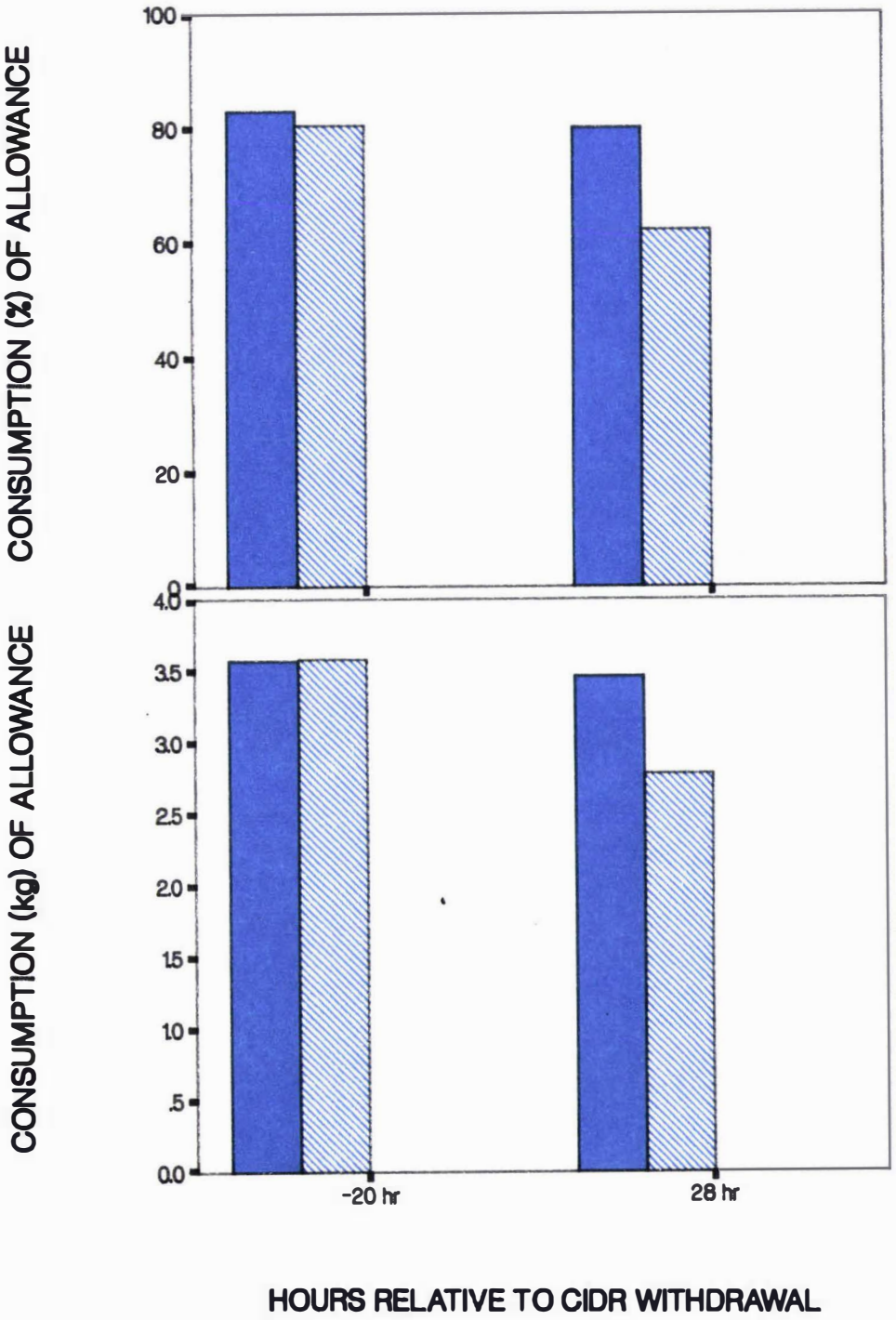
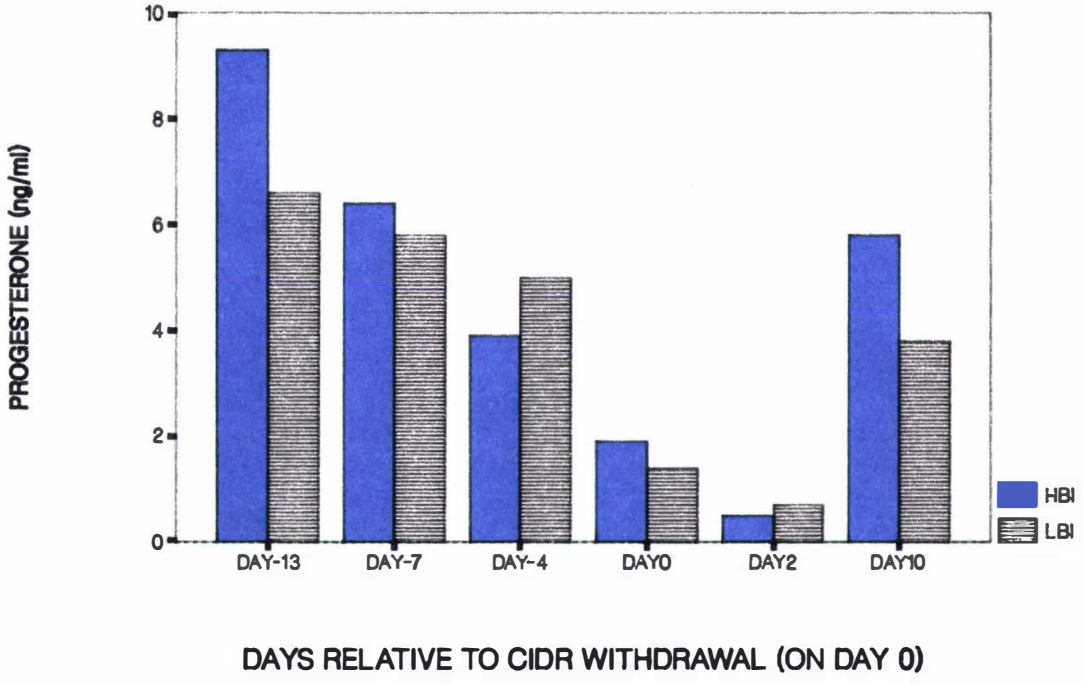


Figure 5.8 The rate of eating, in terms of percentage (upper panel), or amount of allowance consumed (lower panel) measured at 80 minutes after feeding, at 20 hours before and 28 hours after the withdrawal of progesterone-impregnated CIDR in 8 high breeding index (solid bar) and 8 low breeding index (hatched bar) heifers

Figure 5.9 Plasma concentrations of progesterone relative to day of CIDR withdrawal in 8 high breeding index (solid bar) and 8 low breeding index (hatched bar) heifers. CIDRs were inserted 17 days before withdrawal



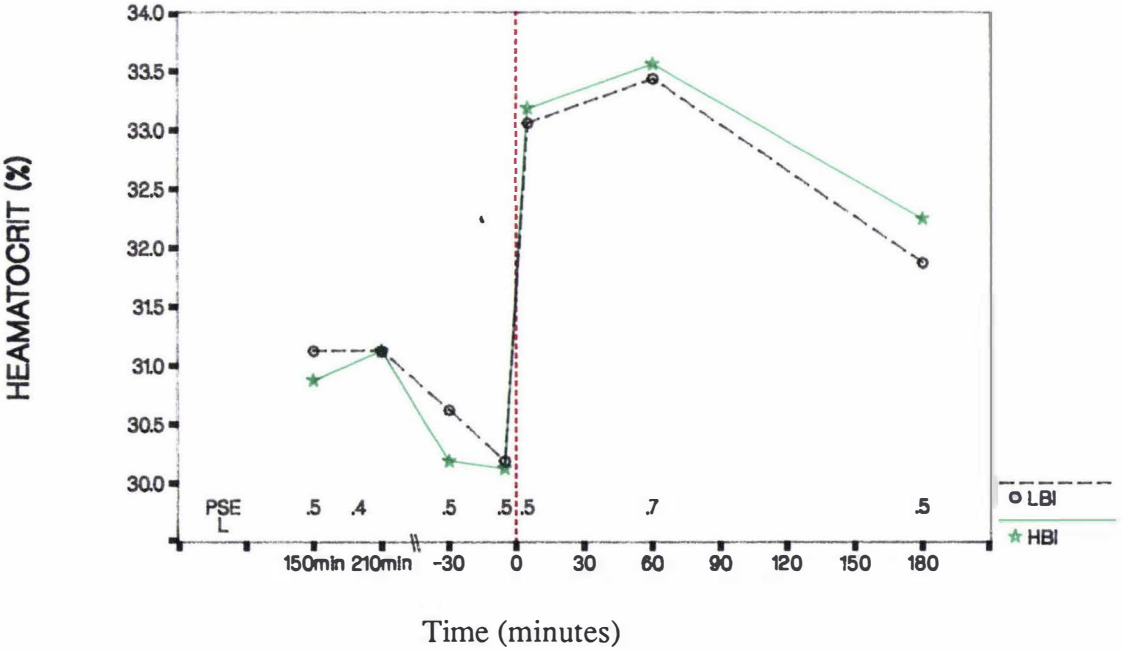


Figure 5.10 Changes in haematocrit in 8 high breeding index (*---*) and 8 low breeding index (o---o) heifers at 150 and 210 minute after the injection of urea, T1824, and thiocyanate, and at the time of refeeding (indicated by the dotted line) that following a period of 72 hour fast.

haematocrit at any time.

DISCUSSION

This experiment examined the effects of genetic selection for milk yield on several physiological parameters in the Friesian heifers, i.e. rate of eating, volume of body fluid compartments, plasma concentrations of metabolites and hormones after feeding and fasting, metabolic responses to challenges of adrenaline administered at different times after feeding, and metabolic responses to glucose challenges given when the animals were treated with progesterone-CIDRs and after CIDR removal.

Volumes of body fluid compartments

Injections of urea, T1824, and NaSCN into blood have been used to measure body fluid distribution and body composition in ruminants (MacFarlane 1975) including cattle (Eley et al. 1981b; Bartle et al. 1987, 1988; Ross et al. 1989). Results of this study showed that plasma volume (estimated using T1824) accounted for about 5% of body weight (Table 5.1) and the calculated extracellular space (by NaSCN) accounted for about 25% of LWT, in line with present knowledge (MacFarlane 1975; Eley et al. 1981b; Ritschel 1986). The urea space were also comparable to other reports (Bartle et al.1987; Bartle et al. 1988).

In Chapter 3, it was hypothesized that differences in volume of body fluid compartments may have caused differences in plasma concentrations of metabolites, especially of urea, between the two lines. This study, however, showed that the volumes of urea distribution, as well as plasma volume (T1824 space), and extracellular fluid volume (NaSCN space), were similar between the HBI and LBI heifers. Furthermore the parameters of the disappearance of the injected urea, T1824 and NaSCN were not affected by selection line, suggesting similar clearance rate of these metabolites in the LBI and HBI animals. Thus, any differences in plasma metabolite and hormone concentrations between the two lines must be due to factors other than variation in volumes of body fluid compartments.

Hormone/metabolite levels during a 29 hour observation period and fasting/refeeding

Urea: The greater plasma urea concentration in the LBI heifers during the 29 hour observation period (Figure 5.1) was consistent with the results during the postabsorptive periods in Chapters 3 and 4. Greater plasma urea levels were also found in low genetic merit calves during fasting (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). In contrast, there were reports that plasma urea levels were greater in fed cows (Barnes et al. 1985) and fasted bull calves (Mackenzie et al. 1988) of high genetic merit. Furthermore, the change in sign of the selection line effect on plasma urea concentration with time after feeding observed in Chapter 3 led to the hypothesis that HBI cattle may differ from the LBI cattle in the volume of urea distribution or dynamics in ingestive/digestive function. Both of the hypotheses were examined in this study. The present results showed no selection line effect on the volume of urea distribution. In addition, the slower rate of eating and simultaneously lower plasma urea in the HBI heifers does not support the hypothesis advanced in Chapter 3 that the greater urea increment in HBI heifers at feeding was linked with a greater ingestive/digestive activity in the HBI heifers.

Acute fasting, which was an effective means of drawing apart the plasma urea concentrations in high and low genetic merit groups in other studies (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnet-Smith et al. 1987), did not induce urea differences between the lines in the present study. In fact the selection line difference in plasma urea became non-significant during the fasting/refeeding period. The discrepancy between these results is difficult to explain, but may have been affected by the levels of energy intake or by difference in energy:protein ratio in the diets fed prior to fasting. Calves fed concentrates plus hay at ad libitum levels prior to fasting had relatively low plasma urea concentrations (3 mmol/l) and fasting caused a substantial rise in plasma urea levels (Tilakaratne et al. 1980; Sejrsen et al; 1984; Sinnet-Smith et al. 1987). In this study, however, intake of hay was restricted to 140% of MER. Moreover, plasma urea concentrations were higher (5 mmol/l) before fasting, and declined

during the fast. In another study, Mackenzie et al. (1988) reported that plasma urea level tended to be greater in fasted HBI bull calves that were previously underfed on hay. Taken together, a picture emerges. Animals well fed prior to fasting exhibit a greater plasma urea level in the low genetic merit calves during acute fasting, whereas a low level of feeding stimulates a greater urea level in plasma of high genetic merit cattle during fasting/refeeding. This notion is supported by the significant line x allowance level interactions observed in Chapter 3. The LBI heifers well-fed (125% MER) in that experiment maintained greater urea levels than other groups during the postprandial period, while the LBI heifers under-fed (75% MER) tended to have the lowest increment in plasma urea after feeding.

Creatinine: The lower plasma creatinine concentration in the HBI heifers than in the LBI heifers was consistent with the observations in Chapter 4, and the HBI heifers fed 125% MER tended to have lower plasma creatinine levels in Chapter 3. In this experiment, fasting and refeeding increased plasma creatinine concentration in heifers, but the line differences remained unchanged. As the extracellular volume into which creatinine was distributed was similar in the HBI and LBI heifers (Table 5.1), the difference in plasma creatinine level between the two lines could have been due to a decreased body protein catabolism in the HBI heifers. However, increased plasma creatinine clearance rate could also have caused lower plasma creatinine concentrations in the HBI line. In a study of two lines of Romney rams divergently selected for fleeceweight, McCutcheon et al. (1987) found that high creatinine clearance rates were associated with low plasma creatinine and urea concentrations. Although the glomerular filtration rates of creatinine were not measured in this study, the slightly greater clearance rates (slow or b-phase) of the injected urea and NaSCN in the HBI heifers (Table 5.2), which are largely due to renal clearance (Ritschel 1986; Bartle et al. 1987), suggested that the difference between the lines in creatinine was not due to differences in renal function. Further studies are, however, required to establish this point unequivocally.

It should be noted that while basal plasma creatinine concentrations were consistently lower in the high genetic merit calves

(the present study) and lactating cows (Peterson et al. 1982; Ma 1985), the effects of genetic merit on basal plasma urea level appeared to be the opposite in calves (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith 1987; the present study) to that in lactating cows (Stark et al. 1978; Peterson et al. 1982; Barnes et al. 1985; Ma 1985). Unlike plasma urea, which can be substantially affected by dietary factors, plasma creatinine in animals fed a creatinine-free diet comes mainly from endogenous protein degradation and thus serves as a better indicator of body protein degradation than plasma urea level. Therefore, the greater plasma urea level in the high genetic merit cows may result from mechanisms other than greater endogenous protein degradation.

Glucose: Greater basal plasma glucose concentrations were found in the HBI cattle in several studies (Flux et al. 1984; Xing et al. 1988; Mackenzie et al. 1988), and in this study (Figure 5.1). Greater plasma glucose levels, presumably due to a difference in entry rate or in gluconeogenesis, in the HBI than in the LBI heifers after feeding, were observed in the baseline-corrected data in Chapter 3. Smaller volumes of glucose distribution, which may also favour a higher glucose concentration in animals with a fixed rate of glucose entry, were observed in the HBI heifers in Chapter 4 (Table 4.3). However, the volumes of glucose distribution were similar between the lines in this study (Table 5.2). Greater glucose output in HBI heifers via glycogenolysis was also observed following the challenge of catecholamines in one study (Bridges et al. 1987), but not in this study (Figure 5.4) nor in Chapter 3. In addition, glycogenolysis in response to glucagon challenge was not affected by selection line (Chapter 3; Mackenzie et al. 1988).

The greater basal glucose level in the HBI heifers of this study may also have arisen from a decreased peripheral glucose utilisation caused by reduced sensitivity to the glucose regulatory effect of insulin. Evidence for this is that basal hyperinsulinaemia and glucose-induced hyperinsulinaemia in HBI heifers did not result in greater glucose disappearance (Figure 5.6). Concomitant hyperglycaemia and hyperinsulinaemia were observed in lactating cows (Flux et al. 1984) and milk-fed calves (Xing et al. 1988).

NEFA: The similar plasma NEFA concentration in the HBI and LBI heifers in the 29 hour observation period was consistent with the results in Chapters 3 and 4 under similar conditions. Fasting tended to result in a slightly greater NEFA level in the HBI heifers. Similar, but more marked, results were reported by Tilakaratne et al. (1980) who attributed this difference to a greater ability to mobilise fat reserves in high genetic merit animals during energy deficit.

Glucagon: The effect of genetic selection on plasma glucagon concentrations in cattle has only been reported in one study in the literature (Barnes et al. 1985). This may reflect the difficulty in raising pancreatic specific glucagon antibody suitable for measuring plasma glucagon level in a radioimmunoassay system. During the course of this study, antisera specific for pancreatic glucagon has been successfully produced by immunizing guinea pigs with BSA-conjugated glucagon and used to measure plasma glucagon in the heifers (Chapter 2).

In view of the regulatory role of glucagon in gluconeogenesis, Flux et al. (1984) implied that plasma glucagon concentrations may have been greater in the HBI than in the LBI cows and therefore responsible for the hyperglycaemia in the selected line. However, the report by Barnes et al. (1985) showed that plasma glucagon was lower in high genetic merit than in low genetic merit cattle. In the present study (Chapters 4 and 5) basal plasma glucagon concentrations were similar in the HBI and LBI heifers in the normal feeding or fasting/refeeding periods despite the persistent hyperglycaemia in the selected line. Therefore the glucagon hypothesis proposed by Flux et al. (1984) is not supported by more recent data.

In this study the glycogenolytic effects of glucagon were similar in the HBI and LBI heifers (Chapter 3). This differed from that of Mackenzie at al. (1988) who reported that the glycogenolytic response to glucagon challenge was reduced in the HBI bull calves . The reasons for this discrepancy are not clear. As glucagon is a hormone involved in gluconeogenesis and thus glucose and urea production (Bergman 1983b; Riis 1983), it is interesting to note that selection for milk production has brought about differences in plasma concentrations of

urea and glucose but not of glucagon (at least in this study).

Metabolic responses to feeding

The rise in plasma urea and creatinine concentration at the onset of feeding/refeeding appears to be very rapid. It is unlikely that the creatinine was of dietary origin as the heifers were fed creatinine-free feed. Altered blood distribution within the body or removal of plasma fluid upon feeding (as indicated by the rise in haematocrit) may be the factors causing the changes in plasma urea and creatinine concentration. Another possibility is that the increased plasma urea and creatinine originated from stimulated muscle activity of the digestive tract and thus increased protein turnover. It is known that feeding is associated with increased catecholamine secretion (Diamond and LeBlanc 1987) which can stimulate muscle protein metabolism in the animal (Nie et al. 1989).

A relatively greater increment in plasma urea concentration at the onset of feeding/refeeding was observed in the HBI heifers. Similar but significant differences were also observed in the baseline-corrected data in Chapter 3 (Appendix 8). Given that the volume of urea distribution and rate of eating were similar between the lines (Chapter 5), then the difference may arise from a greater rate of endogenous urea production in the HBI line. However further research is needed on the rate and control of gluconeogenesis immediately after feeding to determine if the difference between the lines is due to a difference in endogenous urea production.

Increased plasma glucagon at feeding was previously reported for ruminants (Bassett 1972, 1975), and this is thought to be stimulated by a number of metabolic factors as well as the activity of the CNS (Bassett 1981). In this study, HBI heifers on three occasions (Figures 5.2 and 5.4) exhibited very brief but significantly greater increases in plasma glucagon concentrations 15 to 30 minutes after feeding. As hypoglycaemia is an effective stimulus for glucagon secretion, the pancreatic A-cell in HBI heifers may be more sensitive to the decrease in plasma glucose concentration soon after feeding. Alternatively, the greater glucagon secretion may reflect a more active autonomic nervous

system in the HBI heifers which could cause a greater release of glucagon (Bassett 1981;) and increased activity of gluconeogenic enzymes (Woods et al. 1981; Frohman 1983; Woods 1986). Further research is needed to determine if this transient increase in glucagon secretion in the HBI heifers is sufficient to affect either ingestive behaviour or gluconeogenesis.

Haematocrit: The immediate rise in haematocrit (PCV) within 5 minutes at the onset of feeding may partly reflect a release of red blood cells from the spleen into circulation (MacFarlane 197 ; Swenson 1977). Increases in PCV of up to 10-13% can be achieved in sheep within 10-30 minutes of feeding (MacFarlane 197). Reduction of plasma volume, as a result of fluid movement from the plasma into other body fluid compartments upon feeding, may also contribute to an increase in PCV, because splenectomy did not abolish the rise in haematocrit on feeding (MacFarlane 197). Movement of water from the plasma into digestive tract, particularly into the rumen, increased on feeding (Swenson 1977), and the moisture content of adipose tissue also increased on feeding in rats (Carneheim and Alexson 1989). Therefore the changes in plasma metabolite and hormone concentrations in the heifers may have been influenced by a reduction in plasma volume as a result of feeding.

Effect of timing of adrenaline challenge relative to feeding

The magnitude of lipolytic and glycogenolytic responses to the adrenaline challenge was significantly influenced by the timing of the challenge relative to feeding and fasting. Lipolytic response to adrenaline was minimal 7 hours after feeding, and maximal 72 hours after fasting, whereas the reverse was true for glycogenolytic responses. This is in agreement with the observations by Blum et al. (1982) and Frohli and Blum (1988b).

Although the NEFA responses to adrenaline challenge were similar between the selection lines, there were significant line x time of challenge interactions in pre-challenge plasma NEFA concentration. HBI heifers exhibited greater NEFA levels with increasing time of fasting. This is consistent with the result obtained by Tilakaratne et al.

(1980) who found plasma NEFA was significantly higher in the high genetic merit calves after fasting.

Plasma glucose concentrations following adrenaline challenge were generally, but not significantly, higher in the HBI than in the LBI heifers in this study and the differences appeared to increase with time of fasting (Figure 5.4). This is consistent with the observation reported by Bridges et al. (1987) that HBI heifers tended to have a greater glycogenolytic response to adrenaline challenge. However the difference between the HBI and LBI heifers were significant in that study but not in the present investigation.

No significant selection line effects on lipolytic and glycogenolytic response to adrenaline challenge were observed in this study. Results therefore do not support the hypothesis proposed in Chapter 3 that a greater glycogenolytic response at feeding, but a greater lipolytic response during fasting, to adrenaline challenge may have existed in HBI heifers. Thus, the discrepancy between Chapter 3 and the study of Bridges et al. (1987) in greater lipolytic/glycogenolytic response in the HBI heifers to adrenaline challenge remains to be elucidated.

Metabolic responses to glucose challenge and the influence of progesterone on metabolism

The greater plasma insulin concentrations in samples collected before the glucose challenge in the HBI heifers are in line with reports in lactating cows (Flux et al. 1984; Barnes et al. 1985), milk-fed calves (Xing et al. 1988) and bull calves (Mackenzie et al. 1988). Moreover the greater pancreatic insulin secretion in the HBI heifers after glucose challenge was consistent with the results in Chapter 4 when the heifers were treated similarly with progesterone. The results presented in Chapter 4 provided circumstantial support for the hypothesis that variation in the stage of oestrous cycle of the heifers in Chapter 3 masked any differences in insulin response to glucose between the lines. McCann and Reimers (1986) reported that basal insulin levels in heifers was higher during oestrus than during dioestrus. The experiment described in this Chapter was designed to

test the hypothesis that insulin release to glucose challenge would differ between heifers treated with progesterone and immediately after the progesterone withdrawal. However, basal plasma insulin in this study was not affected by the presence or withdrawal of progesterone-CIDR (PWPC) (which should approximate the dioestrous and oestrus phases respectively) (Figure 5.6). Similarly, insulin release to glucose challenges was largely unaffected by PWPC. Therefore, it is still not clear why insulin secretion rates were similar between the lines in Chapter 3 when the oestrous cycles in the heifers were not controlled by progesterone. Nevertheless insulin release in response to glucose challenge in both HBI and LBI heifers in Chapter 3 and in LBI heifers in Chapters 4 and 5 were similar, but lower than that of the HBI heifers in Chapters 4 and 5. This may suggest that progesterone treatment sensitised the pancreas of the HBI heifers to the glucose stimulus. This may be brought about by a differential hypertrophy effect of progesterone on the pancreatic islets in the two lines. Chronic treatment with progesterone or oestradiol alone or in combination resulted in hypertrophy of pancreatic islets in laboratory rodents (Constrini and Kalkhoff 1971; Bailey and Ahmed-Sorour 1980) and induced glucose intolerance in the animals (Metcalfe et al. 1988).

McCann and Reimers (1986) observed that heifers in oestrus had greater basal plasma concentrations of glucose than those in dioestrus. This was consistent with the present result. Plasma glucose before and after glucose challenge were greater in the heifers 46 hours after CIDR withdrawn. Moreover, the significant line x presence/withdrawal of CIDR interactions on pre-challenge plasma glucose concentration indicated a greater rise in the HBI heifers following the withdrawal of progesterone-CIDR.

As selection line differences in plasma concentrations of urea, creatinine, glucose and insulin were observed in this experiment, it is interesting to speculate that interrelationships or cause-effect relationship may exist among these parameters. For instance, the greater basal plasma glucose level in the HBI heifers may be due to an increased glucose sparing effect in this group. This hyperglycaemia may in turn have primed the pancreatic b-cell for a greater response to metabolic stimulus in the HBI heifers. Moreover the hyperglycaemia in

the HBI heifers may have had a inhibiting influence on blood urea concentration. Hyperglycaemia produced by chronic infusion of glucose caused significant reductions in plasma urea in man (Jahoor and Wolfe 1987), sheep (Summer and Weekes 1983) and calves (Petit et al. 1988). Moreover, negative correlations between plasma glucose and urea have been observed in cattle (Tilakaratne et al. 1980; Barnes et al. 1985; Chapter 3 unpublished).

Eley et al.(1981a) reported that plasma progesterone concentration was higher in pregnant cows of high genetic merit prior to parturition. In this study, the endogenous progesterone was only measured after progesterone-CIDR withdrawal, which was relatively (by 50%) but not significantly higher in the HBI heifers (Figure 5.9). As this result was affected by the very low levels of progesterone in two LBI heifers, the effect of genetic selection on plasma progesterone concentration needs further investigation.

Rate of eating:

The eating behaviour, like other behaviours of an animal, is regulated by the hormonal-CNS system and has a strong genetic component (Fraser 1985; Arnold 1985; Caviness 1987). It is known that distinct genetic variation in ingestive behaviour exists between species during the process of evolution (Beilharz 1985; Arnold 1985). Genetic variations in the instincts to explore the environment for food may be a factor affecting the production performance of dairy cattle.

In this experiment, the rate of eating was slightly greater in LBI than in HBI heifers, but this was significant only on the first day of observations (Figure 5.7). It is known that the control of eating behaviour in animals is regulated by a complex of neuropeptides as well as hormones and metabolites (see reviews: de Jong 1986; Baile et al. 1987; Morley 1987; Oomura 1987). In this case, the slower rate of eating in the HBI line may have resulted from a greater plasma glucose concentration in the HBI animals. Falls in plasma glucose levels are known to stimulate ingestion whereas infusion of propionate causes increases in plasma glucose levels (Tilakaratne et al. 1980) and decreases in meal size (de Jong 1986). The higher glucagon level in

the HBI heifers at feeding, although it existed briefly, may have also been associated with the slower rate of eating because increased plasma glucagon concentration is seen as a signal of satiety in various species (Deetz and Wangsness 1981; Forbes 1979; 1985; Morley 1987; Oomura 1987).

The slower rate of eating in the HBI heifers seems to contrast with the observations that HBI cows tended to eat more than LBI cows especially in lactation (Bryant & Trigg 1981; Davey et al. 1983; Holmes 1988). It may be that in those studies the greater food intake by the HBI cattle was due to a longer time spent grazing as high yielding cows tended to spend more time grazing than low yielding cows (Brumby 1959). High yielding cows would also be expected to have a greater nutritional demand whereas the same would not be true in the calves (unless there were substantial differences in maintenance requirement or efficiency of liveweight gain). However, the slower rate of eating in the HBI heifers is not consistent with the hypothesis proposed in Chapter 3 that a greater eating rate may be responsible for the higher plasma urea level in the HBI heifers at feeding. Furthermore, there was no effect of selection line on rate of eating beyond day one of the measurements.

In summary, genetic selection for milk production has resulted in decreased plasma concentrations of urea and creatinine, but increased plasma concentration of glucose in the heifers treated with progesterone. These differences are unlikely to be due to differences in the distribution volumes as the volumes of plasma and extracellular fluid were similar in the HBI and LBI heifers. Adrenaline challenge at different times from feeding resulted in different patterns of glucose and NEFA response, increased lipolysis and decreased glycogenolysis being associated with increasing time of fasting. The HBI heifers also exhibited greater basal glucose and insulin, and insulin secretion to glucose challenge. Whereas the insulin differences were largely unaffected by the presence/withdrawal of progesterone-CIDR, basal glucose was elevated after progesterone-CIDR withdrawal, particularly in the HBI heifers.

CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSIONS

In view of the potential benefits of using metabolic characteristics as genetic markers in dairy cattle breeding (Chapter 1), the present study was conducted to identify differences between the HBI and LBI heifers in some of these characteristics. As noted in Chapter 1, markers should have a number of attributes:

1). They should be easily measured, preferably under field conditions, with low ethical cost of measurement (i.e. not harmful to the animal) and low economic cost of assay. The use of easily-measured metabolic characteristics as markers would allow large numbers of animals to be screened, so ensuring that the potential gene pool available for selection was maximised.

2). They should be expressed in young animals, preferably prior to first mating, so that the generation interval can be reduced to a minimum.

3). They should be genetically correlated with lactational performance and either positively correlated or not correlated at all with other desirable production traits.

In this study, selection line heifers were used as the basis for identifying potential genetic markers. Animals from these lines have the advantage that they are known to be different in average genetic merit even though the genetic merit of individual animals within lines is not known. Results from studies with the heifers may also be compared with results from cows in the same lines. This gives some indication as to whether genetic variation in metabolism of the cows is also expressed in their daughters.

I. Differences in basal plasma concentrations of metabolites and hormones and their responses to feeding/fasting between the HBI and LBI heifers

In this study, one of the most consistent selection line differences was in post-prandial plasma urea concentrations, the HBI heifers maintaining lower levels than the LBI heifers (Chapters 3, 4, and 5). These results were in line with the results of Tilakaratne et

al. (1980), Sejrsen et al. (1984) and Sinnett-Smith et al. (1987) who observed elevated urea concentrations in fasted low genetic merit calves. This may have reflected a difference between the lines in the rate of endogenous protein degradation (Tilakaratne et al. 1980).

The differences in plasma urea level between the lines suggest the possibility that plasma urea might be used as a genetic marker for predicting dairy merit. Plasma urea has been shown to have a moderately high repeatability, heritability, and genetic correlation with milk production (Stark et al. 1978; Peterson et al. 1982; Ma 1985; Woolliams and Smith 1988). However, these parameters were measured in cows, and could be affected by environmental factors with increasing age of the animal. In addition the effect of genetic merit on plasma urea level was positive in cows (Stark et al. 1978; Peterson et al. 1982; Ma 1985) but negative in the calves (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987). Therefore some reservations must exist concerning the possible use of the post-prandial or fasting plasma urea concentrations as a genetic marker for predicting dairy merit. The physiological mechanisms responsible for the genetic difference should be clarified first before wide use of the post-prandial urea level as a marker.

Alternatively one might consider using the urea response to feeding as a genetic marker to predict dairy merit, because greater elevation in plasma urea level immediately after feeding was found in the HBI heifers than in the LBI heifers (Chapter 3, baseline corrected). However, this difference appears to be restricted to a short period after feeding and was most apparent when the animals were underfed. These greater urea levels in the HBI heifers after feeding were similar to the results of Barnes et al. (1985) and Mackenzie et al. (1988). The difference was reduced in the well fed animals, but appears to be repeatable from experiment to experiment (Chapter 3 vs Chapter 5) or from day to day (in Chapter 5). The mechanisms for the post-feeding difference in plasma urea are not known but they do not appear to involve differences between the lines in urea space or rate of food consumption (Chapter 5).

Although the role of plasma urea as a potential genetic marker for

dairy merit is yet to be verified, it is interesting to note that selection line differences in urea concentration have been observed in a number of other situations. These include lines of sheep divergently selected for fleeceweight (McCutcheon et al. 1987; Clark et al. 1989) or lean tissue growth (Bremmers et al. 1988; Carter et al. 1989; Van Maanen et al. 1989) and pig lines divergently selected on the basis of fatness (Mersmann et al. 1984). In each case the high genetic merit lines had low plasma urea concentrations during basal conditions (fed every 2 hours) or fasting. Thus there may be a common genetic relationship between plasma urea concentration and productivity (perhaps protein deposition). Clearly, therefore, the role of urea as a potential genetic marker warrants further study.

The lower plasma creatinine concentrations in the HBI heifers during the postprandial period (Chapters 3, 4 and 5) support the urea results and the concept first proposed by Tilakaratne et al (1980) that selection for milk production in cattle has favoured the mobilisation of other energy reserves (primarily fat) rather than body protein reserves during a period of energy deficit. In addition, the effect of genetic selection on plasma creatinine appears to be consistent in calves and in cows as negative genetic correlations between plasma creatinine levels and milk production have been reported in lactating cows (Peterson et al. 1982; Ma 1985). This consistency of the genetic relationship between plasma creatinine and dairy merit (i.e. its apparent independence of age and physiological stage) could give an advantage to plasma creatinine over plasma urea as the preferred genetic marker. Like plasma urea, plasma creatinine has a relatively high repeatibility and is easily sampled and assayed. Again it is interesting to note that sheep of high genetic merit for fleeceweight have low circulating creatinine concentrations, as well as low urea concentrations, compared with sheep of low genetic merit (McCutcheon et al. 1987; Clark et al. 1989).

If plasma urea and creatinine were to be used as genetic markers it would probably be necessary to control environmental conditions of the animals under test since, as shown in this study, circulating urea and creatinine concentrations, and the between-selection line differences, are markedly influenced by factors such as nutritional

status and time relative to feeding (Chapter 3). These results are also consistant with those in sheep. Thus McCutcheon et al. (1987) showed that the correlation between plasma urea concentrations in sheep held under controlled conditions, and those in the same sheep at grazing, was very low. Therefore, further evaluation of plasma urea and creatinine as potential markers will be required. This would ideally be accomplished by measuring plasma urea and creatinine concentrations (under appropriate conditions) in young bulls which are to be progeny tested, and then determining the size of the genetic correlations with milk production.

Although basal plasma glucose tended to be greater in the HBI heifers the differences were not always significant in this study. This is in line with the results of others (Tilakaratne et al. 1980; Flux et al. 1984; Barnes et al. 1985; Mackenzie et al. 1988; Xing et al. 1988). At present no firm conclusions can be drawn about the suitability of blood glucose as a genetic marker in dairying because its repeatability, heritability, and genetic correlation with milk yield are not well estimated in cattle. In general, one might expect basal glucose concentration to be of limited use as a genetic marker because it is under tight homeostatic control.

Greater basal plasma insulin concentrations, and levels after challenges of glucose, arginine and insulin injections, have been previously reported in high genetic merit cattle (Flux et al. 1984; Barnes et al. 1985; Mackenzie et al. 1988; Xing et al. 1988). In the experiment described in Chapter 3, plasma insulin levels in response to feeding were, however, lower in the HBI heifers than in the LBI heifers. In other experiments reported in this study, differences between the lines in basal insulin were smaller and inconsistent. Thus basal insulin concentration does not appear to have potential as a genetic marker although, as will be discussed later, insulin responses to the glucose challenge appeared to be correlated with dairy merit.

Although selection for milk production has been associated with increased plasma GH concentrations in other genetic groups of dairy cattle (Barnes et al. 1985; Massri et al. 1985; Kazmer et al. 1986; Lukes et al. 1988), the differences were not always significant

in the lines at Massey University (Flux et al. 1984; Mackenzie et al. 1988; Xing et al. 1988). In the experiment reported in Chapter 3 the diurnal plasma GH concentrations were similar between the HBI and LBI heifers, and the magnitude of between-line differences was not affected by the adoption of different energy balances. As GH secretion is pulsatile and the hormone has a short half-life in blood, the results of Chapter 3 may not have been representative of the GH status of the animals. Therefore a 6 hour intensive sampling regimen was further imposed on the animals in the experiment of Chapter 4. The results, however, showed that the number of GH secretion spikes, the magnitude of the secretion spikes, and the percentage increment above the previous nadir, were similar in HBI and LBI heifers. These results would suggest that genetic selection for milk production has had little effect on plasma GH concentrations in the Friesian heifers at Massey University. However, it is still possible that the selection line effect on plasma GH is confined to a particular range of physiological states, as a short-term fast induced a greater elevation in plasma GH level in HBI than in LBI bull calves (Mackenzie et al. 1988). Also relevant to this discussion is the study of Michel et al. (1989) who found that LBI cows were, in absolute and proportional terms, more responsive to the galactopoeitic effects of exogenous GH. This was a short-term study and is yet to be repeated but the apparent difference in response to GH could not be accounted for by a difference between the lines in ability to mobilise body reserves. These results may suggest that the influence of the somatotrophic axis is near-maximal in HBI cows (i.e. they show little response to additional somatotrophic stimulation via exogenous GH), although circulating GH levels in the untreated cows from this study are yet to be analysed. If they are not different, as was the case in the study of Flux et al. (1984) who used cows from the same lines, results may imply that "downstream" components of the somatotrophic axis (e.g. hepatic somatogenic receptors), rather then circulating GH *per se*, have been altered by selection.

II. Response to metabolic challenges in the HBI and LBI heifers

Circulating concentrations of hormones and metabolites reflect the balance between rate of secretion/entry into the plasma pool, rate of removal/clearance from the pool, and size of the pool. The elements of secretion/entry, removal/clearance and pool size cannot be measured simply by assessing circulating levels. In addition some metabolites, particularly those that are central to animal metabolism (e.g. glucose) may be under tight homeostatic control so that little genetic variation is expressed. Whereas metabolic challenges do not meet the criterion of "simple measurement" in a genetic marker, they may provide a method of examining homeorhetic controls that regulate basal metabolite concentrations. Thus metabolic challenges have some advantages as potential genetic markers even though, in their present form, they could not be used to screen large number of animals

An intravenous glucose challenge induced greater insulin release in HBI than in LBI bull calves in one study (Mackenzie et al. 1988). This difference in insulin response was not observed in the heifer calves in the experiment of Chapter 3. However, after the animals were treated with progesterone CIDRs (Chapters 4 and 5), the glucose challenge induced greater insulin release in the HBI line. The reason for the differences in selection line effects in the study reported in Chapter 3 *vs* those in Chapters 4 and 5 is not clear. It does not appear to have been a result of CIDR treatment *per se* given that, in Chapter 5, HBI heifers exhibited greater insulin release to glucose both when they were treated with CIDRs and 46 h after CIDR withdrawal. However, that study permitted a comparison of selection line effects at only two "stages" of the oestrus cycle: during progesterone CIDR treatment (which approximates dioestrus) and at the induced oestrus 46 h later. The possibility still remains that, in Chapter 3, heifers were at variable stages of the cycle and that this compromised the comparison of line differences. A recent study with the Massey University LBI and HBI cows has shown that the HBI cows (at midlactation) also have enhanced insulin release in response to a glucose challenge (A. Michel pers. comm.). The results of these studies therefore constitute one of the few instances in which genetic divergence in metabolic parameters has been exhibited both by lactating

cows and by their daughters. This is an important result because it indicates that genes expressed in the lactating cows are also expressed in their non-lactating offspring, an assumption which is fundamental to the search for genetic markers but which has not been widely demonstrated in practice.

Genetic variation in lipolysis has been found between the HBI and LBI heifers in this study. The greater lipolytic responses to adrenaline challenge in the underfed HBI heifers (Figure 3.8), and the greater basal plasma NEFA levels in the HBI line after prolonged fasting (Figure 5.4), also have the potential to become useful markers of the dairy merit. However, the selection line differences in lipolysis appear to be relatively small as measured by plasma NEFA levels, and the requirement for potentially stressful metabolic stimuli such as high dose adrenaline challenges or chronic fasting may create stress for the animals. The question of variable between-line differences in lipolytic response to adrenaline (significant in Chapter 3 but not in Chapter 5) also remains to be resolved. The selection line differences in lipolysis may be regulated by variation in the activity of certain key enzymes (such as hormone sensitive lipase) which could be assayed by in vitro techniqus using biopsied samples of adipose tissue. Thus one possible avenue for future research is to examine activity of lipolytic enzymes by in vitro techniques. These techniques have the advantages of relatively low cost and ease of sampling (at least from subcutaneous depots). They also permit estimation of lipolytic capacity free of confounding whole-body effects (e.g. changes in blood flow induced by an adrenaline challenge). If such studies were to be undertaken it would be worthwhile also assessing lipogenic capacity of adipose tissue given the observation of McNamara and Hillers (1986a) that lipogenesis is greater in low genetic merit cows during lactation.

III. Conclusions

This study showed that genetic variation exists in nitrogen, glucose, lipid and insulin metabolism between the HBI and LBI heifers at Massey University under appropriate experimental conditions. These include:

1. Plasma urea concentrations during the post-prandial period were lower in the HBI than in the LBI heifers especially when the animals were well-fed. The elevation in plasma urea concentration on feeding was greater in the HBI than in the LBI heifers and this difference appears to be most apparent in conditions of underfeeding.

2. Plasma creatinine concentrations were greater in the LBI than in the HBI heifers and the differences appeared to be increased when the comparisons were made in well-fed conditions.

3. Plasma glucose concentrations were similar between the lines but the glucose entry after feeding appeared to be greater in the HBI than in the LBI heifers in the experiment when the animals received no progesterone. After chronic progesterone treatment, basal plasma glucose concentrations were greater in the HBI than in the LBI heifers fed 140% MER at 12 months of age, but were similar between the HBI and LBI animals fed 70% MER at 6 months of age.

4. Plasma insulin concentrations were greater in the LBI than in the HBI heifers after feeding, and the insulin release to glucose challenge was similar in the HBI and LBI heifers which received no progesterone treatment.

However, the glucose challenge stimulated a greater insulin release in the HBI than in the LBI heifers after the animals were chronically treated with progesterone. This difference was independent of glucose dose, and was exhibited both in heifers fed 70% MER at the age of 6 months and in heifers fed 140% MER at the age of 12 months.

5. Plasma glucagon concentrations were similar in the LBI and HBI heifers (treated with progesterone), except that the elevation in plasma glucagon was briefly greater in the HBI heifers on feeding.

6. Despite a slightly greater mean plasma GH concentration in the HBI than in the LBI heifers, selection line differences in plasma GH, and GH secretion characteristics, were not significant in a 24 hour diurnal period or in an intensive sampling period. Use of different energy balances did not affect these results.

7. Plasma NEFA concentrations were similar in normal feeding conditions between the two lines. Adrenaline challenge induced a greater elevation in plasma NEFA concentrations in the HBI heifers fed 75% MER than in the LBI heifers fed 75% MER or either group fed 125% MER. Prolonged fasting (in the presence of progesterone treatment) caused a greater elevation in basal plasma NEFA concentrations in the HBI heifers but did not affect selection line differences in lipolytic response to the adrenaline challenge.

8. The adoption of different energy balances was effective in separating the selection lines with respect to plasma urea levels and plasma NEFA response to adrenaline challenge.

9. Metabolic challenges of glucose and adrenaline were effective in causing greater insulin secretion and NEFA levels, respectively in the HBI line, at least in some circumstances. Challenges of glucagon and insulin did not produce marked metabolic differences between the lines.

10. Chronic treatment with progesterone may have permitted the expression of differences in insulin release to glucose challenges between the HBI and LBI heifers.

In conclusion, genetic selection for milk production has resulted in differences in plasma concenrations of urea, creatinine, glucose, insulin, and NEFA in the Friesian heifers at Massey University. The expression of these differences, however, appears to require specific experimental conditions, such as an appropriate plane of nutrition, challenge with metabolic stimuli, or control of a physiological process (oestrous activity in this case). As many of these metabolic differences are present in the 6 month old heifers and are simple to measure (such as plasma urea and creatinine), they have the potential to serve as genetic markers for dairy merit in the female cattle. In addition these differences may exist in the bull calves and could be evaluated in a progeny test. However, their true value as genetic markers in dairy cattle breeding will only be known when extensive studies have been performed to estimate genetic correlations between these traits and milk production.

APPENDIXES

APPENDIX 1: Validation of insulin dilution assays

Plasma insulin concentrations after insulin-challenges are often very high and samples therefore have to be diluted before assaying. The dilution procedure has been evaluated in the following three trials during the course of experiments described in Chapter 4.

Trial One: Four bovine plasma pools were spiked to 250 and 60 ng/ml. The 250ng/ml pools were diluted at 1:50, 1:40, 1:25 and 1:20. The 60 ng/ml pools were diluted at 1:16, 1:8 and 1:4. All dilutions were with 7% BSA in phosphate buffer.

Plasma samples collected after insulin challenges of high (10 µg/kg), medium (1 µg/kg) and low (0.1 µg/kg) dose were prepared from 6 heifers and diluted as follows:

1) high dose challenge:
   samples collected 4 minutes after injection: 1:40, 1:25, 1:20, 1:16,
   samples collected 12 minutes after injection: 1:10
   samples collected 20 minutes after injection: 1:6
   samples collected 30 minutes after injection: 1:2

2) medium dose challenge:
   samples collected 4 minutes after injection: 1:8
   samples collected 12 minutes after injection: 1:2

3) low dose challenge:
   samples collected 4 minutes after injection: 1:2

These dilutions were based on simulated clearance curves calculated using the actual dose and published estimates of plasma space and the half-life of insulin in plasma (Land et al. 1983; McCann and Reimers 1985).

Results using the current insulin RIA method (Flux et al. 1984; Chapters 3, 4 and 5) showed a lack of parallelism in the diluted samples. For example, while the dilution of the 60 ng/ml spiked pools at 1:4, and 1:8 gave results close to the expected values, dilution at 1:16 or greater produced results about 50% higher than expected despite the expected concentrations being within the linear range of the assay standard curve. Possible interfering effects of the concentration of BSA used in the dilution were examined by comparing 3.5% BSA and 7% BSA as the working dilution in the following trial.

2) Trial Two: The same pools, spiking methods and dilutions as those in first trial were used, each sample being diluted with both 3.5% and 7% BSA.

The results showed that when the 60 ng/ml pools were diluted at 1:4 and 1:8 with 3.5% BSA, the results were 50% and 25% lower than expected while at 1:16 dilution the result was close to the expected values. Results from the pools spiked to 250 ng/ml and diluted at 1:20, 1:25 were about 20% and 10% lower than expected, while at 1:40 and 1:50 the results were about 15% higher than expected.

Dilution with 7% BSA tended to give higher results than that with 3.5% BSA. In addition, the results from 250 ng/ml pools diluted at 1:40 and 1:50 with 7% BSA were about 50% higher than expected and 30% higher than results from 3.5% BSA dilution.

3) Trial Three: Five pools were spiked to 250, 200, 175, 150, 125, 100, 75, 50 and 25 ng/ml and they were diluted at 1:50, 1:40, 1:35, 1:30, 1:25, 1:20, 1;15, 1:10 and 1:5 respectively with 3.5% BSA so that all diluted pools had the same insulin level of 5 ng/ml.

However, the concentrations estimated by the assay were still consistently greater than would be expected.

Discussion: McCann and Reimers (1985) addressed the problem of high insulin concentrations in samples from insulin-challenged heifers by serially diluting samples with 7% BSA. They reported that diluted samples displaced labelled insulin in parallel with each other and with

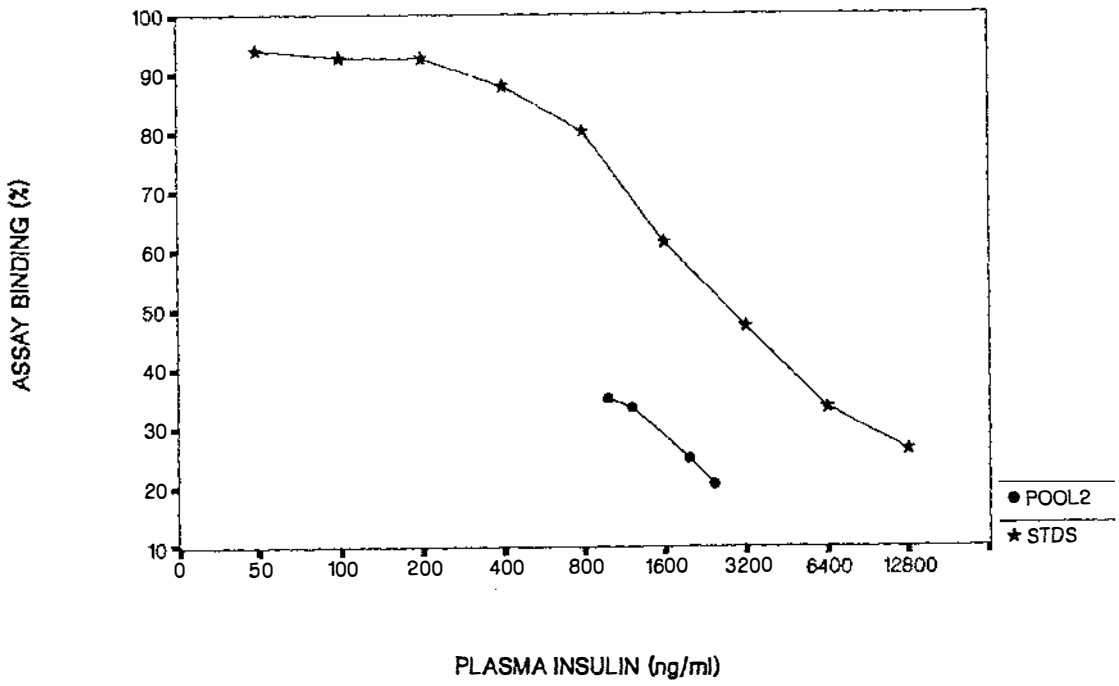
the standard insulin. Exactly how they assessed parallelism (e.g. visually vs comparison of regression slopes) was not stated.

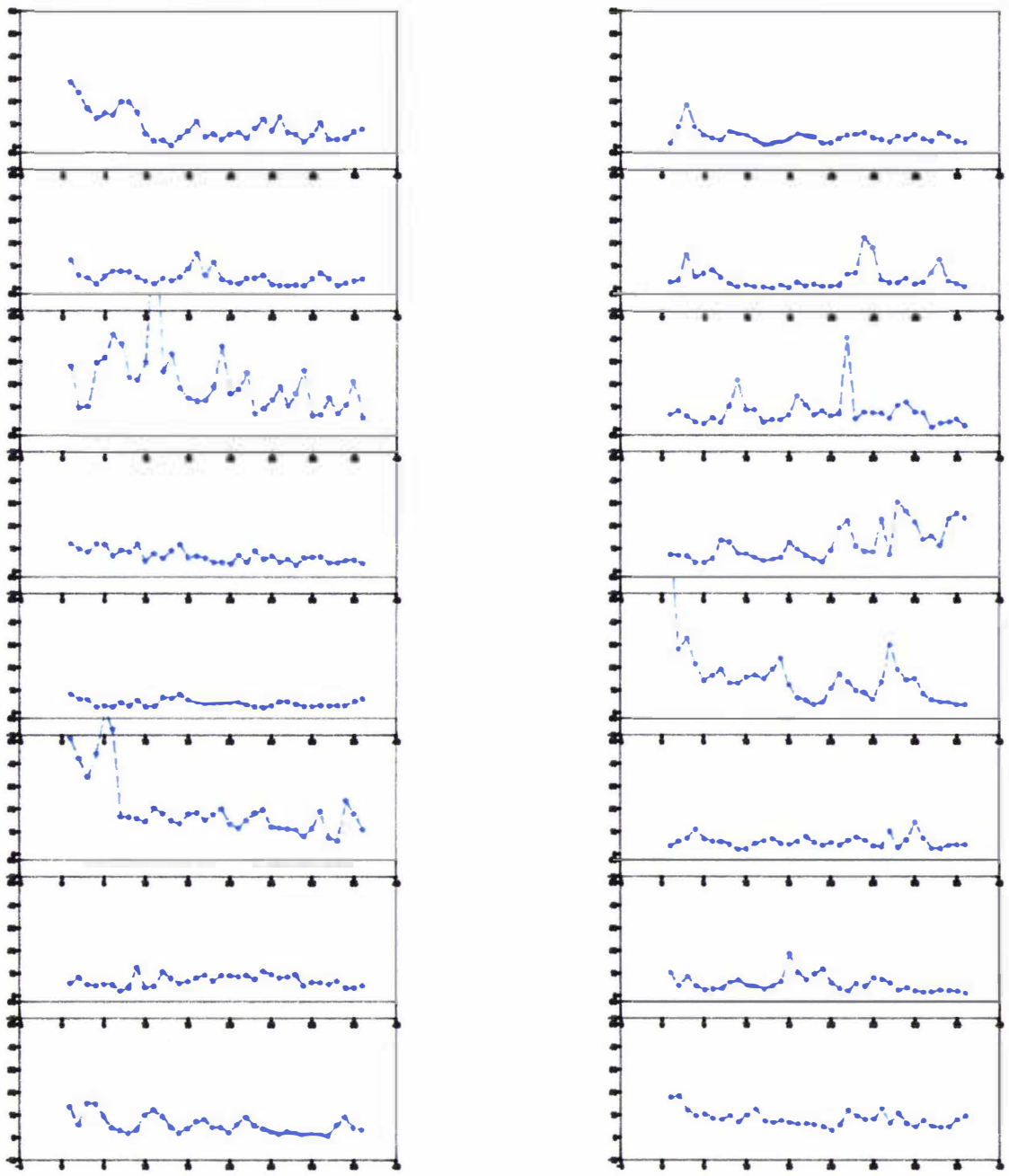
In this study, a similar approach was initially used, the effects of dilution being evaluated both by eye assessment of parallelism and by calculating ratios of observed to expected concentrations (observed= actual assay concentration adjusted for dilution factor, expected= calculated concentration based on insulin content of unspiked pools and amount of cold insulin added to 'spike' the pool). Whereas samples with relatively low insulin concentrations exhibited parallelism and observed/expected ratios close to 1.0 (Trial One), those with high concentrations (e.g. spiked to 250 ng/ml or from high dose insulin challenge) did not. In almost all cases the observed/expected ratio was substantially greater than 1.0 for these high insulin spiked samples. The situation was improved to some extent by using 3.5% as opposed to 7% BSA, suggesting that the BSA influenced binding (even though the BSA itself contained no immunoreactive insulin). This suggestion is reinforced by the fact that observed/expected ratios deviated from 1.0 to the greatest extent in high insulin samples which required the highest dilution to get them on the standard curve. For these samples dilution with 3.5% or 7% BSA substantially over-estimated the true insulin concentration in spiked samples. As a result, dilution of the samples from high dose insulin challenges, as described by McCann and Reimers (1985), could not be relied upon to give accurate estimates of insulin concentrations.

The results of these studies also cast doubt on the methods by which McCann and Reimers (1985) validated the dilution with 7% BSA. Their validation consisted only of an assessment of parallelism in serially diluted samples from their heifers. Because they did not use (spiked) samples of known insulin concentration, they could not assess assayed vs true insulin concentrations. Figure A1 shows the results of serial dilution of one pool (No. 2) in the present study. As described previously, dilutions were made at 1:20, 1:25, 1:40 and 1:50 (in this case using 3.5% BSA). Eye assessment of the displacement curves shown in Figure A1 would clearly lead to the conclusion that the curves were parallel. Furthermore, the estimated insulin concentrations (corrected for dilution factors) in the 1:25, 1:40 and

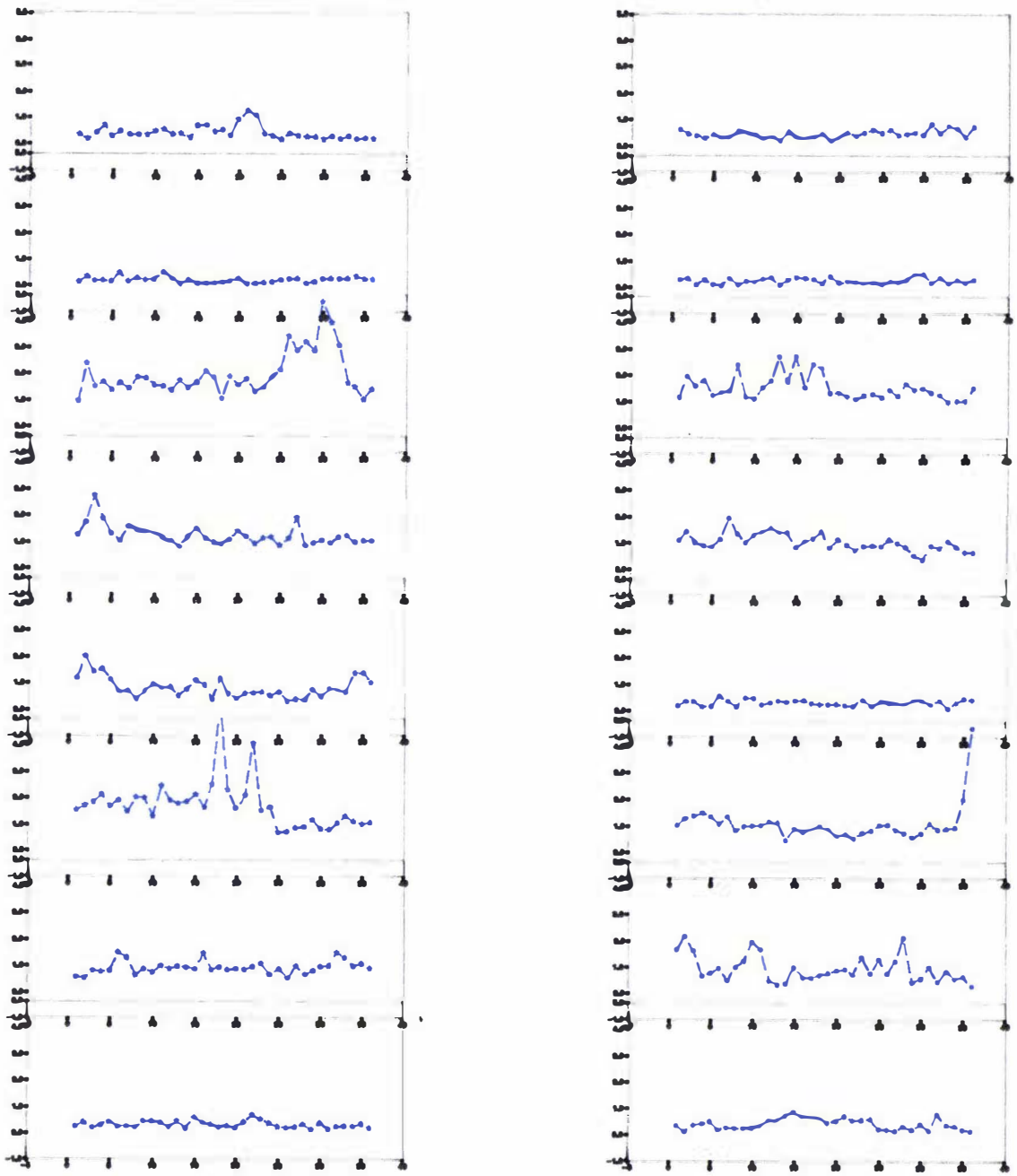
1:50 samples were close to those of the 1:20 dilution (range= 84-98%). Thus, as defined by McCann and Reimers (1985) the BSA dilution appeared to be appropriate. However, because these were spiked pools (i.e. samples with assayed basal insulin levels to which known amounts cold insulin had been added), the actual concentrations of insulin were also known. In the case of pool No. 2, the calculated concentration was 244 ng/ml. Assayed concentrations (corrected for the dilution) and observed to expected ratios were: 1:20, 398.8 ng/ml, ratio= 1.63; 1:25, 360.2 ng/ml, 1.48; 1:40, 335.3 ng/ml, 1.37; and 1:50, 390.2 ng/ml, 1.60. Clearly these results do not support the view that serial dilution with BSA in phosphate buffered saline is an appropriate method of dealing with very high concentrations of insulin such as those which occur after insulin challenge. The reason for this problem is not known but it did mean that the assay could not be relied upon to give accurate results for samples from the high dose challenge in Chapter 4.

FIGURE A1. INSULIN STANDARD CURVE AND THE DISPLACEMENT CURVE OF LABELLED INSULIN BY A SPIKED POOL 2 DILUTED AT 1:20, 1:25, 1:40 AND 1:50.



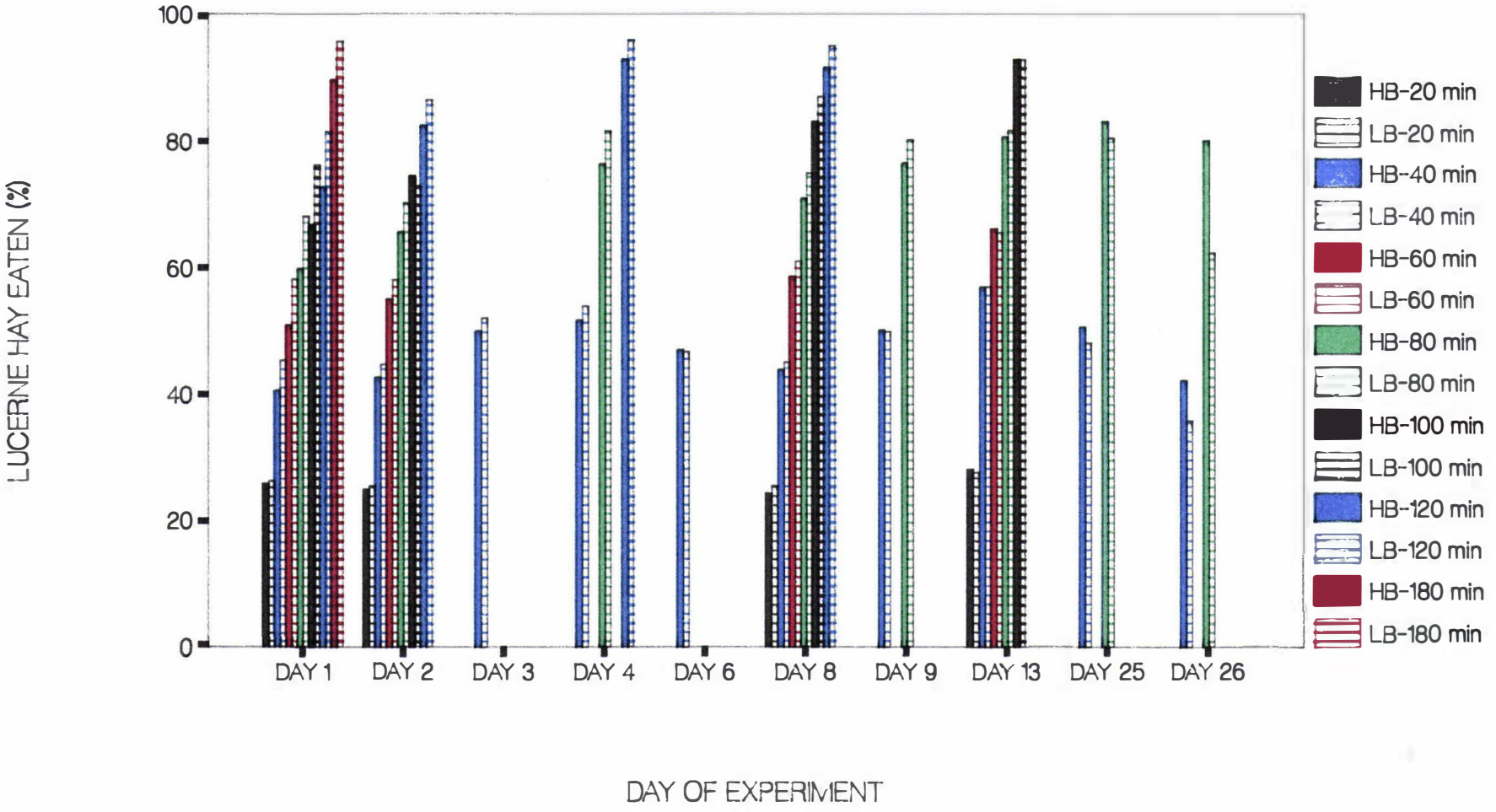


APPENDIX 2: Patterns of plasma GH concentration in 8 HBI heifers (left panels) and 8 LBI heifers (right panels) over a 6 hour period (Chapter 4).

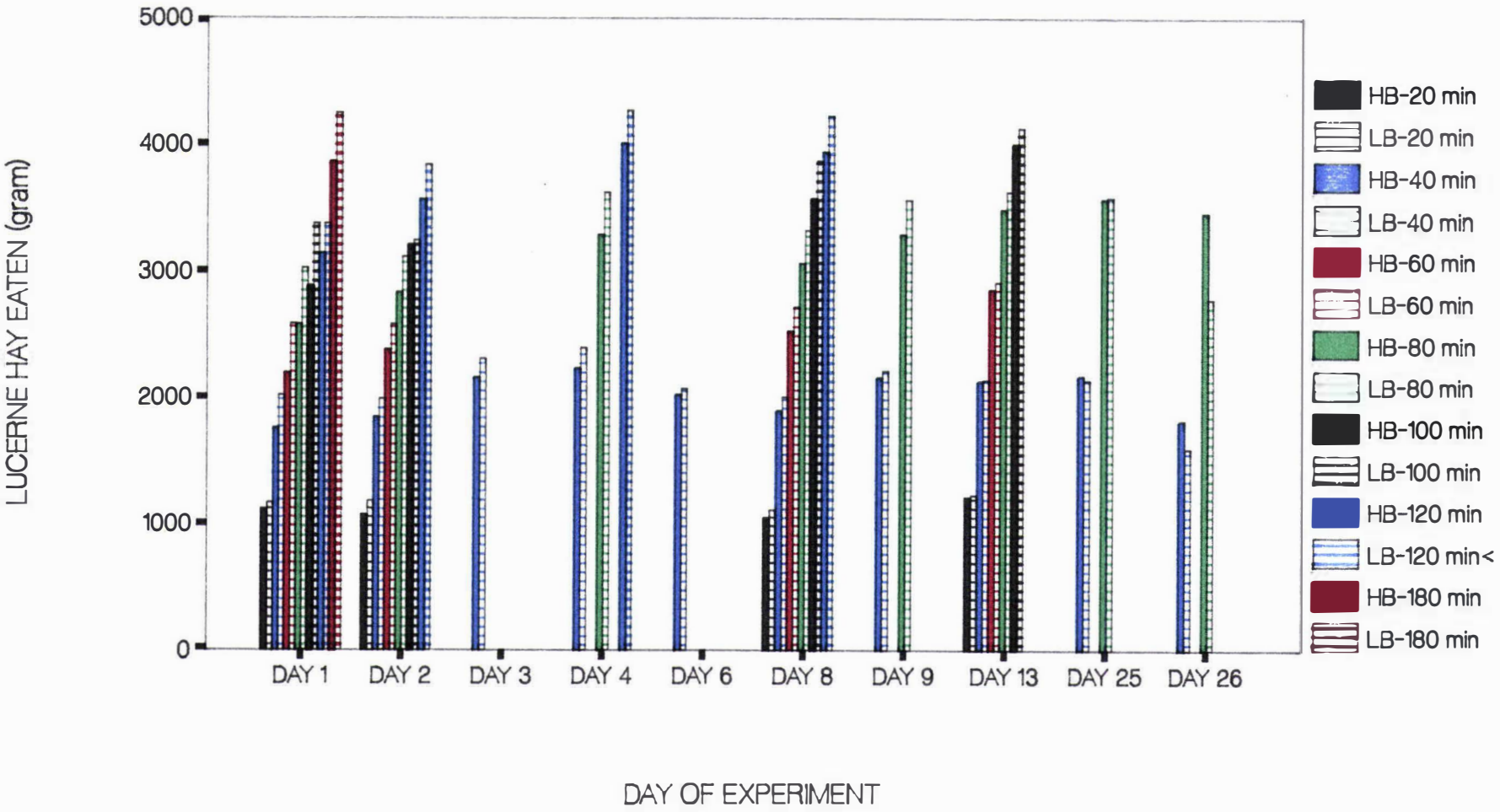


APPENDIX 3: Patterns of plasma insulin concentration in 8 HBI heifers (left panels) and 8 LBI heifers (right panels) over a 6 hour period (Chapter 4).

APPENDIX 4: The rate of eating in terms of percentage (%) of allowance consumed per unit time over the experiment in 8 high breeding index (solid bars) and 8 low breeding index (hatched bars) heifers.



APPENDIX 5: The rate of eating in terms of amount (g) of allowance consumed per unit time over the experiment in 8 high breeding index (solid bars) and 8 low breeding index (hatched bars) heifers.



APPENDIX 6: Determination of the concentration of Evans blue dye (T1824) in plasma

The method described by Consolazio et al. (1964) was used to determine the concentration of T1824 in plasma and is outlined below.

Standards

The standard curve was constituted by diluting the stock solution (4 mg/ml, a proportion of which had been used for the challenge) with physiological saline over a range of 4, 8, 12, and 20 µg/ml. The optical density of the standard solutions was measured at 620 nm using distilled water as a blank. The plot of optical density (O.D) against concentration is shown in Figure A6. The regression equation describing the curve was :

$$Y = 22.19x + 0.33$$

where

Y= T1824 concentration of the sample

X= the O.D of the sample

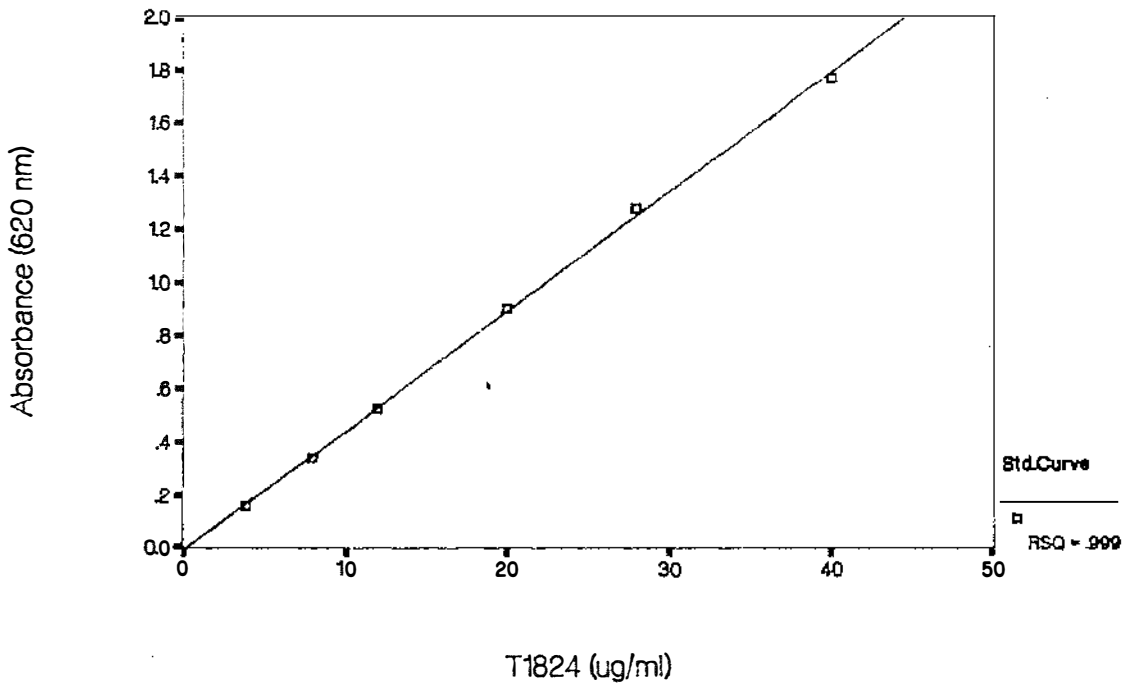
and the r^2 value was 0.9996.

Blood analysis

A plasma blank from the pre-challenge sample (at -5 minutes) of each animal was prepared and set at '0' density at a wavelength of 620 nm. Plasma samples (0.4 ml) were diluted with 0.6 ml saline containing 1% sodium citrate, as the T1824 concentration in a few samples was greater than the highest standard (20 µg/ml). All the diluted samples were then measured in duplicate on the spectrophotometer and the T1824 concentration estimated using the above standard curve. All samples were determined within one assay and the intra-assay coefficient of variation was less than 1.5%.

Since haemoglobin also absorbs at 620 nm and could interfere with the result, it was necessary to check that the experimental procedure had not caused any haemolysis and contamination of the plasma with haemoglobin. Thus the plasma samples from each animal were checked by passing the sample through a column of anionic-detergent washed shredded tissue paper. Under these conditions, the T1824 binds to the paper while haemoglobin passes through the column and is clearly visible in the eluent if present. All the samples were free from haemolysis.

FIGURE A6. STANDARD CURVE OF EVANS BLUE (T1824)



APPENDIX 7: Determination of thiocyanate (NaSCN) in blood plasma

The plasma NaSCN concentration in the samples collected after NaSCN challenge was determined by the method of Consolazio et al. (1964). This method is based on the formation of an orange colour when NaSCN is mixed in acid conditions with $Fe(NO_3)_3$.

Reagents The following reagents were used:
20% trichloracetic acid, filtered before use
Ferric nitrate solution, 80 g of $Fe(NO_3)_3.9H_2O$ dissolved in 2 N nitric acid and made up to 250 ml.

Standards Standard solutions were made up by dissolving 160 mg NaSCN in 200 ml dH_2O, and further diluted to 2.5, 5, 10, 20 and 40 µg/ml.

Methods

1) Plasma proteins were precipitated by diluting 0.2 ml plasma with 1.3 ml H_2O, mixing with 0.5 ml 20% trichloroacetic acid in LP3 tubes and standing for 15 minutes. The mixture was then centrifuged at 3000 g for 15 minutes.

2) 0.6 ml of the supernatant from the samples or of the standard was transferred into LP3 tubes and mixed with 0.6 ml of the ferric nitrate solution. Since the colour developed in the mixture is unstable in the presence of light, the final reaction was carried out under dimmed lighting to delay the decay of the colour and only small batches were processed at a time.

3) The standards and the samples were read on a spectrophotometer at 460 nm within 15 minutes of adding the ferric nitrate solution, with a blank control plasma sample set at 100% T at 460 nm under dimmed light.

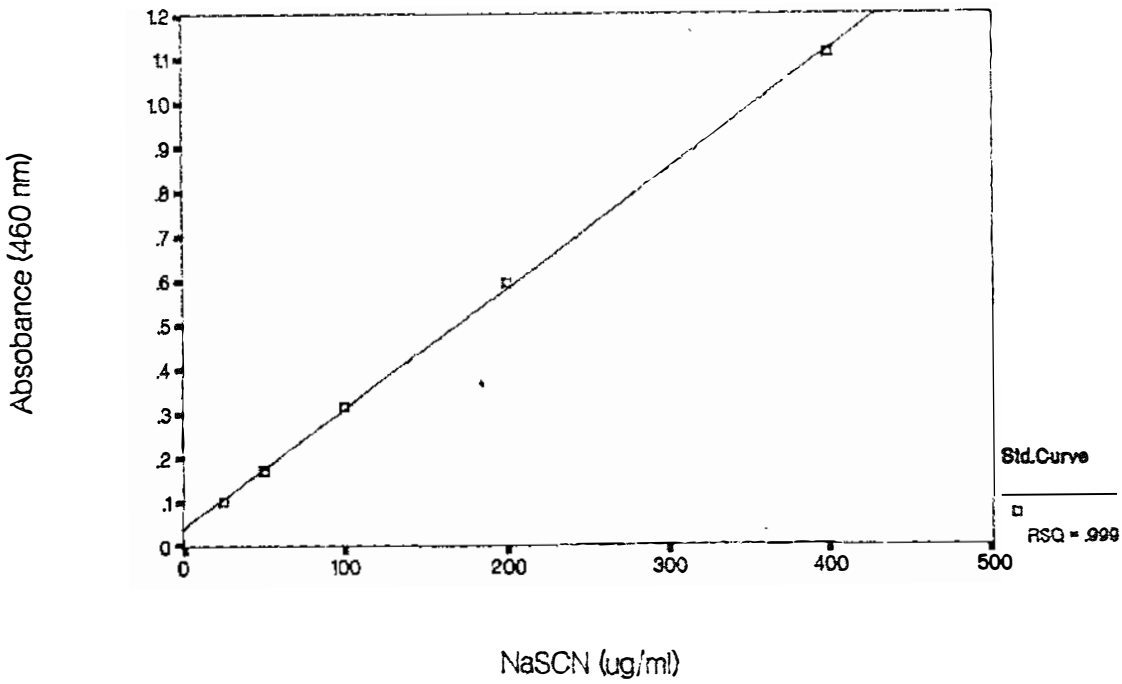
4) The standard curve obtained by plotting the optical density against concentration is shown in Figure A7. The regression equation was:

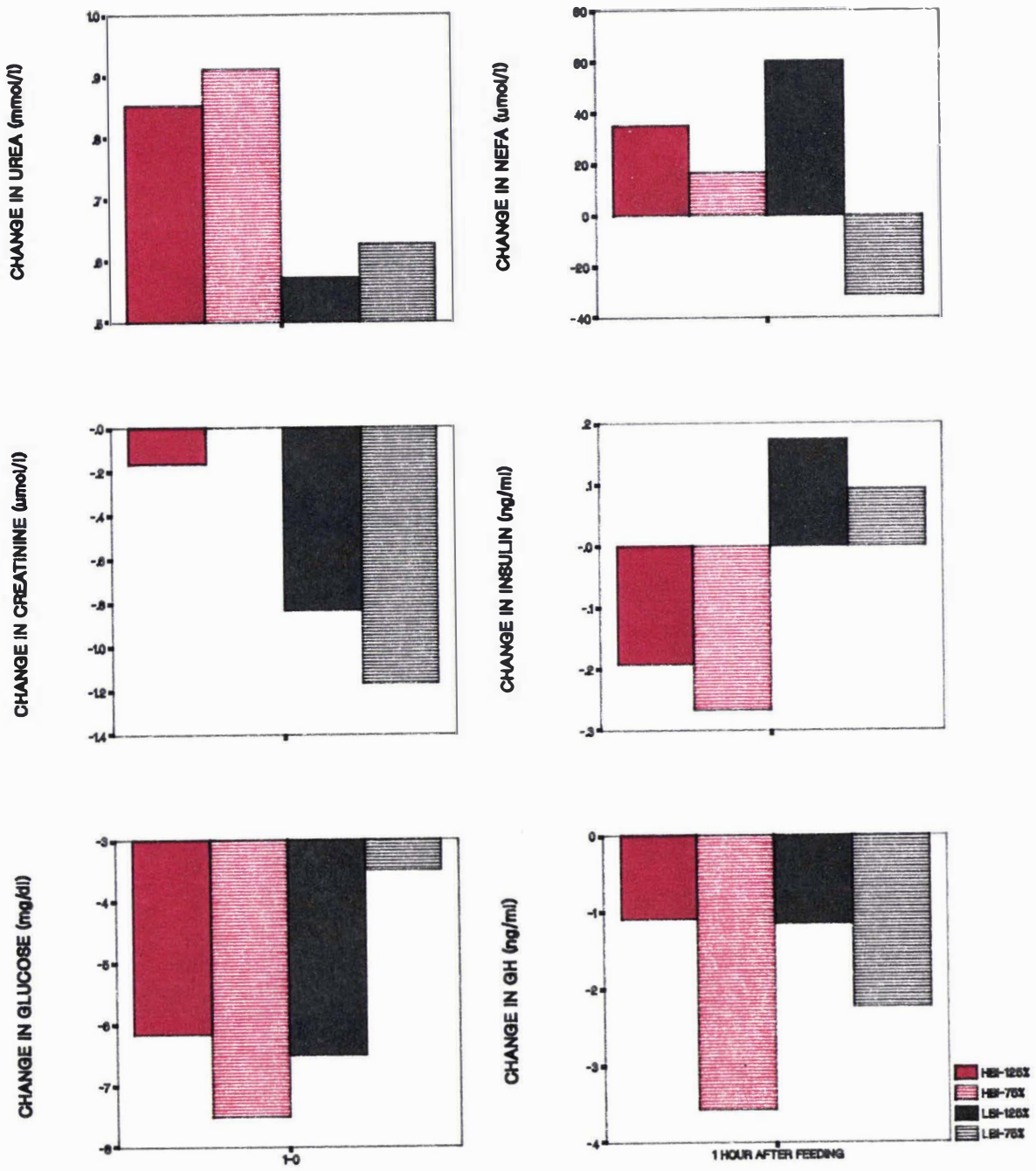
$$Y = 0.036757\ X - 1.4232$$

where

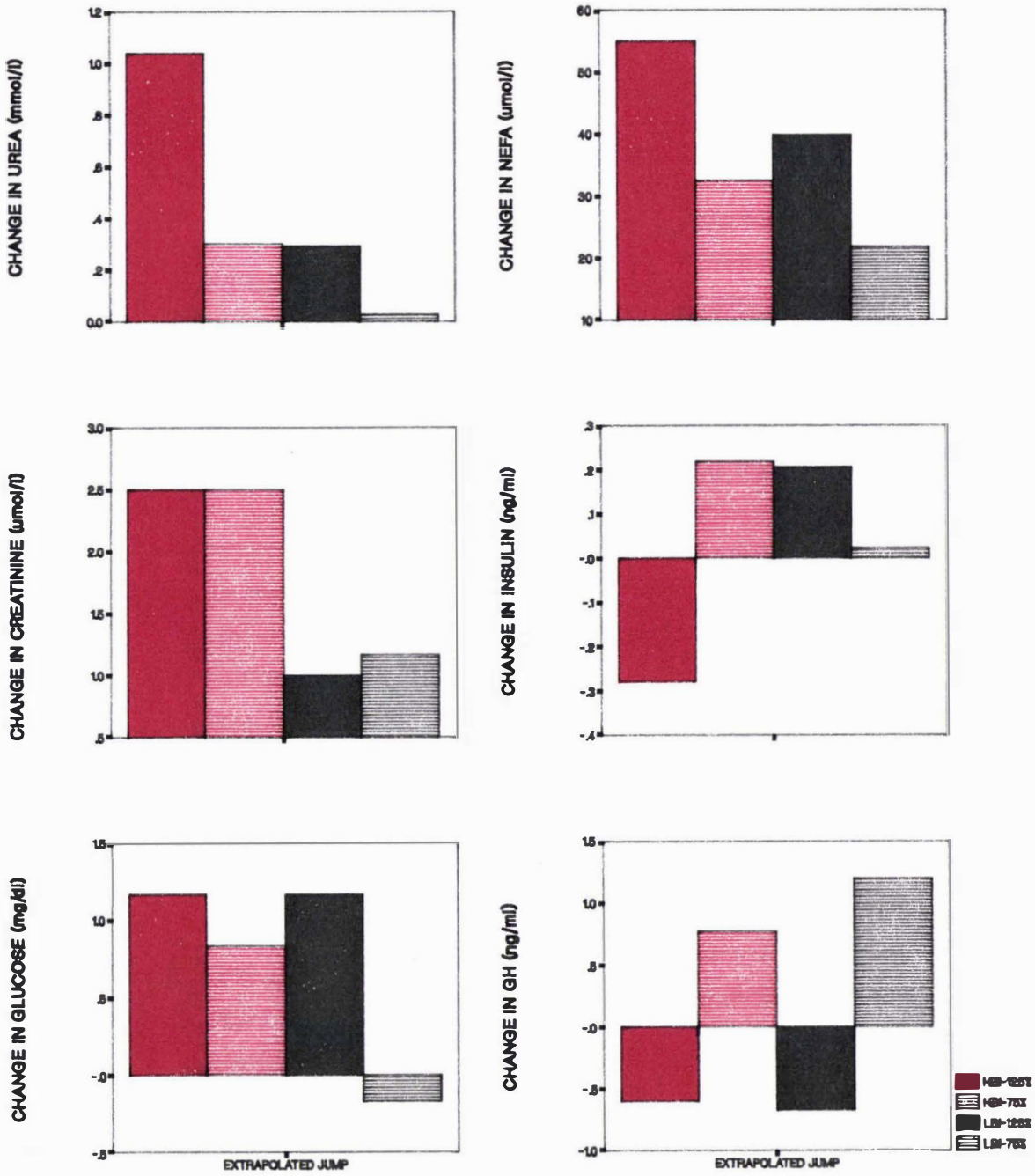
Y= plasma NaSCN concentration (µg/ml) and X is the reading of the absorbance. The r^2 value was 0.9998. All samples were determined in duplicate in one assay and the intra-assay coefficient of variation was less than 3%.

FIGURE A7. STANDARD CURVE OF THIOCYANATE (NaSCN)

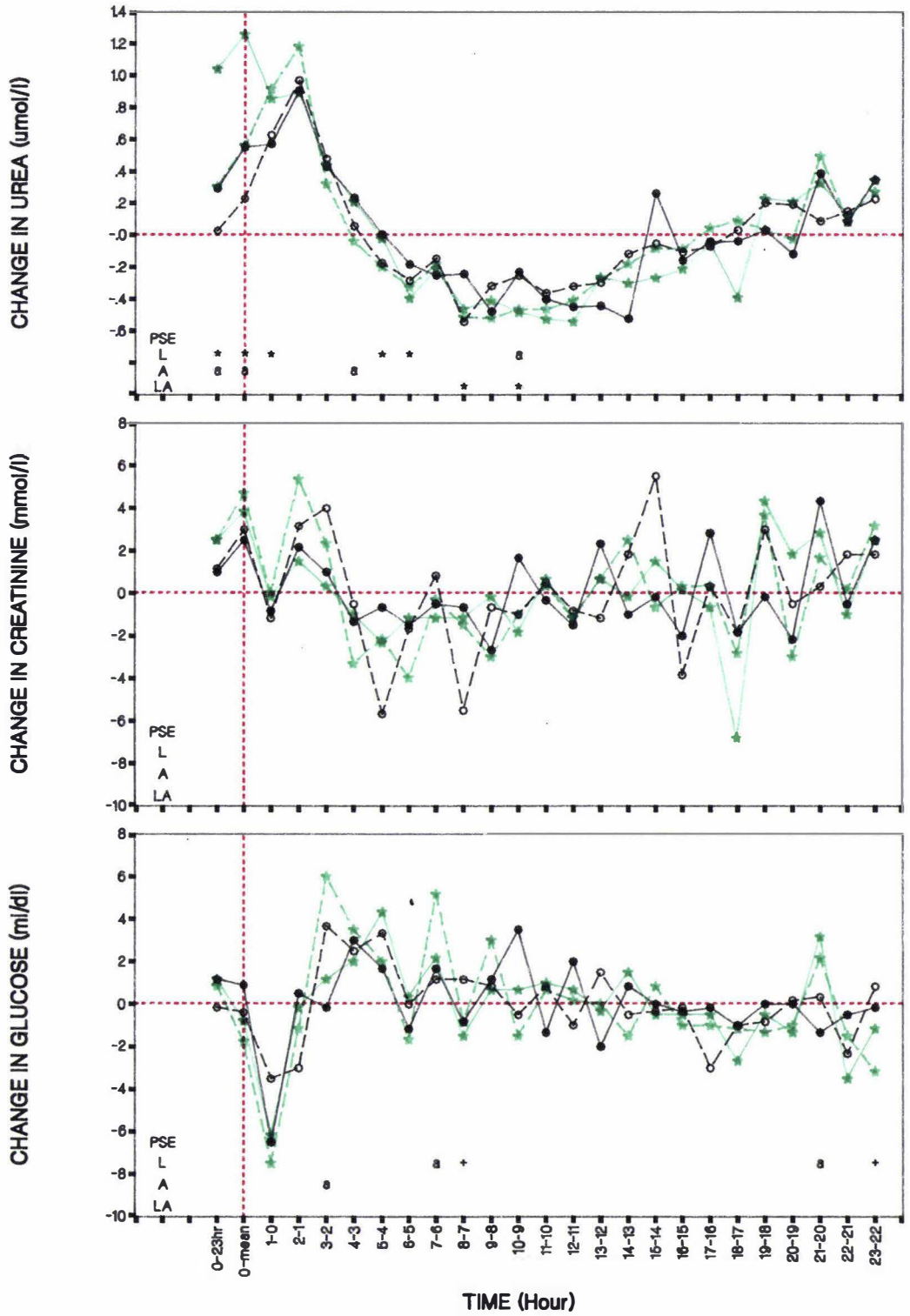




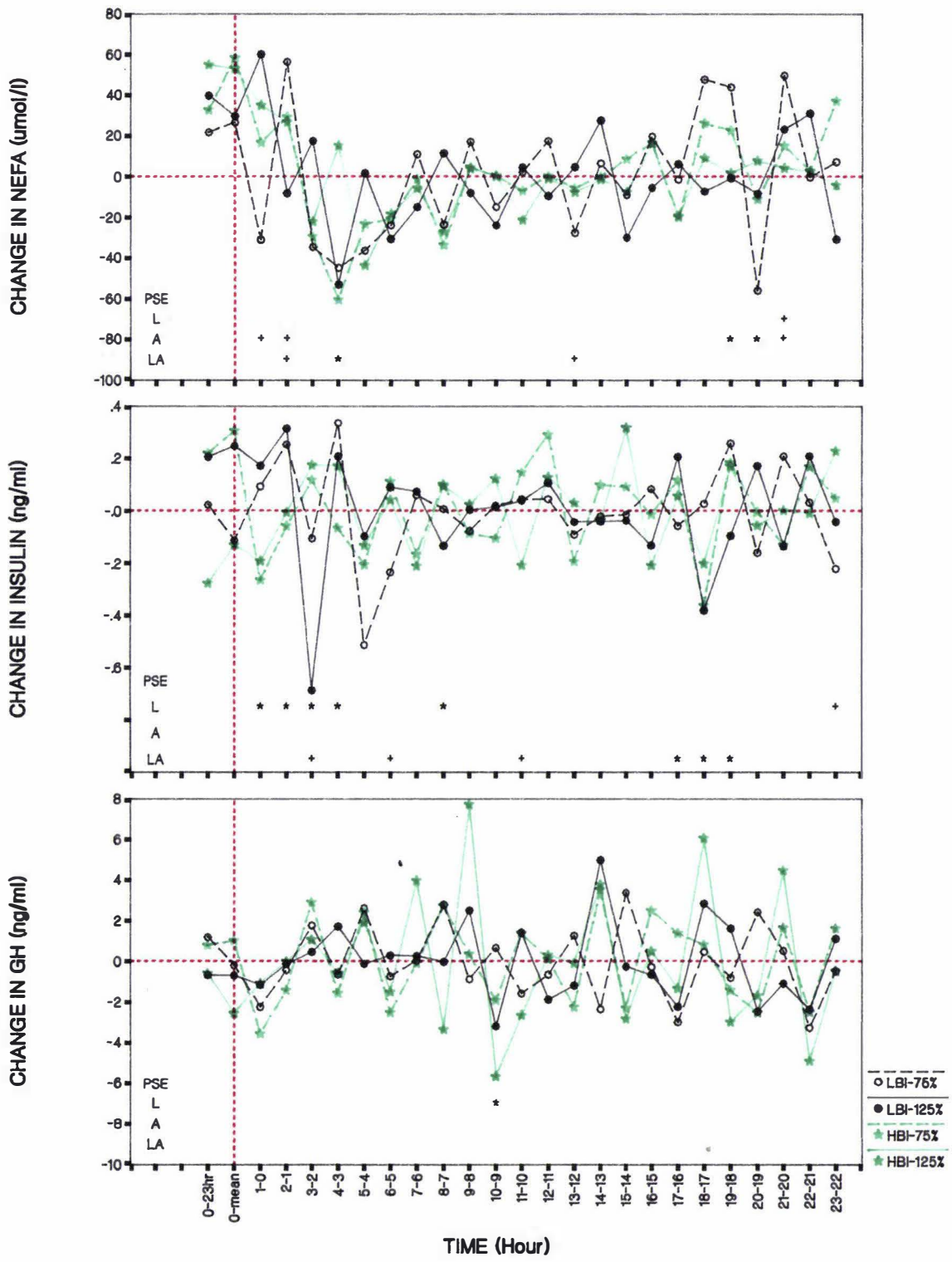
APPENDIX 8a: Feeding-induced changes in plasma concentrations of urea (upper-left panel), creatinine (middle-left panel), glucose (lower-left panel), NEFA (upper-right panel), insulin (middle-right panel), and GH (lower-right panel) in 6 high breeding index (red) and 6 low breeding index (black) heifers offered 75% (hatched bars) and 125% (solid bars) maintenance energy requirement. Changes in concentration = concentration at time 1 (1700h) minus concentration at time 0 (i.e feeding time: 1600h). (Chapter 3)



APPENDIX 8b: Feeding-induced changes in plasma concentrations of urea (upper-left panel), creatinine (middle-left panel), glucose (lower-left panel), NEFA (upper-right panel), insulin (middle-right panel), and GH (lower-right panel) in 6 high breeding index (red) and 6 low breeding index (black) heifers offered 75% (hatched bars) and 125% (solid bars) maintenance energy requirement. Changes in concentration = concentration at time 0 (i.e feeding time: 1600h) minus concentration at time 23 (i.e.1 h before feeding). (Chapter 3).



APPENDIX 8c: Relative changes in plasma concentrations of urea (upper panel), creatinine (middle panel) and glucose (lower-left panel) after feeding in 6 high breeding index (star) and 6 low breeding index (square) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. Changes in concentration = concentration at question minus the concentration of the previous observation.



APPENDIX 8d: Relative changes in plasma concentrations of NEFA (upper panel), insulin (middle panel) and GH (lower panel) after feeding in 6 high breeding index (star) and 6 low breeding index (square) heifers offered 75% (broken line) and 125% (solid line) maintenance energy requirement. Changes in concentration = concentration at question minus the concentration of the previous observation.

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