MANIPULATION OF RUMINANT LACTATION USING PHOTOPERIODIC AND ENDOCRINE TREATMENT

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Contents

LIST OF FIGURES

Chapter three

Chapter four

Chapter five

LIST OF TABLES

LIST OF ABBREVIATIONS

ANCOVA	- analysis of covariance
AUC	- area under the curve
β-HDB	- β-hydroxybutyrate
bST	- bovine somatotropin
CB154	- bromocriptine
CP	- crude protein
E2	- oestrogen
FFA	- free fatty acids
FIL	- feedback inhibitor of lactation
GABA	- γ-Amniobutryic acid
GH	- growth hormone
GHBP	- growth hormone binding protein
GLU	- glucose
GRF	- growth hormone releasing factor
GRL	- glycerol
h	- hour
HGM	- high genetic merit
IGF-I	- insulin-like growth factor-I
IGF-II	- insulin-like growth factor-II
IGFBP	- insulin-like growth factor binding protein
INS	- insulin
kg	- kilogram
l	- liter
LGM	- low genetic merit
MEL	- melatonin
n.s	- non significant
α-MSH	- α-melanocyte-stimulating hormone
OT	- oxytocin
PD	- pars distalis
PL	- placental lactogen
PIF	- prolactin inhibiting factor
PRF	- prolactin releasing factor
PRL	- prolactin
PT	- pars tuberalis
REML	- residual maximum likelihood
RIA	- radioimmunoassay
RMYQ	- relative milk yield quotient
S.E.D.	- standard error of difference
S.E.M.	- standard error of the mean
SRIF	- somatostatin
T4	- thyroxine
TRH	- thyrotropin-releasing hormone
VIP	- vasoactive intestinal peptide

ABSTRACT

This thesis examines some aspects of milk yield manipulation utilizing some factors that can affect the function of the mammary gland.

In the first part of study, the effect of photoperiod on lactation performance in the goats was studied, and particularly to investigate if this response can be potentiated by prior exposure to short periods of short days elicited by melatonin treatment. Long light did not produce a clear stimulatory effect on milk yield, but a small response was seen in autumn and only in goats that were not treated by melatonin. Repeated short cycles of melatonin did not sensitize lactating goats to subsequent long light effect on milk yield regardless of stage of lactation or commencement time of year. Indeed, this treatment might produce a detrimental effect on milk yield when applied in early lactation.

The second part of the study was to determine the maximum metabolic capacity of cows from different genetic merit. We adopted a multiple galactopoietic stimuli, increasing milking frequency, bovine somatotropin and thyroxine, applied in additive stepwise fashion at peak yield to cows from high and low genetic merit. This approach was successfully drove the cows into what we believe their maximum metabolic capacity. Milk yield was increased in an additive fashion at each stimuli. The increase in milk yield capacity was associated with mammary growth which was detected during the maximum stimuli. There was no significant difference in the response to the galactopoietic stimuli between cows from different genetic merit which did not suggest that high genetic merit cows are milking closer to their maximum capacity and, therefore, at greater risk of collapse of metabolic control than low genetic merit cows.

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DECLARATION

The second part of this thesis was part of a major project funded by SOAEFD designed to study the effects of metabolic stress in dairy cows of differing genetic merit. The overall project was performed jointly with others, I was involved with all aspects of the project and with the generation of all of the data. All of the endocrine data reported here are entirely novel. Some of the base data (milk yields, body weights) have previously been reported as part of an M.Sc degree from Copenhagen University (by Annette Sorensen) and are reported here since they are essential to the understanding and discussion of the work. The determinations of blood metabolites reported in chapter 5 were performed by E. Ferguson. With these exceptions, I declare that the work contained in this thesis is my own, undertaken under the supervision and guidance of Dr. C.H. Knight.

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CHAPTER ONE

REVIEW OF LITERATURE

1.1 INTRODUCTION

Milk yield is greatly influenced by the total number of secretory cells of the mammary gland which is determined mostly during mammary development occurring during pregnancy. It has been estimated in the rat that the increase in milk secretion between parturition and peak lactation might be brought about entirely by increase in cell number (Knight *et al.*, 1984). Cellular differentiation which enhance the secretory activity will also contribute to the increase in milk yield (Wilde & Kuhn, 1979). In the goat, cellular proliferation does not continue till peak lactation rather it ceases within 2-3 weeks after parturition (Knight & Peaker, 1984; Wilde *et al.*, 1986) indicating that the increase in cell number may be less important as a determinant of milk yield in early lactation in the goat, and the increase in cellular synthetic capacity contributes mostly to the rise in milk yield up to peak lactation. In the declining phase of lactation cellular activity, as determined by enzyme activities and *in vitro* lactose and casein synthesis rate, is not changed, but it is the loss of cellular number that is the major factor in the gradual fall of the milk yield (Wilde *et al.*, 1986). Initial milk yield and persistency of lactation, which is defined as the rate of decline in milk yield after peak lactation is achieved are important factors affecting the shape of the lactation curve of the lactating cow. A great amount of attention has been made in the period of when milk yield starts to decline looking for ways of manipulating the lactation curve in a manner that will increase milk yield or maintain milk secretion and hence improve persistency which overall results in increase in total milk produced in the lactation cycle. This can be achieved in part by maintaining the milk secretion capacity of the mammary gland by stimulating cellular proliferation and mammary growth, slowing the

rate of cell death (apoptosis), or increasing the secretory activity per cell. A number of stimuli can achieve this improvement in lactation, which includes alteration in systemic hormonal levels (growth hormone or bovine somatotropin, thyroxine), alteration in a local factor that regulates milk secretion rate (frequent milking), or manipulation of the external environment, ie photoperiod (Blaxter *et al.*, 1949; Tucker, 1985; Wilde *et al.*, 1995).

Milk secretion is highly regulated by the endocrine system, which has a major role to play in providing an optimum environment to support the huge metabolic demand for milk secretion in early lactation and for lactation to be maintained. For example, alteration in the secretory activity of lactotrophs and somatotrophs in the anterior pituitary ensures high levels of prolactin and growth hormone in the circulation. Another example which is related to the adaptation of the endocrine system is alteration in the metabolic activity of some organs and tissues in a way that will provide a support for milk secretion. The stimulatory effect of insulin on adipose tissue is reduced or diminished in the lactating animal (Vernon, 1989), for instance. In this chapter, hormones that are closely related to milk production will be reviewed. Galactopoietic factors that have been employed in manipulating milk production, which include local factors (frequent milking), environmental factors (photoperiod) will be discussed. Finally, the most widely used to improve milk production, genetic selection, will be discussed with emphasis on some physiological factors that have been altered in response to genetic selection for high milk production.

1.2 PROLACTIN

1.2.1 Prolactin structure and receptor

Prolactin (PRL) is one member of a family of closely related polypeptide hormones which includes growth hormone, placental lactogen and PRL. Generally, it consists of around 197-199 amino acid residues with a molecular weight of around 23,000 da. Although

the major form of PRL exists in a monomer form, different studies have reported different forms of PRL with different molecular weights and possibly different biological potencies. In mouse serum, Sinha, (1980) reported the existence of a large molecular weight form ("big big"), intermediate ("big") together with the monomeric form ("little"). Another form of PRL which has been detected is the glycosylated form with a possible different biological potency and immunoreactivity from reference PRL (Lewis *et al.*, 1984). The physiological significance of these different forms is not fully understood, but they may be preferentially secreted in response to specific physiological situations. For example, a shift in molecular size distribution in response to milking in cows in which the big form disappeared and the big-big form appeared in the circulation after milking (Gala & Hart, 1980). Also, in the ram PRL variants with higher molecular weights are at significantly higher concentrations in the pituitary of rams maintained in the winter months (Stroud *et al.*, 1992). It can be anticipated that different variants of PRL are secreted by specific stimuli or releasing factors to serve specific biological activities in specific target cells; a concept which is in agreement with the wide biological activity of PRL in different tissues and organs. However, whether a single lactotroph cell has the ability to produce different variants or different cells produce specific variants is not known.

In order for PRL to manifest its effect on target cells, it must first bind to specific binding sites on the surface membrane on these cells. Receptors for PRL belong to the growth hormone/prolactin cytokine family (Wallis, 1992) which is characterized by a transmembrane domain and absence of intrinsic tyrosine kinase. Studies with PRL receptor have indicated the presence of two different forms of PRL receptor; low form with a molecular weight of about 35,000 da and a large one with around 85,000 da and this difference is attributed to the length of the cytoplasmic domain (Kelly *et al.*, 1992). The two forms could be transcribed from multiple mRNA species like the situation in rat liver and

mammary gland (Jahn *et al.*, 1991), but in the rabbit mammary gland they result from post-translational processing of a single long form of the receptor (Edery *et al.*, 1989). In the rat, the molecular form of the receptors showed variation according to the developmental state, and some PRL receptors variants are preferentially present in specific physiological situations. For example, low molecular weight form is present in the mammary gland only during lactation (Guillaumot & Cohen, 1994); the short form predominates in some tissues like liver and mammary gland whereas the long form is present at high concentrations in other tissues like the ovary (Kelly *et al.*, 1992). However, in the cow apparently only one form of receptor of molecular weight of 36,000 is present throughout the developmental stages of the mammary gland (Smith *et al.*, 1993). The structural heterogeneity of PRL receptors might suggest different signal transduction mechanisms which might be involved in mediating the PRL actions in different target tissues and developmental stages.

1.2.2 Control of PRL secretion

Prolactin is secreted by secretory cells, lactotrophs, in the adenohypophysis. A number of neurotransmitters and peptides which are synthesised in the hypothalamus and transported to the anterior pituitary, via portal blood system, exert either stimulatory or inhibitory effects on PRL release. The role of the hypothalamus in mediating inhibitory effects on PRL secretion was demonstrated when the anterior pituitary gland was separated from the hypothalamus, which resulted in steady increase in PRL secretion (Nagy *et al.*, 1979; Lincoln & Clarke, 1995) suggesting the removal of mainly inhibitory factor(s) from the hypothalamus.

Different factors produced mainly by the hypothalamus have the capability to exert, with varying degree, an inhibitory effect on PRL synthesis and release, but dopamine is considered to be the predominant one and widely recognized as the prolactin inhibitory

factor (PIF). Dopamine is a neurotransmitter synthesised in neurons of the arcuate nucleus whose axons terminate in the median eminence capillary, and is then released into portal vessels and transferred to the anterior pituitary (Moore, 1987) where it interacts with D2 subtype of dopaminergic receptors on the lactotrophs causing inhibition of PRL release (Ramsdell *et al.*, 1985; Moore, 1987). Dopamine also inhibits PRL gene transcription and suppresses lactotroph proliferation (Ben-jonathan, 1985). The specificity of the inhibitory effect of dopamine on PRL release is clear since removal of the dopaminergic influence by dopaminergic blockers increase PRL release by competing with dopamine receptors and hence blocking the inhibitory action of dopamine (Lopez *et al.*, 1989; Lincoln & Clarke, 1995). Therefore, dopamine has a major role to play in regulating the PRL release and it has been suggested that chronic tonus of dopamine is responsible for the basal levels of PRL and during periods of physiological stimulation the coordinated action of stimulating factors coupled with a transient decline in the dopaminergic system results in augmenting the PRL release (Ben-jonathan *et al.*, 1980; De Greef & Visser, 1981).

Also, several factors of hypothalamic origin exert some regulatory mechanism on PRL release. The ability of γ-Aminobutryic Acid (GABA) to inhibit PRL release *in vitro* was demonstrated (Lamberts & Macleod, 1978) but only with high doses by acting directly on the anterior pituitary (Grandison & Guidotti, 1979). Somatostatin (SRIF), which is a potent inhibitor of growth hormone release, also has the ability to induce inhibitory effect on basal as well as stimulated PRL release as has been demonstrated in the rat pituitary cells in culture (Hanew & Rennels, 1982), but this inhibitory effect seems to be oestrogen (E2) dependent, since E2 can act directly to regulate the sensitivity of lactotrophs to somatostatin possibly by modifying the number of its receptors (Kimura *et al.*, 1986). The presence of PIF in the posterior pituitary lobe was reported by Ben-Jonathan and Peters (1982), when they observed an elevation of basal PRL after posterior pituitary lobectomy in the rat which

suggested the presence of an inhibitory factor in this area of pituitary. The posterior pituitary lobe participates in PRL regulation most probably by acting as another way of delivering dopamine to the anterior pituitary (Ben-jonathan, 1985); since some of the axones of the neurones of the arcuate nucleus which synthesize dopamine terminate in the posterior lobe.

Although a primary PRL releasing factor has not yet been identified, several factors with PRL releasing activity have been described under different physiological conditions. Several factors have been reported to show stimulatory effects on PRL release with different origin and different potency. Vasoactive intestinal peptide (VIP) which originates mainly from the paraventricular nucleus in the hypothalamus which is transported to the anterior pituitary via the portal blood system (Abe *et al.*, 1985) where it can act directly on lactotrophs to stimulate PRL release (Kato *et al.*, 1978), and the significance of this peptide in PRL regulation has been demonstrated by the finding that passive immunization with antisera against VIP reduced PRL secretion (Kaji *et al.*, 1985). A possible involvement of VIP in the physiological control of PRL during lactation has been suggested in which lactation induces a significant increase in VIP synthesis in the suprachiasmatic nucleus (Gozes *et al.*, 1989). It has been suggested that VIP may regulate PRL release through an intracellular mechanism since the lactotrophs have the capability to synthesis VIP and hence act as an autocrine factor to stimulate PRL synthesis (Arnaout *et al.*, 1986; Balsa *et al.*, 1996).

Thyrotropin-releasing hormone (TRH) is another factor with the ability to stimulate PRL secretion by acting directly at the level of the anterior pituitary to stimulate PRL release. Administration of TRH resulted in a significant increase in PRL in cattle (Vines *et al.*, 1976; Marcek & Swanson ,1984), also, it has been suggested that TRH may be involved in the regulation of suckling-induced PRL release (De Greef *et al.*, 1987).

Serotonin or serotonin precursors exert some stimulatory actions on PRL release from the anterior pituitary (Lu & Meits, 1978). Serotonin has been suggested to mediate its effect on PRL release by modulating dopamine release (Pilotte & Porter, 1981) and it increases TRH and VIP concentrations in the portal system (Jordan *et al.*, 1978; Shimatsu *et al.*, 1982).

The presence of PRF in the posterior pituitary lobe was reported when the posterior lobe was excised from lactating rats which prevented suckling-induced PRL release suggesting the requirement of the posterior lobe for this phenomenon (Murai & Ben-jonathan, 1987). Furthermore, posterior pituitary extracts stimulate PRL release from the anterior pituitary *in vitro* (Hyde & Ben-jonathan, 1988) and *in vivo* (Hyde & Ben-jonathan, 1989), but the nature of this releasing factor and how it mediates its effect on PRL release is still to be determined.

Oestrogen is considered to be a potent factor regulating PRL release since E2 can act directly on lactotrophs to stimulate synthesis and secretion of PRL (Augustine & Macleod, 1975; Kino & Dannies, 1981) and also exerts a mitogenic effect on lactotrophs (Amara *et al.*, 1987). Furthermore, E2 can modulate the hypothalamic PRL inhibitory and stimulatory factors and thereby affecting the responsiveness of lactotrophs to regulatory factors. For example, E2 has been shown to decrease dopamine release and receptors on lactotrophs (Cramer *et al.*, 1979; West & Dannies, 1980) and enhance the stimulatory effect of TRH (Hu & Lawson, 1994) by increasing its receptors on lactotrophs (Gershengorn *et al.*, 1979). Although stimulatory effect of E2 on PRL release has been well documented, under specific conditions E2 can also exert inhibitory effect on PRL secretion (Shull & Gorski, 1989).

Other factors that have been reported to have stimulatory effect on PRL release were oxytocin, angiotensin II, insulin-like growth factor-I, opiates, met-enkephalines and several growth factors (Lamberts & Macleod, 1990).

1.2.3 The influence of season on PRL release

Seasonal influence on PRL release is considered to be important in determining the levels of circulating PRL, with two seasonal factors that are notably involved, photoperiod and ambient temperature. Concentrations of PRL in sera are highest during the long light days and lowest during the short days in seasonally breeding animals like sheep and goats (Lincoln & Ebling, 1985; Emesih *et al.*, 1993) and also in non-seasonally breeding animals like the cow (Koprowski & Tucker, 1973).

Photoperiod influences PRL release via its effect on melatonin (MEL) which is produced by the pineal gland. It has been demonstrated that elimination of MEL from the circulation by pinealectomy abolishes the normal photoperiod-induced changes in PRL in the sheep (Brinklow & Forbes, 1984a) and disrupts the seasonal variation in PRL profiles in the deer (Schulte *et al.*, 1981); in the goats, superior cervical ganglionectomy which perturbs the pineal-photoperiod pathway also disrupts the annual cycle of PRL profiles (Buttle, 1977; Maeda *et al.*, 1986). However, pinealectomy in cattle has little or no effects on PRL induction by long light exposure (Petitclerc *et al.*, 1983; Stanisiewski *et al.*, 1988). Thus, MEL is considered to be a prime candidate for regulating the fluctuation in seasonal PRL profiles in the seasonally breeding animals but to have less role in non-seasonally breeding animal like cattle.

The diurnal rhythm of MEL secretion follows the light-dark cycle. Melatonin profiles during the dark period of the day are characterized by peak values and it is suppressed during the light period (Rollag & Niswender, 1976). It is the duration of nocturnal MEL secretion rather than the amplitude which provides an endocrine index of night length and thus the day length. Consequently, infusion of pinealectomized rams with MEL induced biological responses correlating with the duration of infusion and thus the duration of elevated MEL profile is the mediator of day length on biological activities

affected by season (Lincoln, 1992). Both sheep and goats in temperate climates are short day breeders; their reproductive cycle initiates in response to decreasing day length in the autumn. Therefore, increase in the duration of MEL secretion is the key factor in triggering an endocrine response that leads to start of breeding season (Arendt, 1986; Chemineau *et al.*, 1986). Consequently, MEL has been used to advance the breeding season in sheep and goats (Arendt, 1986; Deveson *et al.*, 1992a). Administration of MEL is an effective way of advancing the breeding season by around two months in the sheep, and mimics the effects of short days on PRL release when administered during long days (Arendt, 1986). Thus, MEL signal is mediating the multiple effects of photoperiod on timing of seasonal reproductive cycle and seasonal variation in PRL release. However, the exact mechanism by which MEL regulates the seasonal PRL profiles is not fully understood, but two pathways have been suggested; by acting through the hypothalamus and hence modulating the PRF or PIF involved in PRL release, or acting directly on the anterior pituitary to regulate the PRL release.

Local administration of MEL within the hypothalamus by microimplants inserted into the mediobasal hypothalamus during long days exposure in the ram induced a short day response effect on PRL release (Lincoln & Maeda, 1992). This suggests that the mediobasal hypothalamus might be a site of MEL action in the photoperiodic influence on PRL release, possibly by affecting the release of neurotransmitters or neuropeptides that are involved in PRL secretion regulation. Although the part of the neural system which might be the specific target for MEL action is not known, it is thought to involve the dopaminergic pathway (Lincoln & Maeda, 1992). Thus, MEL might be acting in the mediobasal hypothalamus area or adjacent areas to stimulate the release of dopamine which consequently delivers to the anterior pituitary an inhibitory signal. However, data from the same laboratory did not support the hypothesis that MEL signal is mediated mainly through

dopaminergic pathway but they did not exclude the involvement of this pathway in mediating the effects of MEL on PRL release (Lincoln & Tortonese, 1995), and the mechanism by which the MEL signal is mediated through the hypothalamus is not clear and it might involve more than one pathway.

The other pathway, in which MEL has been suggested to act directly on the anterior pituitary, is supported from experiments in which the pituitary gland was disconnected from the hypothalamus in rams. This disconnection did not affect the seasonal profiles of PRL as evident by the ability of MEL to exert a short day signal when applied during long day exposure (Lincoln & Clarke, 1994; 1995), suggesting that the MEL signal which encodes the day length may act directly in the pituitary to mediate the effects of photoperiod on PRL release and this photoperiod transduction pathway bypasses the hypothalamus. The presence of MEL binding sites with high density in the anterior pituitary gland is mostly restricted to the *pars tuberalis* (PT)(Dereviers *et al.*, 1991; Piketty & Pelletier, 1993), and the administration of MEL micro-implants in PT has been shown to depress PRL concentrations in the ram (Lincoln, 1994; Malpaux *et al.*, 1995). Taken together, these findings suggest that MEL might be acting directly on PT to modulate the release of factor(s) that act on neighbouring *pars distalis* (PD) cells through a paracrine fashion. However, a direct effect of MEL on PD cannot be excluded. The presence of a low number of MEL binding sites in PD of different species studied which makes it unlikely that MEL is acting through its receptors (Boissinagassee, 1992; Nonno *et al.*, 1995), but may be acting directly by a mechanism which does not involve interaction with membrane binding sites.

The other environmental factor that exhibits some influence on PRL release is the ambient temperature. Increasing ambient temperature above the thermoneutral zone significantly increases serum PRL in heifers and conversely lowering temperature depresses PRL levels (Wettemann & Tucker, 1974) with a tendency for a linear relationship between

PRL levels and ambient temperature. Not only the basal levels are influenced by temperature but also the stimulated levels, since TRH stimulated PRL release was found to be reduced at 10°C and abolished at 4.5°C whereas maintaining heifers at 27°C caused more PRL to be released after TRH injection when compared to heifers exposed at 10°C (Tucker & Wettemann, 1976). This might suggest that temperature regulates PRL release by affecting the lactotrophs responsiveness to PRL releasing factors. The influence of ambient temperature on PRL release is also demonstrated by *in vitro* studies; the secretory activity of lactotrophs taken from piglets reared at hot environmental temperature was higher when compared to that taken from animals maintained at lower temperature (Matteri & Becker, 1996). The prolactin inhibitory factor, dopamine, has been suggested to be involved in this mechanism since a relationship between temperature and the activity of the dopaminergic neurons has been reported. Ambient temperature is positively correlated with the activity of these neurons; exposure to high temperature inhibits the dopaminergic pathway which results in reduction in the concentration of dopamine that reaches the lactotrophs and hence increasing the PRL release (Tucker *et al.*, 1991). Conversely, low temperature increases the activity of dopaminergic neurons terminating in the infundiblum/pituitary stalk (Tucker *et al.*, 1991) suggesting that dopamine release might mediate the temperature induced changes in PRL release. The concentration of the hormone in the blood circulation is affected by secretion rate as well as clearance rate, so the increase in PRL concentration in response to high temperature exposure is likely to be the result of increase in the rate of secretion coupled with a slowing in the metabolic clearance rate (Smith *et al.*, 1977). Since PRL is highly influenced by environmental temperature; it has been suggested that this mechanism might be related to the thermoregulatory mechanism (Salah *et al.*, 1995) and the change in PRL in relation to ambient temperature might constitute a physiological mechanism by which the animal is responding to alteration in

environmental factors.

There has been some evidence to indicate that both temperature and photoperiod interact together to regulate the PRL release. Exposing cattle to lower temperature blocks the stimulatory effect of long-day on PRL release (Peters & Tucker, 1978). Conversely, maintenance at higher temperature stimulated more PRL secretion in response to longer hours of light exposure when compared to natural photoperiod at the same ambient temperature. These findings suggest a strong interaction between photoperiod signal and temperature in regulating the release of PRL. The stimulatory effect of photoperiod was disrupted by low temperature but at higher temperature, photoperiod and temperature act together synergistically to stimulate PRL release. In agreement with what has been suggested in the ram about the lesser role to be played by the dopaminiergic pathway in mediating the photoperiodic effect on PRL release (Lincoln & Tortonese, 1995), this pathway also has not been shown to be involved in relaying the photoperiodic effect on PRL release in calves (Zinn *et al.*, 1991). Thus, photoperiod and temperature can affect PRL secretion, but each of them uses different neural mechanisms to regulate the release of PRL and both acting together as environmental cues to determine the seasonal profiles of PRL.

1.2.4 Prolactin surge in response to milking stimulus

Suckling or milking provokes a rapid PRL secretory burst from the anterior pituitary into blood circulation in different mammalian species. In goats, the PRL rise is initiated within 1-2 minutes of the start of milking and reaches a peak within 2-15 minutes, and then gradually declines to basal values within about 30 minutes (Hart, 1975a). Milking stimulus for PRL release involves the tactile stimulus of the teats by the nursing young or the milking machine which triggers nerve impulses from the sensory receptors in the teats which are

carried via the afferent nerve fibers through the spinal cord and the midbrain to the hypothalamus (Anderson, 1985). This causes a rapid decline in dopamine release (Chiocchio *et al.*, 1979). Apparently this decrease in dopamine secretion sensitizes the lactotrophs to the subsequent releasing factor(s) (De Greef *et al.*, 1987), so the dopamine suppression which is provoked by the milking stimulus is essential for the occurrence of PRL surge as indicated when dopamine agonist treatment prevents the PRL induction in response to milking in lactating goats (Hart, 1973). Several prolactin releasing factors have been reported to have some degree of involvement in this stimulus, but the exact mechanism is still not fully understood and still debated. Some researchers have suggested that TRH may be involved in mediating the suckling stimulus (De Greef *et al.*, 1987), and a recent study suggested a possible role but only in the first days of lactation (van Haasteren *et al.*, 1996). However, others have shown that TRH has a negligible role (Johke, 1978; Riskind *et al.*, 1984). Another PRF, VIP, is also suggested to be involved in the milking-stimulus since passive immunization against VIP severely attenuates suckling-induced PRL release in lactating rats (Abe *et al.*, 1985).

The posterior pituitary lobe may participate in mediating the suckling stimulus release of PRL since excision of this lobe has been shown to abolish the suckling-induced PRL release (Murai & Ben-jonathan, 1987). Although the nature of this factor, its origin and mode of action is not known, some evidence has indicted that it is the intermediate lobe which serves as the source of the active factor involved in PRL stimulus (Hill *et al.*, 1991) which is believed to be α-melanocyte-stimulating hormone (α-MSH), a main product of the intermediate lobe. A supportive line of evidence about the role of this peptide in suckling is indicated when suckling increases the secretory activity of pars intermedia, and antiserum to α-MSH severely attenuated the PRL release after suckling (Hill *et al.*, 1993); furthermore, *pars intermedia* stores of α-MSH are rapidly depleted within minutes of

suckling (Deis & Orias, 1968) supporting the induction of its release by the milking stimulus. The nature of such proposed action of α-MSH on lactotrophs is not fully understood. It has been proposed that it might act as a responsiveness agent by priming the lactotroph to the releasing factor(s) induced by suckling or milking (Hill *et al.*, 1991; Frawly, 1994).

In goats, PRL release at milking declines as lactation advances, but as a consequence of their breeding seasonality it is difficult to separate the effects of stage of lactation from that of season on the concentration of PRL released at milking. The start of lactation coincides with increasing day length in spring and summer, and late lactation with the decline in day length in autumn and winter (Hart, 1975b). The amount of PRL released at milking in spring and summer is higher than that in autumn and winter, but when it is expressed as a percentage of pre-milking values, it is higher when the basal levels is at its lowest, in the fall and winter, (Hart, 1975b) which suggests an inverse relationship between basal levels and the percentage of increase in response to milking stimulus. Using the month of sampling as covariate, to adjust for season effect, in lactating dairy cows, indicated a gradual decline in post-milking PRL release as lactation advances (Koprowski & Tucker, 1973). In fact, there is a decline in the lactotrophs sensitivity to the secretogenic stimuli during the last stages of lactation (Shanti *et al.*, 1994) and this indicates that the decline in milking related PRL release is associated with stage of lactation. Although the stage of lactation is likely to be predominant factor affecting the post-milking PRL stimulus, environmental factors like day length and temperature are also interfering to some degree in this phenomenon (Hart, 1975b).

1.2.5 Prolactin and lactation

Evidence of a role of PRL in mammogenesis in ruminants has been derived from several experimental procedures. Application of the milking stimulus for a long time to virgin goats, which induces PRL rise similar to that seen after milking, also induced udder growth and ultimately lactation (Cowie *et al.*, 1968). Moreover, induced mammary growth by steroid treatment was prevented with simultaneous treatment with bromocriptine (CB154) in virgin goats (Hart & Morant, 1980) and implantation of goats with perphenazine, which increases PRL release, into the median eminence elicited mammary growth and lactation (Vandeputte-van Messon *et al.*, 1976). These studies clearly demonstrate the ability of PRL to serve as a mammogenic factor regulating mammary development in the absence of placental lactogen (PL) produced by the placenta during pregnancy. However, the depletion of PRL during pregnancy from week 8 to week 20 had a small effect on mammogenesis (Forsyth *et al.*, 1985) which suggests less important role of PRL during pregnancy due to the presence of PL.

The requirement of PRL in maintenance of lactation in small animals is well documented. In the rabbit, treatment with bromocriptine causes an immediate fall in milk secretion, which can be restored by PRL treatment (Taylor & Peaker, 1975) and experiments which involves in abolishing of circulating PRL in the rat have indicated that PRL is maintaining lactation by acting as a cell survival factor and maintaining the cellular integrity of the secretory epithelium (Flint & Gardner, 1994). In ruminants, it has been established that PRL plays an essential role in initiation of lactation (lactogenesis) and lesser role in maintaining milk production in established lactation. Prolactin rise around parturition appears to be required for full initiation of milk production in ruminants since blocking this surge delay lactogenesis and depresses milk production for several days in cows and goats (Schams *et al.*, 1972; Johke & Hodate, 1978; Forsyth & Lee, 1993). The decline in milk

yield ranged from 33-72% for some cows and it took an average of six weeks for full restoration of milk production (Johke & Hodate, 1978). Biochemical analysis did not show any effect on cellular number (total DNA) of epithelial tissue, but less RNA, and an apparent inhibition on structural differentiation of alveolar epithelium (Akers *et al.*, 1981a,b). Moreover, PRL has been shown to be required for the reinitiation of lactation in hypophesectomized lactating goats, but it can be withdrawn from hormonal combination without any noticeable effect once lactation has reestablished (Cowie *et al.*, 1964). Taken together, these data suggest that PRL has an important role to play in lactogenesis probably acting to promote cellular differentiation and prepare the mammary gland for maximal milk production.

Once lactation is established, PRL suppression has little or no effect on maintaining milk production in ruminants (Karg *et al.*, 1972; Hart, 1973). However, there have been some reports on the depressive effects of PRL ablation on milk yield; bromocriptine treatment significantly reduced milk yield by at least 20% in lactating dairy goats (Knight *et al.*, 1990a; Forsyth *et al.*, 1995). Also, there is some indirect evidence about the involvement of PRL in the maintenance of lactation. For example, prolactin levels in lactating goats are higher than that of non-lactating ones (Hart, 1975b), PRL is increased in response to milking stimulus and a positive correlation between PRL profile after milking and milk yield in dairy cows was reported (Koprowski & Tucker, 1973).

The failure to see a distinct effect of PRL depletion on ruminant lactation can be explained in several ways. First, PRL depletion was not completely effective and lower concentration may be sufficient for milk maintenance of secretion. Second, CB154 treatment may not be effective in suppressing some PRL variants which can not be detected by radioimmunoassay. Third, there might be a mechanism by which the mammary gland is protected from lower PRL profiles as seen in CB154 treatment or due to seasonal effects on

PRL profiles which results in increase in the accumulation of the hormone in the mammary gland by either increase in mammary uptake of the hormone (Forsyth *et al.*, 1995) or increase in the mammary gland synthesis of PRL since the ability of the mammary gland to synthesis PRL has been demonstrated in rodents as well as in ruminants (Steinmetz *et al.*, 1993; Leprovost *et al.*, 1994; Lkhider *et al.*, 1997). Finally, it has been observed that bromocriptine treatment in lactating goats resulted in depression in PRL release but the GH release in response to milking was significantly increased in a dose dependent manner (Hart, 1975b) because of the ability of dopamine and dopamine agonist, bromocriptine, to stimulate growth hormone release (Harvey, 1995). So, the increase in growth hormone secretion might be compensating for the decline in PRL in maintaining lactation in ruminants.

1.3 GROWTH HORMONE AND THE INSULIN-LIKE GROWTH FACTORS AXIS

Growth hormone (GH) or somatotropin is a polypeptide hormone produced by the somatotrophs of the anterior pituitary gland which exhibits a wide range of metabolic activities like lipolytic, and anabolic activities such as cell division, skeletal growth and protein synthesis.

1.3.1 Growth hormone structure and receptor

Growth hormone principally exists in plasma as a monomer of about 190-191 amino acid residues with a molecular weight of 22,000 da, but several forms of GH have been reported which range from fragments to high molecular weights (Baumann, 1991). The existence of GH or GH-like proteins with different primary sequences, isoforms, which can be produced by different genes, i.e. in humans two GH genes, hGH-N and hGH-V, have

been detected. Alternatively, these isoforms can be attributed to different splicing of GH mRNA. For example, mRNA spliced into two different mRNAs resulting in a 22,000 da hGH with 191 amino acid residues and 20,000 da hGH with deletion of 15 amino acids (32-46) (Lewis, 1992). After synthesis, GH may undergo posttranslational modification to produce a series of variants and these modifications include, dimerization, deamidation, proteolytic cleavage, glycosylation, phosphorylation (Scanes & Campbell, 1995).

Growth hormone receptor is a single chain peptide comprising of 630 amino acid residues, with extracellular domain of 242 amino acids in length and the intracellular domain of 350 amino acids (De Meyts, 1992). Variation may occur in GH receptors during transcription and posttranslational processing. Growth hormone binding studies have indicated that GH is bound to multiple receptors or multiple forms of receptor and different GH action may be mediated by different forms of receptors (Hughes & Friesen, 1985). The GH receptors in the liver are under the control of endocrine system and metabolism. Under situations where feed intake is reduced, GH binding in the liver is also reduced, and normal feeding normalizes the binding process (Maes *et al.*, 1983). In steers, two binding sites in the hepatic membranes with different affinity were detected (Breier *et al.*, 1988a) which can be modulated by nutritional status; high level of feeding is correlated with increase in receptor affinity and the induction of the high-affinity type receptor which is also correlated with the biological activity of GH (Breier *et al.*, 1988b).

The binding of GH to its receptor has been reviewed recently by Wells, (1996). X-ray crystallographic studies have revealed that binding of GH to its receptor results in the formation of a complex of one hormone molecule per two molecules of receptor (De Vos *et al.*, 1992). This receptor dimerization has been proposed to be essential for the activation of GH receptor. The GH uses two different sites (site 1 and 2) to bind to two identical extracellular domains of GH receptor and this dimerization occurs sequentially. hGH binds

to the first receptor through site 1 and then followed by binding to the second receptor through site 2 which results in the formation of a complex which consists of one molecule of the hormone with two molecules of its receptors (Cunningham *et al.*, 1991). Dimerization is thought to be essential for initiation of signal transduction (Chen *et al.*, 1997) and the induction of tyrosine phosphorylation (Silva *et al.*, 1993). Consequently blocking the binding of the second binding site of the hormone to the receptor, ie hGH analogue (G120R) mutated in the second binding surface of the hormone, inhibited the GH receptor dimerization and also has been demonstrated to block the GH-stimulation of lipogenesis in primary adipocyte (Ilondo *et al.*, 1994).

As it is the case for many growth factors which are bound to specific binding proteins in the plasma, the presence of specific binding proteins for GH (GHBP) has been reported. It has been calculated in humans that 30-50% of GH circulates complexed to binding proteins (Baumann *et al.*, 1988). Their production is affected by several factors that include age, sex, pregnancy, nutritional status and endocrine system. For example, feed deprivation results in a drop in GHBP production, which is correlated with GH receptors (Mulumba *et al.*, 1991). The biological relevance of GHBP with respect to GH biological activities is still not entirely understood. However, GHBP can modulate the GH action by inhibiting binding to its receptors (Mannor *et al.*, 1991) and by binding to GH and forming GH-GHBP complexes in the plasma which results in slower metabolic clearance rate of the hormone, protecting it from degradation and eventually increasing its half life in the circulation (Baumann *et al.*, 1987) suggesting that these binding proteins have a significant role in modulating the activity of GH. In cattle, GHBP are detected in plasma with varying molecular sizes and have also been detected in the milk (Devolder *et al.*,1993) which suggests that they can be synthesized in the mammary gland. However, their biological function in the mammary gland function remains to be determined.

1.3.2 Regulation of GH secretion

Growth hormone is secreted from somatotrophs in episodic nature. Its secretion is governed by two hypothalamic hypophysiotrophic factors, growth hormone releasing-factor (GRF) and somatostatin (SRIF). The stimulatory factor, GRF, is synthesized in neurones located in the arcuate nucleus (Werner *et al.*, 1986) released into pituitary portal circulation in the median eminence (Niimi *et al.*, 1989) reaching the anterior pituitary where it interacts with specific binding sites to elicit GH release (Velicelebi *et al.*, 1985). It is believed that GRF is controlling the episodic release of GH since active immunization against GRF diminished the pulsatile release of GH (Moore *et al.*, 1992).

Growth hormone inhibitory factor, SRIF, is produced by neurones in the periventricular and paraventricular nuclei of the anterior hypothalamus (Frohman *et al.*, 1992) and these neurones have their axon terminals in the median eminence where SRIF is released into hypophysial portal circulation and transported to the anterior pituitary gland. The physiological role for SRIF in regulating GH release is indicated by the increase in basal as well as stimulated GH release by SRIF receptor antagonism, or immunization against SRIF (Wehrenberg *et al.*, 1982; Sato *et al.*, 1989). Hypothalamic nuclei may regulate somatotroph function through the release of neurotransmitters that are released into the anterior pituitary and the activity of these neurones are regulated by central nervous system-acting stimuli like stress and humoral feedback from peripheral factors (Ju *et al.*, 1991). Several aminergic and peptidergic factors produced by the hypothalamic neurons possess stimulatory or inhibitory actions on GRF and SRIF secretion, and also act directly in the pituitary to alter the responsiveness of somatotrophs to GRF and SRIF action. Therefore, the hypothalamic control of GH release results from complex interactions at hypothalamic and pituitary sites by numerous stimulating and inhibiting factors (Harvey, 1995).

The amplitude and the frequency of the episodic release of GH are modulated by several factors like nutrition, age, and also by genetic background. The nutritional status can regulate the secretion of GH; reduced feed intake provokes increases in GH secretion and also increases the responsiveness of somatotrophs to secretogenic factors in cattle (Bauman *et al.*, 1979; Breier *et al.*, 1988b) which might be attributed to an elevation in the frequency of GRF release (Armstrong *et al.*, 1993). Conversely, feeding reduces mean GH concentration and also the amplitude of GH episodes (Wheatan *et al.*, 1986; Trenkle, 1989; Mears, 1993).

Milking has been reported to elicit GH release in some mammalian species. In lactating rats, suckling induces the release of GH, whereas the removal of the pups for several hours results in a significant decline in GH (Riskind *et al.*, 1984) and immunization against GRF diminished suckling induced GH rise (Wehrenberg & Gaillard, 1989). Also, in goats, it has been reported that milking stimulates the release of GH (Hart & Flux, 1973; Hart & Linzell, 1977), but the release of GH differs from that of PRL release in the mode of release and that the tactile stimulus is not required for GH, and it appears to be influenced by other factors like feeding and metabolic state (Hart, 1974). Unlike the goats, milking does not provoke GH rise in the dairy cow (Johke, 1978; Lefcourt *et al.*, 1994; Samuelsson *et al.*, 1996) but the suppression of GH after feeding, which normally occurs in cattle, was prevented by the simultaneous feeding and milking (Samuelsson *et al.*, 1996) suggesting that milking might affect the release of GH releasing or inhibiting factors and thus modify the GH release.

1.3.3 GH and lactation

The galactopoietic property of GH has attracted a great deal of attention and there have been numerous investigations of its effects on milk production and its mechanism of

action. The ability of GH to maintain milk production in hypophysectomized lactating goats after the withdrawal of PRL (Cowie, 1964) and the high GH levels in early lactation which fall during late lactation when milk yield is dropping as well and the galactopoietic activity of exogenous GH in lactating dairy cows has justified the need to investigate its role in stimulating milk production. The effect of bovine somatotropin (bST) treatment on lactation performance in lactating dairy cows has been well documented. Milk yield can be increased by bST administration by as much as 40% (Peel & Bauman, 1987), but the response depends on several factors like management practices, e.g. milking regimen, nutrition and environmental conditions (Bauman, 1992). For example, bST treatment is without any effect on milk yield if the cows are not adequately fed. The mechanism by which bST is stimulating milk secretion is still debated, however, two mechanisms have been proposed: a direct way through repartitioning of nutrients towards milk synthesis and indirect pathway, through the stimulation of insulin-like growth factor-I (IGF-I) production. The presence of high bST levels in the circulation alters the partitioning and use of postabsorptive nutrients through the alteration of the metabolism of various tissues and organs like the liver and the adipose tissue (Burton *et al.*, 1994). In general, bST can increase gluconeogenesis in the liver and reduce glucose oxidation by the body tissues resulting in increasing the availability of glucose for the mammary gland (Zhao *et al.*, 1996). Lipid metabolism is also affected by bST. In general, fat mobilization is stimulated by bST (Binelli *et al.*, 1995), but this depends on the energy balance of the cow; if the treatment causes the cow to be in a state negative energy balance, this results in enhancement in lipid mobilization and elevation in the plasma levels of free fatty acids (FFA), which can be used as a metabolic fuel to substitute for glucose. The mobilization of body fat can be increased to an extent related to the state of energy balance (Bauman, 1992). Furthermore, lipogenesis is inhibited by bST regardless of the energy status of the cow probably by alteration in the adipose tissue

responsiveness to lipogenic stimuli like insulin (Peel & Bauman, 1987) resulting in the reduction of the direction of nutrients towards body reserve and increasing nutrients availability for milk synthesis. At the level of the mammary gland, bST causes an increase in mammary blood flow and cardiac output (Davis *et al.*, 1988a) to increase the delivery of partitioned nutrients. Also, it causes increases in the uptake of nutrients by the mammary gland e.g. glucose (Davis *et al.*, 1988b) to support the increase in milk synthesis.

Circulating levels of IGF-I in the plasma is regulated in part by GH so that treatment with bST elicits its release, mainly from the liver. The presence of IGF-I receptors in the mammary gland (Dehoff *et al.*, 1988) and its mitogenic effect on bovine mammary tissues (Baumrucker & Stemberger, 1989) might support the proposed hypothesis that IGF-I mediates the galactopoietic action of bST by acting in the mammary gland. However, a wealth of evidence does not give a lot of support to this contention. In the rat, IGFs did not mimic GH action when administered to lactating rats receiving anti-rat GH (Flint *et al.*, 1992) and a combination of IGF-I, IGF-II, and IGF-binding protein-3 (IGFBP3), which are normally increased by bST treatment, failed to mimic the galactopoietic effect of GH (Flint *et al.*, 1994). Furthermore, in a recent study utilizing the coculture of mammary, liver, and adipose tissues, incubation with IGF-I did not stimulate the synthesis of lipids and proteins by the mammary gland when compared with incubation with GH (Keys *et al.*, 1997). Taken together, these findings may not support the IGF-I theory and the most accepted theory is that bST stimulates milk production via partitioning of nutrients between the mammary gland and the rest of body. It is worth noting that a direct action of GH on the mammary gland is most unlikely, since several conventional binding assays have failed to detect any GH receptors in the mammary gland (Akers, 1983; Gertler *et al.*, 1984; Keys & Djiane, 1988). On the other hand, some investigators have detected mRNA for GH receptors in the mammary gland itself (Jammes *et al.*, 1991).

1.3.4 Insulin-like growth factors and their binding proteins

Insulin-like growth factors (IGF-I, IGF-II) form a family of single chain polypeptide hormones with a molecular weight of around 7,500 da. They are involved in a wide range of activities which include growth, reproduction, lactation and immune system. Although IGFs are synthesized locally by a number of tissues, the IGFs circulating in the blood are mainly produced by the liver. The concentration of IGFs in the circulation varies depending on the physiological state, being mainly regulated by GH and nutritional status. Growth hormone has a stimulatory effect on IGF release, since exogenous bST administration stimulates the release of IGFs and immunization against GRF significantly lower IGFs concentration (Moore *et al.*, 1992). Although bST has been shown to unequivocally increase IGF-I concentration, data for IGF-II are not consistent. Some researchers did not detect any changes in IGF-II in response to bST (Davis *et al.*, 1987) or the increase only occurred during the dry period (Vicini *et al.*, 1991) which suggests that the two IGFs have different regulatory mechanisms or the metabolic status may determine the response to the stimulatory effect of bST on IGF-II.

Nutritional status has a major effect on IGF levels in circulation. For example, it has been reported that in some situation IGF-I is influenced by nutritional status more than by GH. Fasting or reduced feeding which presumably retards growth rate temporarily is associated with lower concentration of IGF-I (Breier *et al.*, 1986; Ronge & Blum, 1989). Moreover, the stimulatory effects of bST on IGF-I synthesis is inhibited by feed restriction in cattle (Breier *et al.*, 1988b; Ronge & Blum, 1989). The mechanism by which the nutritional status affects IGF-I production might involve several possibilities. Some studies in the rat have suggested that it is the decrease in IGF-I mRNA in the liver which bring about the decrease in IGF-I levels (Straus & Takemoto, 1991) and this might be as a result

of reduced GH binding (Baxter *et al.*, 1981; Maes *et al.*, 1983). In steers, this effect might be attributed to the absence of another type of somatotrophic receptor present in the liver which is characterized by high affinity binding to GH (Breier *et al.*, 1988a). Also, postreceptor changes induced by nutritional status might be involved in altered IGF-I secretion (Thissen *et al.*, 1990). Studies in the dairy cow have indicated that the energy status can determine the basal and stimulated IGF-I concentration. In early lactation, the IGF-I levels are low during a time when the cows are usually in a state of negative energy balance because the nutrients from feed intake does not match that in milk (Ronge *et al.*, 1988; Vicini *et al.*, 1991). The levels of IGF-I increases gradually as lactation advances, reaching a maximum value during the dry period when the cow is in a positive energy state (Vega *et al.*, 1991). Furthermore; the stimulated pattern follows the same trend as basal values (Ronge & Blum, 1989; Vicini *et al.*, 1991). Therefore, it has been proposed that serum IGF-I is positively correlated with energy balance in lactating cows. In early lactation, there is a shift in the metabolic state from mostly anabolic during dry period to a catabolic state during early lactation; this process is coordinated by the endocrine system and the GH/IGF-I axis has a role to play in this process. A relatively low IGF-I value in early lactation will favour direction of nutrients away from body stores and conversely higher values is associated with more deposition of nutrients in body tissues.

Two subtypes of receptors for IGFs have been identified. Type I receptor which is characterized by disulfide-linked α-subunits which bind the hormone and β-subunits with tyrosine kinase activity (Leroith *et al.*, 1995) mediates the mitogenic activity of IGFs. The other receptor, type II, is a single polypeptide which lacks the subunits and tyrosine kinase activity (Rechler & Nissley, 1986). Both IGFs bind to the first type with the same affinity but type II preferentially binds IGF-II.

The IGFs in the circulatory system are bound to multiple specific high affinity

binding proteins (IGFBPs) which modulate the biological activities of the IGFs. Their action can be inhibitory or stimulatory and it has been reported that IGFBPs affect IGFs by forming IGF/IGFBP complexes, these limiting the efflux of IGFs from the vascular space, increasing their half life and regulating their metabolic clearance rate. Binding proteins also control the passage of IGFs from the vascular compartment and their transport to target cells, and will modulate the interaction between IGFs and their receptors (Baxter, 1988; Clemmons, 1990; Jones & Clemmons, 1995). Thus, IGFBPs are not acting only as a carrier of IGFs in the circulation, but they have an active role in inhibiting and enhancing the actions of IGFs. Six IGFBPs, 1 to 6, have been identified in several species including the bovine (Cohick *et al.*, 1992; Hossner *et al.*, 1997), and recently this family has been extended to include three more IGFBPs (Rosenfeld *et al.*, 1997). The majority of circulating IGFs are bound to IGFBP3 which forms a complex of 150,000 da by binding to IGFs and a larger protein (acid-labile subunit) (Barreca *et al.*, 1995). The regulation of IGFBPs secretion has not been studied extensively as it has been for their modulatory actions of IGFs. The treatment with bST increases the concentration of IGFBP3 but decreased IGFBP2 (Vicini *et al.*, 1991; Cohick *et al.*, 1992). Like the IGFs, IGFBPs secretion can be modulated by nutritional status. For example, during growth retardation situations like feed restriction or fasting, there is an increase in IGFBP1 and also IGFBP2 but not IGFBP3 in humans (Collet-Solberg & Cohen, 1996). In lactating cows, circulating concentrations of IGFBP2 have been shown to increase in response to feed deprivation but IGFBP3 is less sensitive to nutritional status (McGuire *et al.*, 1995). Therefore, different types of IGFBPs respond differently under different conditions. The modulatory effects of IGFBPs on IGFs activity depend on their concentration, relative proportions in extracellular fluids and distributions between extracellular fluids and cell surfaces (Hossner *et al.*, 1996).

The presence of IGFBPs in milk can be attributed to transfer from blood through the

arterial supply of the mammary gland (Prosser *et al.*, 1991) or *de novo* synthesis in the mammary gland itself (Campbell *et al.*, 1991). The presence of IGFBPs in the milk suggests a potential role to play in the regulation of mammary function. For example, the involuting mammary gland increases its production of IGFBP5 which might serve to block the cell survival activity of IGF-I in the mammary gland (Tonner *et al.*, 1995).

1.4 THYROXINE AND LACTATION

The involvement of the thyroid gland in lactation was recognized more than forty years ago. Thyroidectomy markedly depressed milk yield in lactating dairy cows, which could be restored by thyroid feeding (Graham, 1934a). Also, increasing plasma thyroxine (T4) by means of feeding thyroxine or thyroprotein, an iodinated casein which increases plasma level of thyroxine, or injecting thyroxine stimulate milk production (Graham, 1934a; Shaw *et al.*, 1975; Davis *et al.*, 1987). The milking response, however, varies between individuals and also with stage of lactation; higher response was reported when treatment was performed during the declining phase of lactation (Graham, 1934b). Although milk yield increase can be achieved by T4 treatment, long term treatment has shown to be not successful in maintaining the galactopoietic effects of T4 (Shaw *et al.*, 1975) and the overall increase in milk yield in long term treatments is relatively small. Furthermore, it has been reported that a dramatic decline in milk yield was seen after cessation of thyroprotein feeding (Thomas *et al.*, 1954; Hibbs & Krauss, 1947). Despite the galactopoietic effect of T4 in cattle, the relationship between T4 and lactation is controversial. Selection for high milk production may or may not be associated with significant differences in T4 level in the plasma. Whereas some studies have not been able to establish any difference in T4 in response to genetic selection (Bodah *et al.*, 1972), others have suggested that cows with high milk production capacity have lower plasma T4 levels (Magdub *et al.*, 1979; Bitman

et al., 1984). Also, in rats lactational intensity produced a significant decrease in serum T4 (Jack *et al.*, 1994). This relationship of milk synthesis capacity and T4 implies a negative correlation between the two. This is certainly related to the lower state of metabolism in peripheral tissues which is of great importance in sparing nutrients that can be utilized to support the high rate of metabolism of the mammary gland.

Treatment with T4 has been associated with increases in the metabolic activity in the whole body as indicated by elevation in pulse rate and respiration rate which might lead to body catabolism and consequently reduced body weight when treatment is continued for long time (Hibbs & Krauss, 1947). Short term T4 injection increased milk yield and resulted in an increase in cardiac output and proportion of cardiac output perfusing the udder (Davis *et al.*, 1988a). Moreover, the mammary glucose uptake was increased but the ratio of glucose uptake to lactose output was also increased (Davis *et al.*, 1988b). It can be suggested that increase in mammary blood flow which increases the delivery of nutrients to the mammary gland together with increase in mammary gland metabolism as a consequence of elevation of whole body metabolism might bring about the enhancement of milk yield with T4 treatment.

1.5 LOCAL CONTROL OF MILK SECRETION

It is a normal practice in dairy farms to milk cows twice daily, usually in early morning and late afternoon, but increasing milking frequency results in a significant increase in milk yield (Pearson *et al.*, 1979; Poole, 1982). The increase in milk yield is very rapid so it can be seen within hours in animals milked hourly with oxytocin injections (Linzell & Peaker, 1971). On the other hand, reducing milking regimen to once daily reduces milk yield (Wilde & Knight, 1990). In experiments in which half the udder has been milked more than twice while continuing the other half on twice daily, the increase in milk secretion is

only apparent in the treated gland which shows that the effect is mediated by local rather than systemic factors. Thus, the increase in milk secretion was related to frequent removal of the milk from the udder. The physical distention of the udder is not involved in mediating these effects because when the milk removed from the udder was replaced by an inert solution this did not prevent the stimulatory effect of frequent milking (Henderson & Peaker, 1984). The presence of a local chemical factor that has a negative feedback on milk secretion was first suggested by Linzell & Peaker, (1971) and it is the frequent removal of this factor from the proximity of the secretory cells which leads to increased rate of milk secretion (Henderson & Peaker, 1987) suggesting its direct inhibitory effect on the secretory cells. The increase in milk yield brought about by frequent removal of milk from the udder for a short time is accompanied by increases in the activity of some key mammary enzymes (Wilde *et al.*, 1987b) suggesting an increase in the metabolic activity of secretory cells. After several weeks of continuous frequent milking, a greater number of secretory cells is achieved (Wilde *et al.*, 1987b). Thus, it can be concluded that an early response to frequent milking is associated with increase in rate of secretory cell differentiation, but as a result of long-term response, cellular proliferation appears to be evident. Conversely, less frequent milk removal or incomplete milking reduces the activity of key enzymes and negatively affects the cellular differentiation (Wilde *et al.*, 1989; Wilde & Knight, 1990).

The response to frequent milking is due to the removal of a milk constituent that affects milk secretion by negative feedback on the secretory cells which is known as feedback inhibitory of lactation (FIL). It was identified by screening of goats' milk whey fraction as a protein with a molecular weight of 7600 da (Wilde *et al.*, 1995) which inhibited the synthesis of casein and lactose from explants of mammary tissues in a dose dependent manner (Wilde *et al.*, 1987a; 1995). Furthermore, introduction of FIL into the mammary gland of lactating goats temporarily decreased milk yield (Wilde *et al.*,1995) and

the injection of antibodies against FIL into lactating goats during the declining phase of lactation improved lactation persistency and also decreased the decline in milk secretion in response to once daily milking (Wilde *et al.*, 1996). The inhibitory effects of FIL on milk secretion *in vivo* as well as *in vitro* suggest a role in regulating the rate of milk secretion and it could mediate the effects of frequent milking on milk secretion. Also, FIL is affecting the secretory cells by an autocrine mechanism since FIL is secreted by the same cells on which it exerts its inhibitory effects (Wilde *et al.*, 1995)

1.6 PHOTOPERIOD AND LACTATION

Day length or photoperiod has an influence on a number of biological activities, primarily reproduction, body growth, coat growth and lactation. Several studies on the effects of supplementation of light in lactating ruminants have shown a significant increase in milk production, however, the responses were variable. Increasing the light exposure by artificial lighting during short days in autumn and winter increases milk production by 6-10% in cows and goats (Peters *et al.*, 1978; Peters *et al.*, 1981; Marcek & Swanson, 1984; Terqui *et al.*, 1984; Dahl *et al.*, 1996). The galactopoietic effect of photoperiod on milk yield is not immediate and it requires a minimum of one week before a significant effect on milk yield can be manifested and apparently the response is independent of stage of lactation (Peters *et al.*, 1978). However, the effect is not entirely consistent; some reports in cows (Murrill *et al.*, 1969) and in goats (Hart, 1975b) did not show a stimulatory effect of photoperiod on milk yield.

The mechanism by which photoperiod stimulates milk production is not known and several possibilities have been proposed. It was thought that the increase in milk production in response to exposure to longer hours of light might be the result of increase in nutrient availability which comes from the increase in feed intake (Peters *et al.*, 1981). However,

others reported an increase in milk yield without any change in time spent with eating in dairy cattle exposed to long day photoperiod (Phillips & Schofield, 1989), and long light exposure increases average daily live weight gain without affecting dry matter intake in Holstien heifers (Peters *et al.*, 1978). Furthermore, long light stimulates body growth in heifers even when feed intake is restricted (Petitclerc *et al.*, 1983). Thus, feed efficiency for growth is likely to be increased with exposure to long light. Also, there is evidence that photoperiod may affect behaviour, reduced physical activity being associated with exposure to longer hours of light (Phillips & Schofield, 1989). This could result in reducing the energy expenditure utilized for activity which can be spared for milk production. The other hypothesis that has been proposed for explaining the effect of photoperiod on milk production is that the photoperiod signal is mediated via hormonal releases by the endocrine system. Several hormones have been studied to determine a possible role in mediating the photoperiodic stimulation of milk production, PRL being the primary candidate because it is the only lactogenic hormone that is responsive to changes in photoperiod. Long day exposure is coupled with high PRL values and short day decreases PRL. Also, long light stimulates basal and TRH stimulated PRL release (Peters *et al.*, 1981) indicating that the lactotrophs become more responsive to secretagouges factors.

Skeleton long photoperiod of 6 hours of light and an additional 2 hours of light between 18.00-20.00h significantly increased milk production and PRL release (Evans *et al.*, 1991). Even though the cows were exposed to a total of only 8 hours of light, they were able to pick up the photoperiodic signal of long day and respond to it. This indicates the presence of a photosensitive phase when the cows were responsive to light exposure which occurs between 13-15 hours after subjective dawn. Thus, it is possible that the timing of light periods within a 24 hours light-dark cycle is important rather than the total amount of light exposure. However, not all studies support a role for PRL in mediating the effects of

photoperiod. Exposing heifers to long light stimulated growth in spite of a suppressive effect of low ambient temperature on PRL release (Peters *et al.*, 1980), and others have reported that the galactopoietic effects of long light can be achieved without a significant effect on basal and stimulated PRL release (Marcek & Swanson, 1984). Therefore, it is not proven that increase in feed intake or enhanced secretion of PRL is responsible for the galactopoietic effect of photoperiod in dairy cattle.

Another hormone which might be involved is GH, but most reports indicate that GH is not responsive to photoperiod and therefore cannot be the mediator for the stimulatory effect of photoperiod on milk yield (Peters & Tucker, 1978; Peters *et al.*, 1981; Gustafson, 1994). However, Evans *et al.*, (1991) were able to detect an increase in number of GH peaks in photosensitive phase during light treatment in dairy cows and also, light stimulated GH secretion in lactating goats when lactation was induced by steroid treatment (Terqui *et al.*, 1984) suggesting a possible effect of photoperiod on GH release.

Effects of photoperiod on mammogenesis have been reported for pre- and post-pubertal heifers. Exposure to 16L:8D stimulated growth of parenchyma and increased cellular proliferation (Petitclerc *et al.*, 1985). Also, increased daily exposure of cows to light during the last month of gestation subsequently resulted in enhancement in milk production for the first two months of lactation (Gustafson, 1994) indicating that photoperiod has an effect at the level of the mammary gland during mammary development. Whether the stimulatory effect of long light on milk yield is associated with changes in cellular differentiation or proliferation has not been determined.

Another mechanism that might be related to the galactopoietic effects of photoperiod on milk production is an effect on fat metabolism. Long light exposure affects the body composition by decreasing the rate of fat accretion in both sheep and cattle (Brinklow & Forbes, 1984b; Tucker *et al.*, 1984), and conversely MEL treatment which mimics short

day increases fat deposition in the body (Zinn *et al.*, 1988a). Therefore, it is possible that long light signal might affect fat metabolism by inhibiting lipogenesis or the short day signal might induce lipogenesis and this can be halted by long day exposure. The inhibitory effects of long light on lipogenesis in adipose tissue might increase the portion of nutrients available to the mammary gland and direct nutrients away from body stores. If this the case then the question needs to be answered, how could the long light signal mediate the change in adipose tissue metabolism? Nobody has attempted to address this possibility so far, but one study reported a tendency for lower insulin values during long light exposure in dairy cows (Gustafson, 1994) so it is possible that lower insulin levels might be involved in the direction of nutrients away from body tissues thereby increasing the availability of nutrients towards milk synthesis.

1.7 PHYSIOLOGICAL BASIS IN GENETIC SELECTION FOR HIGHER MILK PRODUCTION

Genetic selection for milk yield in cattle has led to a dramatic increase in milk production per cow. High producing dairy cow can produce about 10,000 kg of milk per lactation cycle and this improved milk yield is the result of accumulated effects of many years of selection for milk yield. With the improved milk production, some traits have not changed with genetic selection whereas others have altered markedly and these may be important determinants of efficient milk production. Digestibility, maintenance requirement, requirement per unit of milk produced have been shown to be similar between cows of different genetic merit (Hart *et al.*, 1978; Bauman *et al.*, 1985b). However, they do differ in their metabolism as nutritional studies have indicated that high yielding cows are characterized by partitioning energy from feed intake toward milk production whereas cows with low genetic merit are not effective in directing their nutrients towards milk. This will

be of particular importance in early lactation, when dairy cows (and especially high yielding ones) are in a state of negative energy balance. Therefore, partitioning dietary energy between milk production and the rest of the body might be the result of inherited trait which might be the main difference between low and high yielding dairy cows. Difference in energy partitioning is regulated mainly by the endocrine system. One important hormone which is highly correlated with milk production and also known for a long time to possess galactopoietic effects in dairy cows is GH, and it was thought that it might have responded to genetic selection. Several studies utilizing cows with different genetic merit have shown consistently that high yielding dairy cows are associated with higher GH concentration (Barnes *et al.*, 1985; Bonczek *et al.*, 1988), and the capability to secrete GH after TRH injection is higher in the high yielding cows (Kazmer *et al.*, 1986) suggesting a higher secretory activity of somatotrophs in the superior cows. Furthermore, the increase in basal GH values and GH response to varying secretogogues with genetic selection has been reported for heifers with potential higher milk production at an early age, before puberty (Barnes *et al.*, 1985; Lovendahl *et al.*, 1991) which suggests that GH might be a physiological trait that can be transmitted by genetic selection. However, some researchers have argued that the difference in GH levels among cows from different genetic lines may be attributed to differences in energy balance resulting from the high milk production in the high genetic merit cows (Hart, 1983; Klemetsdal *et al.*, 1992). When cows of different genetic merit were fed to a similar weight gain, no differences were found between the two groups in GH levels (Hart, 1983) which tends to support this argument. However, in that situation the elevation in plasma GH in the low line could be attributed to the restriction in feed intake, since this has been reported to increase GH concentration in cattle (Kazmer *et al.*, 1985). Also, Kazmer *et al.*,(1986) found a distinct difference between genetic lines in GH levels without any significant dissimilarities in their energy balances.

Insulin (INS) is another hormone which has been shown to be associated with milk production capacity. However, its plasma concentration is low in early lactation and increases as lactation progresses and milk yields fall (Walsh *et al.*, 1980) showing that the relationship between milk production and INS is an inverse one. Also, INS treatment depresses milk yield in lactating dairy cows. The secretion of INS has been investigated with cows of different genetic merit, and most of the studies have indicated a lower INS value in the high line cows (Hart *et al.*, 1978; Bonczek *et al.*, 1988). INS is the major anabolic hormone which mainly promotes nutrients deposition in the body tissues and inhibits the mobilization of nutrients from body stores (Vernon, 1988). Therefore, the lower INS in cows which are characterized by higher milk yield potential will allow for greater availability of nutrients to be directed towards milk synthesis.

Thyroxine also has been determined in studies that were designed to investigate possible hormonal profile differences related to potential milk production. When compared across stage of lactation, T4 is low in early lactation and increases as lactation advances (Bonczek *et al.*, 1988). This led to the conclusion that serum T4 is negatively correlated with milk yield (Walsh *et al.*, 1980) and studies with low and high yielding dairy cows have reported lower serum T4 concentrations in the high yielding cows than that of low yielding ones (Hart *et al.*, 1978; Bines *et al.*, 1983).

Due to the less important role of PRL in ruminant lactation once lactation is established, there is no apparent change in PRL release which might be attributed to genetic selection (Barnes *et al.*, 1985; Bonczek *et al.*, 1988). Since its secretion is influenced by many factors in the dairy cow like photoperiod, temperature, stress, feeding, stage of lactation and milking and also due to its episodic release, it is very difficult to find a sensible correlation between milk production and PRL release.

1.8 AIMS OF STUDY

In dairy cattle, several factors can theoretically be manipulated to affect the shape of the lactation curve. In this study, we attempted to manipulate milk production utilizing several galactopoietic stimuli with different mechanisms of action. Photoperiod is an environmental factor which has been manipulated to stimulate milk production. However, its galactopoietic property is variable and not always evident. Furthermore, how photoperiod stimulates milk production is not known. Since there is evidence that the photoperiodic effect on milk yield may be potentiated by long term previous exposure to chemically induced short days (by melatonin treatment; Knight, 1993), the first objective of this study was to investigate if short term exposure to short days would also prime the goats to subsequent stimulatory effects of long light on milk yield.

Genetic selection for higher milk yield has been used as an avenue of increasing milk production efficiency. Intensive selection for milk production has resulted in a marked increase in the level of production but also increased concerns about the welfare of the cow. Whether high yielding dairy cows are actually milking close to their maximum metabolic capacity and are, therefore, more exposed to the possibility of metabolically related diseases than lower yielding dairy cows, has not been established. To achieve the target of driving the cows into their upmost production, the secretory activity of the mammary gland needed to be manipulated by more than one stimulus. Therefore, manipulation of local factors (frequency of milking) together with systemic manipulation (via endocrine challenges of bST and T4) were aimed to influence the milk secretion capacity through different routes in order to achieve the maximum metabolic capacity. By applying these multiple stimuli to cows from different genetic merit, the relative degrees of risk of metabolic disturbances could be assessed. Another aim of this study was to explore the relative importance of the mammary gland versus the whole body in determining the maximum metabolic capacity.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

In this chapter, collection and preparation of blood samples and methods of hormone determination will be described. Other techniques that were used in particular experiment will be described in the chapter which describes that work. All chemicals used, unless otherwise mentioned, were from Sigma Chemical Company Ltd, Poole, UK.

2.1. BLOOD SAMPLING

Blood samples were collected (approximately 7ml), via jugular venipuncture in goats and from tail vessel puncture in cows, into heparinized vacutainer tubes (Becton Dickinson, Vacutainer Systems Europe, Meylan-Cedix, France). For serial blood samples, blood samples were collected by an indwelling jugular cannula. Intravenous catheterization (using Medicut 14g; Sherwood Medical, Tullamore, Ireland) was performed about 18 hours before the start of blood collection. A cannula (polyethylene tubing; I.D. 1.40mm, O.D 1.90mm; Dural Plastics and Engineering Pty Ltd, Auban, NSW, Australia) was inserted into a jugular vein and sealed with a 3-way tap, and the blood was collected into a vacutainer tube. A saline solution containing heparin (1000 IU/100 ml) was used after each blood collection for flushing the cannula. Samples were centrifuged (1800g, 15 minutes, 4°C) shortly after collection, and the plasma was harvested and stored at -20°C until assayed for hormone concentrations.

2.2. HORMONE DETERMINATION TECHNIQUES

Plasma PRL, GH, and INS were assayed by double-antibody radioimmunoassay (RIA) using the method described by Vernon *et al*, (1981).

2.2.1 Prolactin RIA

Antiserum to ovine PRL was provided by the National Institute of Health (NIH), Bethesda, Maryland, USA. The reference curve (3.12-200 ng/ml) was constructed using ovine PRL (AFP-9221A) supplied also by NIH. RIA buffer (containing 0.05M sodium phosphate, 0.15M sodium chloride, 0.5% w/v bovine serum albumin (RIA grade) and 0.05% w/v sodium azide; pH adjusted to 7.4) was used for sample dilution and the dilution of the antiserum and preparing the standard. For preparing the second antibody, RIA buffer plus an equal amount of polyethylene glycol (16% PEG, from BDH, Thornliebank, Glasgow, UK) and then 0.83% v/v anti rabbit precipitating serum, and 0.03% v/v normal rabbit serum, both donated by the Scottish Antibody Production Unit (SAPU), Carluke, Lanarkshire, UK, were added and mixed thoroughly. An antiserum dilution of 1:60-80,000 and a tracer activity of around 30,000 cpm ^{125}I-oPRL were used to get an average total binding of 30%. The addition of the tracer was delayed for 4-6 hours after the incubation with the first antibody, and then further incubated overnight at room temperature. Then, the second antibody was added and the tubes were centrifuged (3000g, 30 minutes, room temperature) after 2-4 hours of incubation at room temperature. The supernatant was decanted and the tubes were counted by Gamma counter (Packard, Meriden, USA). The PRL levels were determined by comparing the unknown samples to the standard curve. Intra- and inter assay coefficient of variations were determined by running quality control samples containing varying amounts of the hormone at every assay. Inter- and intra-assay coefficient of variation were 15.50 and 9.4% respectively. Sensitivity of the assay determined from estimated concentration at 80% of maximum binding (reference) was 3.35ng/ml.

2.2.2 Growth Hormone RIA

Antiserum to ovine GH (AFP-CO123080, donated by NIH) was used with a dilution of 1:20000, and the standard curve (0.31-40 ng/ml) was prepared from ovine GH (AFP-9220A). The same procedure described for PRL was followed except that the addition of the tracer (20,000 cpm ^{125}I-bST)was delayed 24 hours after the addition of the first antibody to standard curve and sample tubes. Inter- and intra-assay coefficient of variations were 16.5 and 7.2% respectively. Estimated concentration at 80% of maximum binding was 0.382 ng/ml.

2.2.3 Insulin RIA

Anti-porcine insulin (prepared in guinea pig, S115-201, gift from SAPU) was used with a dilution of 1:40,000. Bovine insulin (from Sigma, I-550) was used to construct the standard curve (0.08-5.0 ng/ml). The first antibody and a tracer activity of around 12,000 cpm ^{125}I-bINS were added to standards and unknowns, with the latter added 6 hours after the former. The second antibody was prepared by adding equal amounts of RIA buffer and 16% PEG and then 0.46% w/v EDTA and the pH was adjusted to 7.4 before adding 0.50% v/v anti-guinea pig serum and 0.03% v/v normal guinea pig serum, both donated by SAPU. The assay was performed as that for PRL and GH. Inter- and intra-assay coefficient of variations were 17 and 7.6% respectively. Estimated concentration at 80% of maximum binding was 0.044 ng/ml.

2.2.4 Insulin-like growth factor-I RIA

The method described by Flint & Gardner (1989) was followed for the determination of IGF-I concentration in the plasma after the samples were extracted with acid-ethanol, to separate the IGF-I from its binding proteins. The extraction procedure was done by adding

4 volumes of the extraction medium (2N HCL and ethanol 1:7 v/v) to 1 volume of the samples and the standard tubes and incubating for 30 minutes at room temperature. The tubes were centrifuged at 3000g for 10 minutes; after that, a specific amount of supernatant was removed and an equal amount of neutralizing buffer (4% w/v TRIS : RIA buffer) was added and then the samples were further diluted with RIA buffer. Recombinant human IGF-I (from Bachem, Sulfran Walden, Essex, UK) was used to construct the standard curve (10-2500ng/ml). The first antibody was polyclonal rabbit anti-rhIGF-I (a gift from NIDDK, Bethesda, Maryland, USA) at a dilution of 1:2000. This was added to standards and sample tubes and incubated for 24 hours before adding ^{125}I-IGF-I (approximately 20,000cpm per tube) and then incubating overnight at room temperature. Then, the second antibody (RIA buffer/ 16% PEG with equal volume and 6% v/v anti-rabbit IgG precipitating and 0.4% v/v normal rabbit serum, both from SAPU) was added to the tubes and further incubated for 2-4 hours before centrifuging at 3000g for 30 minutes at room temperature. The pellet was counted as before after tipping off the supernatant. Interassay coefficient of variation was 10.8% and intra-assay was 9%. Estimated concentration at 80% of maximum binding was 8.88 ng/ml.

2.2.5 Radioiodination procedure

Hormones were labelled using the Iodogen technique described by Fraker & Speck, (1978). Radioactive iodine (^{125}I in sodium iodide) was incorporated to the hormone by using Iodogen (Pierce Europe BV, Oud-Beijerland, Netherlands). Approximately 5*u*g of the hormone, 10*u*l 0.5M phosphate buffer (pH 7.3) and 5*u*l of ^{125}I (containing about 0.5 *u*ci) were added to an iodogen coated tube (30*u*l iodogen (0.05 mg/ml in chloroform), evaporated to dryness) and allowed to sit undisturbed for 20 minutes at room temperature. Then, 100*u*l of phosphate buffer was added and the iodogen tube content was transferred

to the top of a sephadex G10 minicolumn. To stop the reaction, 200*u*l potassium iodide (2% w/v) was washed through the tube and into the minicolumn. Then, RIA buffer was added carefully into the top of the column and fractions were collected every 1-2 minutes, and the radioactivity for each fraction was counted. Incorporation of ^{125}I into the hormone was determined by counting a small aliquot before and after precipitation of the protein with 10% trichloroacetic acid. Fractions with a minimum of 80% incorporation were diluted if necessary and stored at -20°C behind lead.

2.2.6 Melatonin assay

Plasma melatonin concentration was determined by direct RIA which was described by Fraser *et al* (1983). The anti-melatonin antiserum (AB/S/021; Stockgrand Ltd, University of Surrey, Guildford, Surrey, UK), and melatonin standard (5-250 pg/ml) was prepared using N-acetyl-5-methoxytryptamine (from Sigma); the dilution medium was melatonin-free plasma which was collected from two goats exposed to bright light (5,000lux) for two hours in early afternoon. Light exposure is a strong suppressor of MEL secretion in different species including the goat and only plasma samples with MEL levels indistinguishable from maximum binding were used to construct the standard curve. The antibody with a dilution of 1:4,000 as well as the tritiated MEL ([O-*methyl*-^{3}H]Melatonin, Amersham International Plc, Buckinghamshire, UK) with an activity of 4,000cpm were added to the tubes containing the samples and the standard which were then incubated overnight at 4°C. The free and antibody bound fractions of MEL were separated by dextran coated charcoal (activated charcoal at 2% w/v in assay buffer which was stirred for 5 minutes before centrifugation at 1,000g for 5 minutes at 4°C; then the supernatant was discarded and the charcoal was resuspended with assay buffer and 0.02% w/v dextran T-70 was added and the solution was stirred for at least one hour at 4°C). The tubes were

centrifuged at 1500g for 15 minutes at 4°C after incubation time of 15 minutes. Then, the supernatant was transferred into vials containing 4ml scintillation fluid, which was prepared by adding 0.5% w/v 2,5-diphenyloxazole and 0.03% w/v dimethyl-popop to toluene (FSA Laboratory supplies, Loughborough, UK). The tubes were shaken for 1h at room temperature before counting the radioactivity. Interassay coefficient of variation was 15.9% and intra-assay was 5.6%. Estimated concentration at 80% of maximum binding was 6.4 pg/ml.

CHAPTER THREE

EFFECT OF REPEATED SHORT CYCLES OF MELATONIN AND LONG LIGHT ON LACTATION PERFORMANCE IN THE GOATS

3.1 INTRODUCTION

Photoperiod has a regulatory influence on a number of biological activities in mammals such as reproduction, body growth and lactation. The effect of photoperiod on lactation performance has been the subject of several investigations in lactating cows, which reported that extended light exposure during the decline in natural day length stimulated an increase in milk yield of 6-10% (Peters *et al.*, 1978; Marcek & Swanson, 1984; Stainisiewski *et al.*, 1985). However, a consistent increase has not always been achieved (Murrill *et al.*, 1969; Hart, 1975b). The mechanism involved in the effect of long light exposure on milk yield is not known. It has been suggested that stimulation of feed intake might be responsible (Peters *et al.*, 1981), but on the other hand, others have shown that the increase in milk yield or body growth was independent of feed intake (Petitclerc *et al.*, 1983; Phillips & Schofield, 1989). Also, it was proposed that a photoperiod signal is acting through the adenohypophesis to stimulate the release of galactopoietic hormones. Although reports on the possibility of GH mediating the photoperiod effects on milk yield were inconclusive (Peters *et al.*, 1981; Evans *et al.*, 1991), PRL might be a prime candidate in mediating the light signal since its secretion is determined by the prevailing day length. This was supported by the association between stimulation of milk yield and the enhanced PRL release by photoperiodic stimulation (Peters *et al.*, 1981; Evans *et al.*, 1991). However, the role of PRL in galactopoiesis of the lactating ruminant has not been established. Manipulation of PRL profiles by inhibiting its release (Beck *et al.*, 1979) or increasing its plasma concentration by exogenous PRL administration (Plaut *et al.*, 1987) were ineffective

in affecting milk yield in lactating cows. Furthermore, some reports have shown that changes observed in milk yield in lactating cows or in growth rate in heifers with long day exposure appeared to be independent of changes in PRL concentrations (Peters *et al.*, 1980; Gustafson, 1994). Therefore, there is no strong evidence to support a role for PRL as a mediator for the stimulatory effect of photoperiod on milk yield.

Melatonin is an indoleamine which is secreted by the pineal gland in response to darkness, so the duration of nocturnal MEL secretion provides an endocrine index of night length and thus day length. MEL has been used in short day breeders like sheep and goats to advance the time of the breeding season (Arendt, 1986; Deveson *et al.*, 1992a,b). Evidence from experiments in which photoperiod manipulation was utilized to modulate the reproductive cycle in sheep or growth rate in heifers suggested that previous photoperiod exposure may influence the response to subsequent, different, photoperiod (Robinson & Karsch, 1987; Zinn *et al.*, 1988b). Most of the studies that employed photoperiod as a galactopoietic stimuli were conducted when the cows had experienced a period of declining day length (autumn and winter) before they were exposed to artificial long days. In lactating goats, there is preliminary evidence to suggest that long term MEL treatment, which mimics short day signal, primed goats to subsequent stimulating effect of long light on milk yield (Knight, 1993). These experiments were conducted to determine if repeated short cycles of MEL would sensitize the goats to subsequent long light effect on milk yield, and if this would be affected by stage of lactation, or the time of year when MEL treatment started.

3.2 MATERIALS AND METHODS

3.2.1 Animals and housing

Twelve Saanen goats kidding for the first time in late March, within four days of each other, were used in the first year's experiment. In the second year's experiment, goats

in their second or third lactation were used. After parturition, mothers were left with their kids for 24 hours before separation and moving to the designated building where they were kept throughout the experiment. Goats were housed in individual pens and they were milked twice daily at 07.00 and 16.00h, and fed 0.75 kg of concentrate diet (goat mix 2, Edinburgh School of Agriculture) at each milking time. Hay and water were made available all time. The goats were milked twice daily at 07.00h and 15.30h.

3.2.2 Experimental design

Cycles of conditioning treatment (2 or 4 weeks) followed by long light (2 or 4 weeks) were started in lactation week 5 (late April). Goats were blocked by milk yield and allocated into two groups where 6 goats were to receive the MEL treatment and the other 6 to receive a vehicle. For MEL treatment, 3mg of MEL (N-acetyl-5-methoxytryptamine, Sigma) were dissolved in 1ml of 50% alcohol and then absorbed into a small amount of concentrate feed which was left to dry at room temperature for about 2h. Fresh treated diet was prepared every 5 days and stored at 4°C before being fed daily to goats. The MEL containing concentrate was fed to goats at 15.00h each day and a similar prepared concentrate but without the indoleamine was also fed to the other group simultaneously. This conditioning treatment was continued for 2 weeks then MEL was stopped before all goats were switched to long light for 2 weeks. Lights were on between 06.00-23.00h daily controlled by a programmed clock timer, and lighting was provided by cool white fluorescent tubes. Light intensity measured by light meter (Illummometer, Kyoritsu Electrical Instrument, Tokyo, Japan) at the eye level of the goats was determined at three times and they were 322.8±15.7, 348±16.7, and 384.2±20.6 lux. While the goats were on normal light (conditioning treatment period) lights were on from 06.00-18.00h daily. The same procedure was applied in a second cycle but the periods of conditioning and

subsequent long light were extended to 4 weeks. It was intended to continue on repeating these cycles throughout lactation, but during the third conditioning period the clock timer was found to be faulty. Since it was not known when the problem with the timer had started, the data from this cycle were excluded, and at early September, cycles of MEL and long light were repeated as they had been performed in early lactation. Part of the experiment was repeated a year later, this time with all treatments commencing in September.

3.2.3 Measurements

Milk yields obtained at each milking were recorded throughout the experiments. Also, milk samples were collected every two weeks and milk fat was determined before defatting (by centrifugation 1000g, 20 minutes, 20°C) and the defatted milk was stored at -20°C before being analyzed for protein and lactose. Body weights were recorded weekly during the experimental periods.

3.2.4 Blood collection and hormonal determinations

Routine blood samples were collected four times weekly (at 14.00 and 16.30h on two different days) throughout the experiment. Also, goats were subjected to frequent blood sampling at two-hourly interval from 09.00-19.00h, and every 5 minutes from 10 minutes before milking to 20 minutes after milking. This was performed during the second week of conditioning and long light cycles in early (April) as well as in late lactation (September) and also during the first week of long light in spring. Blood samples were collected and handled as described in chapter 2.

Weekly samples were assayed for PRL contents, the two-hourly samples were assayed for PRL, GH, MEL, and IGF-I, and samples collected around milking were

quantified for PRL and GH concentrations. Methods of hormonal determinations have been described in chapter 2.

3.2.5 Milk composition

Fat percent

Milk fat percentage was determined by the rapid fat method described by Fleet & Linzell, (1964). Fresh milk samples were incubated in a water bath at 37°C for about 20 minutes and thoroughly mixed before drawing into capillary tubes (Hawksley, UK) and sealing with wax. Then, tubes were centrifuged (1000g,15 minutes at room temperature). The milk samples which were separated into two layers, cream (white) and serum (opaque) layers, were read by microhaematocrit reader and the readings were corrected by multiplying by a correction factor (0.75) to determine the fat percent.

Bradford protein

Protein content was determined by measuring the change in absorbence by a dye binding to protein (Bradford, 1976). A protein standard curve, ranging from 1 to 10μg protein, was prepared from bovine serum albumin. Milk samples were diluted to 1:350 using distilled water. 50μl aliquot of standards and samples were placed in 96-well micro-titration plates, and 240μl Bradford reagent added (20%, Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) and then incubated for 10 minutes at room temperature. Absorbence of the coloured complex formed was monitored at 620nm (Titertek Multiskan MCC340 MKII type 347, Labsystems, Basingstoke, UK).

Lactose

Lactose content was determined using a Sigma kit (510A) for measuring glucose. Milk samples were incubated with β-galactosidase (from Bohringer Manheim) in order to degrade lactose into glucose and galactose. Lactose standard (10mM α-lactose in 0.1M sodium phosphate buffer, pH 7.3) was used to construct a standard curve with concentrations ranging 1-5mM. Milk samples were diluted 1:40 in sodium phosphate buffer. 400 μl of diluted milk sample or standard were transferred into microtube and the following were added:

70μl 0.1M Potassium phosphate buffer (pH 7.3)

20μl 0.1M Magnesium sulphate

10μl β-galactosidase

Then microtubes were vortexed and incubated in the dark at 37°C for 30 minutes. Perchloric acid (100μl 4.2% v/v) was added to stop the reaction. Then, 10μl of the reaction mixture was transferred in a 96-well plate, 300μl of Peroxidase/glucose oxidase/o-dianisidine solution added and incubated for 10 minutes at 37°C. Absorbances were read at 450nm and lactose content was determined by reading from the standard curve.

3.2.6 Statistical Analysis

Average milk yield during each of the MEL or long light cycles was analysed by ANCOVA using the milk yield of the week preceding each cycle as a covariate. Total milk yield during each season was also analysed by ANCOVA and the pretreatment milk means (before the 2week conditioning cycle) as covariate. Hormonal profiles were analyzed by ANOVA or *t* test. For the post-milking PRL profiles, the mean of the two samples collected before milking (10 and 5 minutes before milking) was regarded as basal level and the response area was calculated as the area under the curve minus the basal values. Values of

PRL and GH were logarithmically transformed to get a normal distribution of the data. The data were analysed both on log transformed and original data but the results were similar. Therefore, the results presented here are those on original data.

3.3. RESULTS

3.3.1 Milk yield

Average weekly milk yields for early (spring) and late (autumn) lactation of the first year and the second year experiments are depicted in Figure 3.1. At the commencement of treatment in spring, apparently, the goats did not reach their peak yields since milk yields were still ascending. In MEL-treated goats, peak yield was achieved at lactation week 8.67 ± 0.95 while it continued to increase for an average of further two weeks, in the control group, reaching the peak at week 10.17 ± 0.60, but this variation was not found to be statistically significant ($P > 0.05$, *t* test). Average weekly milk yield for both groups in each cycle of conditioning and long light of the first and second year experiments are presented in Figure 3.2, and they were analyzed by ANCOVA using the milk yield of the week immediately preceding each treatment cycle as covariate which are shown in Table 3.1. Two weeks of conditioning with MEL treatment apparently was not effective in priming the goats to subsequent stimulatory effect of long light on milk yield. So, the conditioning cycle was increased to four weeks before exposure to another four weeks of long light, and this also did not sensitize the goats to long light effect on milk yield regardless of the commencement time of treatment cycles. Indeed, there was no strong evidence of stimulatory effects of extended hours of lighting on milk yield except for a small, but significant, increase during the last long light cycle of the first year experiment and only in goats that did not receive the MEL treatment. In the two-week cycle of long light, in both seasons of the first year experiment, milk yield was not affected by long day exposure in both control and conditioned goats ($P > 0.05$, Paired *t* test). During the spring's second cycle of long light, milk yield decreased in both groups, more sharply in the third week, and this was apparently unrelated to treatment. Average weekly milk yield during the last long light cycle of the autumn of the first year experiment indicated that the group that did not receive MEL

produced more milk than the group fed MEL (P=0.05, ANCOVA). This was also confirmed by Paired *t* test analysis when the average milk yield during long light was compared with that of the week preceded long light cycle (P=0.02). ANCOVA analysis showed a non-significantly lower milk yield in goats that were conditioned by MEL when compared to non-conditioned ones (P=0.08). Despite the tendency for lower yields in MEL treated goats during the conditioning cycles in the first year experiment, ANCOVA did not confirm any significant difference between the two groups at each of 2 or 4 week period of conditioning cycles (P>0.05; Table 3.1). Total milk yield produced throughout the repeated cycles of conditioning and long light analysed by ANCOVA, using milk yield of the week before the commencement of the first conditioning cycle (2-week) as a covariate, has indicated that goats that did not receive MEL treatment produced more milk than MEL-group during spring experiment (for control and MEL-treated, 270.8±13.2 v. 250±8.8 kg, P=0.05, ANCOVA). Also, a similar trend was seen in autumn's experiment, but the difference in milk yield did not reach the significance level (230±9.1 v. 218±13.8 Kg, P>0.05).

Unlike the first year experiment, MEL treatment in the second year experiment did not cause a decline in milk yield both during conditioning or subsequent long light cycles (Figure 3.1c & Figure 3.2c). There was no obvious effect of long day photoperiod on milk yield in both groups; yields were maintained without any significant differences between them (n.s, ANCOVA, Table 3.1). Milk yield fell in the first week of the first long light cycle in both groups and this was clearly unrelated to light treatment. Similar to previous year results, MEL was ineffective in priming the goats to the following long light effect on milk yield. However, unlike what has occurred in the first year experiment, long light exposure in late autumn was without any stimulatory effect on milk yield in either of groups (P>0.05, Paired *t* test).

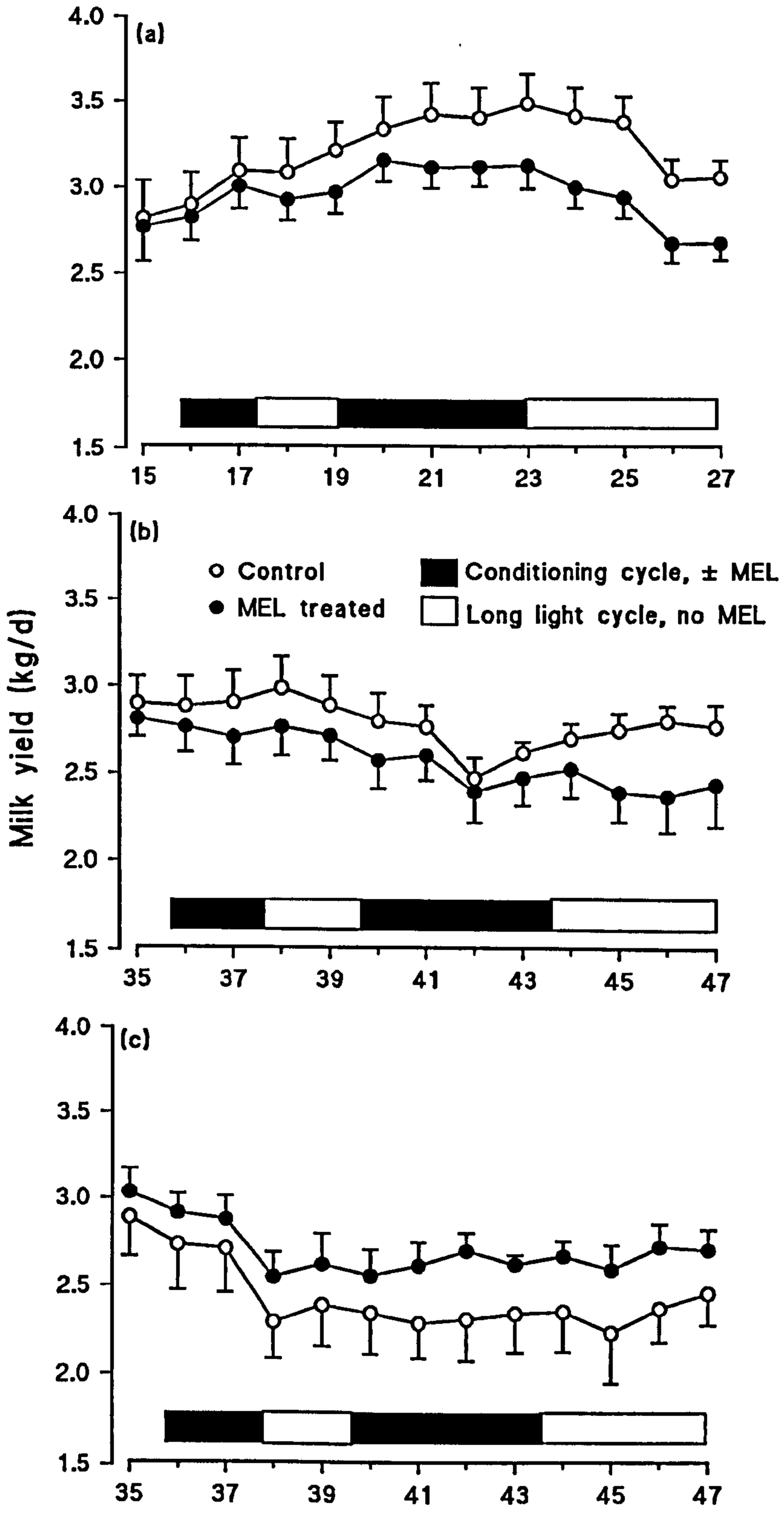

Figure 3.1 Mean milk yields (kg/d) of goats during two and four weeks of conditioning and long light cycles in (a) spring and (b) autumn of the first year experiment and (c) in the second experiment (autumn only). During conditioning cycles, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during long light cycles, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

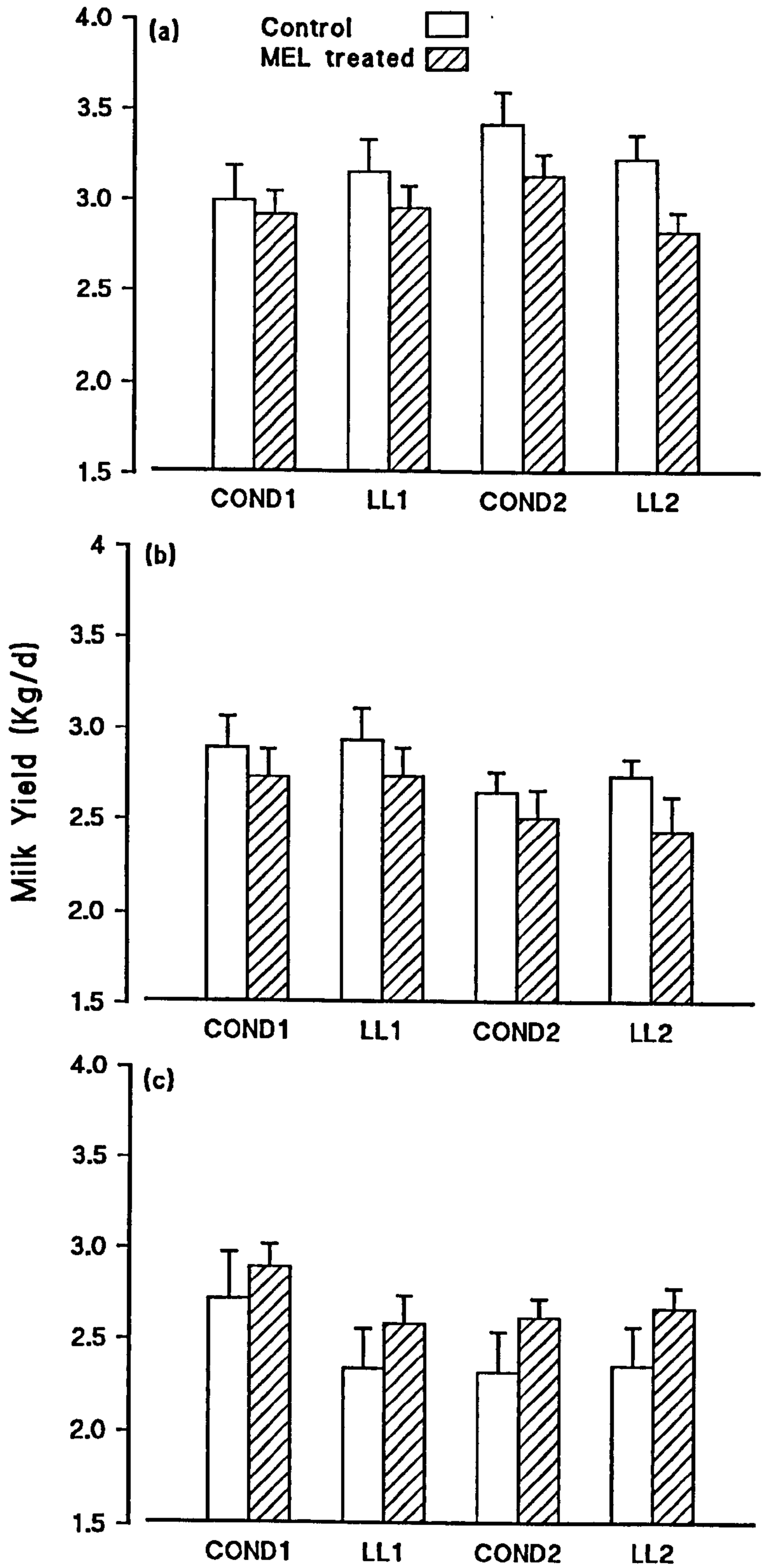

Figure 3.2 Average milk yields (kg/d) of goats during two and four weeks of conditioning cycles (COND1 and COND2), and long light cycles (LL1 and LL2) in (a) spring and (b) autumn of the first year experiment and (c) in the second experiment (autumn only). During conditioning cycles, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during long light cycles, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

Table 3.1. Average milk yields (kg/d) in goats during two (2wk) and four (4wk) of cycles of conditioning and long light in spring and autumn of the first year and second year (autumn) experiments. During the conditioning cycle, goats were either fed 3mg of MEL (MEL-fed) or fed vehicle (control) daily at 15.00h. During long light cycle, lights were on from 6.00-23.00h. Values are adjusted means (using the milk yield in the week immediately before each treatment cycle as a covariate). SED, standard error of difference.

Year	Season	Treatment Cycle	Groups		SED	P Value
			Control	MEL-fed		
First	Spring	Conditioning (2wk)	2.98	2.94	0.05	>0.05
		Long light (2wk)	3.12	2.99	0.07	>0.05
		Conditioning (4wk)	3.29	3.25	0.05	>0.05
		long light (4wk)	3.09	2.95	0.07	=0.08
	Autumn	Conditioning (2wk)	2.85	2.78	0.12	>0.05
		Long light (2wk)	2.85	2.84	0.03	>0.05
		Conditioning (4wk)	2.59	2.58	0.08	>0.05
		Long light (4wk)	2.66	2.53	0.06	**=0.05**
Second	Autumn	Conditioning (2wk)	2.80	2.83	0.09	>0.05
		Long light (2wk)	2.43	2.52	0.06	>0.05
		Conditioning (4wk)	2.42	2.55	0.08	>0.05
		Long light (4wK)	2.50	2.57	0.18	>0.05

Total milk yield produced throughout repeated cycle of conditioning and long light was not lower in the MEL-treated group, rather it tended to be greater than the non-conditioned goats (207.9±18.3 v. 220.5±9.06 Kg, for control and MEL-treated groups respectively, P=0.07, ANCOVA).

3.3.2 Milk composition

Milk composition data are shown in Figure 3.3. MEL treatment did not result in any pronounced changes in milk composition regardless of season. Also, shifting to long light was without any effect on fat or protein content of the milk. There was a small decrease in milk fat percent in both groups during the second week of the second conditioning cycle in autumn (P=0.08, *t* test), the reason for this is not known. Lactose was also not affected by MEL or long light treatment in sample determined in the second MEL and long light cycles in spring (for control and MEL treated, 133.2±2.0, 139.5±6.5 and 138.5±2.5, 133.5±1.2 mM/l during MEL and long light respectively, n.s, ANOVA).

3.3.3 Body weight

Average weekly body weights for both groups during spring and autumn of the first year experiment are shown in Figure 3.4. There was a gradual increase in body weight in spring while it was maintained fairly constant in autumn. There was no evidence of any difference in body weight between the two groups at any time, and both groups exhibited a similar trend in body weight changes except for the final long light cycle in autumn. Average body weight was significantly increased in the control group when compared to that of MEL-treated group (P=0.04, ANCOVA).

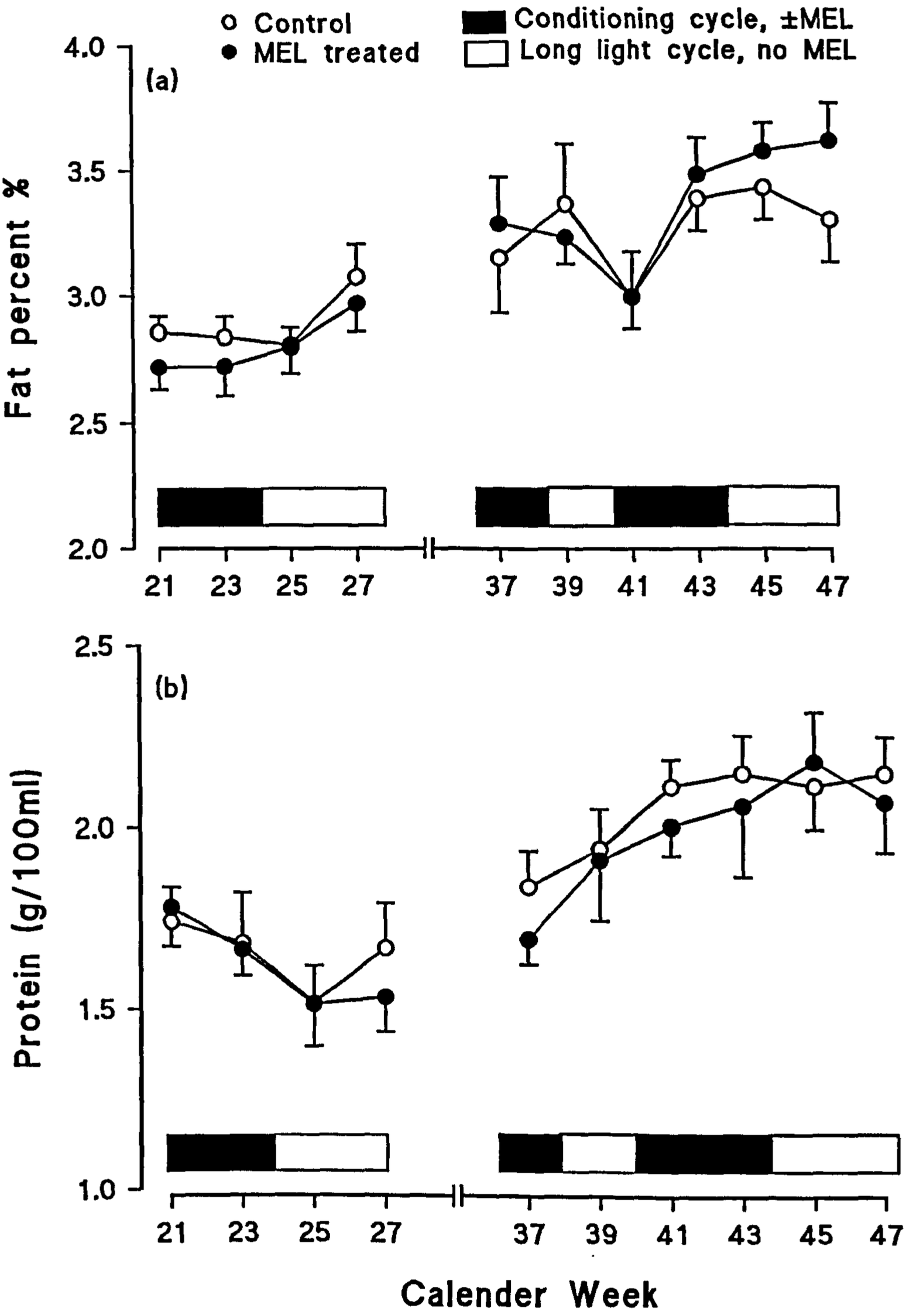

Figure 3.3 Milk composition in milk samples collected biweekly in goats during different periods of conditioning and long light cycles, (a) for fat percent and (b) for protein content in spring and autumn of the first year experiment. During conditioning cycles, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during long light cycles, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

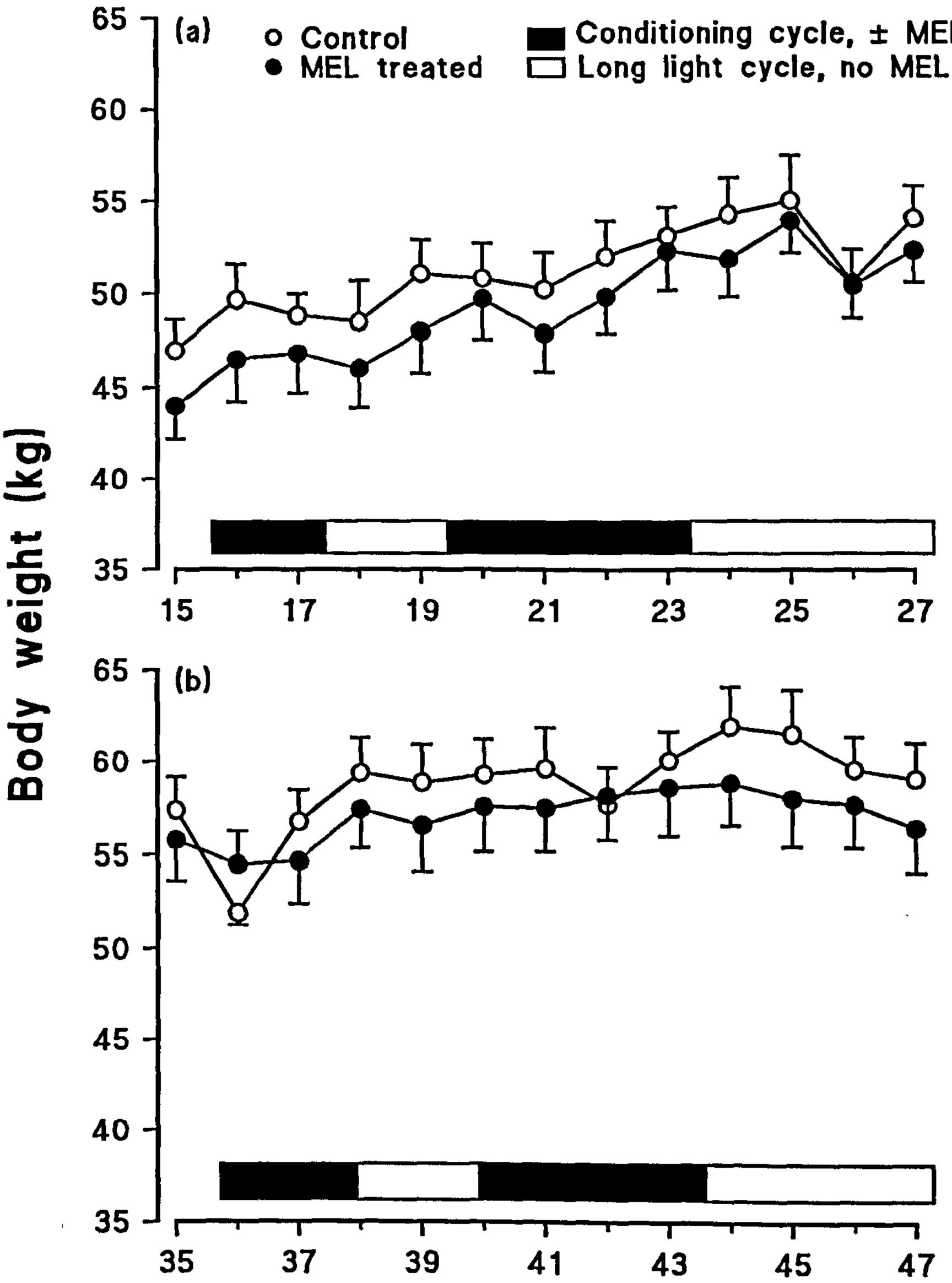

Figure 3.4 Changes in weekly body weights in lactating goats during two and four weeks of conditioning and long light cycles in (a) spring and (b) autumn of the first year experiment. During conditioning cycles, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during long light cycles, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

3.3.4 Hormonal profiles

In the second year experiment, no blood samples were obtained from the goats, and therefore hormonal data from the first year experiment will be only reported.

3.3.4.1 Melatonin profiles

Plasma MEL profiles during the conditioning cycles (second week of the two-week cycle) in both seasons together with that determined during the first week of long light, after cessation of MEL administration, are shown in Figure 3.5. Oral feeding of MEL at 15.00h but not vehicle significantly increased plasma levels of MEL after the feeding reaching a peak at around 30 minutes after the administration, which then gradually declined. Despite this decline, levels of MEL in goats on MEL diet at 17.00 and 19.00h were significantly higher than that in the control group in both seasons ($P<0.05$, *t* test). Height peak of plasma MEL values was not significantly affected by season ($P>0.05$). In spring treatment, MEL concentrations in plasma tended to be elevated during the afternoon (at 13.00 and 15.00h) not only in MEL fed goats but also, to a lesser extent, in the control group ($P<0.01$, at 15.00h, *t* test). Withdrawal of MEL treatment was accompanied by abolishment of the elevation in plasma MEL levels when determined during the first week of long light exposure (Figure 3.5c); MEL levels in goats that had been receiving MEL feeding were similar to that found in goats that had not been treated with MEL ($P>0.05$, ANOVA).

3.3.4.2 Prolactin profiles

Average weekly PRL concentrations for spring and autumn experiments are shown in Figure 3.6. There was no significant difference in PRL profiles between the two groups in pretreatment week ($P>0.05$, ANOVA). Average weekly PRL concentrations were first

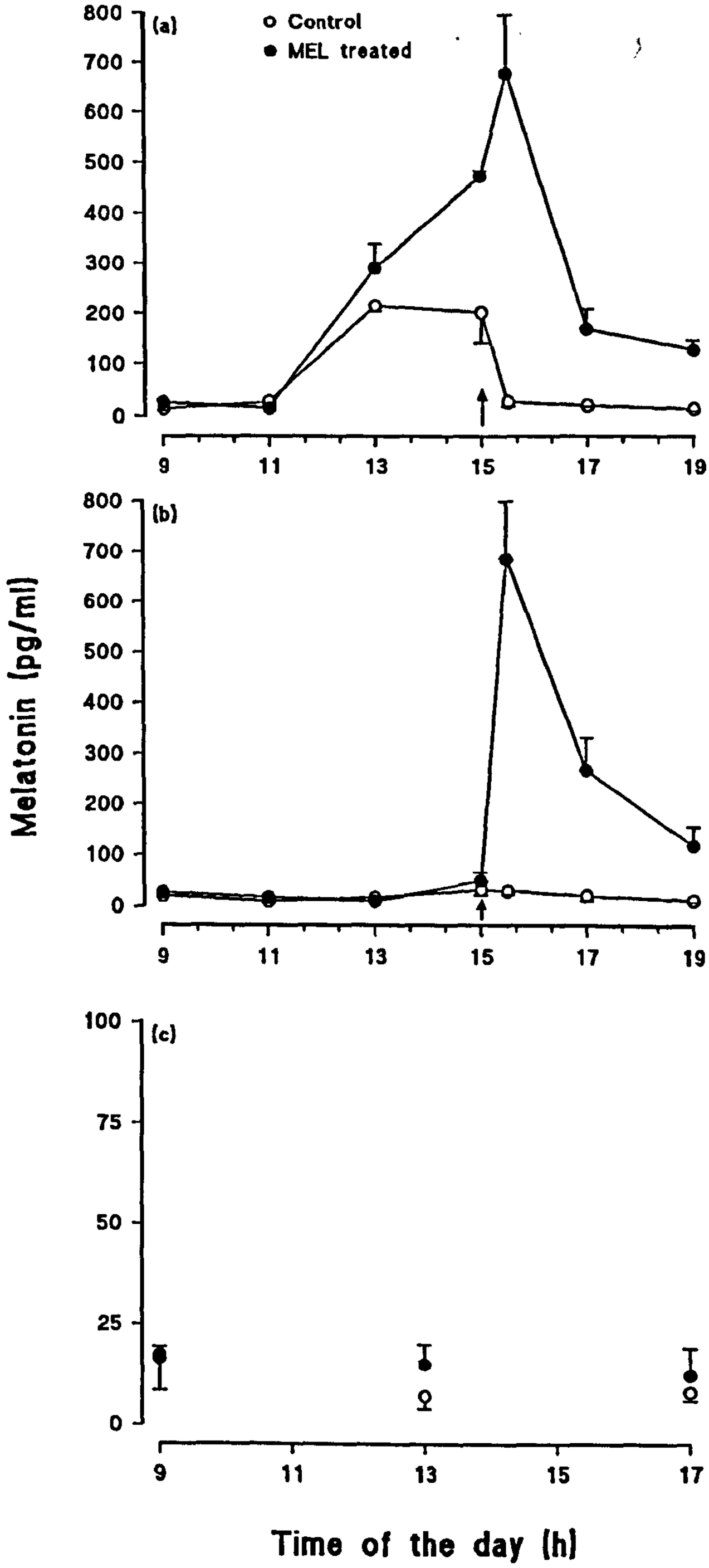

Figure 3.5 Profiles of plasma MEL in lactating goats during the second week of the first conditioning cycle in (a) spring and (b) autumn, and (c) during the first week of the first long light cycle in spring. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrows indicate the time of MEL or vehicle feeding. Values are means with S.E.M.

analysed by ANOVA and the model included group (MEL-treated, control), light treatment (normal, long) and season (spring, autumn) and their interactions. PRL concentration was increased by long light ($P=0.005$), and it decreased during autumn ($P<0.001$). Melatonin treatment did not significantly affect the overall PRL mean nor was there any effect on PRL response to long light. Mean weekly PRL profiles for each group in each treatment cycle which were analysed by ANOVA are presented in Table 3.2. Melatonin treatment when day length was increasing (spring) did not significantly affect PRL concentration, no difference was found between the two groups (>0.05). However, in the second conditioning cycle, PRL levels were increased during the first week in MEL-treated goats ($P=0.09$, Paired *t* test), in contrast to a tendency for a decline in control group ($P>0.05$, Paired *t* test), which then declined as the treatment was carried on but there was no significant difference between the two groups in the cycle mean of PRL. Similar trend in MEL-treated group was observed also during the first week of the second conditioning cycle in autumn ($P=0.04$, Paired *t* test), and the cycle mean of PRL concentration was greater in MEL-treated goats than that of non-conditioned goats ($P=0.01$, ANOVA; Table 3.2). Apparently exposing the goats to only two weeks of long light was not effective in stimulating PRL secretion in both seasons. After shifting to long light in spring, PRL concentration did not increase until at least two weeks of exposure.

Profiles of PRL determined in the two-hourly samples during spring and autumn are shown in Figures 3.7 & 3.8, and area under PRL curve calculated from these samples are in Figure 3.9. The mean of PRL AUC did not indicate that MEL had any significant effect on PRL profiles during the conditioning cycle or alter the long light response; mean of PRL in the MEL-treated goats was not significantly different from that of the control group, confirming the finding from the weekly samples. Frequent samples (two-hourly) during the conditioning cycle did not suggest that MEL suppressed PRL release, but PRL tended to

decline after MEL feeding so that at 19.00h it was almost significantly lower in the MEL-treated group when compared to that in control group (P=0.06, ANOVA; Figure 3.7a). Switching the goats to long light significantly increased PRL profiles (AUC) in both groups, in the first week (P=0.01) and second week (P= 0.004, *t* test) when compared to that of the conditioning cycle (Figure 3.9a). There was no evidence of any difference in response to long light between the two groups in the second week, and both groups followed a similar pattern of diurnal pattern change (Figure 3.7c). Prolactin was relatively low in the morning and exhibited a marked increase in the afternoon (P<0.01, *t* test) which was maintained till the last sample. Seasonal effects on PRL profiles were also evident in the two-hourly samples, AUC of PRL release was markedly declined in autumn compared to spring's values. MEL treatment during autumn was without any effect on basal PRL profiles (Figure 3.8a). Although AUC was higher in MEL-fed goats during MEL treatment, it was not statistically different from that in the controls. Prolactin concentration was not affected by previous MEL treatment since there was no significant difference between the two groups. PRL secretion in goats was increased when they were maintained at long light (P=0.04, *t* test) regardless of MEL treatment.

PRL profiles in samples collected at 5 minutes interval around the afternoon milking in spring are presented in Figure 3.10. Goats were milked 20-50 minutes after MEL or vehicle feeding. PRL levels before milking (basal) tended to be greater in the MEL treated goats; nevertheless, they released less amount of PRL in response to milking stimulus than that of the control goats (Figure 3.10a). Furthermore, total AUC for PRL profiles calculated from samples collected post-milking was lower in MEL-fed goats but it did not reach the significance level because of high variability in the control group (961±407 v. 3347±1814, n.s, ANOVA).

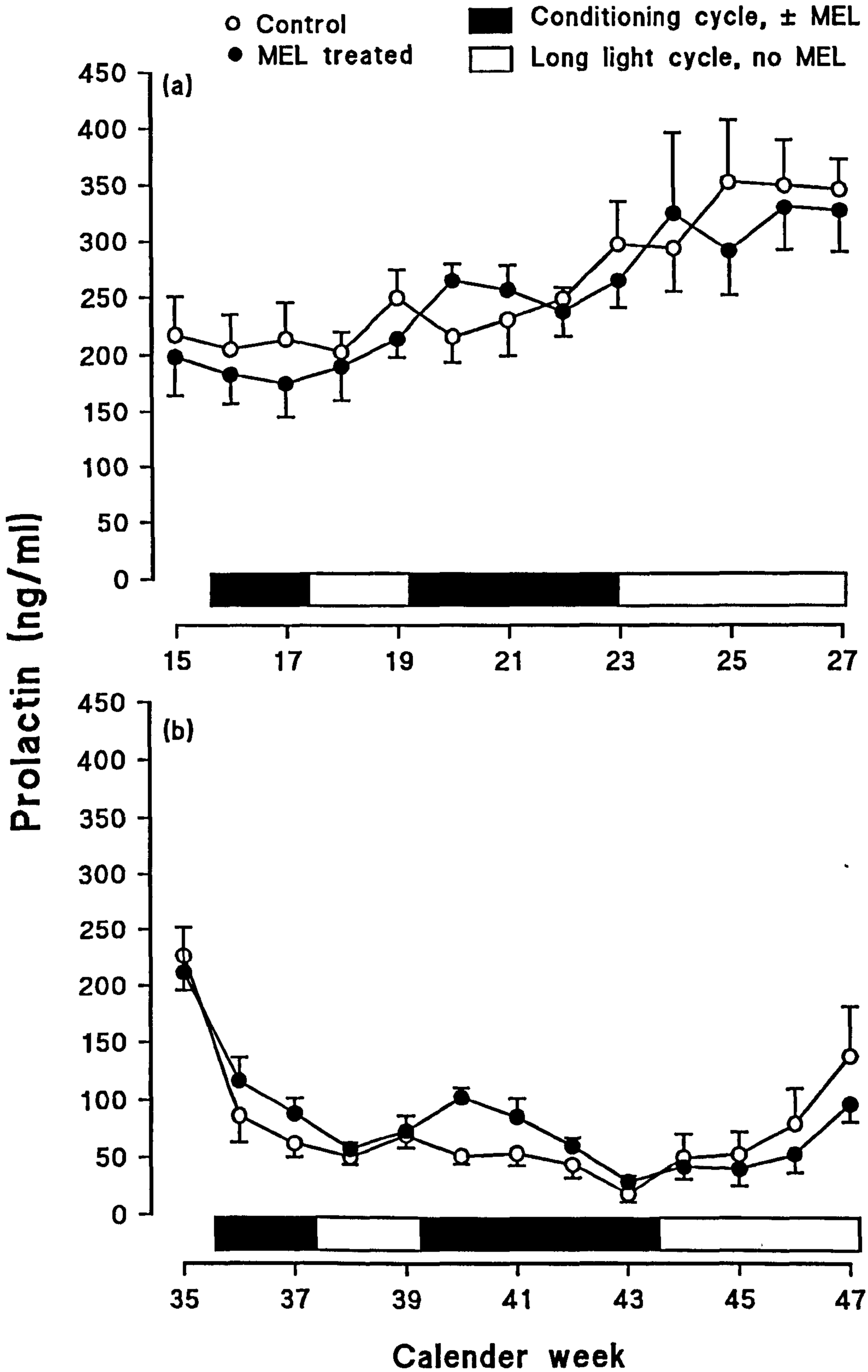

Figure 3.6 Mean weekly concentrations of plasma PRL (ng/ml) in lactating goats during two and four weeks of conditioning and long light cycles in (a) spring and (b) autumn of the first year experiment. During conditioning cycles, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during long light cycles, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

Table 3.2 Average prolactin levels (ng/ml) in goats during two (2wk) and four (4wk) of cycles of conditioning and long light in spring and autumn of the first year experiment. During the conditioning cycles, goats were either fed 3mg of MEL (MEL-fed) or fed vehicle (control) daily at 15.00h. During the long light cycles, lights were on from 06.00-23.00h. Results were analysed by analysis of variance and values are means. SED, standard error of difference.

Season	Treatment cycles	Groups		SED	P Value
		Control	MEL-fed		
Spring	Conditioning (2wk)	208.8	178.1	38.3	>0.05
	Long light (2wk)	225.3	201.3	29.5	>0.05
	Conditioning (4wk)	247.5	250.7	26.0	>0.05
	long light (4wk)	334.1	317.0	39.3	>0.05
Autumn	Conditioning (2wk)	73.1	101.6	18.8	>0.05
	Long light (2wk)	58.8	64.3	10.4	>0.05
	Conditioning (4wk)	41.1	69.2	8.9	**=0.01**
	Long light (4wk)	80.0	58.0	30.2	>0.05

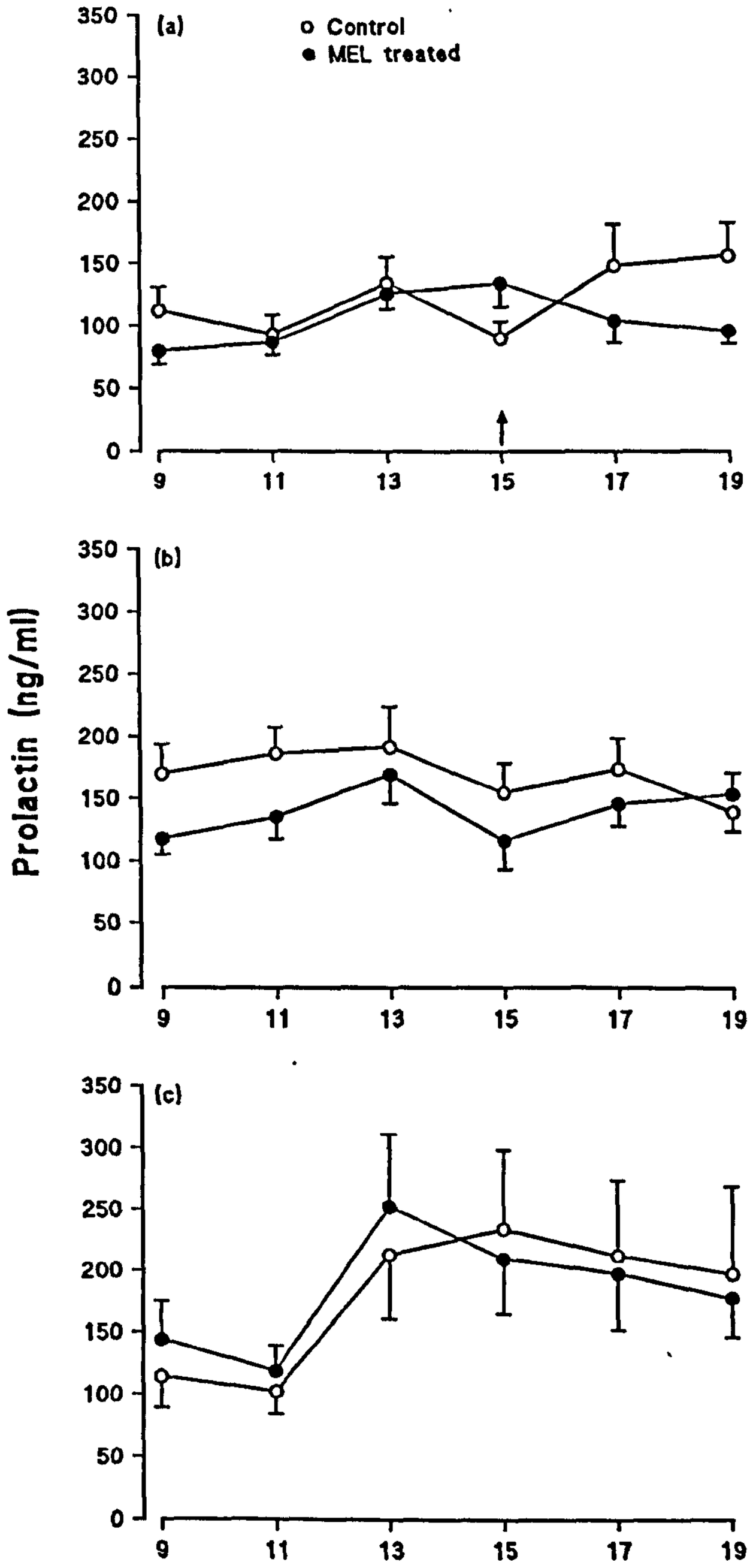

Figure 3.7 Patterns of PRL secretion (ng/ml) in lactating goats during (a) the second week of conditioning cycle, (b) during the first and (c) the second week of long light cycle in spring. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrow indicates the time of MEL or vehicle feeding. Values are means with S.E.M.

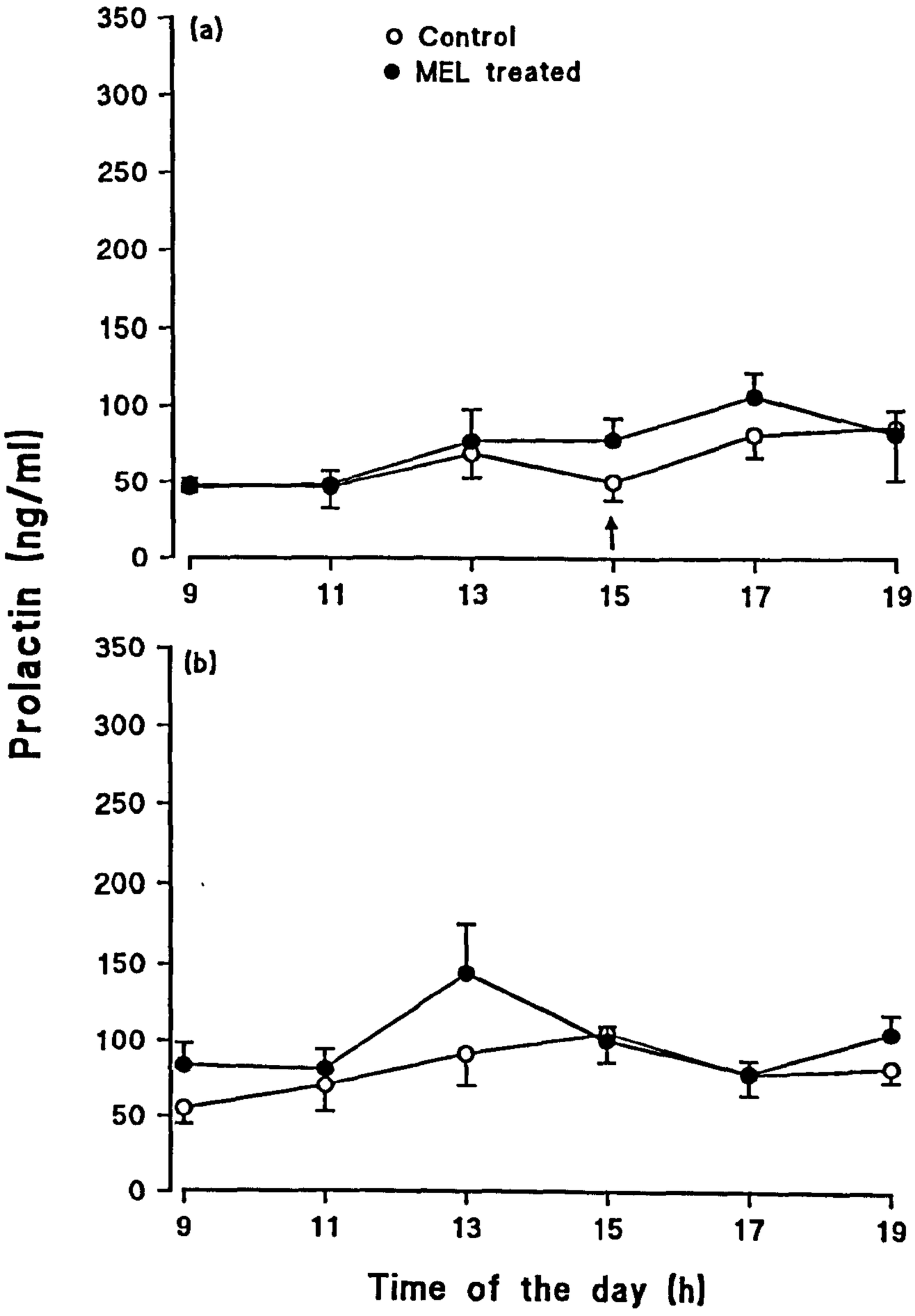

Figure 3.8 Patterns of PRL secretion (ng/ml) in lactating goats during (a) the second week of conditioning cycle, (b) during the second week of long light cycle in autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrow indicates the time of MEL or vehicle feeding. Values are means with S.E.M.

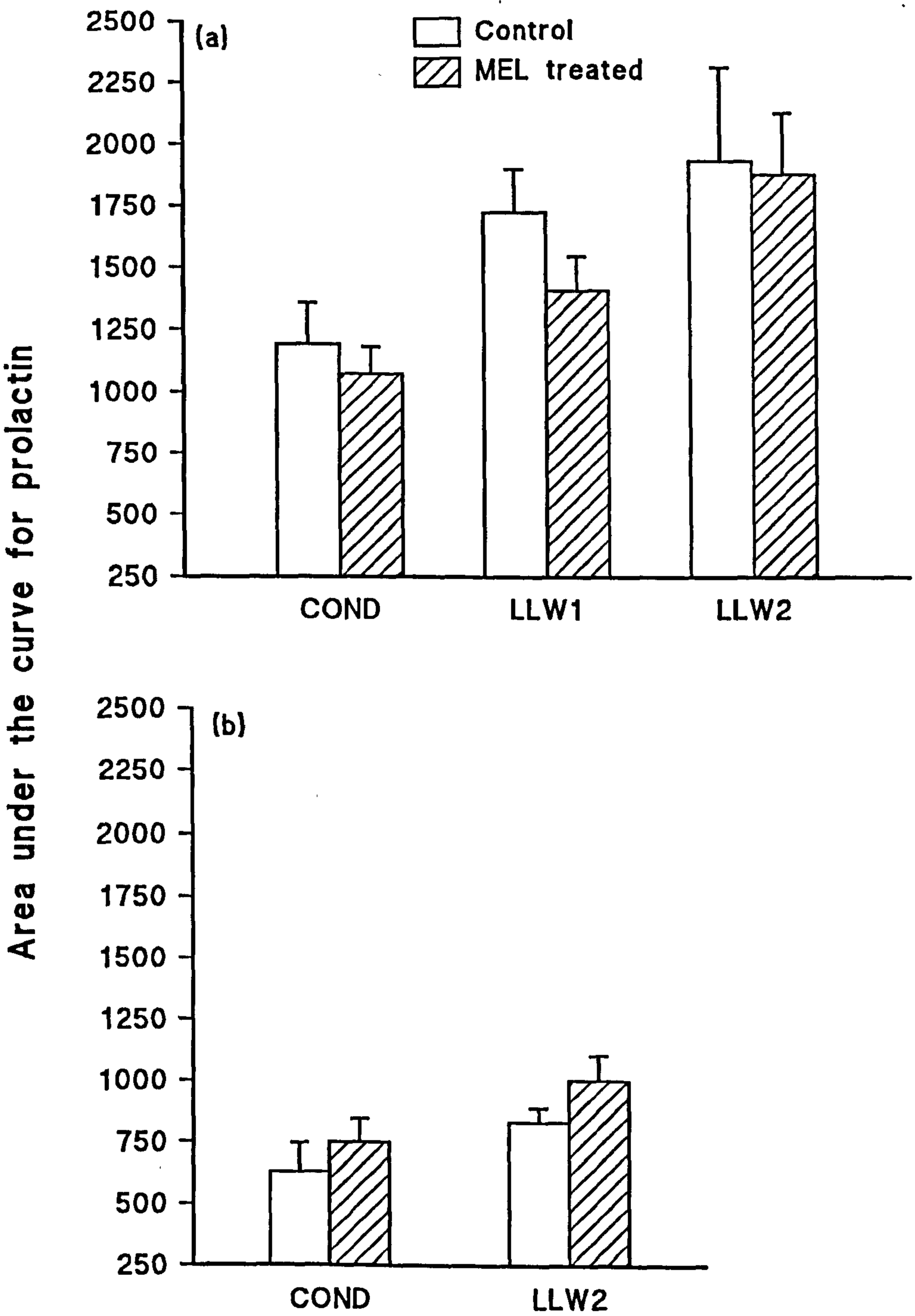

Figure 3.9 Plasma PRL area under the curve (calculated from the two-hourly samples) of lactating goats during the second week of the two-week conditioning cycle (COND) and the first (LLW1) and the second (LLW2) week of the long light cycle in (a) spring and (b) autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

In the first week of long light, AUC was increased in both groups but was still lower in the goats that had been treated with MEL than in controls (1084±155 v. 3254±1251, n.s, ANOVA). In the second week of long light, MEL-fed goats reached their peak value earlier than control group but no significant difference was found in AUC between them (for MEL-group and control, 2775±844 v. 4306±1757, n.s, ANOVA).

Milking-related PRL profiles in autumn are presented in Figure 3.11. Total AUC for postmilking PRL profiles were non-significantly decreased in control goats in autumn compared to in spring ($P>0.05$, *t* test), however, MEL-treated group had greater PRL profiles in autumn than that of spring during the conditioning cycle ($P=0.04$, *t* test). Profiles of PRL in the MEL-group were unexpectedly higher than that found in control group over all sampling times resulting in a significantly difference in AUC (5096±851 v. 1749±242, $P<0.01$, ANOVA). This effect was abolished once MEL feeding stopped and both groups were maintained on long light and AUC was significantly reduced ($P<0.01$, ANOVA) in goats with MEL resulting in no significant differences between the two groups (1568±526 v. 2000±474, n.s, ANOVA).

3.3.4.3 Growth hormone profiles

Profiles of weekly GH levels determined during pretreatment indicated that there was no significant difference between the two groups (for MEL-treated 2.90±0.59 and control 2.73±0.66ng/ml, $P>0.05$, *t* test). Two-hourly profiles of GH in spring are presented in Figure 3.12. During the second week of conditioning cycle, the pattern of GH secretion in MEL-treated goats differed from that in controls; basal levels were remained at lower levels. Plasma GH AUC are presented in Figure 3.14. The difference between treated and control goats was significant ($P=0.006$, ANOVA). In the first week of long light, goats treated with MEL showed no changes to their previous GH secretion pattern or mean. On the other

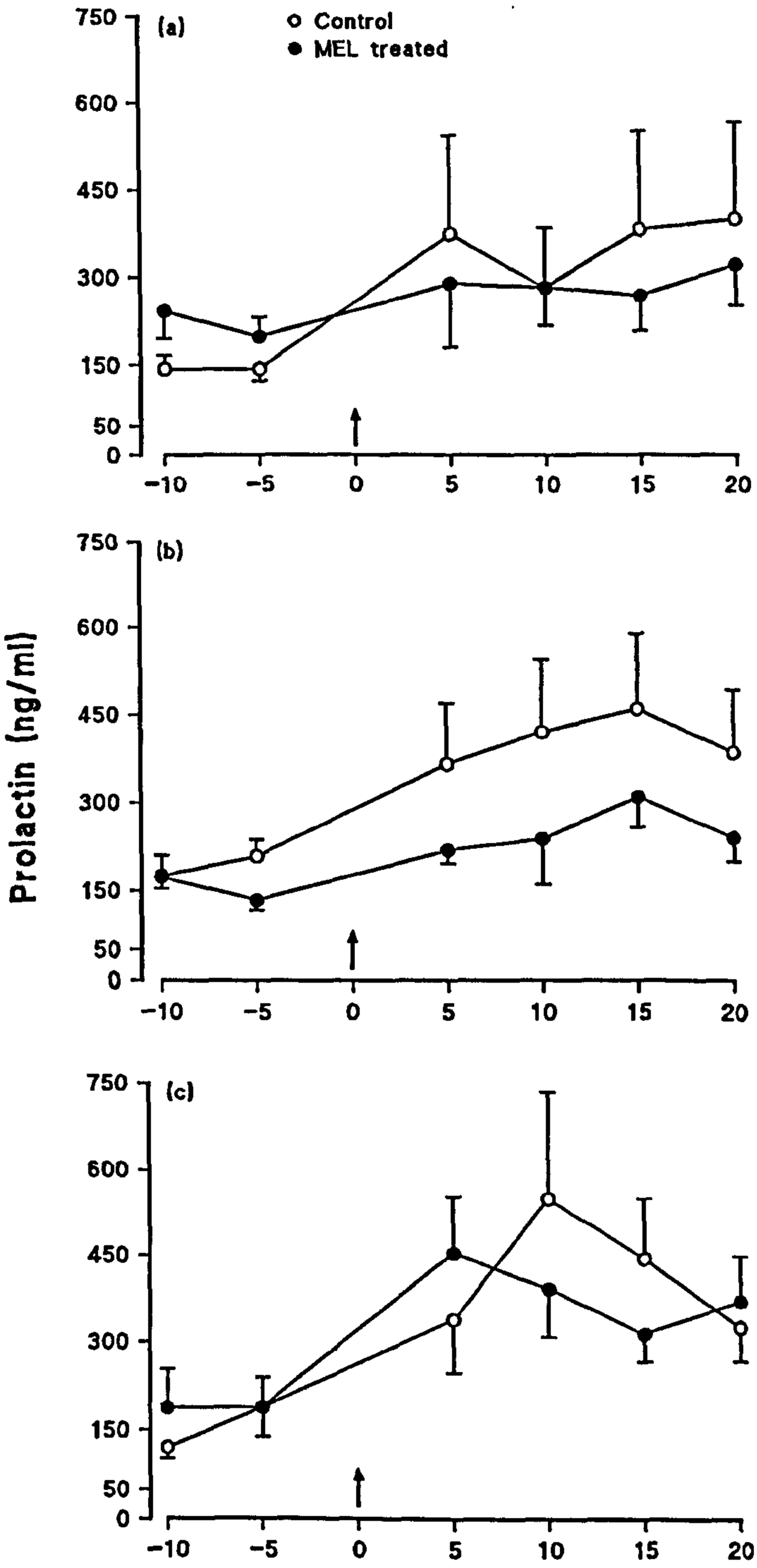

Figure 3.10 Profiles of plasma PRL concentrations (ng/ml) in lactating goats determined around milking time during (a) the second week of conditioning cycle, (b) during the first and (c) the second week of long light cycle in spring. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrows indicate the time when goats finished milking. Values are means with S.E.M.

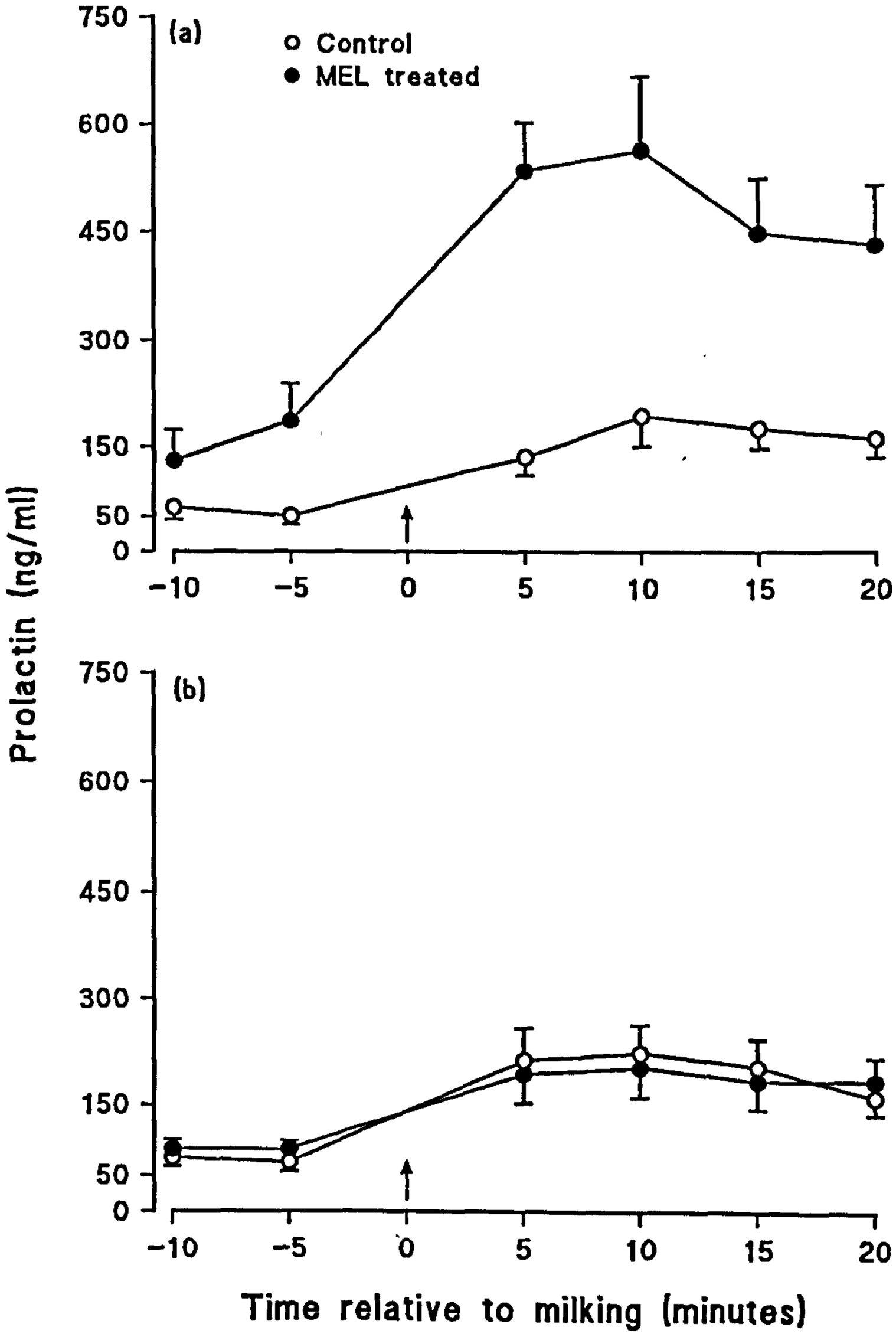

Figure 3.11 Profiles of plasma PRL concentrations (ng/ml) in lactating goats determined around afternoon milking time during (a) the second week of conditioning cycle and (b) during the second week of long light cycle in autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrows indicate the time when goats finished milking. Values are means with S.E.M.

hand, in control goats distinct peaks were diminished and also AUC tended to be reduced (P=0.13), generally both groups followed a similar pattern and there was no significant difference in GH levels between them. In the following week of long photoperiod, both groups were characterized by higher number of secretory bursts and the peak values tended to be higher than that found in the two preceding weeks. Despite these changes in the pattern of GH release, no effect of long light could be detected in the control group, but AUC was only significantly higher in the MEL group (P=0.02, *t* test) when compared to that in the week before long light exposure.

Profiles of GH during autumn are presented in Figure 3.13. Plasma concentrations of GH in MEL-fed goats were maintained at lower levels than controls in all samples collected except in the sample taken immediately before treatment (at 15.00h; Figure 3.13a). This resulted in a lower plasma GH AUC for this group when compared to control group (P=0.10, ANOVA, Figure 3.14b). Subjecting goats to long photoperiod resulted in a small increase in GH concentration, significant in MEL-group only (P=0.01, *t* test). However, peak values always tended to be higher in control goats.

Profiles of GH determined around milking are shown in Figure 3.15 for spring and in Figure 3.16 for autumn treatments. The data are largely confirmatory of the two-hourly profiles. Milking stimulated GH release to a limited extent (less than PRL). Total AUC of GH profiles determined from post-milking samples indicated that MEL suppressed GH release in spring (for MEL-group and control, 33.7±9.4 v. 82.3±20, P=0.06) as well as autumn (15.0±2.9 v. 66.7±19, P=0.04, *t* test). Long light did not cause any dramatic change on post-milking GH release in controls, but it was significantly higher in MEL-group when compared to that found during the conditioning period (P<0.01). There was no obvious seasonal effect on AUC as comparison between conditioning cycles of spring and autumn did not reveal a significant difference in either of groups. However, there was an

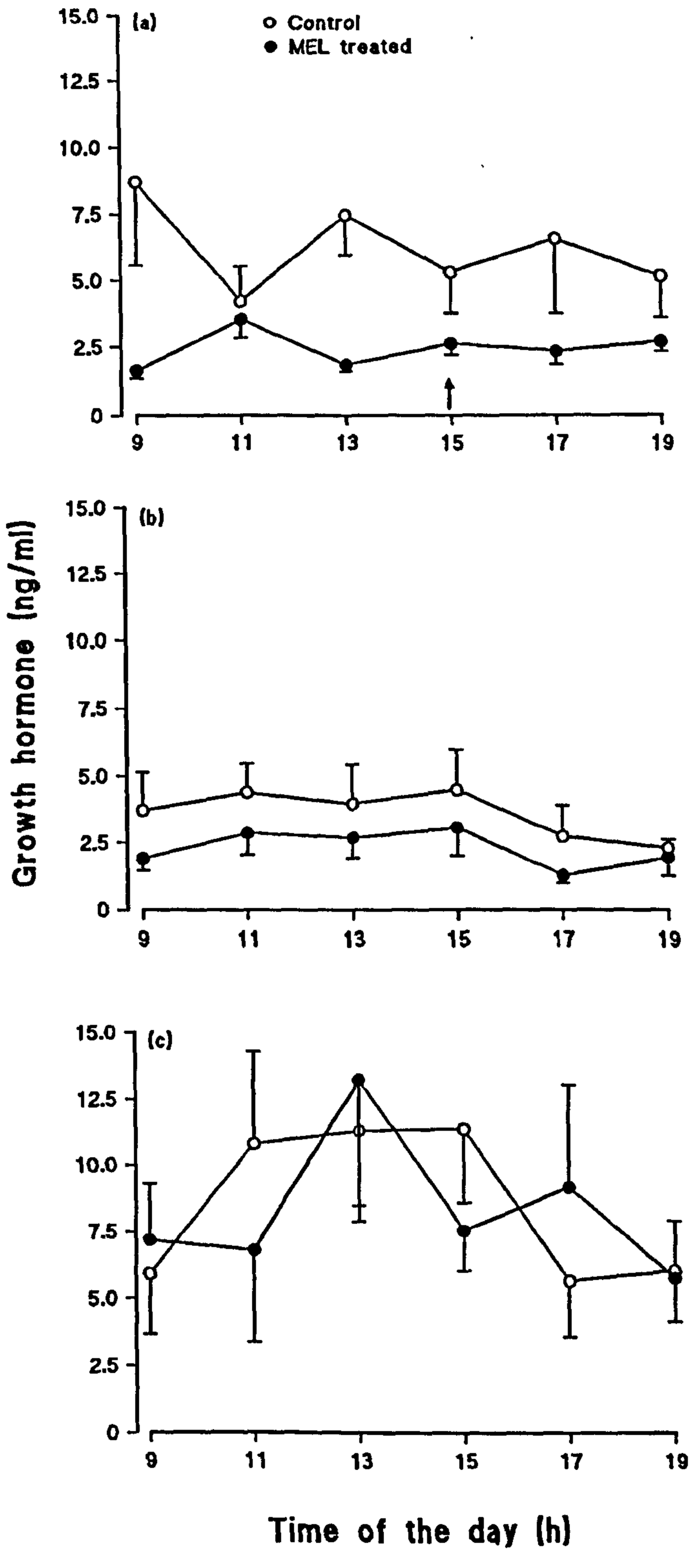

Figure 3.12 Patterns of GH secretion (ng/ml) in lactating goats during (a) the second week of conditioning cycle, (b) during the first and (c) the second week of long light cycle in spring. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrow indicates the time of MEL or vehicle feeding. Values are means with S.E.M.

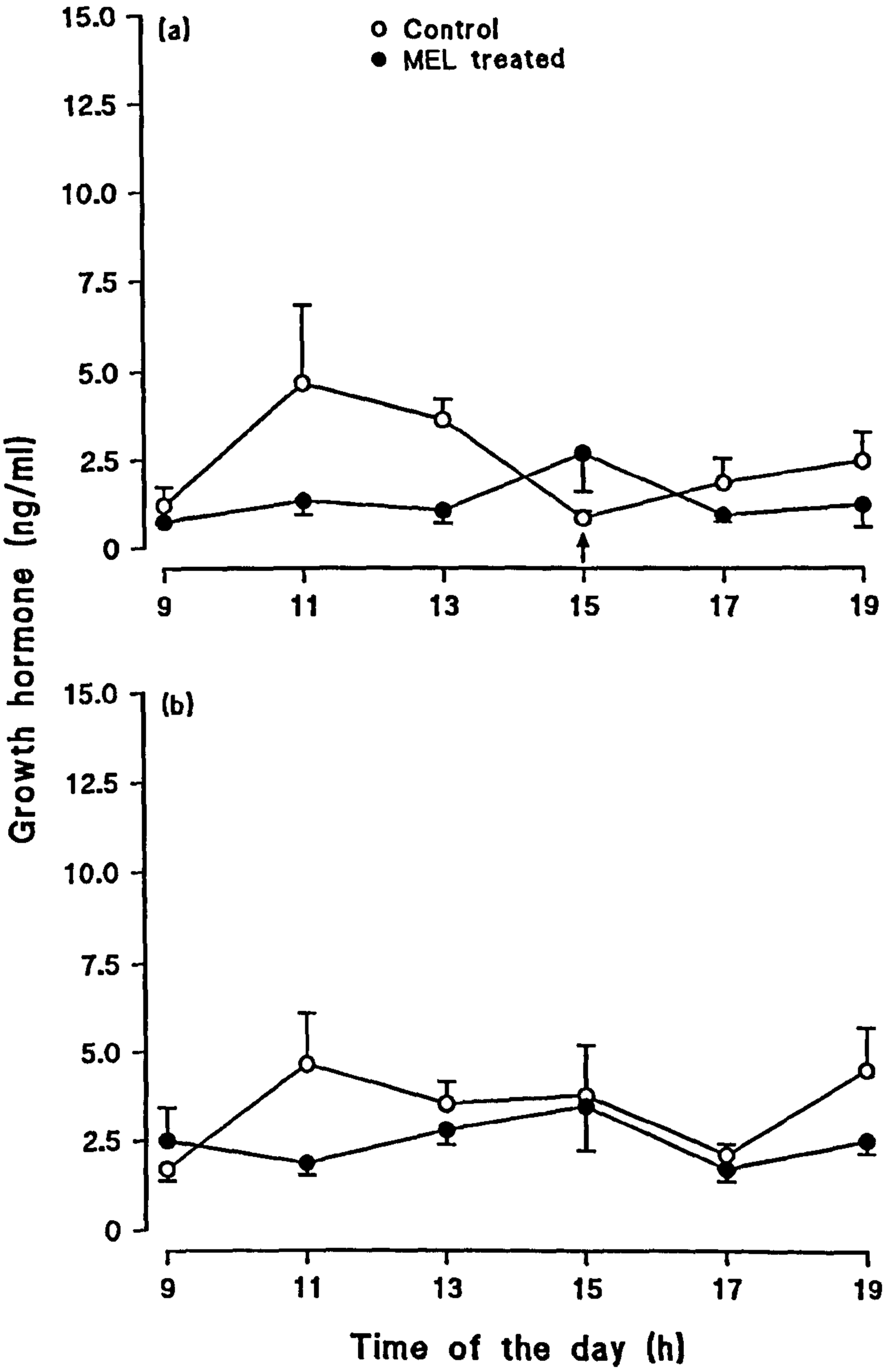

Figure 3.13 Patterns of GH secretion (ng/ml) in lactating goats during (a) the second week of conditioning cycle, (b) during the second week of long light cycle in autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrow indicates the time of MEL or vehicle feeding. Values are means with S.E.M.

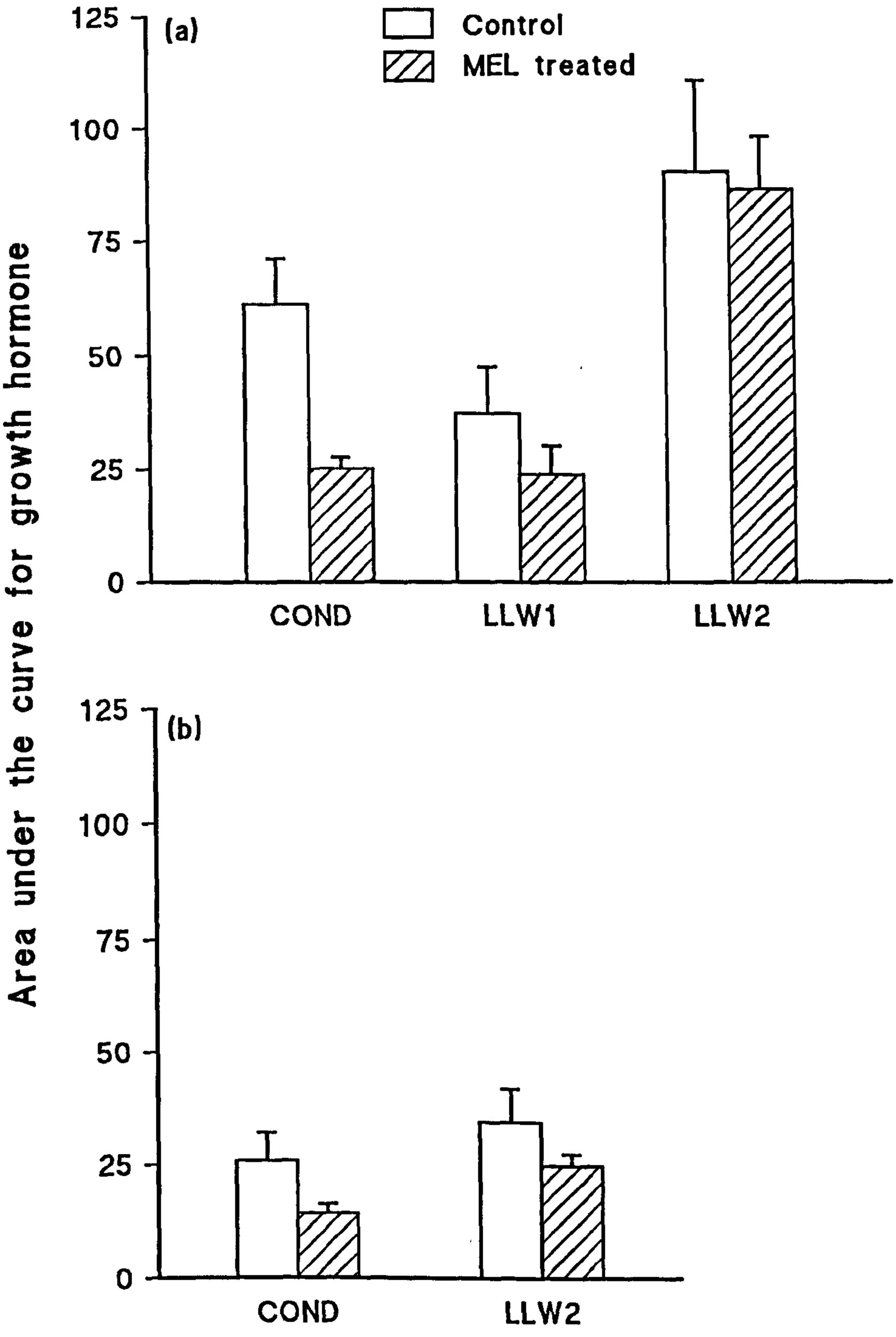

Figure 3.14 Plasma GH area under the curve (calculated from the two-hourly samples) of lactating goats during the second week of the two-week conditioning cycle (COND) and the first (LLW1) and the second (LLW2) week of the long light cycle in (a) spring and (b) autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

apparent variation in long light cycles between spring and autumn; average AUC of all goats was significantly higher in spring than that in autumn (110.9±22 v. 39.8±3.2, $P<0.01$, *t* test).

3.3.4.4 Insulin like growth factor-I profiles

Plasma concentration of IGF-I determined in the two-hourly samples collected during the first two cycles (MEL and long light) in spring and autumn are shown in Figure 3.17. There was no evidence of any modulatory effect exerted by MEL on plasma IGF-I concentration in spring or autumn, there was no significant difference between MEL-group and controls ($P>0.05$, *t* test, Figure 3.17a, b). While switching to long light was without any effect on IGF-I levels in spring, it was slightly reduced, but significant, in autumn in both groups ($P=0.05$, *t* test).

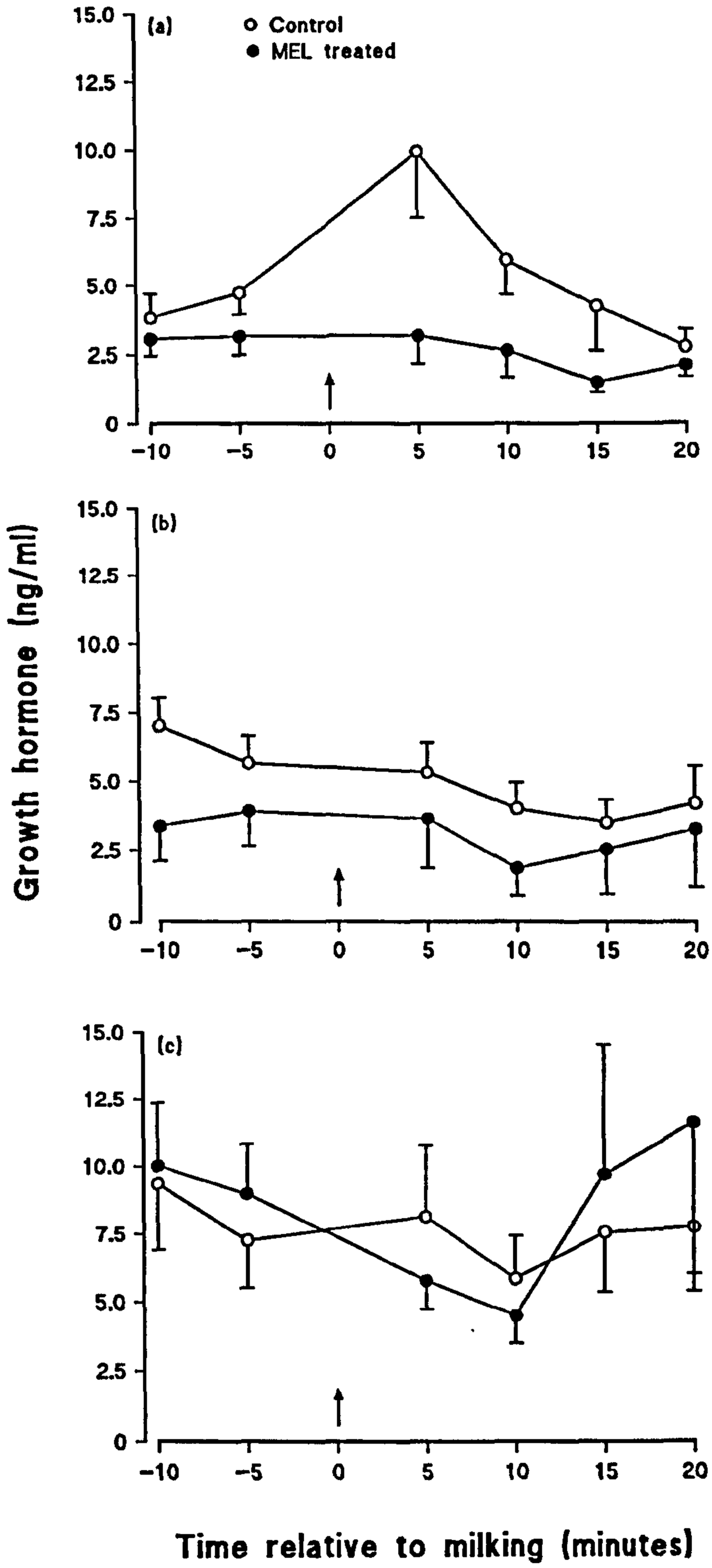

Figure 3.15 Profiles of plasma GH concentrations (ng/ml) in lactating goats determined around milking time during (a) the second week of conditioning cycle, (b) during the first and (c) the second week of long light cycle in spring. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrows indicate the time when goats finished milking. Values are means with S.E.M.

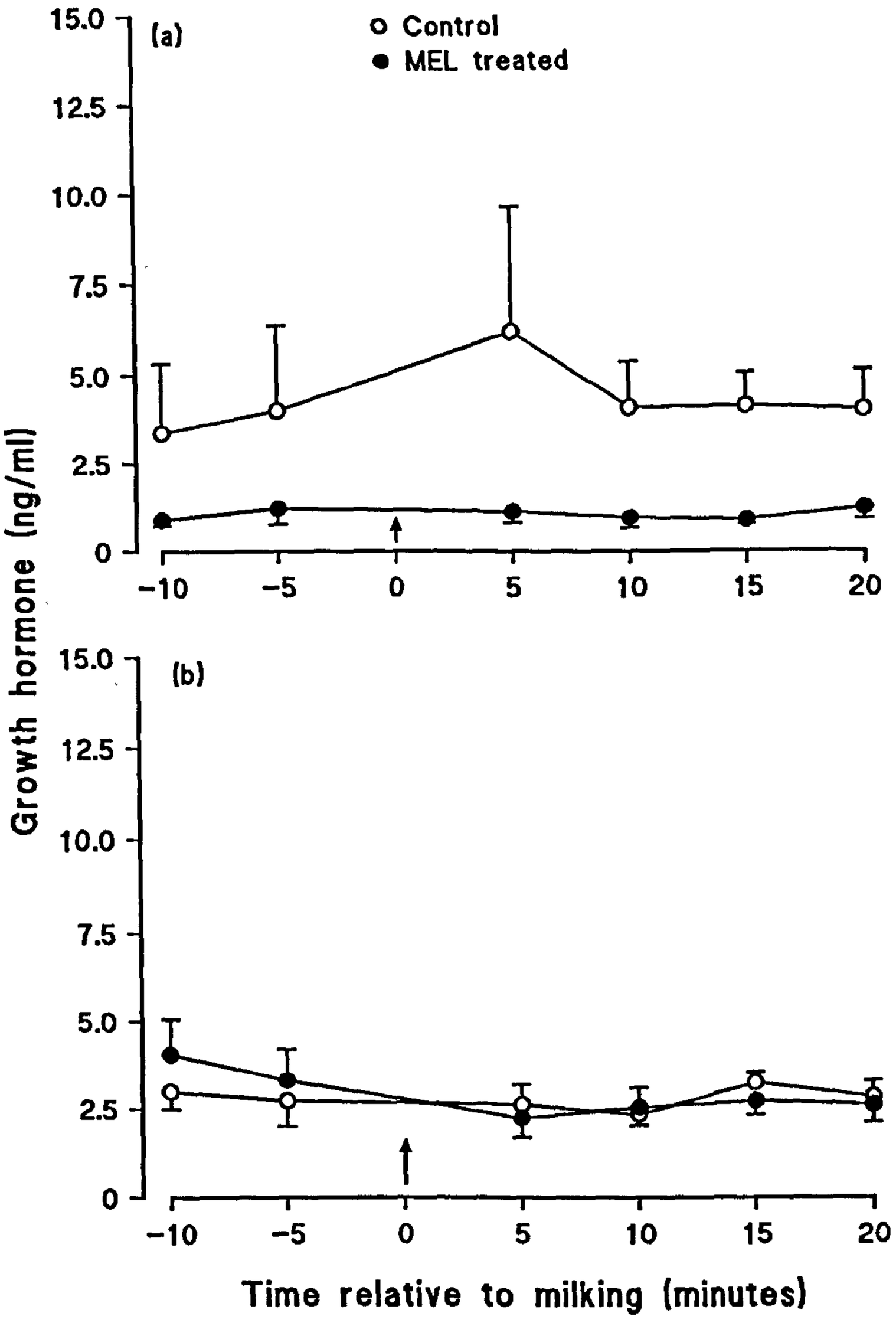

Figure 3.16 Profiles of plasma GH concentrations (ng/ml) in lactating goats determined around afternoon milking time during (a) the second week of conditioning cycle and (b) during the second week of long light cycle in autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Arrows indicate the time when goats finished milking. Values are means with S.E.M.

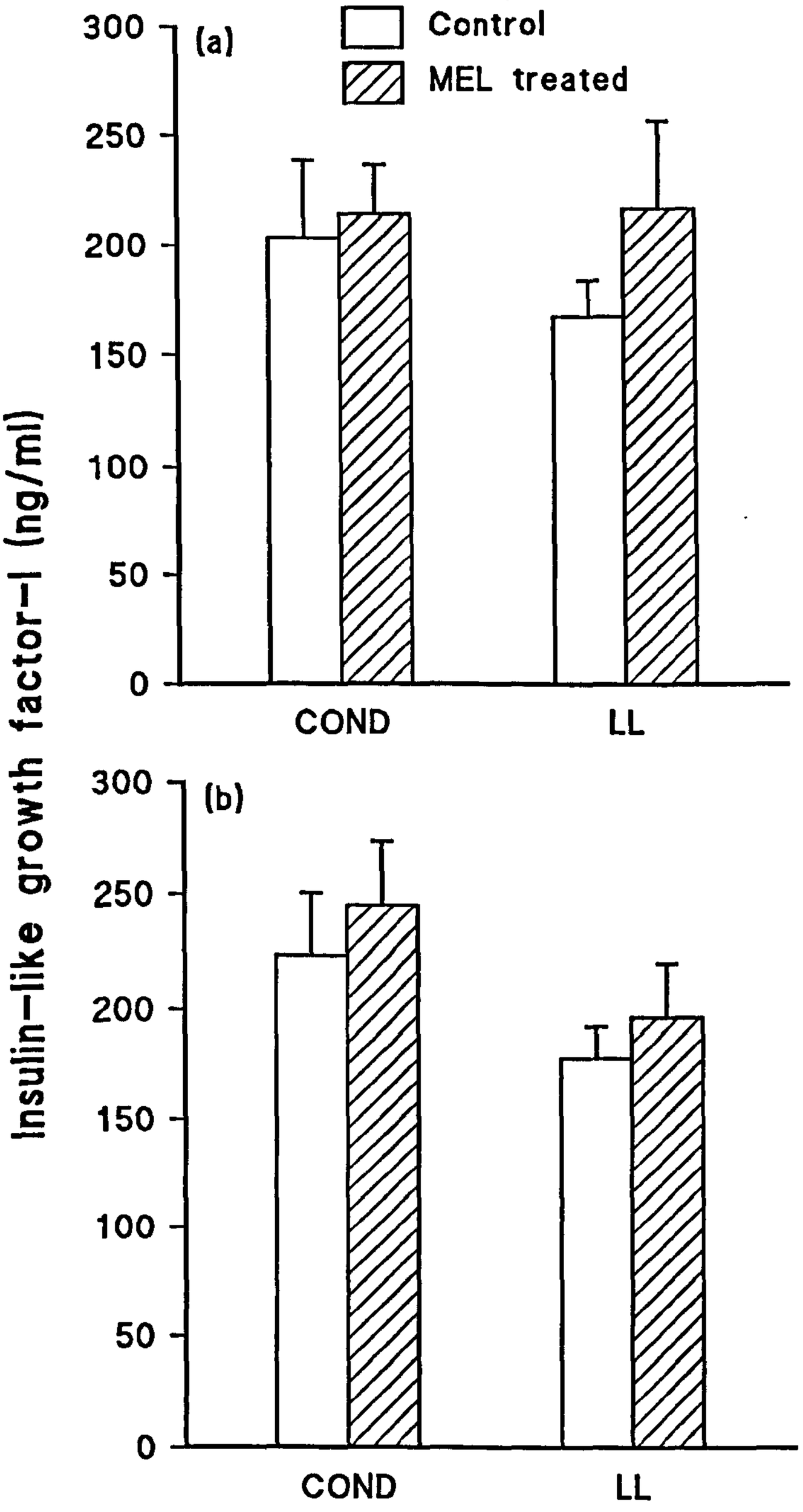

Figure 3.17 Profiles of plasma IGF-I concentrations (ng/ml, dtermined from the two-hourly samples) in lactating goats during the second week of coditioning cycle (COND) and long light cycle (LL) during (a) spring and (b) autumn. During the conditioning cycle, goats were either fed 3mg/d of MEL (MEL-treated) or fed vehicle (control) at 15.00h, and during the long light cycle, lights were on between 06.00-23.00h daily. Values are means with S.E.M.

3.4 DISCUSSION

3.4.1 Milk yield

Short term MEL treatment in the middle of the day to mimic short day signal was not effective in priming goats to subsequent effect of long photoperiod on milk yield, regardless of the time, commencement or duration of MEL treatment. Indeed, milk yield tended to be reduced when the MEL treatment was started in early lactation, when the day length was increasing. Milk yield was still increasing at the time of the start of the experiment, at week 5 of lactation. It continued to increase in the control group and reached a peak higher levels while goats with MEL treatment, it apparently reached peak about two weeks earlier and lower milk yield. The main factor, at the mammary gland level, responsible for the increase in milk production at peak lactation is the enhancement in the secretory activity of the epithelial tissues (Wilde *et al.*, 1986). Therefore, a tendency for MEL-treated goats to achieve peak yield at an earlier stage might suggest a possible disruption of the differentiation process. Alternatively, maintaining the control goats at a minimum of 12h of light might have some stimulatory effects on metabolic activity of the secretory epithelium. In this study, no group of goats was maintained outdoors at natural light to be compared with the two studied groups, and to clarify if light has caused any stimulation on milk yield of the control group. Although average milk yield of MEL-treated goats during each MEL or long light was not found to be significantly different from milk yield in control goats, total yield during the whole early lactation period was significantly lower in the MEL group. This clearly suggests that repeated cycles of MEL and long light are exerting an inhibitory effect on the lactation performance. Melatonin is involved in the regulation of many biological processes in mammals including reproduction and body growth and may also exert some influence on mammary gland development and function. It can be proposed that MEL might affect milk secretion indirectly, by altering the release

of pituitary hormones which are important for the function of the mammary gland. In this study, PRL released post-milking tended to be suppressed by MEL in spring treatment. Also, GH was maintained at a lower level during the MEL treatment. Thus, the reduction in the amount of PRL that the mammary gland was exposed to (postmilking) while under MEL treatment coupled with lower circulating GH at this stage of lactation might have affected the secretory activity of the epithelial tissue which in turn affected milk yield. Although PRL may not be essential for maintenance of established lactation in ruminants (Karg *et al.*, 1972; Hart, 1973; Beck *et al.*, 1979), it definitely has a role to play in the initiation of lactation (Schams *et al.*, 1972; Forsyth & Lee, 1993) which has been indicated to be required for full structural differentiation of alveolar epithelium (Akers *et al.*, 1981a,b). The galactopoietic effects of GH in ruminant is well known, and there is some evidence in goats which suggests a requirement for GH in maintenance of normal lactation (Cowie, 1964). Therefore, it is possible that the lower GH caused by MEL might have contributed to the decline in milk yield.

A possible modulatory effect of photoperiod on mammary gland development in ruminants has been suggested. Maintenance of prepubertal heifers on 16h of light: 8h of darkness has been shown to stimulate growth of the mammary parenchyma (Petitclerc *et al.*, 1985) suggesting that long light exposure, which also reduces the duration of the nocturnal MEL release, might be involved in this process. This hypothesis has been confirmed by Sanchez-Barcelo *et al*, (1991) when they observed a reduction in mammary parenchymal growth in prepubertal heifers treated with MEL at the middle of artificial long days. Also, MEL treatment during pregnancy appeared to affect mammary gland development as reflected by the reduction in mammary gland weight and total DNA (Mediavilla *et al.*, 1993). It has been proposed that part of the action of MEL is mediated directly at the level of the mammary gland (Sanchez-Barcelo *et al.*, 1990) and the presence of MEL binding

sites in mammary gland membranes of mice support this contention (Recio *et al.*, 1994). However, in spite of this evidence of interference of MEL with mammary gland development, there is no equivalent evidence to indicate that MEL is affecting mammary gland function during lactation. Treatment of lactating cows with MEL for eight weeks or for 20 weeks in goats (after the summer solstice) was without any effect on milk yield (Buchanan *et al.*, 1991; Knight, 1993). In addition, maintaining lactating ewes on short days (8.5h light: 15.5h of darkness) for four weeks starting at lambing, did not affect milk yield compared to others maintained on long light (Bocquier *et al.*, 1990). The divergence in milk yield between MEL and control groups during the spring treatment was not exhibited during the first conditioning cycle but rather started after the cessation of MEL treatment and exposure to long photoperiod, during the first long light cycle. Occasionally in the first year experiment the milk yield tended to decline with long light exposure in goats that had been on MEL before switching to long light. Taken together, these findings did not support that the deleterious effect on milk yield was attributed to MEL *per se* but to the rapid and progressive changes in day length which might have resulted in an external desynchronization and shift in the phase of the circadian rhythms involved in milk production. Self sustained oscillations with periods of 24h, the circadian rhythms, are entrained by the synchronizing effects of environmental cues such as light and dark cycles (Takahashi & Zatz, 1982). Repeated cycles of MEL (short day signal) and long light might have resulted in desynchronization of the circadian system. Short photoperiodic cycles of one month of long days followed by one month of short days abolished seasonality of reproduction in male goats (spermatogenetic activity was maintained at a high rate throughout the year; Delgadillo *et al.*, 1995). Also, the tendency for higher PRL concentrations during MEL treatment in autumn is another example of the disturbance of the circadian rhythm involved with PRL release in these goats. This study and that of

Knight, (1993) demonstrated that MEL treatment did not affect milk yield when treatment was initiated later in the year, after exposure to a long duration of long hours of lighting. This may be not simply related to seasonal or stage of lactation related sensitivity to MEL because the second year experiment was performed exactly like that in autumn of the first year yet the milk yield was not reduced in MEL group during the second year experiment. This suggests the importance of photoperiod cue while the day length is increasing in spring so that any consecutive rapid alteration in day length might be a determining factor to milk yield. Once the goats had experienced a period of long days and the day length was decreasing, then MEL was ineffective in disturbing the innate circadian rhythm involved in milk secretion. In fact the relationship between environmental factors such as photoperiod and milk production in goats is somewhat peculiar; when goats are kept to lactate for longer period of time, milk yield exhibits seasonal variation. Yield is high in spring-summer and low in autumn-winter, which might suggest a possible correlation between day length and lactation cycle so that a manipulation of light regimen will affect the yield. However, maintaining goats at extra hours of lighting during autumn-winter was ineffective in changing the normal decline in milk yield (Linzell, 1973; Hart, 1975b). Linzell, (1973) has suggested the existence of an innate rhythm for milk production in goats which may be independent of environmental cues like photoperiod. This may not be entirely true, since stimulatory effects of photoperiod on milk yield in goats have been achieved (Terqui *et al.*, 1984; Knight, 1993) and also in this study milk yield tended to increase during the last long light period in autumn of the first year experiment in the control group. The difficulty in explaining why effects of photoperiod on milk yield were inconsistent emerges from the fact that the mechanism by which photoperiod affects milk production is far from understood and still debated (Tucker, 1985; Dahl *et al.*, 1997). This inconsistency in the effect of photoperiod on milk yield has also been reported in cows. Despite the number of reports

which indicated that more hours of lighting during the winter is stimulatory to milk production (Peters *et al.*, 1981; Marcek & Swanson, 1984; Stainisiewski *et al.*, 1985), others could not detect any effect (Murrill *et al.*, 1969; Tanida *et al.*, 1984). It is possible that other environmental factors like ambient temperature might interfere with the light effect or factors related to lighting regimen such as source or intensity of light may be factors in determining the photoperiod response.

3.4.2 Melatonin profiles

The increase in plasma MEL concentration at 13.00 and 15.00h in control group during the first cycle of MEL in spring was an unusual observation. Samples were collected during the day time, a situation which would be expected to be circumstances for MEL suppression. In goats, as with other mammals, MEL secretion is suppressed by light treatment and it has been shown that 150 lux is sufficient to suppress MEL levels by at least 80% in Saanen goats (Deveson *et al.*, 1990) and the light intensity in this study (at least 300 lux) should have been more than enough to suppress MEL concentration throughout the day. The increase was only evident in the two samples collected before the feeding of the designated diet and in fact MEL levels fell to normal diurnal levels in samples collected thereafter in control goats, suggesting that the rise in MEL was unlikely to be related to an exogenous source. Since this was the first time of frequent sampling by catheter for these goats and a possible stress related response was suspected, it is possible that the elevation of MEL levels might have been also elicited by stress related factor. Rats subjected to chronic stress showed a significant increase in plasma MEL which could not be suppressed by constant illumination of bright light (Persengiev *et al.*, 1991). Thus, stress related increase in plasma MEL can overcome the suppressive effects of light on MEL secretion.

Plasma MEL in the treated group increased after MEL feeding and reached a maximum level about 30 minutes after the time of administration, which then declined but it was sustained at higher levels than that found in controls until the last sample at 19.00h. Administration of MEL in sheep by means of oral feeding has been reported to increase after feeding in a similar manner to that observed in this study (Kennawy *et al.*, 1982).

3.4.3 Prolactin profiles

Seasonal variation in PRL release is controlled by environmental factors, photoperiod and temperature. The levels of PRL are normally high during spring-summer and decline as day length decreases in autumn-winter months in the goat (Buttle, 1977). This seasonality in PRL profiles was not disrupted very much by this progressive light changes cycles; PRL was higher in spring and declined in autumn and also the goats were able to respond to photoperiod stimulation. It was believed that the duration of the nocturnal rise in MEL secretion may relay the effects of photoperiod on PRL release; high PRL levels are associated with short duration of physiological levels of plasma MEL (long day signal) and conversely longer duration of nocturnal rise in MEL (short day signal) are associated with lower circulating PRL (Maeda *et al.*, 1988). In this study, MEL treatment in spring did not result in a significant changes in PRL secretion and had only a marginal effect on the response to subsequent long light treatment. Extending the duration of higher plasma levels of MEL during the day by MEL feeding in the middle of the day, while the day length was increasing, apparently did not produce a significant decline in basal PRL concentrations as has been reported by others in sheep (Kennaway *et al.*, 1982; Symons *et al.*, 1983). The failure of MEL to exert an inhibitory effects on PRL levels can not be attributed to efficiency of treatment to elevate plasma MEL since MEL was elevated in all goats shortly after feeding and remained elevated until the end of the subjective day. The time of year of

the commencement of MEL treatment may account for the lack of a significant difference in basal PRL levels between MEL treated and control goats. In goats and sheep, animals have to be maintained on a sufficient duration of long days in order for the MEL to transmit its photoperiodic message (short day signal) on the hypothalamo-pituitary axis to modify the PRL release, presumably to remove the photorefractoriness caused by exposure to short day in winter (Prandi *et al.*, 1987; Deveson *et al.*, 1992b). In ewes, MEL treatment did not affect PRL concentration when treatment started in early spring, April, but PRL concentration was significantly reduced when MEL treatment began two months later in June (Poulton *et al.*, 1986). Alternatively, photorefractoriness can be removed by exposure to long light in the winter for some time before MEL signal became effective in reducing plasma PRL in early spring (Forsyth *et al.*, 1997). This indicates that MEL effects on suppression of PRL cannot be achieved without sufficient prior exposure to long days presumably to remove the short day refractoriness. However, the two hourly sampling assessed for PRL content might suggest a slight influence of MEL treatment on PRL profiles in spring, at least in the first cycle of MEL. There was a tendency for PRL levels to be reduced after the feeding with MEL but not at other times, suggesting that MEL feeding partially suppressed PRL secretion and this suppression was dependent on the presence of high levels of plasma MEL. This was also supported by the tendency for attenuation of post-milking PRL surge since milking was performed shortly after MEL feeding at a time when plasma MEL was at highest levels. There is no available data on the effect of MEL on post-milking PRL release in ruminants, however, evidence from the rat has indicated that MEL caused a strong inhibition to the suckling induced PRL surge (Juszczak & Stempniak, 1997). The mechanism by which MEL participates in the neuoroendocrine regulation of PRL is not fully understood. MEL might be acting on hypothalamic regions by modulating the production of releasing or inhibiting factors that subsequently will affect PRL synthesis and

secretion in the pituitary gland (Lincoln & Maeda, 1992). However, this may not be the whole story; strong evidence in the ram indicated that the hypothalamus, and consequently releasing or inhibiting factors, may not be required for the MEL signal on PRL release (Lincoln & Clarke, 1995). This suggest that MEL can act directly in the pituitary gland, and a direct effect of MEL on PRL release from pituitary cells *in vitro* has been demonstrated in the rat (Griffiths *et al.*, 1987). So, how does MEL exert its direct action in the pituitary gland to modulate PRL release? It is unlikely that MEL is acting directly in the *pars distalis* to modulate PRL secretion since it does not possess strong MEL receptors expression (Boissinagasse, 1992; Nonno *et al.*, 1995). Indeed, a great amount of evidence suggests that the main site of MEL action in the pituitary gland is in the *pars tuberalis* as indicated by high density of MEL binding sites (Dereviers *et al.*, 1991; Piketty & Pelletier, 1993) and the demonstration of suppressive effects of MEL on PRL release when implanted in *pars tuberalis* in sheep (Lincoln, 1994; Malpaux *et al.*, 1995). It can be anticipated, therefore, that MEL might be acting on some cellular components of the *pars tuberalis* in a way that affects neighbouring *pars distalis* cells in a paracrine fashion. The exact mechanism is far from understood, but this might involve changes in the sensitivity of lactotrophs to PRL releasing or inhibiting factors. The existence of PRL releasing factor from *pars tuberalis* can not be excluded since such a factor has been identified recently (Hazlerigg *et al.*,1996), but whether this releasing factor might account for the action of MEL on seasonal fluctuation of PRL concentrations is not known.

Treatment of MEL in autumn produced some effects on PRL which were different from those exhibited in spring. Plasma PRL levels tended to be higher during the first conditioning cycle and the first two weeks of the second cycle; there was no evidence of any tendency of lower PRL levels after MEL feeding in the frequent sampling and indeed the PRL surge in response to milking stimulus was potentiated. The response of MEL treatment

in autumn might be related to photorefractoriness elicited by rapid changes in day length signal on the mechanism of PRL release. During the first week of the first conditioning cycle, a sharp fall in PRL concentration in both groups was seen and this was attributed to switching the goats from long photoperiod to normal light (control) or short day (MEL treated). However, PRL profiles in MEL group were at higher levels than that of the controls, and a similar trend was also seen in the first week of the second conditioning cycle. Similarly alternating short cycles of four weeks of short days and long days in bucks caused a tendency for elevation in PRL levels during the first two weeks of short day exposure (Delgadillo & Chemineau, 1992). Secretion of PRL which undergoes marked circadian and seasonal changes is controlled by the circadian pacemaker system and that pineal gland transmiting day length information to the neuroendocrine axis via MEL secretion. Therefore, it can be suggested that abrupt and progressive changes in day length signals (short and long light) might have resulted in desynchronization in the endogenous circadian rhythm involved in the regulation of PRL secretion. The entrainment of the rhythm by photoperiod message would require some period of time before photoperiodic message can be expressed on PRL release. For example, PRL levels tended to decline on continued MEL administration (second conditioning cycle), and also, stimulatory effect of long light on PRL release was not immediate and it required some time to occur.

3.4.4 Growth Hormone

The ability of MEL to affect GH has been demonstrated since GH plasma levels in MEL-treated goats were lower and this response appears to be independent of season. MEL treatment also has been shown to suppress GH secretion in hamsters (Vaughan *et al.*, 1993). MEL has been proposed to affect the release of pituitary hormones by acting in the mediobasal hypothalamus (Lincoln & Maeda, 1992; Malpaux *et al.*, 1995) presumably to

modify the release of factors that exert stimulatory or inhibitory actions on the release of pituitary hormones. Evidence from the rat indicates that MEL stimulates somatostatin release from rat medial basal hypothalamus *in vitro* (Richardson *et al.*, 1981) which then reduces the secretion of GH from somatotrophs, but direct effects of MEL on the pituitary to affect GH release can not be excluded (Griffiths *et al.*, 1987). The tendency for a decline in the basal GH profiles seen in the first week of long light exposure in the control group was somewhat difficult to explain, but it was also maintained at lower levels in MEL-fed group. It is possible that the higher plasma GH concentrations during normal light exposure were not related to light treatment, GH may be elicited by other factors. This was the first time that the goats were subjected to frequent bleeding by means of catherization, so stress might has contributed to the elevation in GH secretion during the first conditioning cycle. Simms *et al*, (1978) have observed higher plasma GH in hourly samples when collected for the first time by means of catherization in goats which then declined on repeated sampling. Goats in this study were catheterized on the day before the day of sample collection but they had been subjected to weekly bleeding by venipuncture at least two weeks before the commencement of the experiment. Nevertheless, this may not be related directly to catherization or sample collection effects but may be as consequence of stress related disturbances in the daily activities of the goats during the day of sampling. The changes in GH during light treatment in the control goats, no significant difference between normal light and the second week of long light exposure in GH levels in both seasons, did not support a stimulatory effect of photoperiod on release of GH. The effect of photoperiod on GH secretion in ruminants is equivocal. In heifers and lactating cows, there is no evidence to indicate that photoperiod exerts a modulatory effect on basal GH release (Peters & Tucker, 1978; Peters *et al.*, 1980, 1981; Gustafson, 1994), but it might affect the number of episodic pulse releases of the hormone (Leining *et al.*,1980; Evans *et al.*, 1991). Also,

seasonal variation in GH levels in circulation was not evident (Petitclerc *et al.*, 1983). In lactating ewes and growing lambs, GH plasma concentrations were not altered by light manipulation (Bocquler *et al.*, 1990; Francis *et al.*, 1997). In contrast, Barenton *et al*, (1987) reported that plasma GH levels were associated with the prevailing day length in the ram, being high during long photoperiod and low in short days. In male as well as female goats, there is no evidence of a regulatory role of environmental factors such as photoperiod and temperature on the release of GH (Hart & Buttle, 1975; Kloren *et al.*, 1993) which suggests that GH levels do not exhibits a seasonal variation such as that found for PRL. In this study, light might participate in the regulation of GH release. Treatment with MEL which mimics short day signal depressed GH secretion and subsequent exposure to long light stimulated its release in this group regardless of season. On the other hand, there was no convincing evidence for a strong stimulatory effect in the control group in which the increase in GH did not achieve the significant level. However, four out of the six control goats exhibited an apparent rise in plasma concentration in spring and, interestingly, the same goats also showed the same trend in autumn. Had there been more animals in each treatment group, a significant effect might have emerged. Indeed, comparison between the first and second weeks of long light indicated a significant difference between the two means in the control goats, but not when GH levels during normal light were compared with that of the second week of long light.The decline in GH concentration in August can be attributed mostly to stage of lactation; as lactation advances so GH level declines. Repeated cycles of short day (or normal day) and long light did not attenuate the decline in GH.

There was no clear rise in GH concentrations after milking and it was increased after 5 minutes of milking in only three goats out of the six controls. The mode of release is different from PRL and may not be related to the tactile stimulation of the mammary gland (Hart & Linzell, 1977).

3.4.5 Insulin-like growth factor-I

Treatment with MEL did not affect IGF-I concentration despite the tendency for depressive effect on GH levels. The finding in this study of a lack of a correlation between IGF-I and GH levels in plasma changes in response to photoperiodic manipulation was not something unexpected. Several reports which indicate that GH level is not correlated well with plasma IGF-I in cattle (Breier *et al.*, 1986; Ronge & Blum, 1989) despite the significant role of GH as a regulator of IGF-I synthesis. Moreover, photoperiodic manipulation may result in changes in IGF-I which are independent of GH changes in lactating cows and lambs (Dahl *et al.*, 1997; Francis *et al.*, 1997). In fact, several factors might be involved in regulation of hepatic IGF-I synthesis and hence its circulation levels which includes nutrition, metabolic status and the thyroid status (McGuire *et al.*, 1992; Rodriguez-Arnao *et al.*, 1993). The decline in IGF-I in response to long photoperiod in the autumn might constitute a paradox, since shifting to long light has been associated with promotion of growth (Tucker *et al.*, 1984) together with stimulation of feed intake (Peters *et al.*, 1981). Therefore, IGF-I concentrations would be expected to increase with long light not decline. Stimulatory effect of exposure to extended hours of lighting on IGF-I levels have been demonstrated in lambs (Francis *et al.*, 1997) and in lactating cows (Dahl *et al.*, 1996) but not in female deer (Adam *et al.*, 1996). In agreement with what has been seen in this study, Sarko *et al*, (1994) observed a decline in serum IGF-I concentration in calves when shifted to 16h of light. There was no change in live body weights during the second week of long light to be accounted for this decline in IGF-I. However, caution is needed in drawing any conclusion from this finding since plasma levels of IGF-I was determined in frequent samples from only one day which might have been affected by factors other than photoperiod. Unfortunately, voluntary food intake was not measured during the experimental periods, although goats did not show a tendency for lower live weight during

long photoperiod. This indicates that photoperiodic effects on IGF-I were probably not a result of differences in nutritional status.

3.5 GENERAL DISCUSSION

In this study, stimulatory effect of photoperiodic manipulation on milk yield has not been well established. Maintaining lactating goats on long light at different stages of lactation were ineffective in increasing milk yield except for one occasion, in the last long light cycle of the first year experiment. Nonetheless, this response was modest and did not occur in all goats, it was only evident in control goats. This inconsistency in stimulatory response to photoperiod is not novel and has been reported previously in ruminants. This suggests the existence of other factors that might limit or prevent this response. For example, ambient temperature might be a possible factor interfering with the galactopoietic effect of photoperiod.

The main objective of this investigation was to test if short term MEL treatment was effective in sensitizing the goats to the following long light stimulation on milk yield. There was no supportive evidence to suggest that exposure to such a conditioning factor with this length resulted in increasing the capability of milk production once they were switched to long light. Indeed, there was a tendency in MEL treated goats to produce less milk once they were on long light in the first year experiment, suggesting that short term MEL treatment might block or attenuate the long light effect. The goat is considered a seasonal breeder and its lactation cycle starts in early spring and ends in late winter, and there is a parallelism between changes in milk yield and day length. Several biological functions which exhibit cicannual rhythms are entrained by changes in ambient photoperiod. Therefore, it is possible that mammary gland function which clearly shows a well defined seasonal rhythmicity is entrained by day length changes, and MEL is the factor which is responsible for the transmition of photoperiodic message to the neuroendocrine system. Starting the treatment regime of short cycles of MEL and long light while the day length was still increasing might have disrupted the normal seasonal (photoperiod) effect to entrain

the endogenous rhythmicity involved in milk production. However, delaying the onset of cycles until goats had passed the longest day (summer solstice) did not result in any determinant effect on endogenous rhythmicity of milk yield. This was possibly related to the fact that the goats needed to pass a critical period (longest day) before any rapid and abrupt manipulation in day length was without any deleterious effect on milk production. Although there was no evidence to indicate a possible stimulatory effect of long light on milk yield in the second year experiment, there was a tendency for conditioned goats to produce more milk than the non-conditioned ones in the overall cycles. This, at least, confirmed the proposed hypothesis about the timing of starting MEL manipulation and also agrees with what was found in the previous year regarding the lack of priming effect of MEL on subsequent response to long light exposure. Also, the absence of any effect of long light on milk yield supports the contention of interference of other factors with this process.

Melatonin treatment by itself resulted in a tendency for a non-significant reduction in milk yield as compared to controls, provided that the treatment was started before summer solstice. It was possible that the inhibitory effect of MEL on GH may account for this decline in milk production. However, the tendency for lower milk yield after cessation of MEL treatment and start of long light in the first year supports the hypothesis of disruption in the seasonality caused by rapid alternating changes in day length signal at this critical period. This study did not indicate that short term MEL treatment sensitized the goats to subsequent long light effect on milk yield regardless of time of the commencement of treatment cycles. In fact, applying treatment cycles before the longest day may produce some detrimental effects on milk yield thus indicating the importance of seasonality in lactation performance in goats. There was a lack of definitive and consistent increase in milk yield by long light which suggests the possibility of involvement of other factors that might interact with the galactopoietic effect of photoperiod.

CHAPTER FOUR

EFFECT OF MELATONIN ON THE EFFICIENCY OF UDDER EMPTYING AT MILKING IN THE GOAT

4.1 INTRODUCTION

In the previous chapter, it has been shown that short cycles of melatonin (MEL) followed by long light might, in some cases, produce an inhibitory effect on milk yield. The overall conclusion suggested that this effect may not be related directly to MEL, but may be related to the disruption in the normal increase in day length caused by abrupt and alternating exposure to MEL and long days. However, some evidence pointed to a possible involvement of MEL in this response. For example, in the MEL-fed goats, plasma GH levels were lower and there was also a tendency for suppression of post-milking PRL release in early lactation treatment. This suggests that MEL might be acting on the hypothalamo-pituitary axis to affect the release of some hormones which are involved in the regulation of mammary gland function. This is supported by the existence of MEL binding sites in several areas in the brain indicating the multiple activity of this hormone. Oxytocin (OT) is a hormone released from the neurohypophysis upon the suckling or milking stimulus which acts on the myoepithelial cells surrounding the alveolus to induce milk ejection by contracting them. Some evidence from the rat and hamster has shown that the pineal indoleamine, MEL, exerts some regulatory mechanism on OT release; MEL reduced OT release from rat hypothalamus explants (Yasin *et al.*, 1993) and from the neurointermediate lobe of the pituitary in hamster (Juszczak *et al.*, 1995). It is not only the basal release of OT which has been affected by MEL, but also the stimulated release of OT (by KCL) has been shown to be reduced by MEL (Yasin *et al.*, 1996). There is also some evidence to suggest a diurnal variation in the modulatory influence of MEL on OT release, for example, OT

release was not suppressed by MEL treatment when it was applied at midnight, in fact it was stimulated (Yasin *et al.*, 1995).

If the MEL treatment is causing an inhibition of milking-elicited OT release, this could result in incomplete milking. Incomplete milking can lead to a reduction in milk yield and reduction in the metabolic capacity of secretory epithelium through increased activity of a locally-active autocrine inhibitory protein, the feedback inhibitor of lactation (Wilde *et al.*, 1989), confirming the contention of inverse relationship between residual milk volume and rate of milk secretion (Peaker & Blatchford, 1988). Accordingly, this experiment was conducted to investigate the impact of MEL on the completeness of milking of the udder in the goats and if this would be affected by the changes in day length at which MEL was started.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Saanen goats which kidded in spring and of different parities were used. The determinations of the impact of MEL on residual milk volume were performed during early May (lactation week of 1.86 ± 0.34) and in July, after the summer solstice when the goats were in lactation week of 10.40 ± 0.98. The two experiments were conducted at different years with different animals at each year. Housing and management were the same as that described in chapter 3.

4.2.2 Experimental design

In both trials, goats were not treated with MEL or experienced artificial long days before the start of the experiment. The concept of the experiment was to determine the effect of MEL on milking-induced OT release indirectly through the determination of the

volume of milk remained in the udder after normal milking (residual milk). MEL was fed to goats for 14 days and estimations of the residual milk volumes were done at three periods, immediately before MEL treatment (pretreatment), during the first days of treatment (early MEL) and during the last days of treatment (late MEL). At each interval, the estimation was repeated on three successive days and the average of these determinations was used for the evaluation. The MEL feed was prepared as described in the previous chapter, and fed to goats daily at 15.00h, approximately 30-60 minutes before milking. The assessment was done only at the afternoon milking, and the goats were machine-milked in the parlour as normal followed by thorough hand stripping, and this was carefully maintained at each milking. After milking the goats, they were moved to an area near their pens where the determination of the residual milk was carried out. Goats were then hand milked 1-2 minutes after injection of 0.2IU of OT (in 1ml of 0.9% NaCL) administered via one of the jugular veins. This was followed by another dose of OT and hand milking, the collected milk fraction was recorded to the nearest 0.5gm. The milking procedure before and after OT injection was kept the same throughout the determination periods.

4.3 RESULTS

Milk yield volumes during P.M. milking obtained by normal machine milking (machine) and that recovered after OT injection followed by hand milking (residual) are presented in Table 4.1. Neither milk volume obtained before OT nor the total milk volume (machine + residual) were affected by MEL treatment, and regardless of the time of the year ($P>0.05$, *t* test). Administration of MEL shortly before P.M. milking apparently did not affect the efficiency of udder emptying as indicated by a lack of any differences in residual milk volume ($P>0.05$, *t* test) and again without any interaction between MEL and changes in day length. The residual milk which was expressed as a proportion of the P.M. milking are presented in Figure 4.1. During spring treatment, proportion of residual milk tended to be higher during pretreatment period when compared to that determined later in the year (16.50 ± 5.6 v. 8.27 ± 1.0, $P>0.05$, *t* test). Proportion data agree with absolute residual volume in the absence of any effect of MEL on the udder emptying of milk during normal milking ($P>0.05$, *t* test) at both intervals of MEL treatment (early and late MEL). In early lactation (spring), there was a trend for a drop in residual milk values during the MEL application ($P=0.08$, Paired *t* test), whereas, it was remained fairly steady in the group which was examined later in the year.

Table 4.1 Milk volume (l) of goats obtained during normal P.M. milking (Machine), and after intravenous injection of 0.4 IU of oxytocin (Residual) which were determined (A) before and (B) after the summer solstice. Values are average (± S.E.M) of three days determinations before MEL treatment (Pre-trt), during the first three days (Early-MEL) and last three days (Late-MEL) of MEL treatment which was fed daily at a dose of 3mg at 15.00h for 14 days. The goats were milked within 30 minutes of MEL feeding.

	Milk volume (l)	Pretreatment	Early-MEL	Late-MEL
(A)	Machine	1.29 ± 0.06	1.52 ± 0.10	1.54 ± 0.07
	Residual	0.24 ± 0.06	0.18 ± 0.04	0.12 ± 0.05
	Total	1.53 ± 0.09	1.70 ± 0.13	1.66 ± 0.10
(B)	Machine	1.67 ± 0.08	1.55 ± 0.09	1.82 ± 0.12
	Residual	0.14 ± 0.02	0.13 ± 0.02	0.14 ± 0.02
	Total	1.81 ± 0.09	1.68 ± 0.09	1.96 ± 0.12

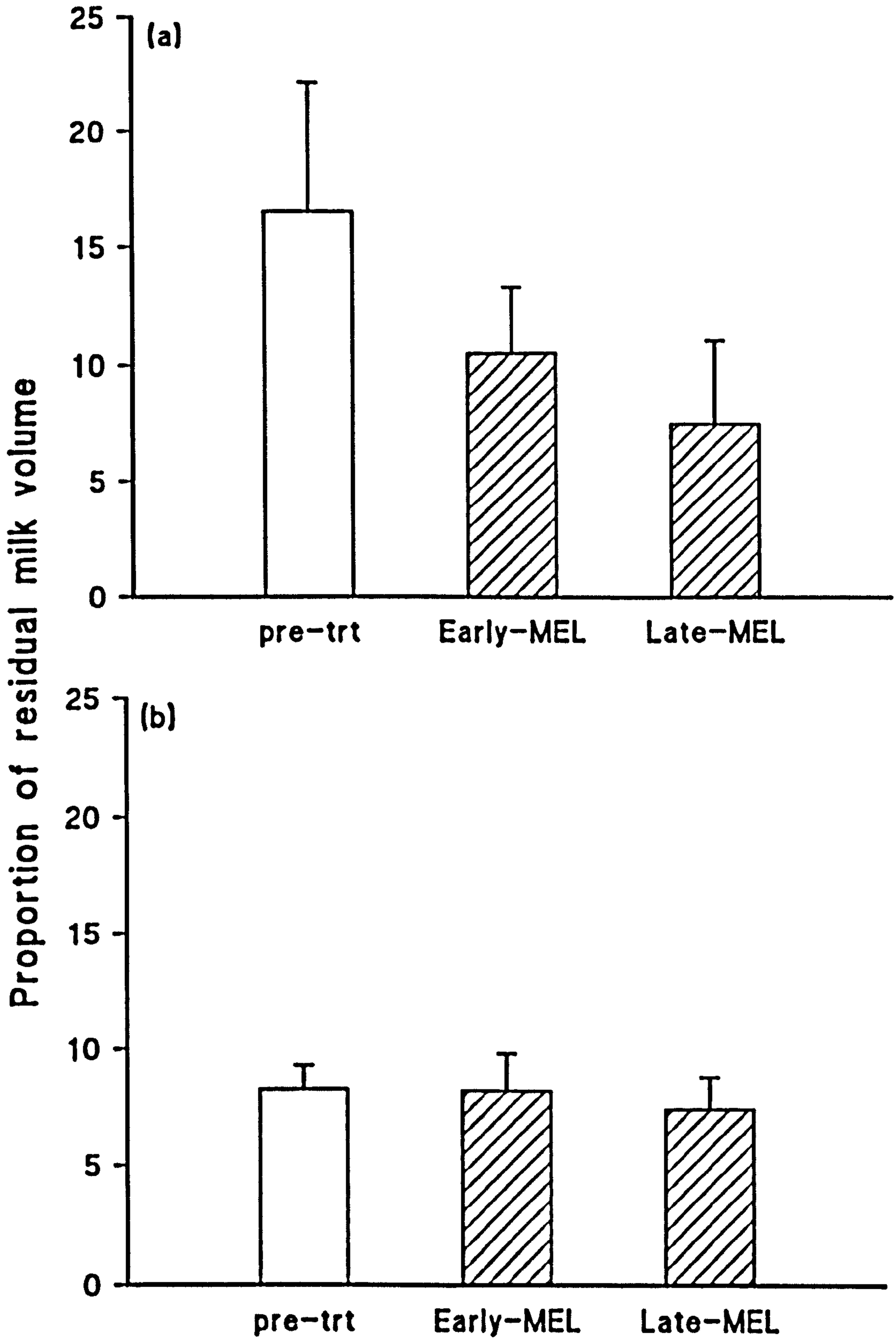

Figure 4.1 Proportion of residual milk volume during P.M. milking in goats recovered after intravenous injection of 0.4 IU of oxytocin which were determined (A) before and (B) after the summer solstice. Values are average (with S.E.M) of three days determinations before MEL treatment (Pre-trt), during the first three days (Early-MEL) and last three days (Late-MEL) of MEL treatment which was fed daily at a dose of 3mg at 15.00h for 14 days. The goats were milked within 30 minutes of MEL feeding.

4.4 DISCUSSION

The results undoubtedly show that short term MEL treatment did not affect the efficiency of milk removal at normal milking notwithstanding of changes in day length. Milking or suckling elicits the release of OT from the neurohypophysis which acts to facilitate the expulsion of formed milk from the alveoli and small ducts to gland cistern. Thus, the milk ejection reflex elicited by OT is prerequisite for completeness of milk removal at milking. In this study, OT concentrations at milking were not determined, but the response of the residual milk suggests that MEL did not effect OT release. *In vitro* experiments have shown that MEL inhibits the release of OT from hamster neurointermediate lobe (Juszczak *et al.*, 1995) and rat hypothalamus (Yasin *et al.*, 1993). The involvement of MEL in the modulation of pituitary hormones release is well known and the existence of MEL binding sites in the hypothalamo-pituitary region supports the role of MEL. The radioligand 2-[^{125}I]iodomelatonin has been used extensively to localize binding sites in peripheral and brain tissues. Determination of target sites where MEL acts is a prerequisite for understanding the physiological mechanism of MEL action. In the goat, MEL, apparently, is not acting directly in the neurohypophysis to affect the release of OT since MEL was not found to bind in this pituitary region (Deveson *et al.*, 1992c). MEL might be acting in the hypothalamus to modify OT release, and, the presence of high affinity MEL binding sites in the suprachiasmatic nucleus (SCN) has been reported in goats (Deveson *et al.*, 1992c). There is an association between SCN and the paraventricular nucleus (Swanson & Cohen, 1975), so that the output of the paraventricular nucleus could modulate the activity of the cells which synthesis OT, the magnocellular neurones. Alternatively, MEL could modify the uptake and release of neurotransmitters such as serotonin, norepinephrine, dopamine and glutamate (Cardinali *et al.*, 1975) which in turn modulate the release of OT from the neurohypophysis.

Despite the reported inhibitory effects of MEL on OT release, there are two factors which might determine the modulatory effect of MEL on OT release, dose and light:dark cycle. It has been shown, recently, that responsiveness of the hypothalamo-neurohypophyseal axis to MEL treatment depends on the time of the day, so that MEL suppressed both basal and KCl-stimulated release of OT at the end of the day, whereas, it enhanced the OT stimulated release during the dark period (Yasin *et al.*, 1996). This diurnal variation in the effect of MEL on the activity of OT releasing cells is likely to be related to changes in the density of MEL binding sites in the hypothalamo-pituitary axis. Several studies have demonstrated that MEL binding sites in SCN and PT were lowest in the end of the dark period and highest later in the day (Piketty & Pelletier, 1993; Gauer *et al.*, 1993). In this experiment, MEL administration and the determination of residual milk were performed at a time (late afternoon) which was postulated to be of high MEL binding sites. In addition, the tendency of a modulatory action of MEL on both GH and PRL levels shown in the previous chapter may not suggest low binding sites could have been accounted for the lack of effect on milk ejection caused by MEL. It is possible that the time of milking at which the levels of MEL anticipated to be extremely high may not have provided an appropriate condition for MEL to suppress the release of OT. In the rat, MEL administration in the intracerebroventricular region inhibited the suckling-induced OT release when given at a dose regarded to be in the physiological range, while it was without effect with high doses (Juszczak & Stempniak, 1997). Indeed, plasma MEL concentrations determined around the time of milking were considered to be a pharmacological levels (chapter 3). Also, MEL treatment by means of feeding with a similar dose increased plasma MEL levels which reached high values 30 minutes after feeding, and this was significantly higher than that found during the dark period (Kennaway *et al.*, 1982). Therefore, this suggests that MEL effect on OT release is dependent on its concentration. The putative

multiple MEL pathways, diurnal variation in MEL binding sites, together with species related differences in pathway of OT synthesis and release and a possible interaction between MEL and this pathway all make it difficult to analysis and interpret this relationship.

The tendency for a decline in the proportion of residual milk during treatment in the early stage of lactation treatment might suggest an improvement of milk removal caused by MEL. However, this is unlikely to be the case. Values during pretreatment were high in three goats which then declined in the next determination, and two of these goats were in their first lactation. This was not something unexpected for goats which had not been accustomed to milking procedure, particularly at this very early stage of lactation. The release of OT elicited by milking in the goats is variable, not only among animals but also among individual milking episodes within the same animal (McNeilly, 1972)

It can be concluded that MEL has no effect on milk ejection reflex as assessed by volume of residual milk. Although no attempts were made to measure the levels of plasma OT released at milking, a possible partial suppression of OT cannot be excluded, however, the overall outcome did not support a possible effect of MEL on the efficiency of completeness of emptying of the udder at normal milking.

CHAPTER FIVE

METABOLIC CAPACITY IN COWS DIFFERING IN GENETIC MERIT

5.1 INTRODUCTION

The strategy that has been adopted to improve the efficiency of milk production in dairy cattle is to increase milk production per cow which can be achieved by genetic selection. Milk yield is a phenotypic trait that can be inherited and has been widely used in breeding programs to produce cows with high milk production capability. Therefore, many years of genetic selection has resulted in a dramatic increase in milk yield per cow. Several studies have investigated the factors that are closely related to milk secretion which might have been altered in response to genetic selection for high milk yield. It has been indicated that selection for high milk yield did not result in changes in digestion, maintenance requirement per unit metabolic live weight or efficiency in milk synthesis (Bauman *et al.*, 1985b). The ability of the high yielding dairy cows to increase their level of milk production has arisen mainly from their nutrients partitioning between milk synthesis and the other biological functions of the animal. They preferentially partition more nutrients towards the mammary gland and away from body tissues to satisfy the high metabolic demand of the udder. In early lactation, the slow increase in feed intake lags behind the peak in milk production in superior cows, which put the cows in a negative energy balance situation. Therefore, part of the milk energy is derived from the body stores to meet the energy deficit and it has been estimated that during the first four weeks of lactation, the mobilization of body reserves were energically equivalent to about one third of total milk produced (Bauman & Currie, 1980). Thus, high merit cows are also characterized by rapid and great ability of utilizing their body stores to increase the concentrations of milk constituents precursors.

This physiological adaptation is coordinated by the endocrine status and the hormones that have a major regulatory role in metabolism are GH and INS. The ratio of GH to INS constitutes a key element in determining the milk production efficiency in lactating cows (Herbein *et al.*, 1985). The high levels of GH and lower values of INS which are typical characteristics of high yielding dairy cows especially in early lactation facilitate the availability of energy-yielding metabolites by increasing the rate of lipolysis, inhibiting lipogenesis and increasing gluconeogenesis in the liver. Thus, in the high genetic line, nutrients from the diet are directed to milk synthesis at the expense of body stores, whereas more of the diet's nutrients are directed toward body stores in the low yielding cows. Another factor which has been suggested to be influenced by genetic selection is feed intake, in which the high merit cows consume more feed than the low merit ones (Bauman *et al.*, 1985b). Another factor which has not received much attention is the extent of mammary gland development and the total number of secretory cells and possibly the secretory activity of the epithelial tissue, which are of great importance in determining the milking capacity of the udder.

Milk secretion is regulated systemically by galactopoietic hormones and locally within the mammary gland by feed back inhibitor of lactation. Lactating cows particularly at peak lactation are at high metabolic pressure imposed on them because of the preferential flow of great amount of nutrients towards mammary gland at the expense of other body tissues. In fact, this might result in some compromise in other body functions like the immune system or increase the risk of metabolic disturbances. Whether the high yielding dairy cows are operating closer to their maximum and hence are more at such risk of metabolic disturbances than low yielding cows has not been determined. The response to a combination of endocrinological manipulation and frequent milking to estimate the maximum capacity of dairy cows differing in genetic merit is an important means in

evaluating the level of risk that well managed high yielding dairy cows are exposed to.

The objectives of this experiments were: 1) To define and compare the maximum metabolic capabilities of genetically superior and average dairy cows. 2) To establish if this metabolic capacity is determined at the mammary gland level or elsewhere in the body.

5.2 MATERIALS AND METHODS

5.2.1 Cows

Twenty-four primi-and multiparous lactating Holstein-Friesian dairy cows of different genetic merit were used in this experiment. The cows were from the Blythbank and Langhill herds and all were the progeny of at least three generations of selection using semen from 100% Holstein bulls. Twelve cows were of a high genetic merit, defined as being within the top 5% of UK national herd (H), and twelve cows of a lower genetic merit which were comparable to the UK average (L). Calving dates ranged from September 9th 1995 to January 14th 1996.

After calving, the cows were housed in a cubicle yard and fed a total mixed ration composed of silage and a complete mix (21% crude protein (cp) and 13 MJ/kg DM) *ad libitum*. In the parlour, the 12 cows assigned to treatment groups were fed 8 kg of a 22% cp concentrate, while the untreated groups received 5 kg of the same concentrate, and the feed ration was divided into 4 meals each day.

5.2.2 Experimental design

The experimental cows were in a 2 X 2 factorial design (treatment X genetic merit). Commencing in lactation week 6, 6 H and 6 L cows were allocated to treated groups (HT, LT) and the other 6 cows from each genetic line remained untreated control groups (HC, LC). Within genetic lines, the distribution between control and treatment groups took into

account age (parity), source, calving date, milk yield, body weight and body condition score. The two weeks preceding this were used as pretreatment, period 1. After that, HT and LT were treated on consecutive two-week periods while HC and LC remained on the management just described.

The treated groups received the treatment in an additive stepwise fashion as follows. Period 2, in which the frequency of milking was increased to four-times (4X) daily milking at intervals of approximately 6h. Period 3, a further milk secretion stimulus of recombinant bovine growth hormone in a delayed release vehicle (500 mg bST, Posilac, Monsanto, St Louis, USA) was injected subcutaneously at the beginning of this period. The combination of 4X and bST treatment for another 2 weeks was regarded as period 4. In period 5, 50mg of thyroxine (Sigma, Poole UK) in 15% polyvinylpyrollidone delay vehicle applied as a subcutaneous injection every second day for 2 weeks. In period 6, while the galactopoietic stimuli (4X+bST+T4) were continued, half of the udder (two diagonally-opposed mammary glands) was maintained at four times milking (4X) but milking frequency was reduced to once a day (1X) in the other half for two weeks; in order to determine if the 4X half increases its rate of milk secretion as a result of reduced milk secretion caused by reduced milking frequency in the 1X half.

Following period 6, treatments were stopped and cows in treatment groups were returned to twice (2X) daily milking, and final observations (period 7, recovery period) were made on all cows during a two week period which commenced two weeks after treatment termination, or equivalent stage of lactation in controls.

The treatment periods can be summarized as follows:

Period 1. 2 weeks pre-treatment

Period 2. 2 weeks 4X milking

Period 3. First 2 weeks bST plus 4X milking

Period 4. Second 2 weeks bST plus 4X milking

Period 5. 2 weeks T4 plus bST plus 4X milking

Period 6. 3 weeks 4X/1X milking plus bST plus T4

Period 7. 2 weeks recovery (starting 2 weeks after last treatment)

During the third week of 4X/1X milking, a mammary biopsy was performed for the treated cows and this resulted in decline in milk yield, so the data collected in this week were not included in the statistical analysis. One of the cows from HT group died from acute peritonitis shortly before the recovery period, so HT group ended up with only 5 cows in period 7.

5.2.3 Measurements

In order to determine the effects of treatments on the function and the characteristics of the mammary gland, and to assess the concentrations of circulatory hormones and metabolites, the following measurements were performed.

5.2.3.1 Milk yield

The effect of genetic line and treatments with a possible interaction between the two factors on milk yields were determined by measuring the half udder yields at every milking to a precision of 100g.

5.2.3.2 Body weight and body condition score

Body weight and body condition score were measured weekly. Heart rate was also determined weekly by palpation.

Body condition score, to determine the change in the fat mobilization, was assessed

by a scoring system which was developed by the East of Scotland College of Agriculture (1976). It is based on measurements of the loin area between the hip bone and the last rib as well as around the tail head which are considered important areas in determining the mobilization of the fat tissue. The body condition score was determined by using a 1 to 5 scale, where 1 is extremely thin cow and 5 excessively fat cow. The assessment was done by two assessors throughout the experimental periods.

5.2.3.3 Udder characteristics

Determination of the udder volume

To determine the effect of genetic merit on the size of the udder and also to determine if treatments have produced any effect on mammary growth, mammary gland size was determined non-invasively by polyurethane foam cast as described by Dewhurst *et al*, (1993). The udder was completely emptied by 20 units of oxytocin injection (Oxytocin-S; Intervet, Cambridge, UK) which was followed by milking out the gland immediately before performing the casting. Cows were introduced into a crush where they were mildly sedated with an injection of 0.25ml Rompun (Bayer, Ltd. UK) and their hind legs held apart by tightening them to the crush wall. Udder was clipped and an udder cream (Coopers Pitman Moore, UK) was applied to ease the release of the cast. Polyurethane foam (Froth-Pak^{R}, Foampax Scotland, Newmilns, UK) was applied to the udder and the cast was removed after hardening. The total volume of the udder was obtained by filling the cast with material of known density and weighing. The gross udder volume was determined for all cows at three times, during pre-treatment (period 1), maximum stimulation (period 5) and finally in the recovery period (period 7).

Measurements of cisternal and

alveolar storage capacity

The alveolar and cisternal milk volumes were determined by catheter drainage based on the method described by Knight *et al*, (1994). On the day of determination, cows were moved to an unfamiliar area and far away from the parlour shortly after morning milking, to avoid milk ejection which might be stimulated by hearing the milking machine. The cows were kept tied in stalls until drained. The drainage was started about 8 hours after the last milking. Teats were cleaned with 70% alcohol-2% hibitane solution and sprayed with a local anaesthetitic (Xylocaine spray, Astra Pharmaceutical, Kings Langley, UK). After that, a sterile plastic catheter (14g Medicut Catheter, Sherwood Medical Industries, Tullamore, Ireland) was introduced via the teat canal of the two front glands, allowing the milk to flow freely from the gland, and the milk was collected until flow of milk ceased which was considered to be the cisternal milk. Immediately after that, the alveolar milk volume was collected by further drainage of the gland with the administration of oxytocin (20 units i.v.). Both milk fractions were weighed to determine the milk volumes of cisternal and alveolar portions. The assessment was performed in the pretreatment period (period 1), during the maximum stimulation (period 5) and then in the recovery period (period 7).

5.2.3.4 Blood collection and hormonal analysis

Blood samples were collected three times weekly immediately after the mid-day milking (for the treated cows, but for the control cows, they were put into the parlour and fed the concentrate without being milked). This sampling regime was maintained throughout the experiment. Additional frequent samples were taken. At the time of frequent sampling, the cows were moved to individual stalls immediately after the morning milking and bled every hour, from 9.00h to 19.00h. For the time around milking, the blood samples were

collected every 5 minutes, with a total of 7 samples from each cow. The cows were first moved to the parlour and bled twice before milking, then another sample was collected about 2 minutes after the application of the milking machine, which was then followed by 4 further samples started 5 minutes immediately after milking finished when the cows were moved back to their stalls. Processing of samples and determination of plasma hormones were described in chapter 2. The thrice weekly samples were used for determination of PRL, GH, IGF-I and INS, while the hourly as well as around milking samples were assayed for PRL. The frequent sampling was performed before treatment (period 1), then it was repeated during the maximum stimulation (period 5) and then during the recovery period (period 7).

5.2.3.5 Determination of blood metabolites

Plasma glucose was determined by glucose oxidase and peroxidase (Bergmeyer & Besnt, 1974) and free fatty acids (Wharton, 1974). β-hydroxybutyrate by colorimetric method (Williamson & Mellanby, 1974) and glycerol by alkaline hydrolysis (Eggstein & Kuhlmann, 1974).

5.2.3.6 Statistical analysis

To compare the pre-treatment differences between genetic lines, analysis of variance (ANOVA) was used. Effects of treatment and a possible interaction between line and treatment were tested on period mean values (milk yield, body weight, body condition score and heart rate) by analysis of covariance (ANCOVA) using pre-treatment means (period 1) as a covariate to adjust for the pre-treatment values. To analyse cumulative effects between period means, differences between consecutive periods were analysed by ANCOVA using pre-treatment values as a covariate. Paired and unpaired *t* tests were used to compare individual mean values or between experimental periods.

Unilateral effects of a change in milking frequency in the test gland (1X) were detected by calculation of a relative milk yield quotient (RMYQ) as described by Linzell and Peaker, (1971); RMYQ: (t2 x c1)/(t1x c2) where c is the yield of the control gland (4X) and t is the yield of the test gland (1X), 1 is the period before treatment and 2 is the period of treatment. Values more than or less than unity indicate a positive or negative change, respectively, evaluated statistically by paired *t* test. Minitab program was used in performing the above statistical analysis.

Due to the distribution of cows into five groups based on their calving dates (from September to January), which might have introduced a seasonal factor that could have affected the release of some hormones, notably PRL, hormonal data were analysed by Genestat (Genestat 5: Lewes Agricultural Trust, 1993), using Residual Maximum Likelihood (REML). This procedure was designed for un-balanced designs, to adjust for any differences that might have been related to group effect (month of sampling). The model consists of two parts, fixed effects which include the effects of line + treatment + their interaction, and a random effect which includes group effect.

5.3 RESULTS

5.3.1 Milk yield

During pre-treatment period, milk yield was significantly higher in the cows of the high genetic merit (29.25±2.0 and 37.04±2.2 kg/d for low and high genetic merit groups respectively, P=0.016, *t* test), but there was no significant difference between the two groups within each line (P>0.05).

Milk yields of the control as well as treated cows from the two genetic lines during experimental periods are presented in Figures 5.1 and 5.2, and Table 5.1. In HC cows, milk yield gradually declined throughout the experiment, whereas the decline in LC cows did not commence until period 3. In contrast to the controls cows, the treated cows yields increased from period 2 to period 5 and were significantly higher than those of the control cows (Figure 5.2). Period mean milk yields were analysed by covariance analysis (ANCOVA), using the pretreatment period as covariate to correct for pre-existing genetic line differences. This indicated that application of treatment stimuli significantly increased milk yields in both treated groups (Table 5.1). In all treatment periods, with the exception of periods 6, 4X/1X milking, and recovery period, a significant positive treatment effect was evident.

Increasing milking frequency to 4 times a day significantly increased milk production (P=0.01) in HT and LT groups (Table 5.1). There was a significant line*treatment (L*T) interaction (P=0.02), suggesting a better response to 4X in HT cows, and this was also supported by significant difference between the two lines in the absolute increase analysed by *t* test (0.78±0.51, and 2.90±0.78kg/d; P=0.05, for LT and HT, respectively).

Treatment with bST significantly increased milk secretion ($P<0.01$) in both treated lines (Table 5.1). The milk yield data in Figure 5.2 gives the impression of higher response in the low line group, however, this was not confirmed statistically; there was no significant interaction between line and bST treatment ($P>0.05$, ANCOVA). Thyroxine further increased milk yield ($P<0.001$, ANCOVA, Table 5.1) without any significant differences in milk yield response between the two treated groups.

During the 1X/4X period the yield declined in the treated groups to an extent that abolished the difference between treatment and control cows ($P>0.05$, ANCOVA). Comparison between the control and treated groups indicated significantly lower milk yields in the treated cows ($P<0.001$, ANCOVA) two weeks after the treatments had ceased, i.e. in the recovery period (Table 5.1).

To determine the cumulative effects of treatments on milk yield, differences between consecutive period means were analysed, again using pre-treatment mean as a covariate (ANCOVA; Table 5.2). When the yields of the treated cows during the 4X period were compared to that of pre-treatment they were significantly higher ($P=0.04$), but the response in HT cows was greater than LT cows as indicated by a significant L*T interaction ($P=0.02$). Following the first bST injection, although there was a tendency for a small response in the low line group neither HT nor LT increased their yield above 4X values ($P>0.05$). However, in the second period of bST a significant additive effect was observed ($P<0.05$) without any significant differences in the response related to genetic line ($P>0.05$). Unlike bST, T4 produced an immediate and rapid highly significant further increase in yield (Table 5.2) which started from the first week of treatment and was maintained in the second week (data not shown). Reducing milking frequency in half of the udder to 1X daily produced a significant inhibitory effect on total milk yield ($P<0.001$, ANCOVA) without any difference between the two lines. Yield declined by about 25.5%

in both lines within the two-week period, and declined further in both groups by almost 40% three weeks after the termination of treatments. In the control cows, yield gradually declined during the experiment as indicated by negative mean difference values in Table 5.2.

Increases in yield for LT and HT during each of the stimulatory period (4X, bST and T4), expressed relative to pre-treatment yield are in Table 5.3. As indicated earlier, the absolute increase in response to 4X was greater in HT compared to LT cows, however, this was reversed with the other stimuli; the greater absolute increase as well as percent of pretreatment was in LT cows but these were not statistically different ($P>0.05$, *t* test).

Half udder milk yields for the two treatment groups during T4, 1X/4X and recovery periods are shown in Figure 5.3. During period 6, half of the udder was milked once-daily (1X) whilst half continued on four-times daily milking (4X). This clearly produced a marked depression in milk yield for the 1X milked udder-half but there was also a slight decrease in milk yield for the 4X milked half. Changes in 1X relative to 4X were analysed using RMYQ. In both control groups values were close to unity, but they were significantly less than unity in the treated groups (Table 5.4). This was due entirely to reduced yield in the 1X half since paired *t* test analysis revealed no significant increase in milk yield for the half-udder which continued on 4X milking (overall mean decrease 0.25 ± 0.32 kg/d, $P>0.05$). In the second week of 4X/1X milking no further significant decrease in milk yield in the 1X half was found (data not shown) without any difference in the response between LT and HT.

Milk yield dropped very markedly in LT and HT between 4X/1X milking period and the recovery period, when milking was twice daily and no treatments were administered. There was a significant negative difference between treated and control cows during this period, in complete contrast to the earlier positive differences (Figures 5.2 and 5.3, and

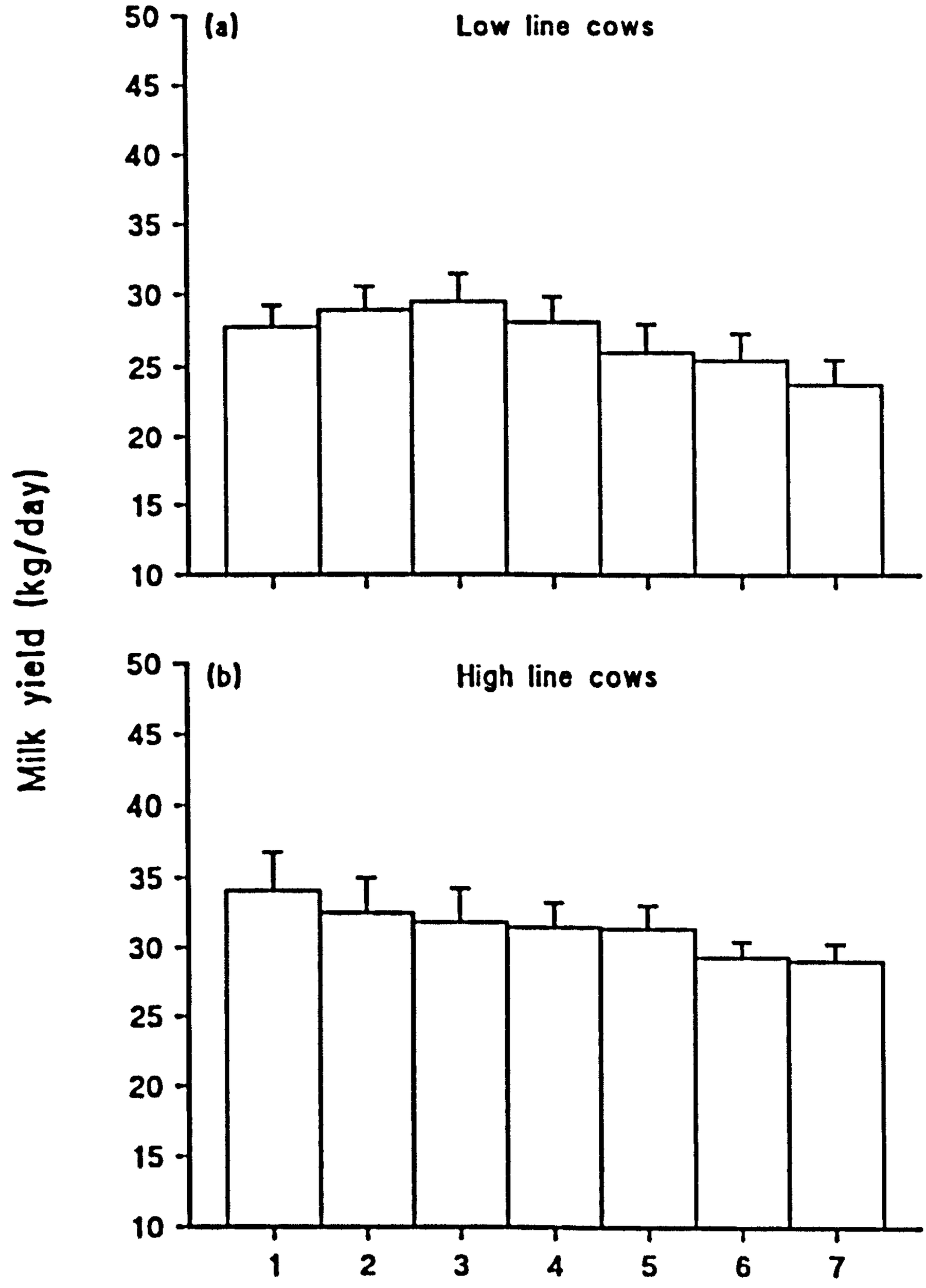

Figure 5.1 Mean milk yields (kg/d) of (a) low genetic merit and (b) high genetic cows maintained at standard management level in early lactation. Values are means of two week periods which commenced at week 5 of lactation (period 1). Vertical bars represent S.E.M.

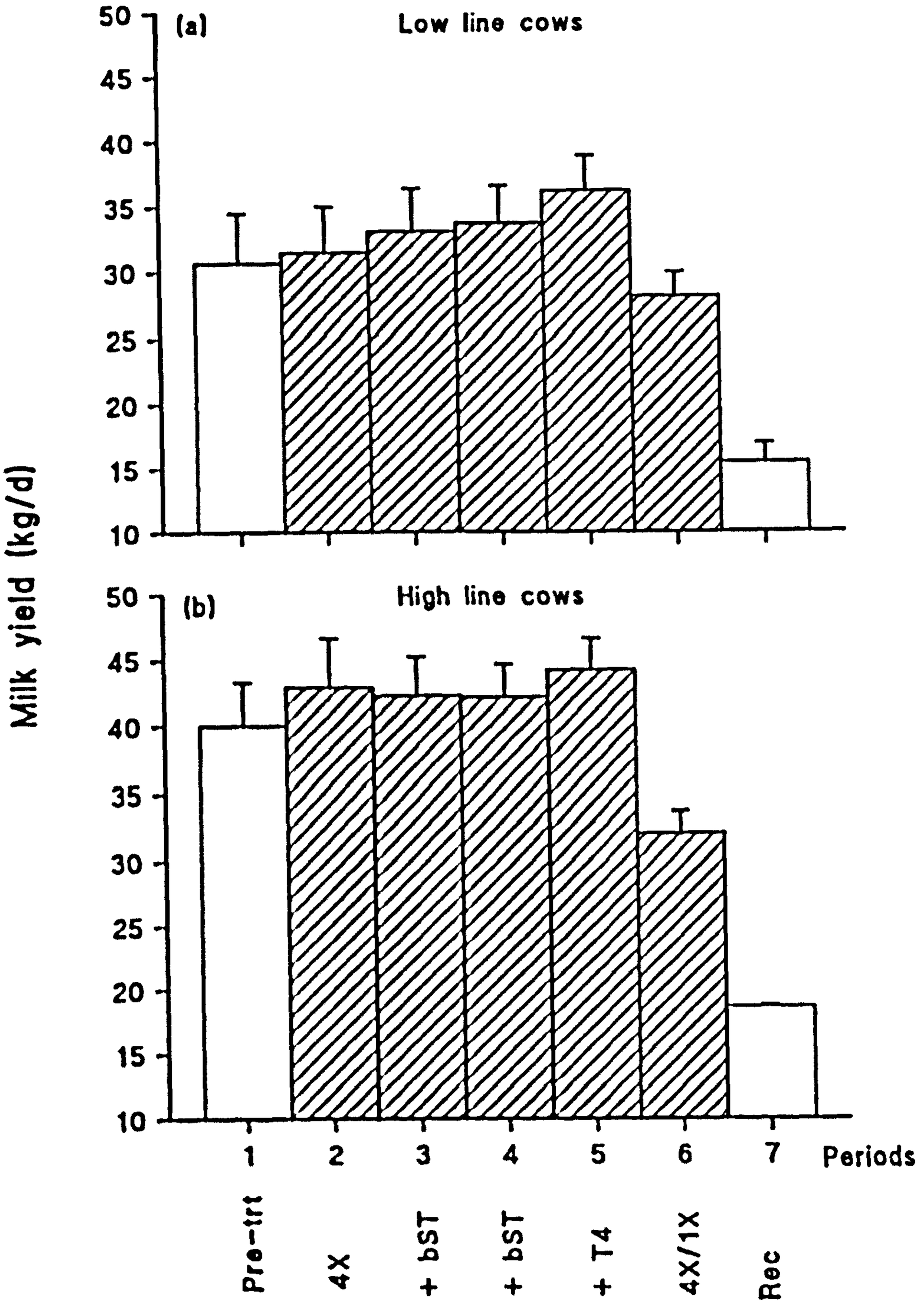

Figure 5.2 Mean milk yields (kg/d) of cows from (a) low genetic merit and (b) high genetic merit treated at peak lactation with galactopoietic stimuli in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.1. Mean milk yields (kg/d) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean (period 1) as covariate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), ANCOVA. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Treatment	Line	T * L
2 (4X)	33.5	31.6	34.2	36.6	1.48	P=0.04	n.s	P=0.02
3 (bST)	33.7	30.7	35.6	36.5	1.67	P<0.01	n.s.	n.s.
4 (bST)	31.4	30.9	35.6	37.6	1.60	P<0.001	n.s.	n.s.
5 (T4)	29.2	30.9	38.1	39.7	1.41	P<0.001	n.s.	n.s.
6 (4X/1X)	27.6	28.6	29.3	29	1.48	n.s.	n.s.	n.s.
7 (Rec)	24.8	28.8	16.1	18.0*	1.99	P<0.001	P=0.04	n.s.

Table 5.2. Milk yield differences between consecutive periods (kg/d) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Values are means which are adjusted for pretreatment period mean (period 1) as covariate. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashions follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), ANCOVA. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Treatment	Line	T x L
2 (4X)	0.56	-1.36	1.25	3.61	1.48	P=0.04	n.s.	P=0.02
3 (bST)	0.14	-0.87	1.36	-0.04	1.10	n.s.	n.s.	n.s.
4 (bST)	-2.28	0.21	0.08	1.05	1.13	P<0.05	n.s.	n.s.
5 (T4)	-2.15	-0.02	2.41	2.13	1.13	P<0.001	n.s.	n.s.
6 (4X/1X)	-1.61	-2.27	-8.75	-10.72	0.78	P<0.001	P<0.05	n.s.
7 (Rec)	-2.85	0.13	-13.25	-10.59*	1.61	P<0.001	P=0.02	n.s

Tables 5.2 and 5.3). Part of this difference was due to continued depression in the udder half milked once daily, despite both halves now being on twice daily milking (Figure 5.3, Table 5.4). RMYQ values continued to be significantly less than unity in the treated cows. Paired *t* test also revealed a significant (LT; $P < 0.01$, HT; $P < 0.001$) decrease in the half which had been milked 4X daily. Responses were very similar in LT and HT cows: no interaction between treatment and line was evident.

5.3.2 Body weight

Body weights of control and treated cows are illustrated in Figures 5.4, 5.5, and Table 5.5. Genetic lines showed no significant effect on the initial weights (for low and high line groups, 590.3 ± 12, 589.2 ± 15kg, $P > 0.05$, ANOVA) and there was no difference between groups within line ($P > 0.05$, ANOVA). While the body weights for HC were maintained without any major changes throughout the experimental periods, they gradually increased in the LC group; Paired *t* test analysis between pretreatment and recovery periods indicated a significant body weight gain in LC 45.75 ± 11.68kg, $P = 0.01$, but not in HC group, 17.5 ± 26.02kg, $P = 0.16$.

In the treated cows, 4X and bST did not affect body weights in either line (ANCOVA, using period 1 mean as covariate; Table 5.5). However, during the following periods, the body weights were rapidly depressed in a stepwise fashion, so T4 treatment triggered body weight loss ($P = 0.02$) which was further depressed in the 1X/4X treatment ($P < 0.01$, *t* test). By this time the cows had lost about 8% of their initial weights. The return to normal management practices after termination of treatments did not prevent the loss in body weight, and it was estimated that the cows lost an average of 15% of their pre-treatment body weights when measured during the last period of measurement (recovery). Once again there was no significant interaction between line and any treatment, so treated

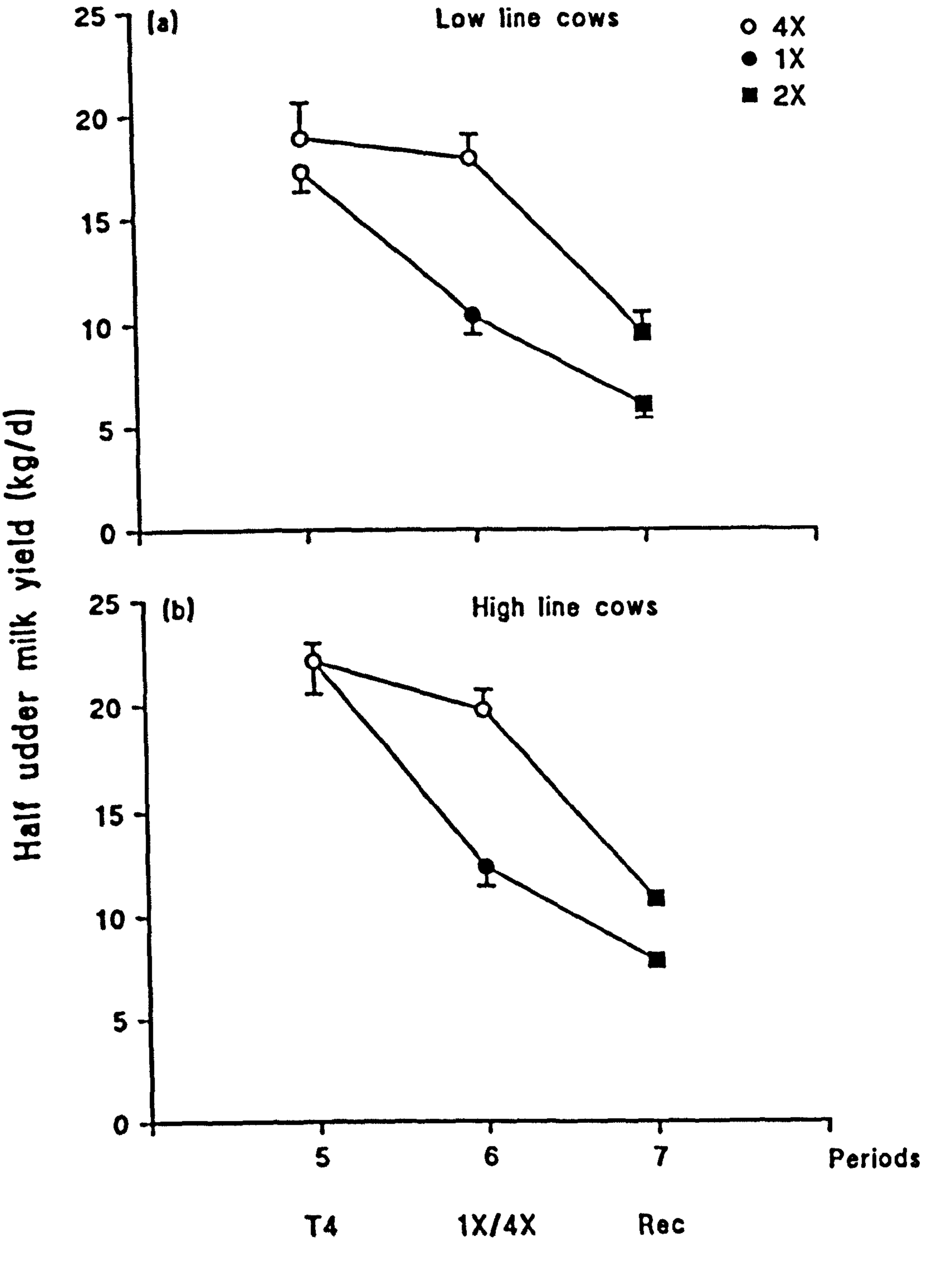

Figure 5.3 Half udder milk yields (kg/d) of cows from (a) low genetic merit and (b) high genetic merit. Values are means for 2 wk periods of maximum stimuli, milking four times daily + injection of slow-release formulation of 500mg of bovine somatotropin every 14d + injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder four times and the other half one time daily (4X/1X), and 3 wk after the cessation of all treatments (Rec). Vertical bars represent S.E.M.

Table 5.3. Increase in milk yield of treated cows from low (LT) and high (HT) genetic merit cows during stimulatory periods. Treatment which started at peak lactation (week 5 of lactation) in stepwise fashion, milking four times daily (4X), injection of 500mg of slow-release formulation of bovine somatotropin treatment every 14d (bST), injection with 50mg of thyroxine on alternate days (T4). Values are mean ± S.E.M. n=6. bST is second bST period.

	LT	HT
Absolute increase (kg/d)		
4X	0.79 ± 0.51	2.90 ± 0.78
bST	3.02 ± 1.07	2.11 ± 1.72
T4	5.47 ± 1.41	4.15 ± 1.19
Yield, percent of pretreatment		
4X	103.6 ± 1.96	107.2 ± 1.98
bST	112.9 ± 5.03	106.5 ± 4.32
T4	122.2 ± 7.3	111.63 ± 3.74

Table 5.4. Relative milk yields quotient (RMYQ) values (mean±S.E.M) for transition to 4X/1X and between T4 and recovery period. n=6. Significance represents difference from unity, paired *t* test.

Start of 4X/1X		
LC	1.01±0.01	n.s.
HC	1.05±0.03	n.s.
LT	0.66±0.04	P<0.001
HT	0.64±0.02	P<0.001
Carry-over to recovery period		
LT	0.76±0.08	P<0.05
HT	0.75±0.07	P<0.01

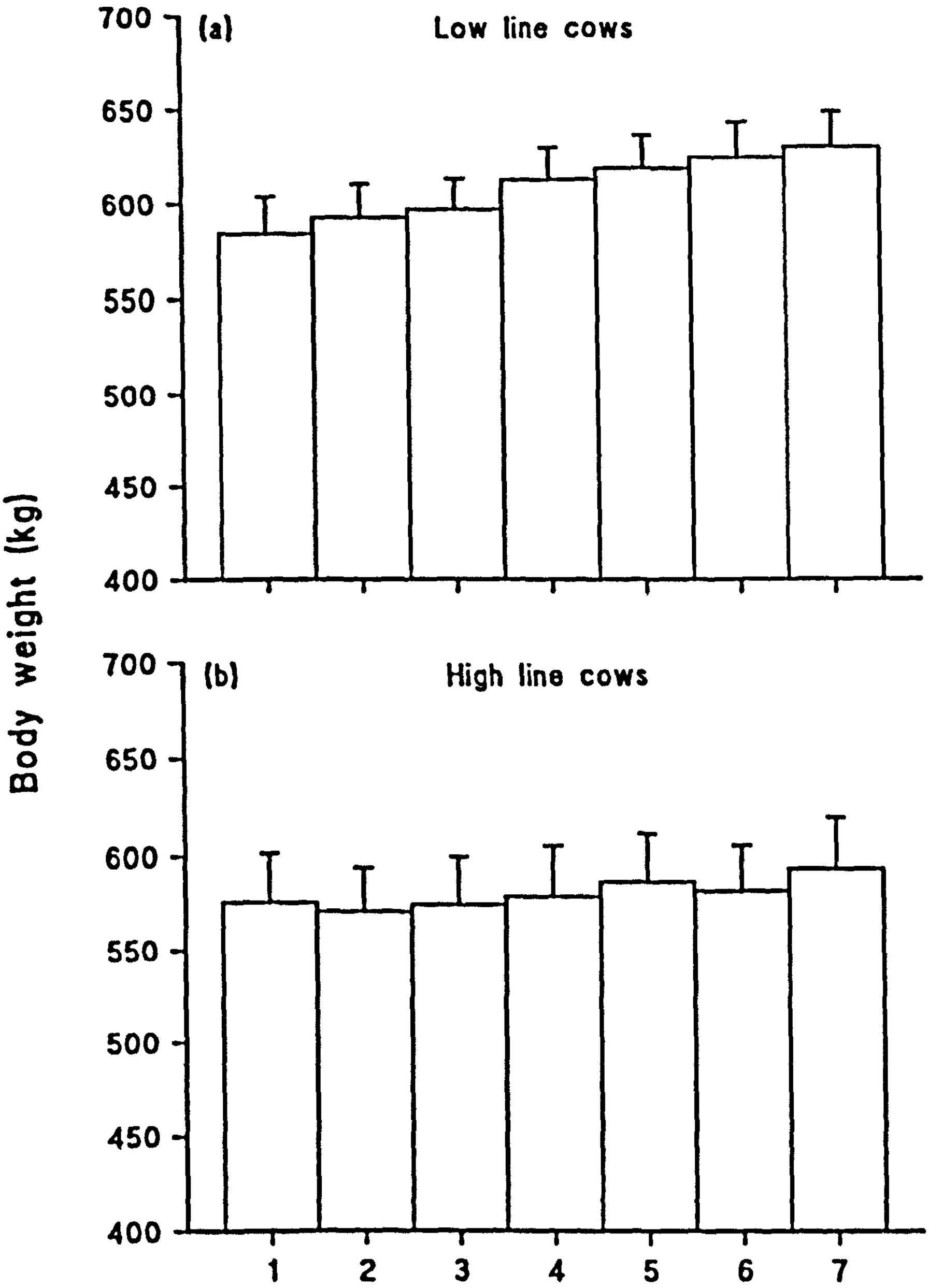

Figure 5.4 Changes in body weights of cows from (a) low genetic merit and (b) high genetic maintained at standard management level in early lactation. Values are means of two week periods which commenced at week 5 of lactation (period 1). Vertical bars represent S.E.M.

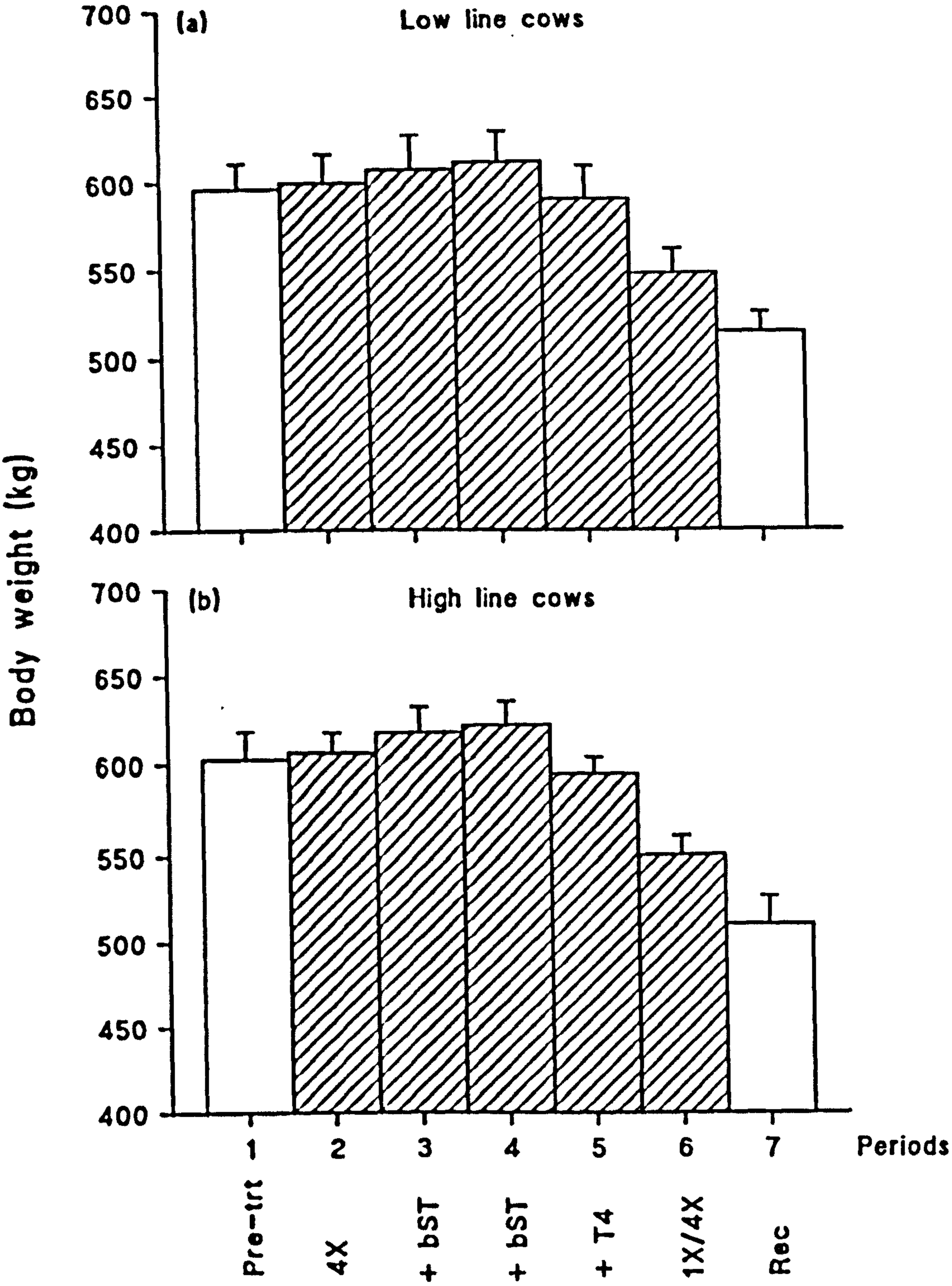

Figure 5.5 Changes in body weights of cows from (a) low genetic merit and (b) high genetic merit treated at peak lactation with galactopoietic stimuli in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.5. Period mean body weights (kg) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean (period 1) as covariate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), ANCOVA. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Treatment	Line	T * L
2 (4X)	597.3	583.2	594.2	595.5	9.3	n.s	n.s	n.s
3 (bST)	601.8	587.2	601.3	606.2	12.9	n.s	n.s	n.s
4 (bST)	617.4	591.4	605.8	610.8	12.7	n.s	n.s	n.s
5 (T4)	622.7	597.9	584.7	583.5	14.4	P=0.02	n.s	n.s
6 (4X/1X)	628.3	592.2	542.6	541.1	14.6	P<0.001	n.s	n.s
7 (Rec)	630.5	600.1	511.2	504.9*	17.7	P<0.001	n.s	n.s

cows responded in the same way to the different stimuli regardless of genetic background.

5.3.3 Body Condition Score

Comparison between the low and high line cows before the start of the experiment indicated a significantly higher body condition score value for the low line group (2.6±0.36 v. 1.8±0.21, P=0.02, ANOVA) but no difference between groups within line. Body condition scores for control and treated cows are shown in Figures 5.6, 5.7 and Table 5.6. There were a tendency for increase in body condition score during the course of experimental periods for both control groups, particularly at the last measurement period in LC cows. Like the response of body weight, body condition score was not affected by 4X or bST treatment (P>0.05, ANCOVA, using period 1 mean as covariate). However, T4 administration significantly reduced the body condition score values in both lines (P=0.01, ANCOVA) with no significant interaction between line and T4 treatment. This depressive effect was further continued during the next period (1X/4X) and also during the recovery period (P<0.001, ANCOVA). The body condition score of the treated cows was about one third of their control groups at the last measurement period (recovery) and both HT and LT showed a similar trend.

5.3.4 Heart rate

There was no significant pretreatment difference in heart rate of cows from different lines (90.4±3.9 and 87.5±2.25 beats/minute, P>0.05, ANOVA, for low and high line cows respectively) or between groups within line. Data averaged for treatment periods are shown in Figures 5.8, 5.9 and Table 5.7. Heart rate values did not manifest any major variations between periods in control groups. In the treated groups, heart rate was not affected by 4X and bST treatments (P>0.05, ANCOVA, using period 1 mean as covariate),

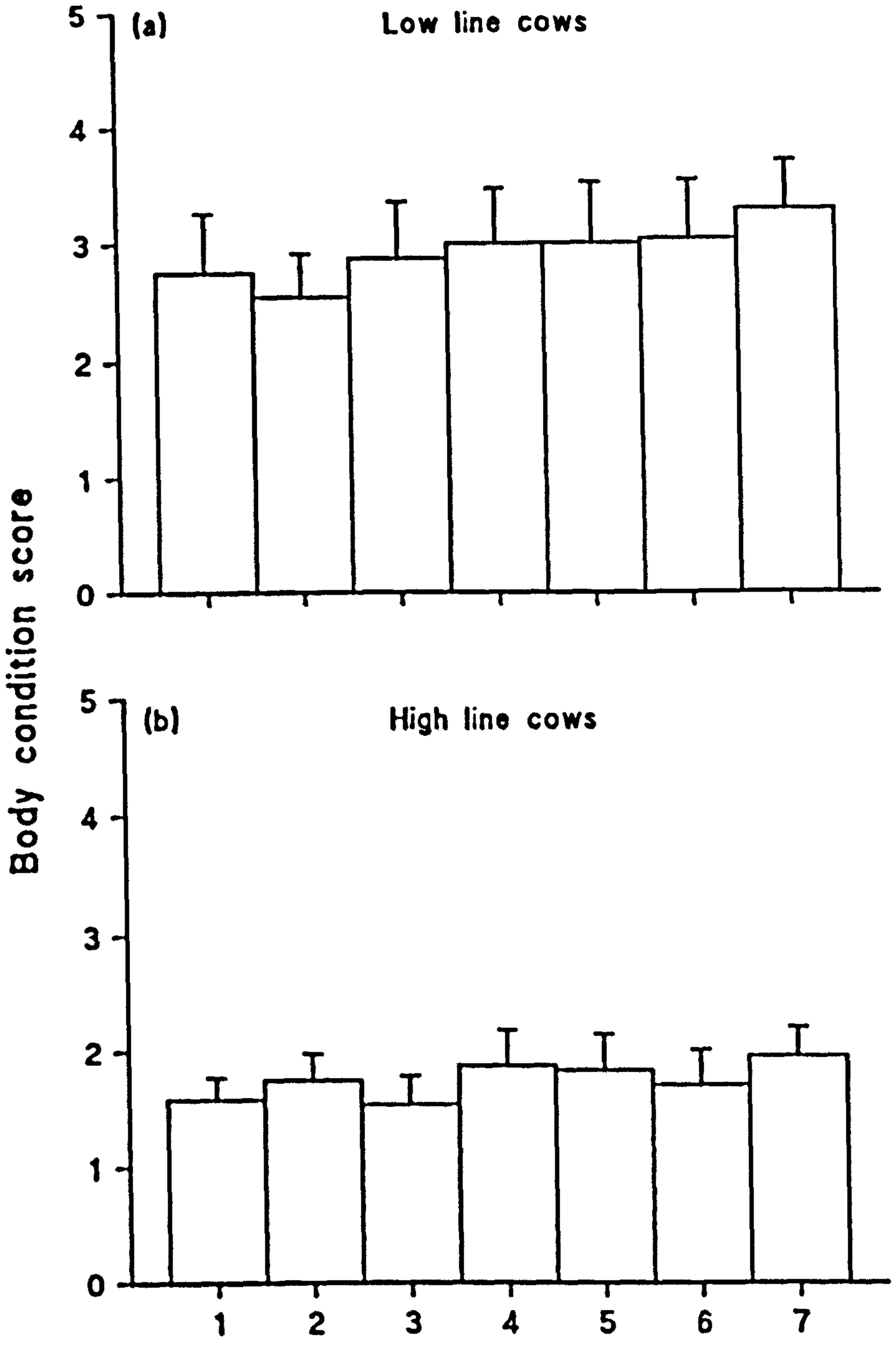

Figure 5.6 Changes in body condition scores of cows from (a) low genetic merit and (b) high genetic merit maintained at standard management level in early lactation. Values are means of two week periods which commenced at week 5 of lactation (period 1). Vertical bars represent S.E.M.

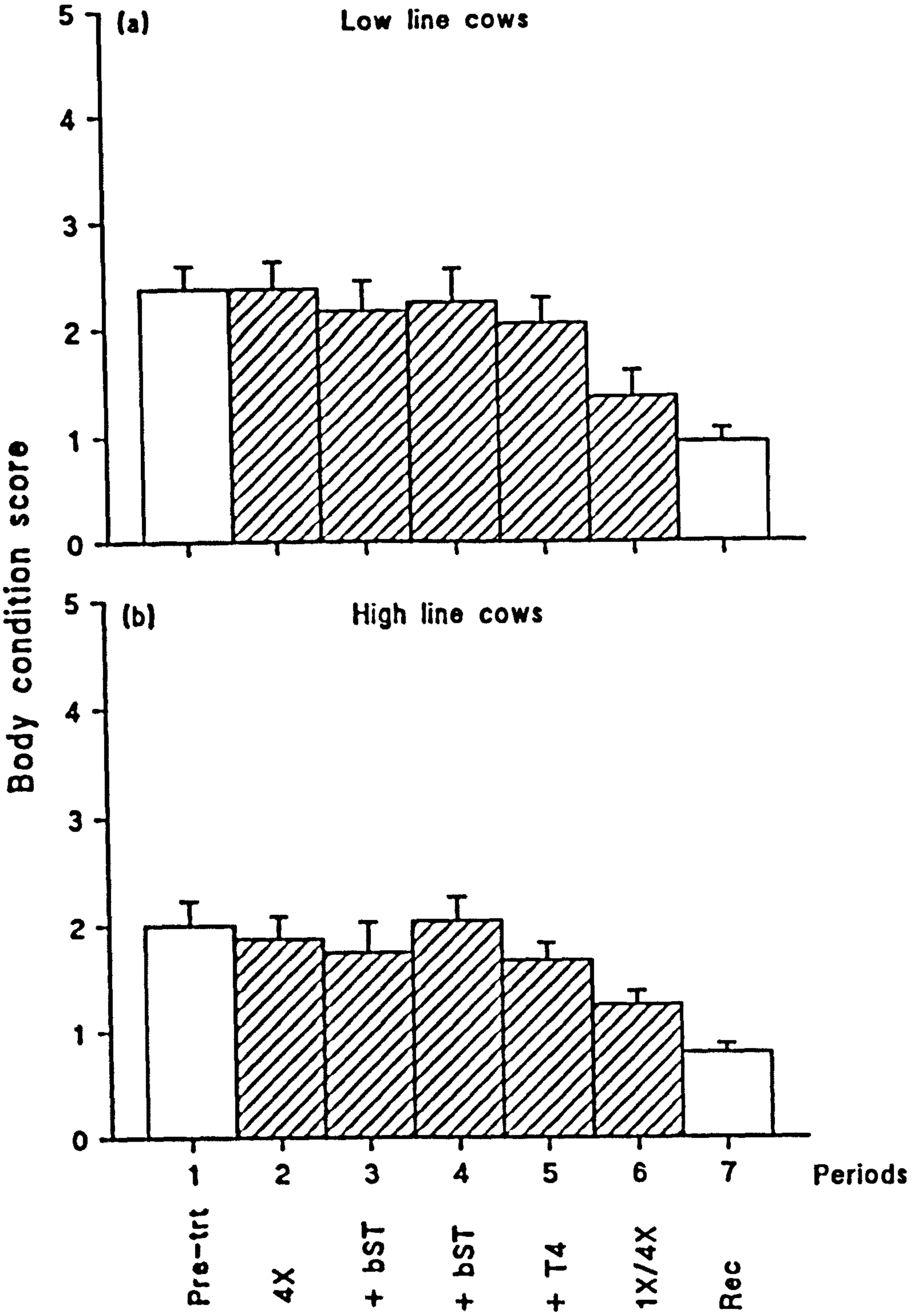

Figure 5.7 Changes in body condition scores of cows from (a) low genetic merit and (b) high genetic merit treated at peak lactation with galactopoietic stimuli in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.6. Period mean body condition scores for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean (period 1) as covariate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), ANCOVA. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Treatment	Line	T * L
2 (4X)	2.1	2.2	2.2	2.0	0.2	n.s	n.s	n.s
3 (bST)	2.3	2.1	2	1.9	0.2	n.s	n.s	n.s
4 (bST)	2.5	2.4	2.1	2.2	0.3	n.s	n.s	n.s
5 (T4)	2.5	2.3	1.9	1.8	0.3	P=0.01	n.s	n.s
6 (4X/1X)	2.6	2.1	1.2	1.4	0.4	P<0.001	n.s	n.s
7 (Rec)	2.9	2.3	0.8	0.9*	0.2	P<0.001	n.s	n.s

however it was increased considerably during T4 treatment to a value 35% higher than the preceding period (P<0.001, ANCOVA, Table 5.7). This high value was maintained during 1X/4X period with a tendency for a higher heart rate in the LT group, indicated by a significant line X treatment interaction (P<0.03, ANCOVA). Also, *t* test analysis confirmed the significantly higher heart rate in LT group (P=0.03). Despite falling back towards control values, heart rate during the recovery period remained significantly elevated in both lines (P<0.05, Table 5.7).

5.3.5 Udder characteristics

Gross udder volume

Pre-treatment udder volume measurement revealed a significantly higher udder volume in the high line group (18.1±1.8 v. 13.6±1.7 l for the high and low line cows respectively, P=0.02, ANOVA) without any significant difference between groups within line (P>0.05). Udder volume for control and treated cows during the three measurement periods are illustrated in Figure 5.10. Control cows maintained udder size throughout experimental periods, however, LC tended to show a small increase but HC showed a small decrease from pre-treatment to recovery period. To assess treatment effect on udder volume, the differences between each of two consecutive periods were calculated and tested for significance by ANOVA, which are presented in Table 5.8.

The cumulative effects of treatment combination culminated in a significant mammary gland growth in both treated groups (P=0.02, ANOVA), without any difference between the two treated groups (no significant line*treatment interaction, P>0.05, ANOVA). However, when the increase in udder volumes were expressed as a percentage of that measured during pre-treatment period, LT cows showed a non-significant tendency for higher response (120.14%±5.25, 113.27%±4.40, for LT, HT respectively; P>0.05,

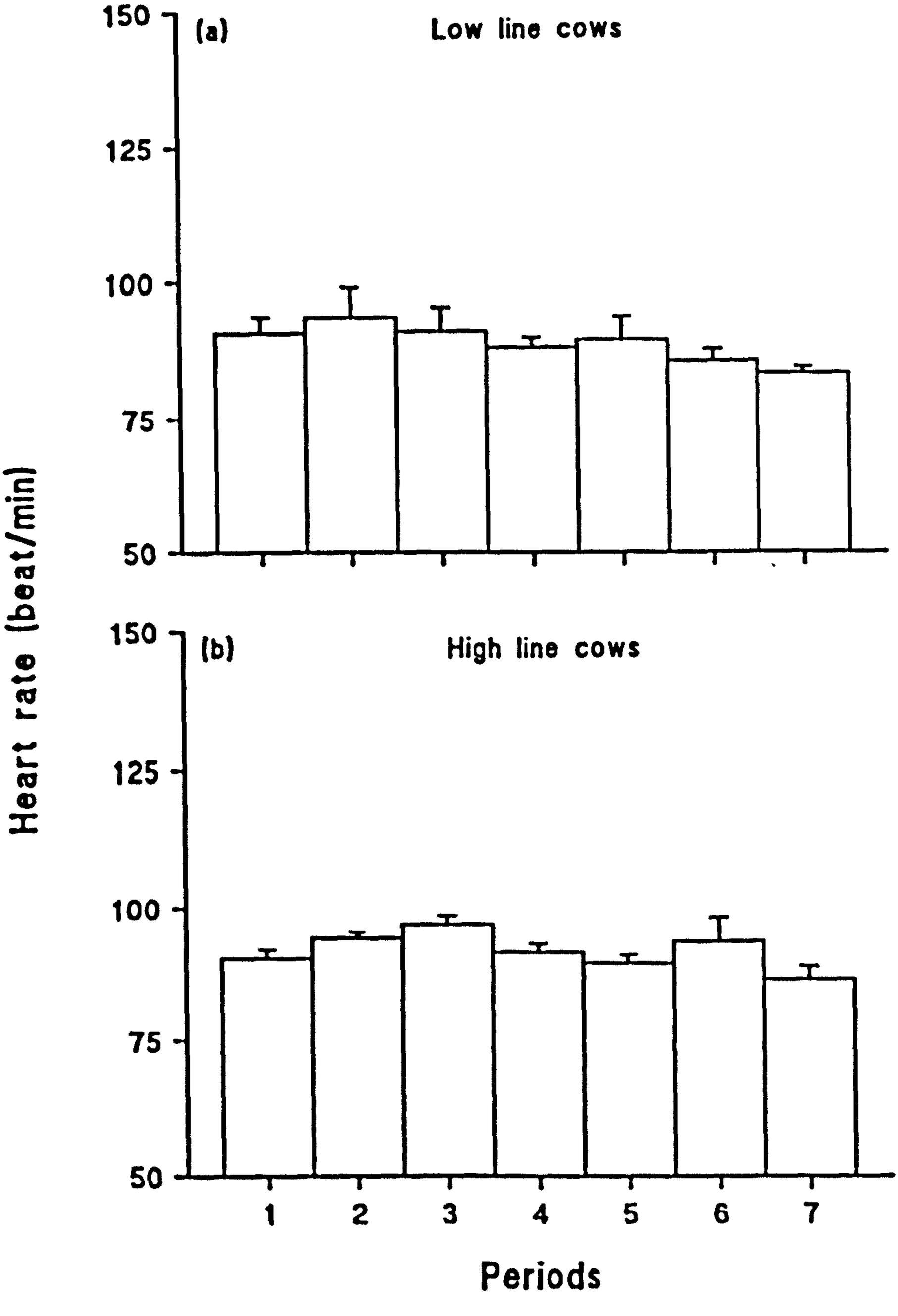

Figure 5.8 Changes in heart rates (beat/minute) of cows from (a) low genetic merit and (b) high genetic maintained at standard management level in early lactation. Values are means of two week periods which commenced at week 5 of lactation (period 1). Vertical bars represent S.E.M.

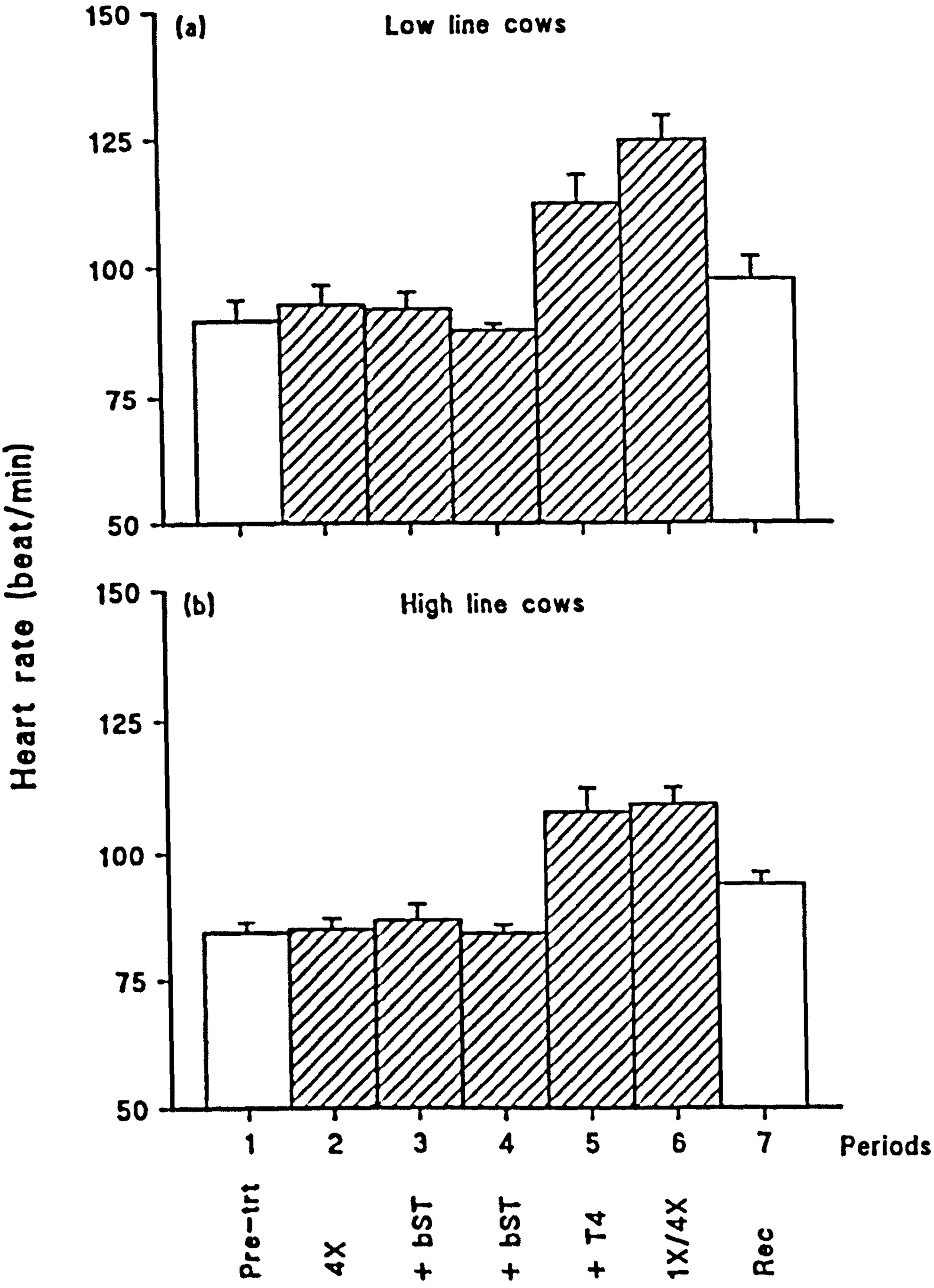

Figure 5.9 Changes in heart rate (beat/minute) of cows from (a) low genetic merit and (b) high genetic merit treated at peak lactation with galactopoietic stimuli in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.7. Period mean heart rate (beats/min) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean as covariate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), ANCOVA. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Treatment	Line	T * L
2 (4X)	92.1	93.2	92	89.5	4.4	n.s.	n.s.	n.s.
3 (bST)	89.5	95.8	91.1	91.5	3.5	n.s.	n.s.	n.s.
4 (bST)	87.5	91.7	88.2	84.4	1.9	n.s.	n.s.	n.s.
5 (T4)	89	90.4	118	114.2	5.8	P<0.001	n.s.	n.s.
6 (4X/1X)	84.8	92.8	123.8	109.2	5.7	P<0.001	n.s.	P<0.03
7 (Rec)	83.5	86.2	100.3	97.8*	3.1	P<0.001	n.s.	n.s.

ANOVA). This increase in udder volume was not maintained during the recovery period; treated cows ended up with udder volumes that were not statistically different from their counterpart control cows. The decline in udder volume during the recovery period measurement for the treated cows was significant (P=0.01, ANOVA), which was confirmed by paired *t* test (P=0.005).

Sites of milk storage

Only the front quarters were drained in order to determine the storage sites of the udder, and analysis between the right and left front quarters did not result in a significant difference between them. Therefore, data from both quarters were pooled for each cow and used to assess genetic and treatment effects on milk storage sites.

Pre-treatment alveolar milk volume showed a tendency for higher values in the high line group (4743±480 v. 3581±422 ml/8h; P=0.07, ANOVA, for high and low line cows respectively). Neither the cisternal volume (for low and high line, 1068.9±191, 1371.8±251 ml/8h) nor the percentage of milk stored in the cistern (24.23% ±5.0., 23.53% ±5.0) during 8h period was affected by genetic selection (P>0.05, ANOVA). Alveolar and cisternal milk volumes for control and treated cows are illustrated in Figures 5.11 and 5.12. Over the experimental periods, control cows showed a gradual decline in alveolar milk volume, with a tendency for higher decline in LC cows. Differences between pre-treatment and maximum treatment in milk storage capacity are shown in Table 5.9.

At maximum treatment, treated cows exhibited a significant increase in alveolar milk volume (P=0.01, ANOVA), also analysing the change in alveolar volume between pre-treatment and T4 periods indicated a significant increase in response to treatment (P=0.05, ANOVA). In general all cows maintained their cisternal milk portion in the max period without any significant difference either between groups or across stage of lactation.

Percentages of cisternal milk volume for the control and treated cows are given in Figure 5.13. There was no significant difference between lines in cisternal portion ($P>0.05$, ANOVA). The maximum treatment period was maintained for all groups of cows without any treatment effect ($P>0.05$, ANOVA, Table 5.9).

Differences between maximum treatment and recovery periods in milk storage capacity are presented in Table 5.10. Alveolar portion was decreased in the recovery period for all cows and ANOVA indicated a significant reduction in the treated groups ($P=0.002$) and when compared to the previous measurement (max) for all cows, it was significantly reduced during the recovery period ($P<0.006$, paired *t* test) in all except the HC group, where the decline was small and not statistically significant ($P>0.05$). All cows showed a decline in cisternal milk volumes during the recovery period with a tendency for the rate of decline to be higher in the treated cows ($P>0.05$, ANOVA). Cisternal percentage during the recovery period was reduced in the control groups while it was maintained for treated groups, however no differences were found in response to treatment or between maximum and recovery period ($P>0.05$, ANOVA). Although the cisternal milk volume was reduced during the recovery period for treated cows, when expressed relative to the total yield (half udder yield) it showed the percentage of milk stored in the cisternal was maintained during this period. This was mostly related to the decrease in the alveolar volume as a result of the deleterious effect of treatments on the milk secretion in the last stages of treatment.

5.3.6 Hormonal profiles

Routine blood samples collected thrice weekly were assayed for PRL, GH, IGF-I, and INS, while the frequent samples (hourly and post-milking) were subjected to PRL determination only.

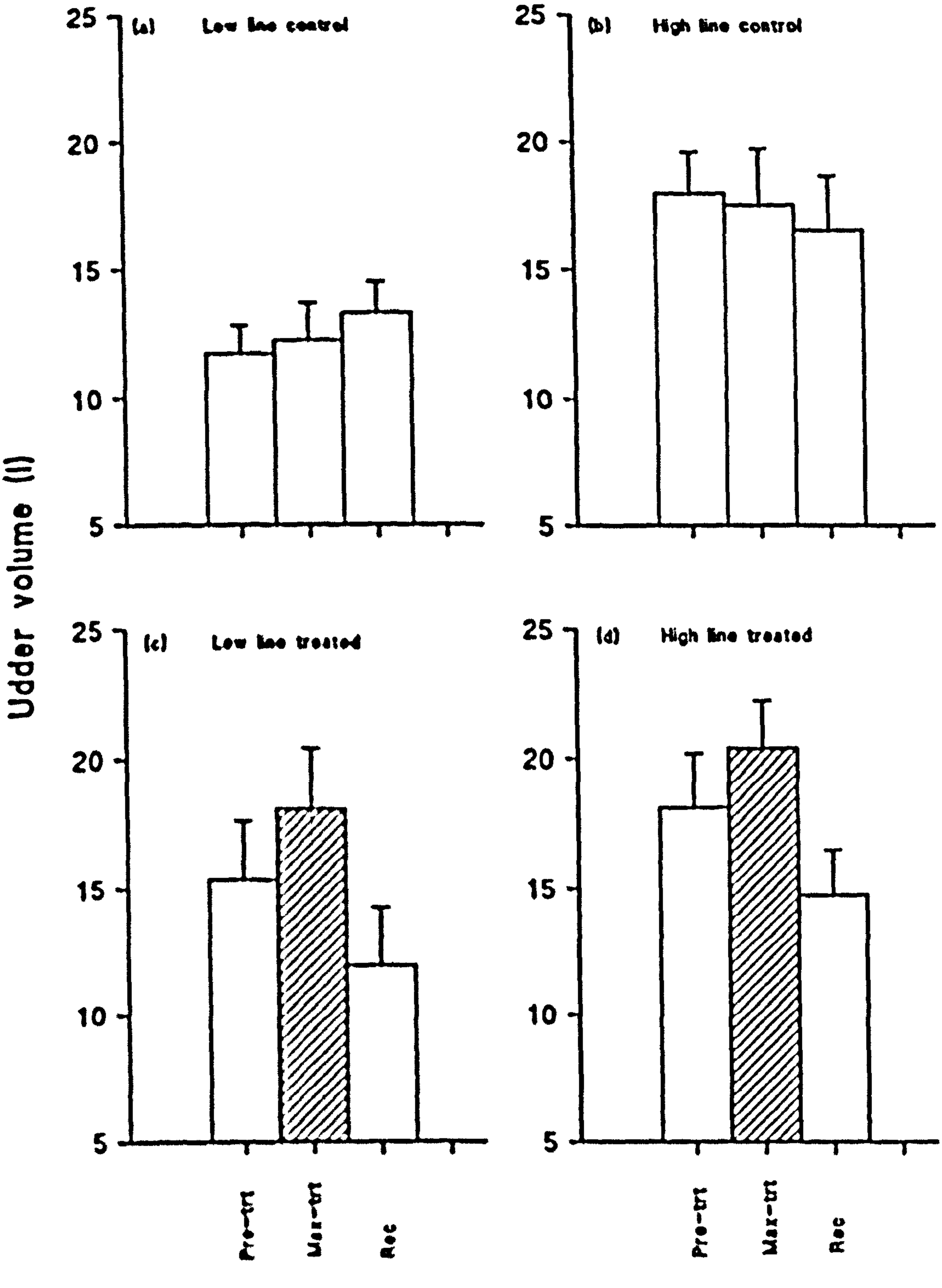

Figure 5.10 Changes in gross udder volumes in control cows of (a) low genetic merit, and (b) high genetic merit, and treated cows of (c) low genetic merit and (d) high genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (Max-trt), and 3 wk after cessation of all treatments (Rec). During maximum stimuli treated cows were milked four times daily + injected with 500mg of slow-release formulation of bovine somatotropin every 14d + injected with 50mg of thyroxine on alternate days. Vertical bars represent S.E.M.

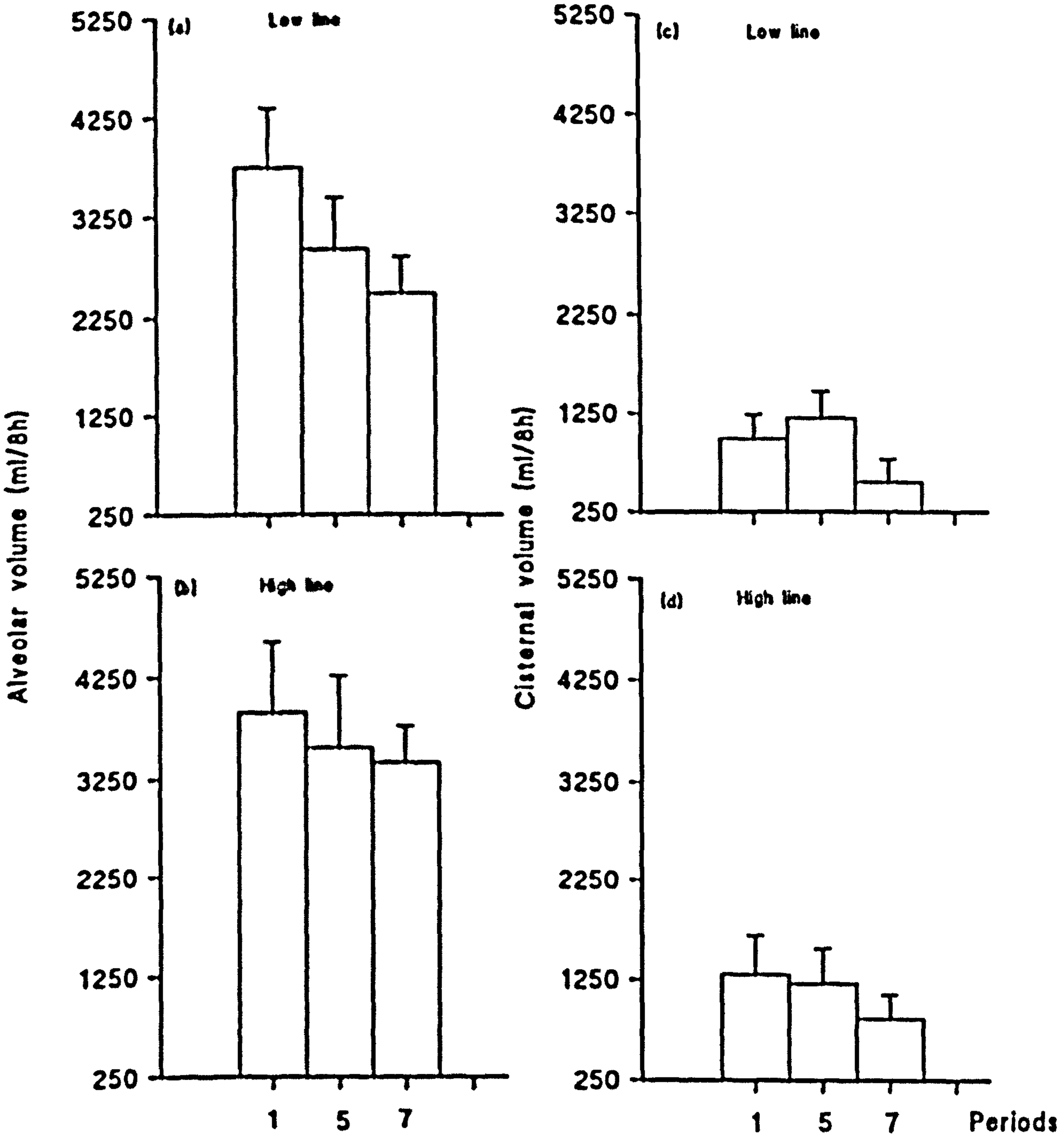

Figure 5.11 Changes in Alveolar milk volume (ml/8h) in cows from (a) low genetic merit and (b) high genetic merit, and cisternal volume (ml/8h) of (c) low genetic merit and (d) high genetic merit maintained at standard management level. Determinations were performed at week 5 (period 1), week 13 (period 5), and week 20 (period 7) of lactation. Vertical bars represent S.E.M.

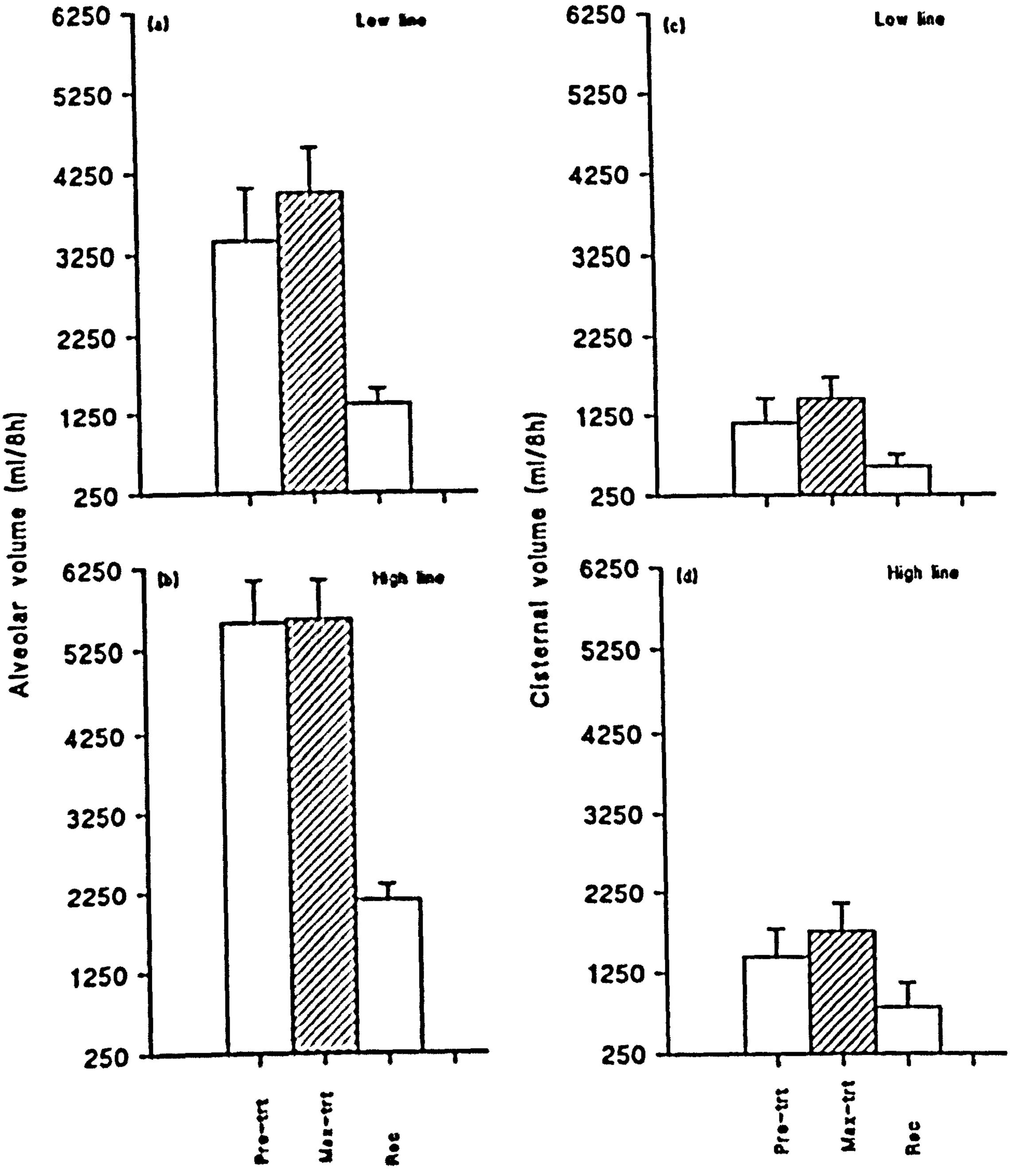

Figure 5.12 Changes in alveolar milk volume (ml/8h) in cows of (a) low genetic merit and (b) high genetic merit, and cisternal milk volume (ml/8h) of (c) low genetic merit and (d) high genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (Max-trt), and 3 wk after cessation of all treatments (Rec). During maximum stimuli, treated cows were milked four times daily + injected with 500mg of slow-release formulation of bovine somatotropin every 14d + injected with 50mg of thyroxine on alternate days. Vertical bars represent S.E.M.

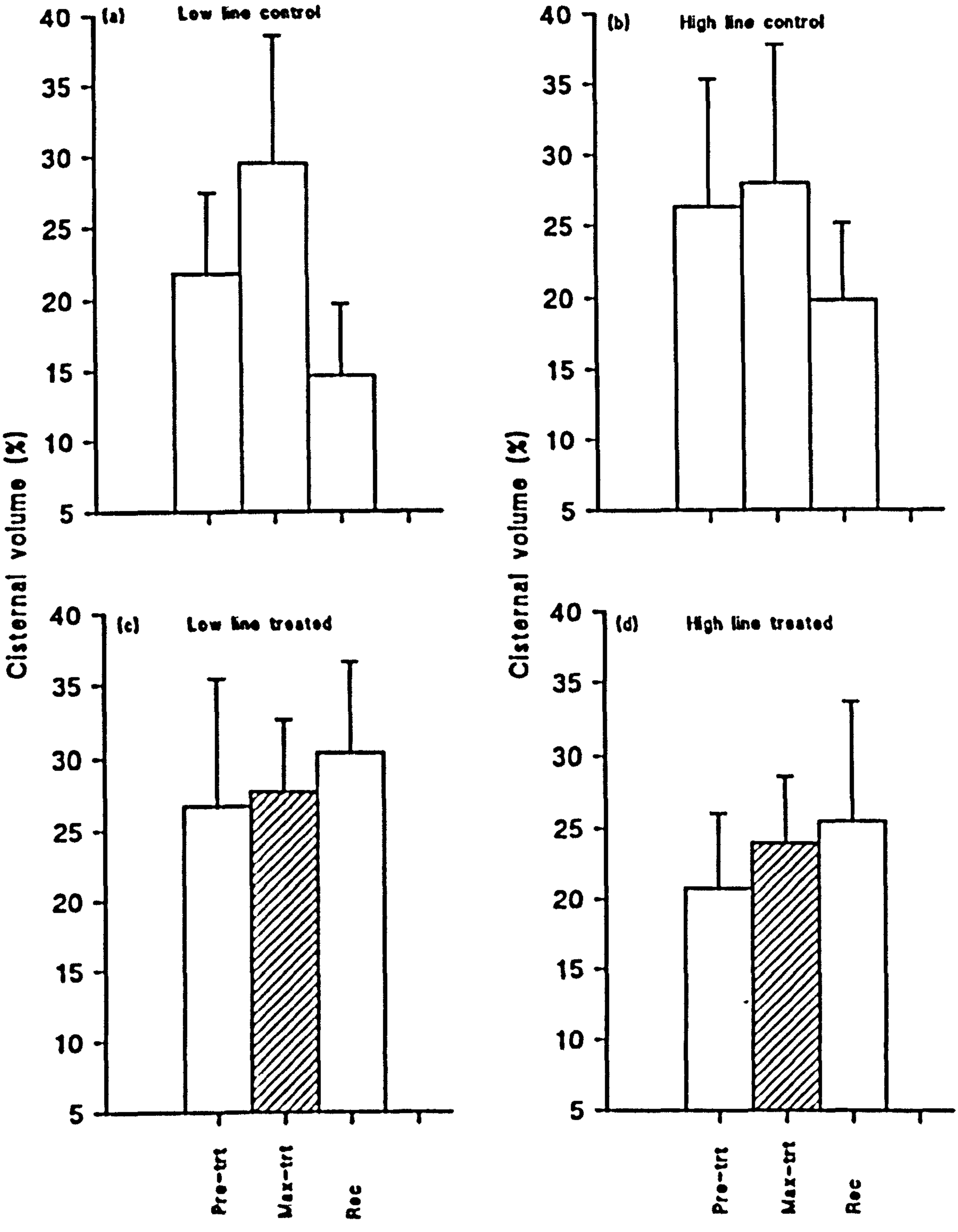

Figure 5.13 Changes in cistern proportion, at 8h after normal milking, in control cows of (a) low genetic merit, and (b) high genetic merit, and treated cows of (c) low genetic merit and (d) high genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (Max-trt), and 3 wk after cessation of all treatments (Rec). During maximum stimuli, treated cows were milked four times daily + injected with 500mg of slow-release formulation of bovine somatotropin every 14d + injected with 50mg of thyroxine on alternate days. Vertical bars represent S.E.M.

Table 5.8. Differences in gross udder volumes (litres), in control and treated cows, between pretreatment and maximum stimuli (4X+bST+T4; Pretrt-T4), and between maximum stimuli and recovery period (T4-Rec). Values are differences between means, n=6 or (*)5. Significance tests for effects of treatment (T), Line (L) or interaction (T*L), ANOVA.

Differences between periods	LC	HC	LT	HT	SED	Treat	Line	T*L
Pretreat - T4	0.52	-0.48	2.75	2.18	1.4	P=0.02	n.s	n.s
T4 - Rec	1.07	-1.0	-6.1	-5.6*	1.4	P<0.01	n.s	n.s

Table 5.9. Differences in alveolar and cisternal volumes (ml), and cisternal proportion, in control and treated cows, between pretreatment and maximum stimuli (4X+bST+T4). values are differences between means measured 8h after normal milking, n=6. Significant tests for effects of treatment (T), Line (L) or interaction (T*L), ANOVA.

	LC	HC	LT	HT	SED	Treat	Line	T*L
Alveolar Volume	-825	-345	583	38	613	P=0.05	n.s	n.s
Cisternal Volume	217	-90	304	317	336	n.s	n.s	n.s
Cisternal Proportion	7.8	1.7	1.0	3.2	7.3	n.s	n.s	n.s

Table 5.10. Differences in alveolar and cisternal volumes (ml), and cisternal proportion, in control and treated cows, between maximum stimuli (4X+bST+T4) and recovery period. Values are differences between means measured 8h after normal milking, n=6 or (*)5. Significance tests for effects of treatment (T), Line (L) or interaction (T*L), ANOVA.

	LC	HC	LT	HT	SED	Treat.	Line	T*L
Alveolar Volume	-848	-143	-3100	-3313*	606	P<0.001	n.s	n.s
Cisternal Volume	-490	-348	-873	-908*	398	n.s	n.s	n.s
Cisternal Proportion	-6.6	-6.2	6.3	1.4*	9.8	n.s	n.s	n.s

5.3.6.1 Prolactin profiles

Period average PRL profiles

Average PRL concentrations for both control and treated cows during the experimental periods are presented in Figure 5.14. Because of the range of calving dates (from September to January) cows were arranged for treatment into 5 groups according to their calving dates. This resulted in the introduction of a seasonal factor that might have an influence on the secretion of PRL. Analysis of variance indicated a significant effect of the month of sampling on PRL concentration ($P<0.05$). Therefore, month of sampling at each period for every group was used as the random factor in the REML statistical model. Results of REML analysis with the adjusted period means are in Table 5.11. Pre-treatment basal PRL profile was not affected by genetic selection for high milk yield (27.04 and 25.38 ng/ml, SED=6.2, for low and high line respectively; $P>0.05$, REML) without any difference between groups within line.

For the comparisons between any two consecutive periods, PRL period means were analysed by ANCOVA with the month of sampling at each period for each group as a covariate. In the control groups, PRL did not vary across the experimental periods and also between groups within period ($P>0.05$, ANCOVA), although there was a tendency for a small increase as lactation advanced which was more obvious during the last stages of the experiment. PRL profiles were significantly increased from pre-treatment to 4X in the treated cows ($P<0.01$, ANCOVA) with a tendency for the response to be higher in HT cows (not significant). There was a tendency for elevation in PRL concentration between 4X and first bST periods ($P>0.05$) which was further increased during the second bST injection ($P=0.05$, ANCOVA). PRL concentration was significantly reduced during T4 administration compared to earlier treatment periods ($P=0.05$, ANCOVA) for both treated groups at a similar rate. Despite this decrease, PRL concentrations in the treated cows

remained significantly higher than in the control cows (P<0.01, REML). Continued bST and T4 treatment while shifting to 1X/4X milking did not affect PRL levels, but they were significantly reduced in the recovery period (P=0.01, ANCOVA), when there was no significant treatment effect on PRL profiles.

Hourly average PRL profiles

The frequent bleeding (hourly samples) were quantified for PRL to give a better estimate of its pattern of secretion. Peaks were seen for some cows especially around milking time which might have been attributed to milking stimulus, so the hourly average means were determined by establishing mean hormone concentrations for each cow and subtracting those points greater than 2 standard deviation from the mean.

The average hourly PRL profiles calculated from frequent sampling which were performed during pre-treatment, maximum, and recovery periods are presented in Figure 5.15 and Table 5.12. Basal PRL profile was not affected by genetic selection, since statistical analysis by REML did not reveal any significant pretreatment difference between the two lines (13.06 and 13.26ng/ml, SED=3.82, P>0.05; for the low and high line, respectively) without any difference between groups within line. Maximum treatment resulted in a significant increase in average hourly PRL concentration in both treated lines (P<0.01, REML). During the recovery period, PRL in treated cows fell back towards control cows values, although it was still high in the low treated cows but this was not statistically different (P>0.05, REML). Control cows maintained basal PRL levels during the three sampling times without any major changes. Comparison between the consecutive periods in the treated cows indicated a significant increase in the average hourly PRL values during the maximum treatment which was significantly decreased during the recovery period when compared to their respective previous measurements (P<0.05, ANCOVA, using

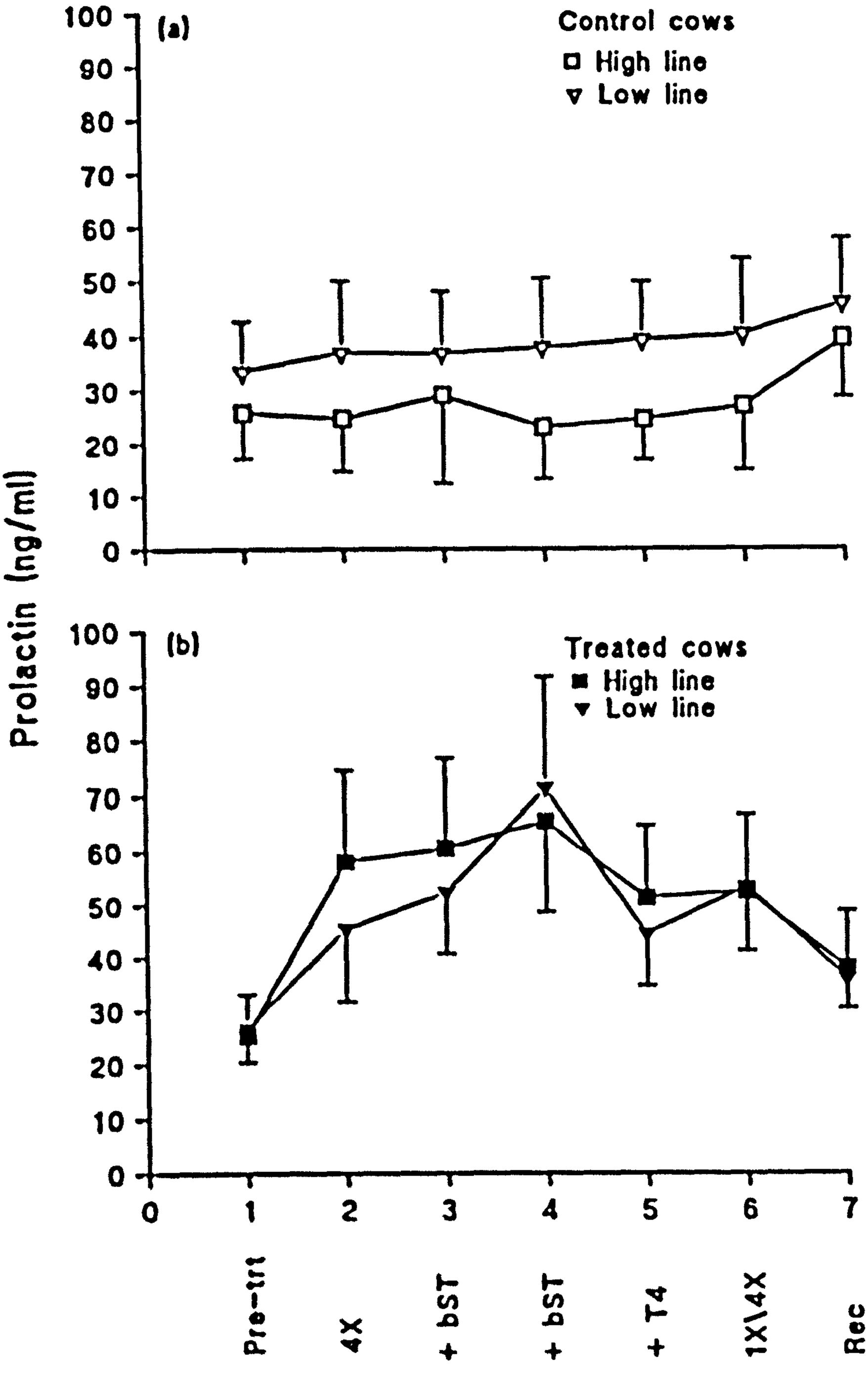

Figure 5.14 Prolactin profiles (ng/ml) in control cows of (a) low and high genetic merit and treated cows of (b) low and high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.11. Period mean prolactin profiles (ng/ml) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for month of sampling as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
2 (4X)	25.04	25.68	41.80	57.62	16.35	n.s	P<0.05	n.s
3 (bST)	20.97	26.54	47.80	57.22	14.94	n.s	P<0.01	n.s
4 (bST)	25.93	27.33	71.44	64.63	16.44	n.s	P<0.01	n.s
5 (T4)	28.36	23.44	48.72	54.02	12.62	n.s	P<0.01	n.s
6 (4X/1X)	30.04	28.40	51.80	52.61	16.35	n.s	P=0.05	n.s
7 (Rec)	40.83	39.76	37.61	39.46*	15.11	n.s	n.s	n.s

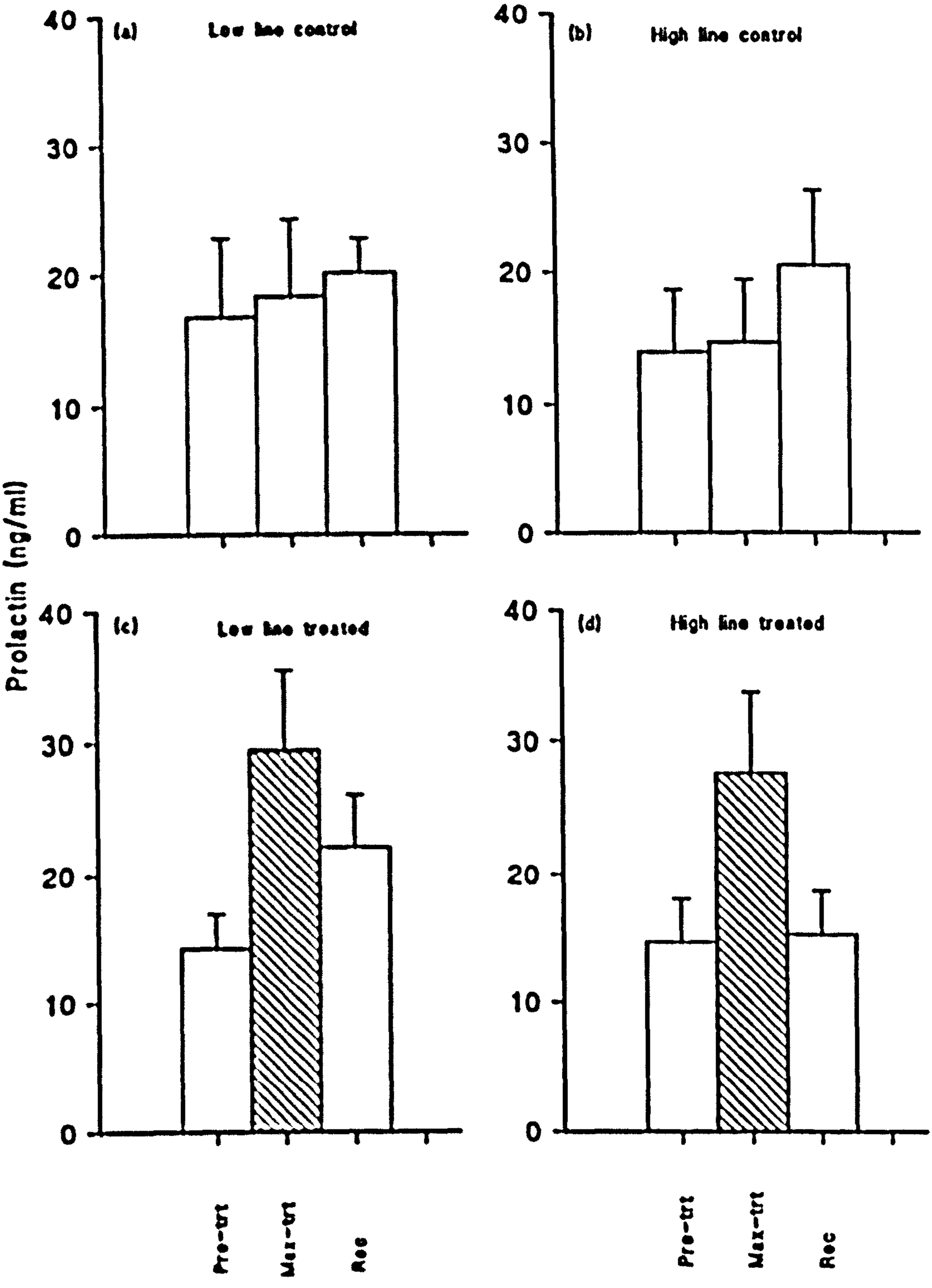

Figure 5.15 Average hourly prolactin profiles, un-adjusted for season, (ng/ml) in control cows of (a) low genetic merit, and (b) high genetic merit, and treated cows of (c) low genetic merit and (d) high genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (max-trt), and 3 wk after cessation of all treatments (Rec). During maximum stimuli treated cows were milked four times daily + injected with 500mg of slow-release formulation of bovine somatotropin every 14d + injected with 50mg of thyroxine on alternate days. Vertical bars represent S.E.M.

month of sampling as covariate).

Post-milking PRL profiles

The area under the post-milking PRL profile curve (Areas under the curve, AUC) was calculated from the samples collected after milking. Post-milking AUC for PRL (uncorrected) are illustrated in Figure 5.16. and Table 5.13 presents the values after correction for the month of sampling.

Genetic line did not affect the ability of cows to release PRL in response to milking stimulus. During the maximum stimulation, comparison between AUC of control and treated groups indicated a small but significantly higher value in the treated groups ($P=0.04$). All groups apart from HC showed a decline in AUC in the recovery period, which was only significant for the treated groups ($P=0.02$, ANCOVA, using month of sampling as covariate)

5.3.6.2 Growth hormone levels

Plasma GH concentrations of control as well as treated cows for the experimental periods are in Figures 5.17a and 5.17b, and Table 5.14. There was a significant pre-treatment genetic effect on plasma GH concentration which was significantly higher in the high line cows (2.80 v. 1.24 ng/ml, SED=0.44; $P=0.003$, REML). Increasing milking frequency to 4 times per day did not affect GH levels ($P>0.05$, REML). However it was significantly increased in response to bST injection ($P<0.001$) with a significant line X treatment interaction ($P<0.05$, REML). GH increasing more in HT than in LT ($P=0.04$, t test). The levels of GH in the treated groups were maintained above that of the control cows from the commencement of bST treatment till the last period (recovery) ($P<0.01$, REML). Injection of the second bST (period 4) increased GH levels in both treated cows

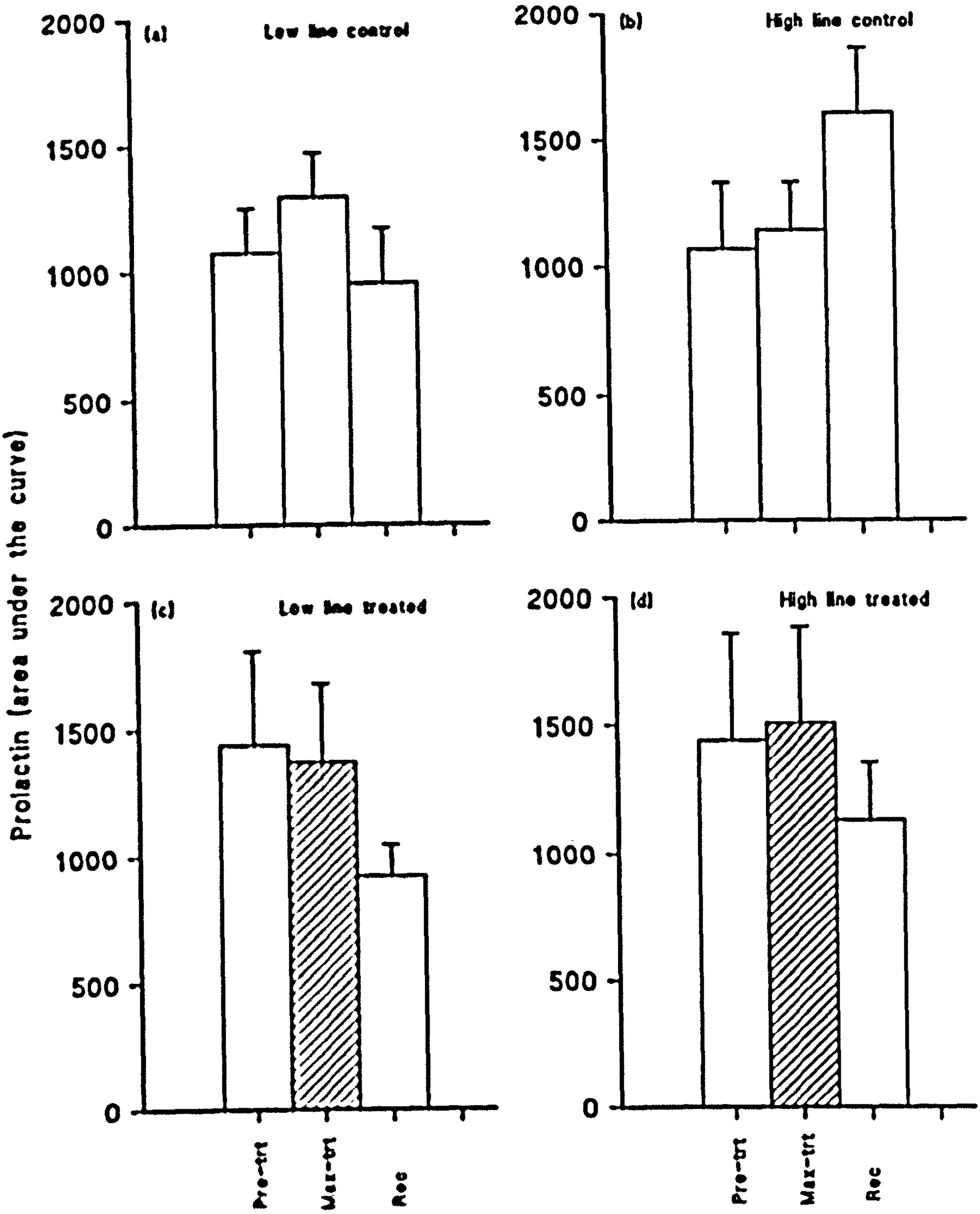

Figure 5.16 Total area under the curve for prolactin profiles, un-adjusted for season, determined from 5 samples collected post-milking at 5 minutes interval in control cows of (a) low, and (b) high genetic merit, and treated cows of (c) low and (d) high genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (Max-trt), and 3 wk after cessation of all treatments (Rec). During maximum stimuli, treated cows were milked four times daily + injected with 500mg of slow-release formulation of bovine somatotropin every 14d + injected with 50mg of thyroxine on alternate days. Vertical bars represent S.E.M.

Table 5.12. Hourly average prolactin profiles (ng/ml) and Table 5.13 total area under the curve for post-milking prolactin release in untreated (c) and treated (T) cows from low (L) and high (H) genetic merit determined during pretreatment period (Pre-trt), maximum galactopoietic stimuli (T4) and 3 weeks after cessation of all treatment (Rec). During maximum stimuli, treated cows were milking four times daily + injected with slow-release formulation of 500mg of bovine somatotropin every 14d + injected with 50mg of thyroxine every alternate days. Values are means which are adjusted for month of sampling. n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Table 5.12.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
1 (Pre-trt)	13.43	13.33	12.7	13.2	5.43	n.s	n.s	n.s
5 (T4)	13.08	14.38	28.61	26.79	6.56	n.s	P<0.001	n.s
7 (Rec)	15.65	20.11	23.11	14.73*	5.87	n.s	n.s	n.s

Table 5.13.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
1 (Pre-trt)	911	1258	1426	1426	303	n.s	n.s	n.s
5 (T4)	1017	1207	1480	1610	280	n.s	P=0.04	n.s
7 (Rec)	818	1692	881	1228*	301	P=0.02	n.s	n.s

above the levels in the first injection, with a greater increase in LT group. During the thyroxine treatment, GH concentrations increased in HT group when compared to the previous value ($P>0.05$, *t* test) while it was not changed in LT group. Nonetheless, weekly means indicated that during the first week of T4, GH tended to increase in both lines after the third bST injection ($P=0.07$, *t* test) but GH levels decreased in the second week and *t* test analysis indicated that this decrease was only significant in LT group ($P=0.02$). Plasma GH was significantly reduced three weeks after the last bST injection ($P<0.05$, ANCOVA) but it did not drop to the control levels; a significant treatment effect was still evident ($P=0.001$, REML, Table 5.14).

5.3.6.3 Insulin-like growth factor-I profiles

Concentrations of IGF-I for all treatment groups are illustrated in Figure 5.18 and Table 5.15. Plasma concentration of IGF-I was significantly lower in the high line cows compared to the low line ones (27.81 and 45.73ng/ml, SED=7.87; $P=0.02$, REML) during the pre-treatment period. Increasing milking frequency (4X treatment) did not affect the concentration of the hormone, but the line effect was still evident ($P=0.01$, REML). When bST was administered, it caused a significant rise ($P<0.01$) in IGF-I plasma levels in both treated groups, with higher levels in LT cows as indicated by a significant line X treatment interaction during the second bST ($P<0.05$) which was confirmed by *t* test analysis between the two lines ($P=0.04$). Thyroxine treatment reduced the stimulatory effect of bST on IGF-I concentration resulting in a steep drop in IGF-I levels during T4 treatment in both genetic lines ($P<0.01$, paired *t* test), which was further significantly decreased in the following period ($P<0.01$, paired *t* test). At 1X/4X treatment, HT had the lowest IGF-I levels among cow groups which was also lower than their pre-treatment levels. The suppressive effect that was seen upon the administration of T4 was abolished after the termination of all treatment,

resulting in a rise in the IGF-I concentration to match the control groups in the recovery period, and the effect of line was evident at this stage of lactation ($P<0.02$, REML). In both control groups, IGF-I was maintained across each stage of the experiment without any large changes, but there was a tendency for a small increase as lactation progressed.

5.3.6.4 Insulin profiles

Average period concentrations of INS for control and treated cows are shown in Figure 5.19 and Table 5.16. Pre-treatment INS concentration was significantly higher in the low line cows compared to the high line cows (0.16 v. 0.11 ng/ml, SED=0.027; $P=0.05$, REML). Statistical analysis did not reveal any treatment effect on INS plasma concentration during all treatment periods ($P>0.05$, REML) except for a tendency for a non-significant effect during T4 treatment (period 5) when the treated cows had lower INS concentration than the control groups ($P=0.06$, REML). This reduction was significant when tested by paired *t* test ($P<0.01$). During the 1X/4X period, INS increased in both treated lines ($P>0.001$, paired *t* test). The increase in INS levels in treated cows was continued towards the recovery period ($P=0.01$, paired *t* test). Both control groups exhibited a similar pattern for INS; it was maintained for the most of the experiment with a tendency for a gradual elevation which was more evident during the last stages of the experiment.

5.3.7 Blood metabolites

Plasma concentrations of glucose (GLU), free fatty acids (FFA), β-Hydroxybutyrate (β-HDB), and glycerol (GRL) were determined in one sample of the three samples collected weekly.

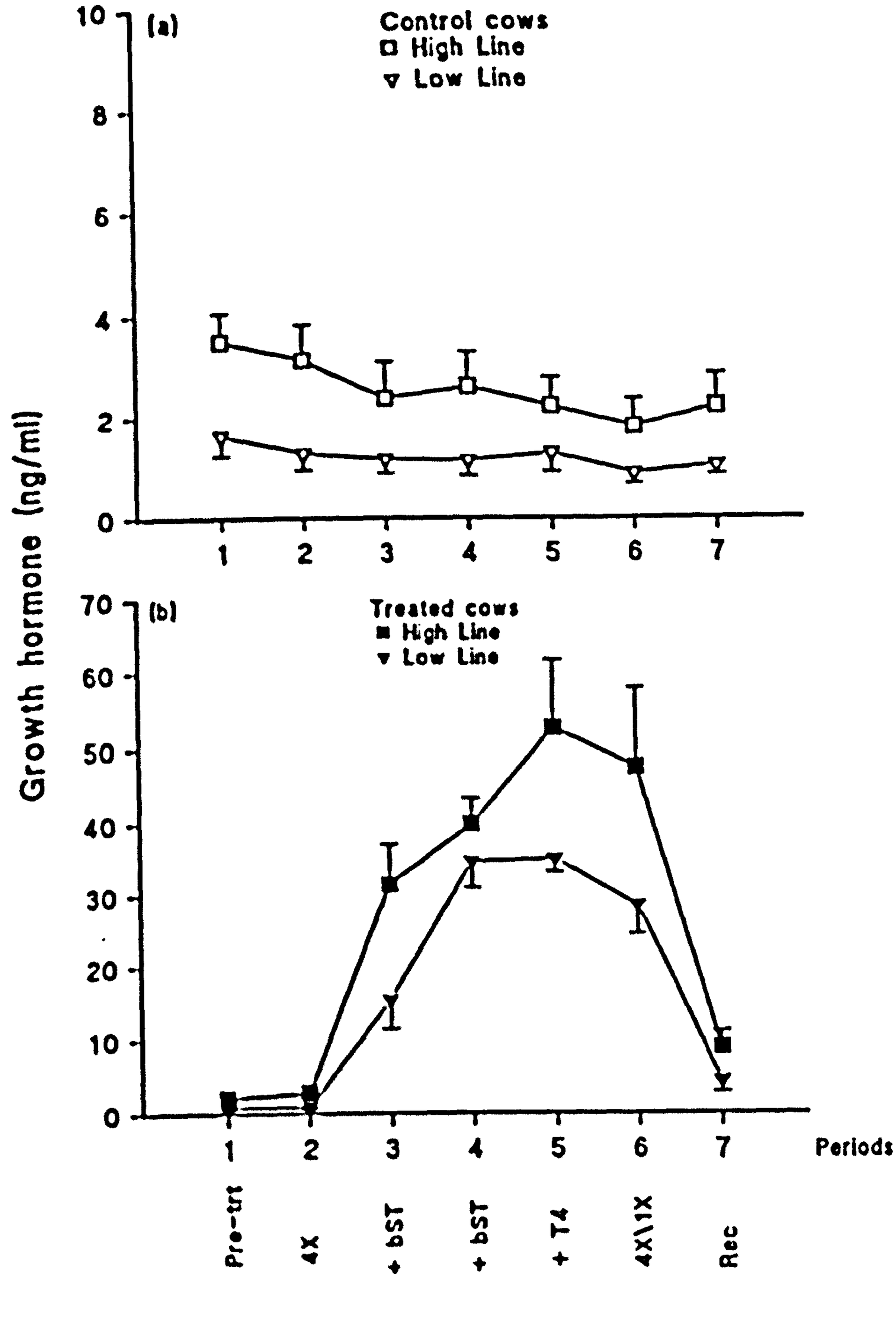

Figure 5.17 Growth hormone profiles (ng/ml) in control cows of (a) low and high genetic merit and treated cows of (b) low and high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec). Note the difference in scale between a and b.

Table 5.14. Period mean GH concentrations (ng/ml) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, using group as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
1 (Pre-trt)	1.63	3.5	0.84	2.11	0.62	P=0.003	n.s	n.s
2 (4X)	1.0	3.2	0.98	2.83	0.83	P=0.003	n.s	n.s
3 (bST)	1.19	2.4	15.4	31.63	4.87	P<0.02	P<0.001	P<0.05
4 (bST)	1.01	3.14	34.83	41.54	3.27	n.s	P<0.0001	n.s
5 (T4)	1.31	3.41	35.42	54.01	6.36	n.s	P<0.0001	n.s
6 (4X/1X)	0.58	2.22	28.55	48.78	7.78	n.s	P<0.001	n.s
7 (Rec)	0.27	2.92	4.95	9.56*	1.83	P=0.05	P=0.001	n.s

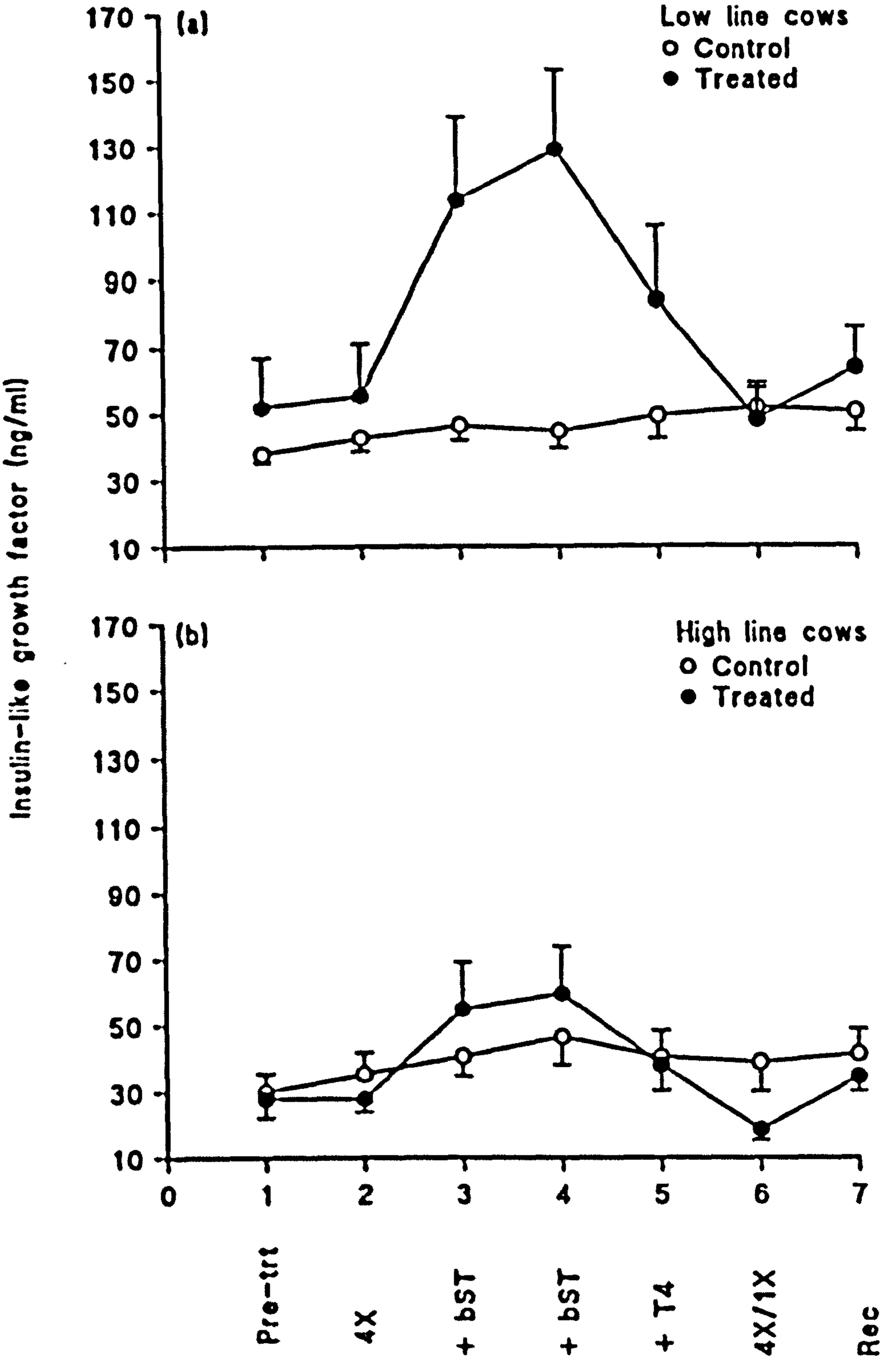

Figure 5.18 Insulin-like growth factor-I profiles (ng/ml) in both control and treated cows of (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.15. Period means of insulin-like growth factor-I levels (ng/ml) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, using group as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
1 (Pre-trt)	41.54	28.98	49.92	26.64	11.12	P=0.02	n.s	n.s
2 (4X)	48.20	34.73	53.20	25.85	10.61	P=0.01	n.s	n.s
3 (bST)	55.30	37.04	108.38	52.63	17.28	P<0.01	P<0.01	n.s
4 (bST)	51.31	42.19	123.68	58.13	18.06	P<0.01	P=0.003	P<0.05
5 (T4)	55.48	38.90	81.04	34.10	16.91	P=0.01	n.s	n.s
6 (4X/1X)	53.69	38.11	46.90	18.24	10.62	P<0.01	n.s	n.s
7 (Rec)	53.13	39.66	60.94	34.17*	11.63	P<0.02	n.s	n.s

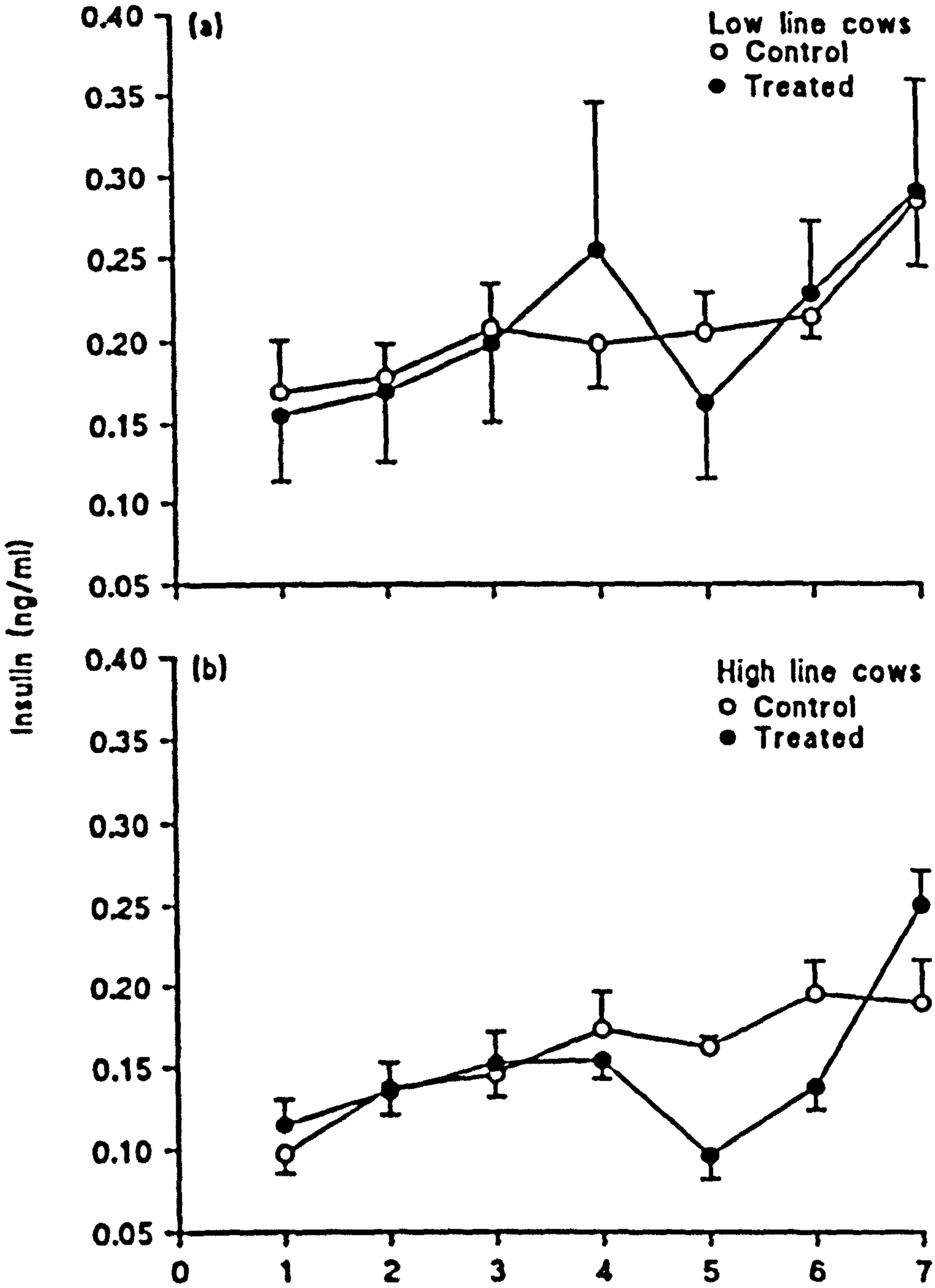

Figure 5.19 Insulin profiles (ng/ml) in both control and treated cows of (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.16. Period mean insulin concentrations (ng/ml) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, using group as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
1 (Pre-trt)	0.17	0.11	0.16	0.11	0.04	P=0.05	n.s	n.s
2 (4X)	0.17	0.14	0.17	0.13	0.03	n.s	n.s	n.s
3 (bST)	0.21	0.14	0.19	0.15	0.04	P=0.05	n.s	n.s
4 (bST)	0.19	0.17	0.25	0.16	0.07	n.s	n.s	n.s
5 (T4)	0.21	0.15	0.15	0.09	0.04	P=0.05	P=0.06	n.s
6 (4X/1X)	0.22	0.19	0.22	0.14	0.04	P=0.05	n.s	n.s
7 (Rec)	0.28	0.19	0.29	0.25*	0.07	n.s	n.s	n.s

5.3.7.1 Glucose concentration

The period mean concentrations of plasma GLU in the four groups of cows at the various experimental periods are shown in Figure 5.20 and Table 5.17. Plasma levels of GLU did not differ significantly between the high and low line groups for samples determined during the pre-treatment period (5.61±0.29 and 5.78±0.36 mM/l for low and high genetic merit cows respectively, $P>0.05$, REML), and there were no differences between groups within line. Values reported here tended to be higher than what have been normally reported for lactating dairy cows. Neither increasing milking frequency nor bST treatment had any effects on plasma GLU levels; it was maintained for both treated and control cows during the periods of 4X and bST treatment. However, T4 administration triggered a significant increase in GLU levels ($P=0.05$, REML). During 1X/4X treatment, plasma GLU concentrations were further increased in both treated cows ($P<0.01$, *t* test), and the significant line*treatment interaction was indicative of higher GLU levels in LT group compared to HT group, which was confirmed by *t* test ($P=0.01$). Termination of all treatments resulted in a significant drop in GLU concentration in the treated cows ($P<0.01$, *t* test), but the average concentration did not drop to the level found in the control groups when the cows were in the recovery period; REML analysis revealed that treated cows still had a significantly higher GLU levels ($P<0.001$) and again with a tendency for higher plasma GLU levels in LT cows when compared to HT cows ($P=<0.01$, *t* test). In the control cows, GLU levels remained relatively constant throughout the experimental periods without any differences between the two lines.

5.3.7.2 Free fatty acids concentration

Period mean concentrations of FFA are illustrated in Figure 5.21 and Table 5.18. Due to lower FFA levels in HC cows compared to other groups during the pre-treatment

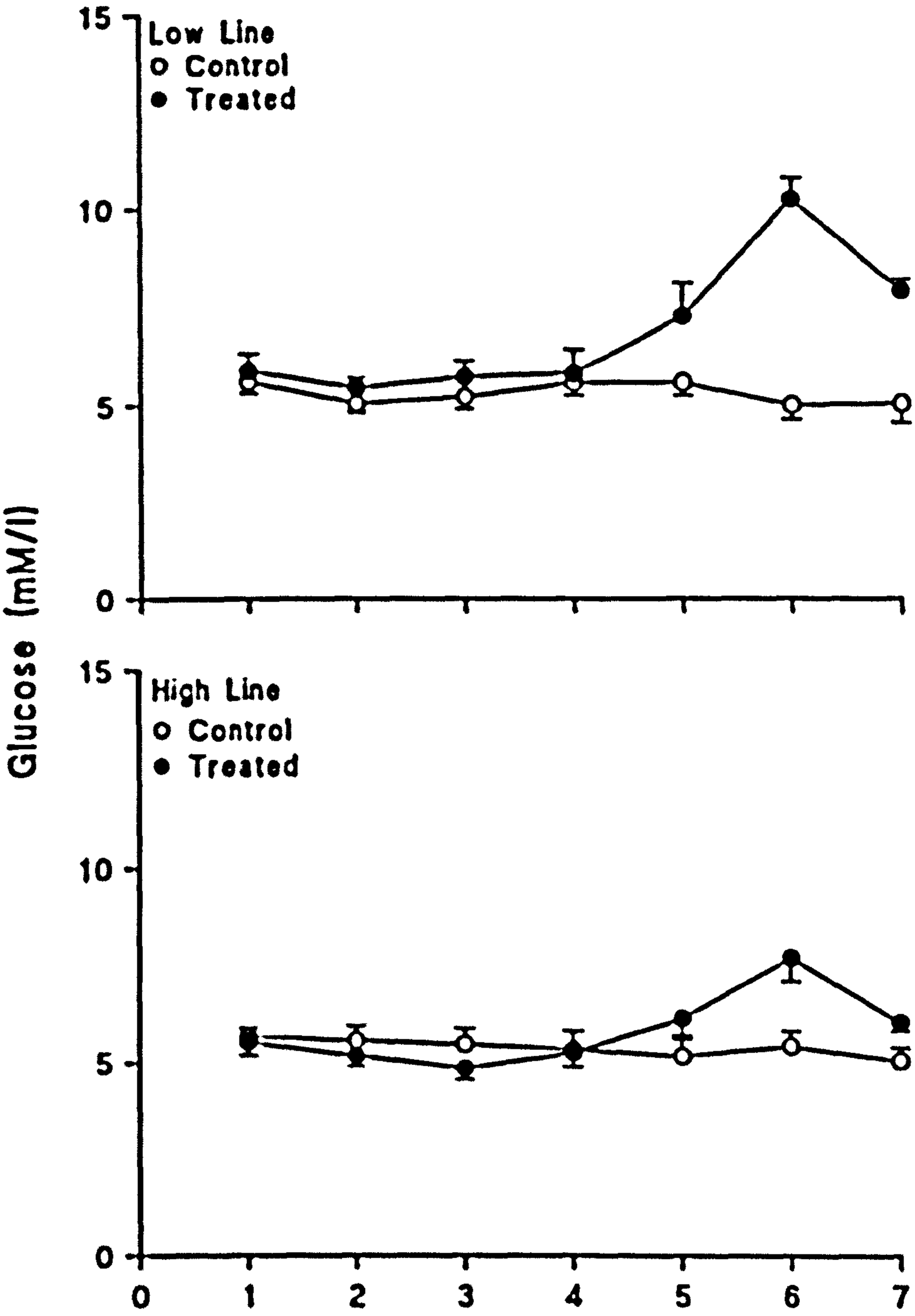

Figure 5.20 Concentrations of glucose in plasma (mM/l) of both control and treated cows from (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.17. Period mean plasma glucose concentrations (mM/l) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, using group as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
2 (4X)	5.05	5.58	5.48	5.18	0.40	n.s	n.s	n.s
3 (bST)	5.43	5.44	5.76	4.87	0.45	n.s	n.s	n.s
4 (bST)	5.72	5.40	5.92	5.30	0.58	n.s	n.s	n.s
5 (T4)	5.88	5.14	7.26	5.98	0.74	P=0.05	P=0.05	n.s
6 (4X/1X)	5.16	5.33	10.20	7.73	0.66	P<0.01	P<0.001	P<0.01
7 (Rec)	4.89	4.97	7.81	6.14*	0.53	n.s	P<0.001	P<0.05

period, the mean of the pre-treatment was included in the statistical model as a covariate to increase the precision of the analysis. Plasma levels of FFA did not differ significantly between high and low yielding dairy cows ($P>0.05$, REML). Frequent milking (4X) did not affect FFA levels but they were increased slightly during bST treatment which was not found to be significant when compared to period 2 levels (n.s, Paired *t* test). Due to the large variation between individuals, a large changes in FFA levels was needed to detect a significant difference. Administration of T4 resulted in a large increase in FFA concentration in both treated lines ($P=0.027$, *t* test) which was slightly declined in the period of 1X/4X (n.s, *t* test) so there was no significant differences between treated and control group ($P>0.05$, REML). Treated cows manifested a further drop in FFA concentrations in the recovery period ($P=0.02$, *t* test) to levels lower than control cows ($P=0.06$, REML). FFA levels in the control cows remained constant across all periods (HC) or increased during the last two periods (LC) and the increase between period 6 and the recovery period was significant in LC group ($P=0.05$, Paired *t* test).

5.3.7.3 β-Hydroxybutyrate concentration

Plasma concentrations of β-hydroxybutyrate for both control and treated cows are presented in Figure 5.22 and Table 5.19. Because of the tendency for higher levels of plasma β-hydroxybutyrate in both treated groups before the commencement of treatment, pre-treatment mean was used as a covariate to adjust for these higher levels and increase the accuracy of the statistical analysis. Initial β-HDB plasma concentration was not found to vary significantly between the two genetic line groups (1.35 ± 0.17 and 1.50 ± 0.40 mM/l for low and high genetic merit cows respectively, $P>0.05$, ANOVA). Values reported here tended to be higher than what have been normally reported for lactating dairy cows. Adjusted period means in the treated groups were slightly higher, but significant ($P=0.05$),

during 4X and the first bST periods. However, neither *t* test nor paired *t* test have confirmed a significant change between pretreatment and the following periods in the treated groups. This was related to the tendency for a decline in β-HDB levels in period 2 of the controls while it was maintained in the treated groups. Levels of β-HDB from period 4 to the recovery period were maintained without any significant changes between treated groups within the period or across periods when tested by *t* test.

5.3.7.4 Glycerol concentration

Plasma means of GRL for control and treated groups are in Figure 5.23 and Table 5.20. Plasma GRL levels were not affected by genetic selection, since there was no significant line effect during the pre-treatment period ($P>0.05$, REML). The significant line*treatment interaction in period 4 was because of the rise in plasma GRL in HC group, but the rise from period 3 to period 4 in HC group was not significant ($P>0.05$, paired *t* test), and also, when LT group was compared with HT group, it was significantly higher in LT cows ($P<0.04$, *t* test). Also, in the following period (period 5) there was a highly significant L*T interaction ($P<0.001$, REML) and comparison between groups within line has indicated that it was only significant in the low line group ($P=0.05$, *t* test). Although GRL levels were relatively higher in the treated cows in period 6 (1x/4x treatment), REML analysis did not reveal any differences between treated and control groups and also there was no significant change between the levels of period 5 and period 6 in the treated groups ($P>0.05$, *t* test). Plasma levels of GRL in the treated groups declined in the recovery period without any significant difference between treated and control groups (n.s., REML). None of the comparisons between successive periods or between line within the period were found to be significant in the control groups ($P>0.05$, *t* test).

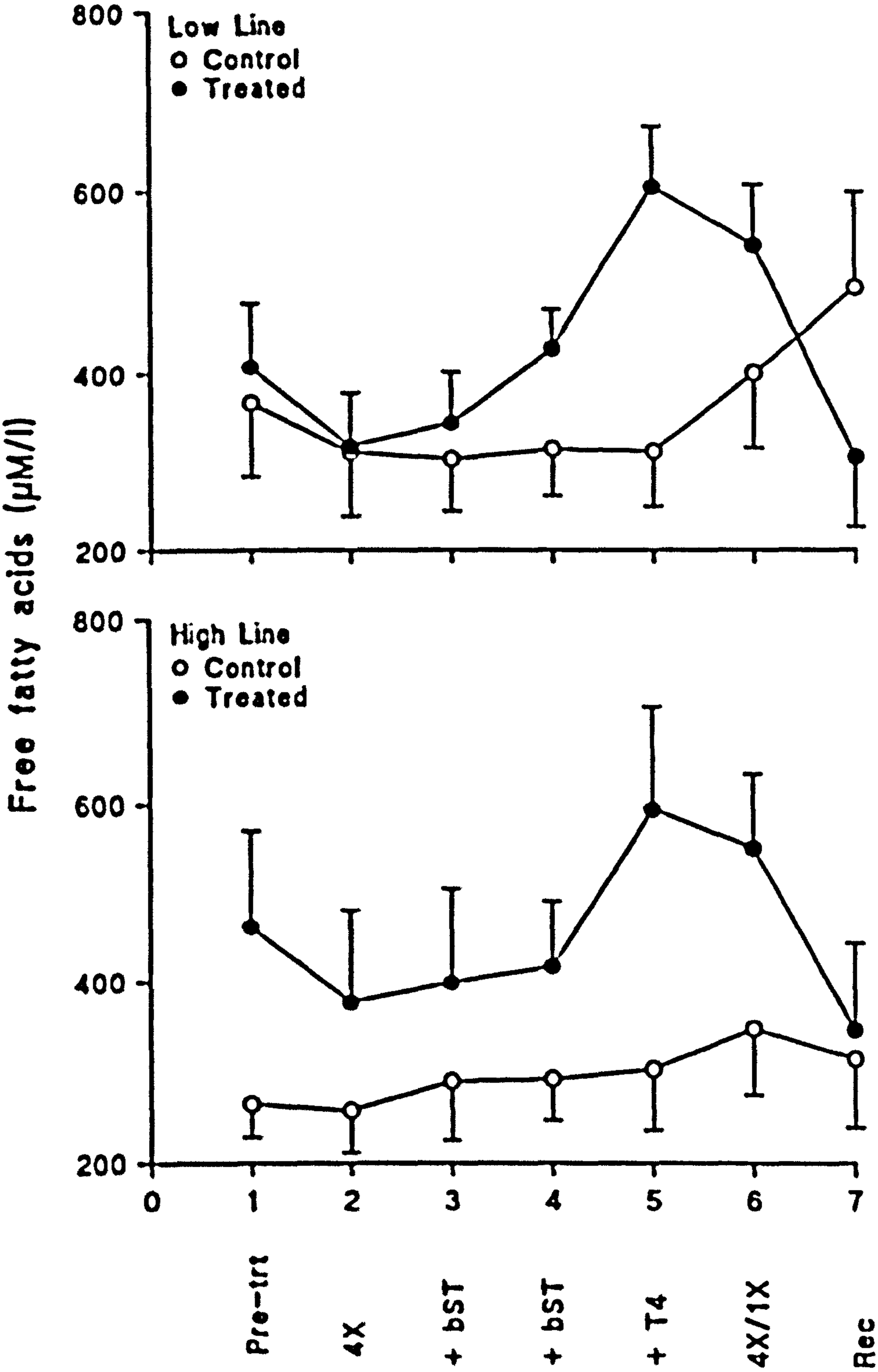

Figure 5.21 Concentraions of free fatty acids in plasma (μM/l) of both control and treated cows from (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.18. Period mean plasma free fatty acids (uM/l) for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean (period 1) as covariate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
2 (4X)	317	343	292	310	62	n.s	n.s	n.s
3 (bST)	309	363	323	342	79	n.s	n.s	n.s
4 (bST)	318	343	413	378	62	n.s	n.s	n.s
5 (T4)	318	387	579	528	77	n.s	P=0.002	n.s
6 (4X/1X)	405	412	521	502	91	n.s	n.s	n.s
7 (Rec)	504	396	272	290*	116	n.s	P=0.06	n.s

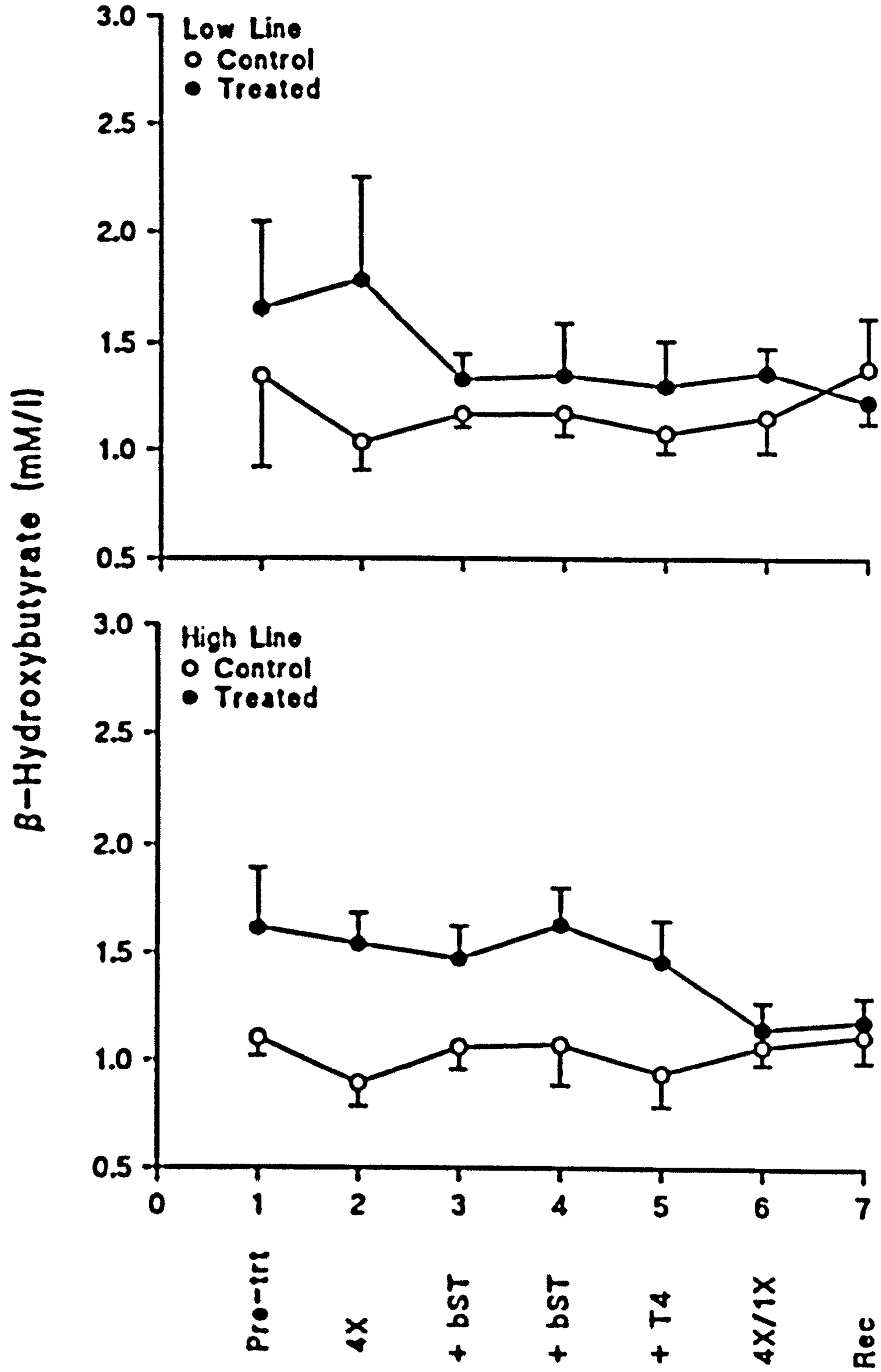

Figure 5.22 Concentrations of β-hydroxybutyrate in plasma (mM/l) of both control and treated cows from (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.19. Period mean values for plasma β-hydroxybutyrate (mM/l) in untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, which are adjusted for pretreatment period mean (period 1) as covriate, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
2 (4X)	1.07	1.03	1.68	1.46	0.33	n.s	P=0.04	n.s
3 (bST)	1.18	1.11	1.31	1.49	0.15	n.s	P=0.05	n.s
4 (bST)	1.20	1.13	1.31	1.60	0.19	n.s	n.s	n.s
5 (T4)	1.08	0.97	1.28	1.44	0.21	n.s	n.s	n.s
6 (4X/1X)	1.16	1.1	1.35	1.14	0.19	n.s	n.s	n.s
7 (Rec)	1.39	1.1	1.21	1.18*	0.24	n.s	n.s	n.s

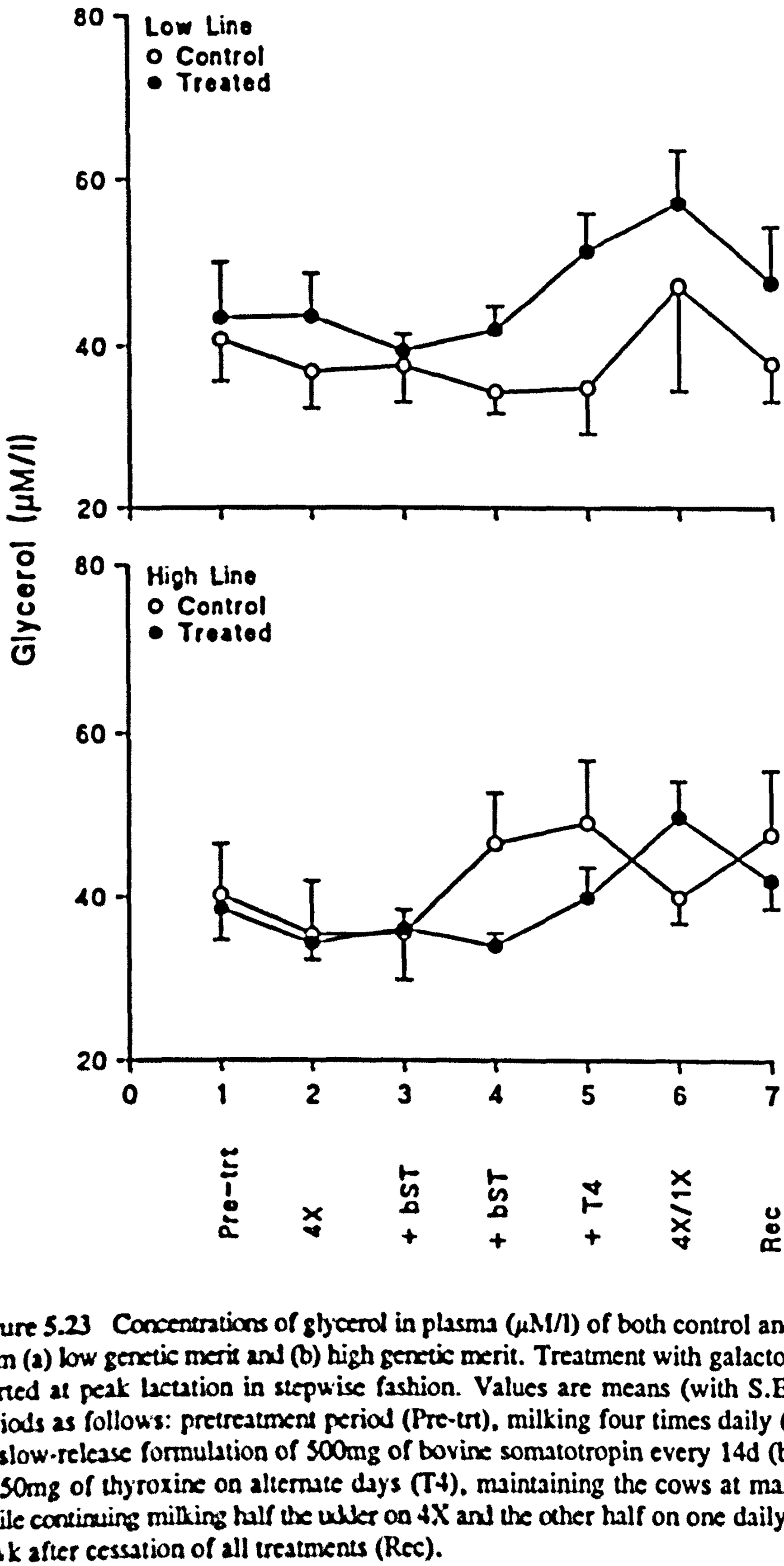

Figure 5.23 Concentrations of glycerol in plasma (μM/l) of both control and treated cows from (a) low genetic merit and (b) high genetic merit. Treatment with galactopoietic stimuli started at peak lactation in stepwise fashion. Values are means (with S.E.M) for 2 wk periods as follows: pretreatment period (Pre-trt), milking four times daily (4X), injection of slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine on alternate days (T4), maintaining the cows at maximum stimuli while continuing milking half the udder on 4X and the other half on one daily (4X/1X), and 3 wk after cessation of all treatments (Rec).

Table 5.20. Period mean values for plasma glycerol (μM/l) in for untreated (c) and treated (T) cows from low (L) and high (H) genetic merit. Treatment started at peak lactation (week 5 of lactation) and applied in stepwise fashion. Values are means for 2 weeks periods, using group as random factor, as follows: milking four times daily (4X), injection with slow-release formulation of 500mg of bovine somatotropin every 14d (bST), injection of 50mg of thyroxine every alternate days (T4), maintaining the cows at maximum stimuli while milking half the udder on 4X and the other half on one daily (4X/1X), and 3 weeks after cessation of all treatments (Rec). n=6 or (*)5, and significance tests for effects of treatment, line or interaction (T*L), REML. SED standard error of difference.

Period	LC	HC	LT	HT	SED	Line	Treatment	L*T
2 (4X)	36.40	34.12	46.43	38.77	5.91	n.s	n.s	n.s
3 (bST)	37.66	35.71	40.29	37.42	5.52	n.s	n.s	n.s
4 (bST)	34.37	46.67	41.97	33.97	5.40	n.s	n.s	P=0.01
5 (T4)	33.53	50.28	57.24	48.54	5.41	n.s	P<0.01	P<0.001
6 (4X/1X)	48.26	40.06	56.85	49.30	10.64	n.s	n.s	n.s
7 (Rec)	36.15	47.01	53.41	47.89*	7.56	n.s	n.s	n.s

5.4 DISCUSSION

The cows used in this investigation were daughters of bulls selected as sires of cows with high or low genetic merit. They were the progeny of two to six generations of selection with a mean difference in predicted breeding value for the output of fat plus protein of about 78kg (Woolliams *et al.*, 1993). Pretreatment milk yields indicated that high genetic merit cows (HGM) produced an average of 30% more milk than low genetic merit cows (LGM) without any effect on milk composition. So what is the biological basis for this difference in milk production? In several studies, estimated transmitting ability for higher milk production was not related to efficiency in digestion or nutrient absorbtion (Bauman *et al.*, 1985b; Belyea & Adams, 1990). Also, maintenance requirement per unit metabolic body size or partial efficiency for utilization of nutrients for milk synthesis are not linked to predicted genetic merit (Bauman *et al.*, 1985b). Genetic selection for high milk yields may not always be associated with changes in body weight, as indicated by our data and also in other studies (Barnes *et al.*, 1990; Michel *et al.*, 1990). However, others have reported significantly higher body weights in cows of high genetic merit (Davis *et al.*, 1983; Belyea & Adams, 1990). This inconsistency which probably relates to the difference in breeding programs suggests that this trait is not an essential component for the potential of milk production. Perhaps other factors related to body growth may be more important than body weight *per se*, such as the extent of development or growth of some organs. Although there was no difference in body weight between HGM and LGM cows in this study, there was some evidence to suggest possible differences in proportions of some body components which are related to milk production capacity. For example, udder volume was shown to be higher in HGM cows, and gut capacity may have been higher, since HGM cows have a high rate of food consumption which implies higher capacity of the gastro-intestinal tract. On the other hand, LGM cows had higher fat stores as indicated by their body condition

score. Therefore, the extent of growth of some body components might be more important in adaptation for the increased milk production than an increase in body weight *per se*.

Pretreatment udder size determination indicated a significantly higher udder volume in high line cows. Davis *et al.*, (1983) also reported higher udder volume in Jersey cows of high genetic merit when compared to lower genetic cows. The variation in gross udder volume reported here apparently associated with differences in milk yields; correlation between milk yield and udder volume was significant ($r=0.76$, $P=0.01$). Therefore, the difference in milk production potential between cows from different genetic background can be partly explained by the extent of udder development which in turn reflects a variation in the total number of the secretory cells. Calculation of milk secretion efficiency (amount of milk produced per amount of secretory tissue per day) did not reveal any differences between the two genetic merits (2.23 ± 0.17 and 2.09 ± 0.11 for low and high line cows respectively, n.s, ANOVA). However, gross udder volume is not a precise estimate of secretory tissue, since it includes connective tissue, adipose tissue, blood vessels and secretory tissue, but the majority of this volume is occupied by the secretory epithelium.

The most essential and principal criteria which is highly linked to genetic selection for milk production is the distribution of the available nutrients between the mammary gland and body tissues (Bauman *et al.*, 1985b). Superior cows are more efficient in milk production because they partition greater amounts of nutrients towards milk synthesis and away from deposition in body stores. The variation in energy partitioning between the mammary gland and body stores is genetic in origin and is mediated by differences in endocrine balance. Several hormones are involved in the regulation of body metabolism such as GH, INS, thyroxine, and glucagon but the two of most relevant to the dairy cow are GH and INS (Bines & Hart, 1982). Differences in GH and INS concentrations in plasma

between HGM and LGM cows were detected in this study; higher GH levels coupled with lower levels for INS in HGM cows as compared to LGM cows.

The fall in circulating plasma INS at the onset of lactation is considered one of the adaptive processes to increase the availability of nutrients for the purpose of milk synthesis. INS is an anabolic hormone which promotes the utilization of glucose by peripheral tissues, inhibits gluconeogenesis and glycogenolysis, promotes the storage of nutrients in adipose tissue (lipogenesis) and inhibits the release of nutrients from adipose tissue, lipolysis (Vernon, 1989). As a consequence, high yielding dairy cows are usually characterized by lower INS levels during lactation (Walsh *et al.*, 1980; Barnes *et al.*, 1985; Bonczek *et al.*, 1988). The difference between cows from different genetic merits is likely to reflect difference in secretion rate (Hart *et al.*,1980). Lower INS levels in high line cows is associated with metabolic adaptation in carbohydrate metabolism which will preferentially divert energy to the mammary gland at the expense of the rest of the body tissues (Hart, 1983; Collier *et al.*, 1984).

The involvement of GH in nutrient partitioning during the lactation cycle is well recognized because of the positive correlation between milk production and plasma levels of GH. Its role in altering the energy partitioning is by increasing the availability of nutrients by promoting gluconeogensis and lipolysis of adipose tissues, and inhibiting the utilization of nutrients by peripheral tissues and lipogenesis in the adipose tissue (Collier *et al.*, 1984). GH thus antagonizes the anabolic activities of INS. The higher GH levels we observed in HGM cows is in agreement with others who provided evidence suggesting that GH levels would likely increase with genetic selection for milk yield (Barnes *et al.*, 1985; Bonczek *et al.*,1988). However, whether this difference in GH is a true genetic trait linked to genetic inheritance or related to the net energy balance of the lactating cow has been debated. Hart (1983) proposed that GH variation between cows from different genetic merit

may not be a cause of, but a consequence of variation in energy balance between high and low genetic merit cows. However, Kazmer *et al.*, (1986) have reported that both basal levels and responsiveness of GH to TRH were greater in cows selected for higher milk yield in the absence of any difference in energy balance, supporting the genetic effect on GH secretion. Our treated HGM and LGM cows exhibited similar rates of lipolysis and also maintenance of body condition score and body weight for several weeks (before T4 administration) suggesting similar energy balance and little fat mobilization to cover for any energy deficit. In fact, greater GH release has been shown in the same high genetic line at an early age (Woolliams *et al.*, 1993) suggesting that GH release is genetically programmed in the HGM cows utilized in this study.

So, one of the reasons for greater milk production in HGM cows is the ability to reduce the amounts of nutrients deposited in the body tissues, thereby increasing the availability of nutrients available to support higher milk yield. This variation can be attributed to difference in circulating levels of GH and INS between the two genetic lines. The variation in plasma levels of IGF-I between the two groups was in accordance with the difference in allocation of available nutrients. Like INS, IGF-I is an anabolic hormone which is involved in many processes of body growth. Therefore, lower IGF-I concentration in HGM was probably related to the need to reduce tissue synthesis elsewhere in order to support the high nutrient requirement for milk synthesis. An inverse correlation exists between milk production and serum IGF-I in lactating cows; IGF-I which reaches its minimal levels in early lactation will increase gradually when milk yield falls as lactation advances (Ronge *et al.*, 1988). The lower plasma IGF-I concentration in the high genetic merit cows was one of the physiological adaptations to support the high milk synthesis by reducing body anabolism.

It is well known that the dramatic increase in milk production at the onset of lactogenesis is not accompanied by a concurrent increase in feed intake, which in turn drives the cow into a period of negative energy balance. The difference between output and input is covered by nutrients released from body-reserve mobilization (Bauman & Currie, 1980; Vernon, 1989). The lower body condition score in HGM cows, when compared to that of LGM cows, was a consequence of higher rate of fat mobilization to adjust for the higher level of energy deficit during the first few weeks of lactation cycle. Unfortunately, net energy balance was not assessed in this study because this required the determination of feed intake in individual cows which was not possible without restraining the cows into stalls, a situation which could have resulted in the introduction of stress factor to the cows. Hydrolysis of triacylglycerol in the adipose tissue during mobilization will liberate FFA and glycerol (GRL) (Vernon, 1989) so their plasma levels may reflect the energy status of the animal; high levels would presumably suggest an energy deficit. In this study there were no differences in FFA or GRL concentrations between the two lines despite the expected variation in their metabolic status. The lack of any significant difference suggests that both of them had passed their acute negative energy balance which prevails immediately at the onset of lactation and this might also suggest that the cows were in a state of positive energy balance during the pretreatment period. The cows were fed an adequate and balanced ration, therefore, the anticipated higher intake of high energy density diet might has been sufficient to cover the majority of the nutrients requirement of HGM cows in this study. Hart *et al*, (1978) have found higher FFA concentrations in high yielding cows in early lactation which was positively correlated with GH, but body stores mobilization was very high as judged by decrease in body weights. On the other hand, Flux *et al*, (1984) could not detect any variation in plasma FFA between cows from different milk production potential at week 10 of lactation when the cows were fed *ad libitum*, but FFA were increased in response to

restricted feeding. However, the suggestion that HGM and LGM were both at a positive energy balance during pretreatment period should be treated with caution because this suggestion is based on plasma levels of FFA and GLR which were determined from only two samples during two weeks period and their levels are known to be variable throughout the day and affected by some factors like time of feeding (Flux *et al.*, 1984). Nonetheless, this does not mean that both groups were at the same level of energy status; low line cows might have been at a state of higher energy balance than high line cows. This was supported by IGF-I results in which low genetic merit cows had greater plasma levels than high genetic merit cows suggesting that due to their lower milk yield, low line cows were associated with surplus nutrients that can be directed towards body tissues and the reverse was true for the high genetic merit cows. Despite the significant differences between HGM and LGM cows in some of the hormones which are involved in metabolism regulation, plasma glucose concentrations were at similar levels in the two genetic groups. This is in agreement with other reports (Barnes *et al.*, 1985). Glucose availability as a precursor of lactose in the mammary gland is a limiting factor for milk synthesis. The absence of a difference in glucose levels between the two genetic lines may not reflect a similarity in glucose kinetics, glucose partitioning is not likely to be the same in the two groups. The higher requirement of glucose for lactose synthesis in HGM cows would necessitate an increase in its availability by means of increase in its rate of production and also in its diversion away from non-mammary tissues, which are both facilitated by higher GH levels together with lower INS concentrations (Chalupa & Galligan, 1989). Thus, higher rate of gluconeogenesis coupled with higher rate of glucose uptake by the mammary gland in the high genetic merit cows would result in maintenance of plasma glucose at levels similar to that in low genetic merit cows.

Circulating β-HDB is derived mainly from transformation of butyrate during its absorption through the rumen wall, and also can be synthesised from FFA in the liver during the time of low availability of glucose for oxidation by peripheral tissues (Vernon, 1989). Apparently blood β-HDB levels were not different between the two genetic lines. Also, other studies with cows from different genetic background did not report any differences in β-HDB levels between high and low genetic cows (Flux *et al.*,1984; Michel *et al.*, 1991). However, Hart *et al.* (1978) reported higher levels in high yielding cows as compared to cows of lower genetic merit because the high genetic merit cows were in state of high energy deficit and also their plasma GLU was low.

Prolactin is linked with lactation in many species, but results from this study did not show any differences in PRL levels (basal and postmilking) which can be attributed to genetic selection. This is in agreement with several reports which did not suggest that PRL was altered as a result of intensive breeding for high milk production (Barnes *et al.*,1985; Bonczek *et al.*, 1988). This is not surprising, since the galactopoietic effects of PRL have not been established in ruminants. Neither reduction of basal levels nor increasing plasma levels by means of injection of bovine PRL have been shown to significantly affect bovine milk yield after the establishment of lactation (Karg *et al.*, 1972; Plaut *et al.*, 1987). However, it cannot be simply concluded that PRL has no role in maintenance of lactation in ruminants. There is a mechanism by which the mammary gland is protected from fluctuations in PRL concentration, which includes increase in intra-mammary accumulation of the hormone either by increase in transfer from blood circulation or *de novo* synthesis, as the ability of the mammary gland to synthesize PRL has been demonstrated (Steinmetz *et al.*, 1993). The ratio of milk PRL:plasma PRL concentration was increased after experimentally lowering PRL level by bromocriptine in ruminants (Beck *et al.*, 1979; Forsyth *et al.*, 1995), this suggests that PRL might be protected from intracellular lysosomal

degradation, there may be an increase in the transfer of PRL from circulation to the mammary tissue (against concentration gradient), or it could be as a result of the increase in *de novo* synthesis of PRL, all of which might be a contributing factor in ensuring that PRL will be readily available to the mammary gland. Some evidence in the rat suggests that PRL plays a role in metabolic adaptation in maternal tissues during lactation to support nutrient requirement for milk synthesis (Bauman & McCutcheon, 1986). If PRL is involved in such processes in ruminant lactation it may not be reflected by variation in levels of the hormone between cows differing in their milk production potential.

Like the basal levels, the amount of PRL released after milking was not significantly correlated with genetic merit, although HGM cows did tend to release more. High variation between cows within each group as well as the interference of seasonal factors also contributes to the difficulty in interpreting this data. The capacity of lactotrophs to release PRL *in vivo* as assessed by TRH stimulation was not found to be affected by genetic merit (Kazmer *et al.*, 1986). However, some evidence suggest that post-milking PRL surge might be more closely related to milk production than basal levels. The amount of PRL released at milking is highest at peak lactation and gradually declines as lactation advances in lactating cows (Koprowski & Tucker, 1973) but even then a correlation between milk production and amount of PRL released at milking was not demonstrated (Koprowski & Tucker, 1973). Also, infusion of PRL after milking in goats had little or no effect on milk production (Jacquemet & Prigge, 1991) which did not support a clear correlation between post-milking PRL surge and milk yield.

Increasing milking frequency from twice to four times a day significantly increased milk yield in both groups. The response was immediate and the average increase in milk was about 1.85 kg/d. A similar absolute increase in milk yield in response to increasing

milking frequency has been reported by others (Hillerton *et al.*, 1990; Knight *et al.*, 1992). The milk yield response to increasing milking frequency was not accompanied by significant changes in milk composition in this study. The increase in rate of milk secretion by frequent milking is mediated by local rather than systemic factors, since increasing milking frequency in one half of the udder significantly increases milk yield in that half only (Dewhurst & Knight, 1994). Moreover, we observed no significant changes in the endocrine profiles during frequent milking although there was a tendency for higher PRL concentration. This was probably a consequence of the sampling regime used in the experiment, in which all cows went through the parlour before bleeding but it was only the treated cows that were being milked. Therefore, the significantly higher PRL levels cannot be attributed to treatment factors because samples were taken while PRL level was elevated in response to milking stimulus in the treated cows. Physical distention of the udder does not appear to be involved in the response to frequent milking (Henderson & Peaker, 1984). Rather, the effect involves the existence of an autocrine regulatory protein that has been identified in goat's milk whey fraction and has been demonstrated to exert an inhibitory action on secretory activity of both *in vivo* and *in vitro* (Wilde *et al.*, 1987b). The inhibitor has been demonstrated to be synthesized by primary cultures of goat mammary epithelial cells and has been named the feedback inhibitor of lactation (FIL; Wilde *et al.*, 1995). As milk accumulates in the udder between milkings, a gradual increase in the concentration of FIL leads to a decline in the rate of milk secretion. Therefore, more frequent removal of milk, from the alveolar compartment, and hence FIL from the vicinity of secretory cells will result in an increase in the rate of milk secretion. It is most likely that the inhibitor acts through receptors located on the apical membrane of the secretory cells to regulate their secretory activity. After secretion, milk is stored in the alveolar lumen and small ducts (alveolar milk) or in the cistern and large ducts (cisternal milk). The relative importance of

this distribution is related to the proportion of milk that is stored away from its secretion site, since FIL is inactive in this area. Cows that store higher proportion of milk in the cisternal compartment might be anticipated to be more efficient in milk secretion. It has been recommended in the dairy cows that an interval of 8h after normal milking was an appropriate time for assessing milk storage sites (Knight *et al.*, 1994). Proportion of milk volume stored in the cistern (cisternal percentage) did not vary between HGM and LGM cows. It has been postulated in the goats that cisternal percentage was positively correlated to secretion efficiency (Peaker & Blatchford, 1988). In this study, there was no difference in secretion efficiency between the two lines and it would not be expected to find a trend for a correlation between secretion efficiency and cisternal percentage.

The increase in milk yield seen in response to increasing milking frequency must have involved an increase in the supply of nutrients to support the extra milk produced. The source of such nutrients must be from either increase in dry matter intake or mobilization of body stores. Feed intake cannot be assessed because of the lack of feed intake measurement in this investigation, but several reports which utilized milking frequency as a means for increasing milk production did not indicate that feed intake was stimulated by frequent milking (Pearson *et al.*, 1979; DePeters *et al.*, 1985; Kazmer *et al.*, 1986). There was no evidence from this study to suggest that body fat mobilization was activated since body weights and body condition scores were maintained during 4X period. This was also supported by the nonsignificant change in rate of lipolysis as judged by levels of plasma FFA and GLR. It is most likely that the extra nutrients came from the ingested feed, since the feed was supplied unlimited, but the increased in intake was too small to be noticed from the limited measurements made.

The plasma levels of β-HDB were greater in the treated cows during 4X period when compared to controls. Increase in ketone bodies is usually associated with energy drain

imposed by high levels of milk yield particularly during the first few weeks of lactation cycle when GLU levels are reduced. The decline in plasma levels of GLU triggers an increase in FFA levels and this metabolite can be converted to ketone bodies by the liver (Amaral-phillips *et al.*, 1993). Plasma levels of FFA and GLU were not significantly different from that of controls which suggests another mechanism way causing the rise in β-HDB. It is possible that this may have been related to an increase in ruminal production of butyrate, which is converted to β-HDB in the rumen wall and liver. Treated cows were receiving higher amounts of concentrate ration than controls and it is known that feeding higher protein diet may increase the production of β-HDB (Kronfeld, 1982).

The prolonged release formulation of bST utilized in this study resulted in a significant increase in milk yield over and above frequent milking. Unlike the milk yield response to bST injected daily which can be seen within 6 days of administration, the milk yield response was not seen until the second bST injection. The delayed-release formulation administered every 14 d has been known to show a cyclical pattern of milk yield (Bauman *et al.*, 1989). Knight *et al.* (1992) observed a better milk yield response in the second injection of delayed formulation compared to the first one. Although tremendous amounts of published studies describe the response to bST treatment, few studies have utilized bST in frequently milked cows. Treatment of cows milked four times daily with slow-release formulation of bST significantly increased milk yield by 3.9 kg/d (Armstrong *et al.*, 1990), and in another study a daily injection of 25mg of bST in cows milked three times daily for 127 days starting at week 7 of lactation increased milk yield by 6.1 kg/d (Jordan *et al.*, 1991). However, these studies did not include different milking frequencies so the magnitude of increase of milk yield above frequent milking cannot be assessed. Knight *et al.*, (1992) have reported a 14.2% increase in milk yield above that of milking four times

when cows were injected with 14 d bST preparation. Milk yield was increased by about 1.30 kg/d (5.7%) above that of 4X milking in this study. The lower response seen here was probably related to the nutritional status of the cows at the time of treatment. In this study, the treatments commenced at peak yield when the cows are normally in great demand of nutrients and there might be a limited availability of nutrients to further increase milk yield, whereas in the other study the treatments started several weeks after the cows had passed lactation peak and milk yield was declining, so that the cows had presumably shifted to a more positive energy state.

The mechanism by which bST stimulates milk yield is not fully understood, but it has been widely accepted that bST exerts its galactopoietic effects by altering the partition of postabsorptive nutrients between milk synthesis and body tissues synthesis (Bauman & Currie, 1980). Homeorhetic control involves the coordinated alteration of the metabolic processes of many body tissues that leads to the preferential direction of nutrients towards milk synthesis and inhibits the storage of nutrients in body tissues (Bauman *et al.*, 1985a; Peel & Bauman, 1987). The extra nutrients needed for milk yield response during bST treatment in the short term may be provided by decreased in body tissue synthesis if the cow is in positive energy balance before and during treatment, or by the release of nutrients by the mobilization of body stores if the cow is in negative energy balance or if the treatment forces the cow to be in negative energy balance (Bauman & McCutcheon, 1986). However, bST can eventually increase feed intake several weeks after the commencement of treatment so that the cows are able to a adjust their level of intake to match their additional nutrient requirement for milk production (Peel & Bauman, 1987). As the dry matter intake was not measured here, the assessment of bST effect on the increase in feed intake cannot be determined. Body weight as well as body condition score were not significantly changed during bST administration, which indicates that no major body reserve mobilization took

place during this period. Body condition score is only indicative of the amount of subcutaneous fat and, therefore, may not be a sensitive enough way to determine changes in adipose tissue mobilization within such a short period of time. Plasma levels of FFA and GRL provide additional information. Treatment with bST did not result in a significant elevation of plasma levels of FFA or GRL, supporting the conclusion that substantial lipolysis had not been elicited by bST. Treatment with bST may not cause an acute increase in plasma levels of FFA, but it can elicit a chronic elevation when treatment causes the cows to be in negative energy balance (Bauman *et al.*, 1988). In this study, there was a small tendency for increase in FFA during both periods of bST injection suggesting a trend towards increase in the rate of adipose tissue lipolysis. Milk fat tended to be higher in treated cows (P=0.03) and protein percent was significantly lower (P=0.03) and these changes in milk composition are typical of cows treated with bST when the treatment results in negative energy balance (Peel *et al.*, 1983). The lower milk yield response to bST seen in this study was mostly related to the lower ability of bST to induce fat mobilization which was related to time of treatment; cows were most likely just recovering from an acute state of negative energy balance in which they mobilized most of their fat reserves. Lipolytic effects of bST may be dependent on the energy status of the cows at the start of treatment, so in cows at peak lactation with lower body reserves, treatment with bST may not change the plasma levels of FFA when compared to treatment during mid lactation when the cows presumably shift to a positive energy balance (McDowell *et al.*, 1987). Although there might not be a substantial increase in the rate of adipose tissue mobilization, the metabolism of the adipose tissue must have been altered in a way that a major inhibition or suppression of lipogenesis has occurred (Bauman, 1992). Perhaps nutrient density of the ration coupled with inhibition of nutrient deposition in body tissues might have accounted for the supply of the extra nutrients required for milk production during bST treatment. The bST effects

on carbohydrate metabolism in the dairy cow is well established, it reduces glucose oxidation by peripheral tissues and increases the rate of glucose synthesis by the liver. Irreversible loss rate of glucose has been shown to be increased in bST treated cows together with a reduction of glucose oxidation to CO_2 but plasma glucose concentration was not affected by treatment (Bauman *et al.*, 1988) which constitute one of the coordinated alterations of metabolism in non-mammary tissues to supply the glucose needed for the increase in milk synthesis. Despite this alteration in GLU kinetics, glucose homeostasis was not affected by bST treatment in the present study as there are no changes in plasma levels of GLU during bST periods.

Stimulation of milk production by bST is not only through increasing the availability of milk precursors, but also involves an increase in the cardiac output and blood flow perfusing the udder (Davis *et al.*, 1988a,b). However, this is not the end of story; how does GH enhance the ability of secretory tissues to produce more milk? Is there any direct action of GH on mammary gland tissues? It is unlikely that GH exerts a direct action, since GH receptors cannot be detected in the mammary gland by conventional binding assays (Akers, 1983; Gertler *et al.*, 1984). It has been proposed that GH might affect mammary gland function indirectly through IGF-I, as the presence of IGF-I receptors in the mammary gland has been demonstrated (Dehoff *et al.*, 1988) and the mitogenic activity of IGF-I on bovine mammary tissues has been reported (Baumrucker & Stemberger, 1989). Treatment with bST significantly increased plasma concentrations of IGF-I in both treated groups compared with controls. Although IGF-I is produced by various tissues, the liver is the major source of circulating IGF-I and its synthesis is partially under the control of GH. Treatment with bST in cows has been known to stimulate IGF-I production (Davis *et al.*, 1987). This is partially related to an increase in the rate of IGF-I synthesis, as has been shown by an increase in hepatic IGF-I mRNA (Sharma *et al.*, 1994). However, Newbold *et al.*, (1997) have shown

that the increase in IGF-I in response to slow release formulation of bST, similar to that administered in this study, was not due to any increase in the concentration of hepatic GH binding sites. Also, another study where bST was injected daily to lactating dairy cows did not result in any changes in the abundance of mRNA for GH receptors or the number of free binding sites for GH despite the increase in plasma levels of IGF-I (Vanderkooi *et al.*, 1995). Therefore, the mechanism related to the increase in IGF-I production elicited by bST treatment may not involve an alteration in the number of GH binding sites, and it is possible that alteration in the function of the receptors that leads to an enhancement of GH binding might be involved. The increase in IGF-I plasma levels during bST administration is consistent with the suggestion of a role for IGF-I in mediating the milk production response. Systemic infusion of IGF-I to lactating goats did not mimic the effects of GH administration on milk yield stimulation (Davis *et al.*, 1989), but IGF-I had some stimulatory effect on milk secretion when it was given via close-arterial infusion into the mammary gland (Prosser *et al.*, 1990) although this response was subsequently shown to be attenuated by increasing milking frequency (Prosser & Davis, 1992). So, the effect of IGF-I on milk secretion is inconclusive. In the rat, IGF-I, IGF-II, and IGF-binding protein-3 administered concurrently failed to mimic the galactopoietic effect of GH on milk secretion (Flint *et al.*, 1994). Utilizing coculture of mammary, liver, and adipose tissues, the presence of IGF-I did not stimulate lipid or protein synthesis by the mammary tissues (Keys *et al.*, 1997) which does not support a direct galactopoietic effect of IGF-I.

Treatment with bST did not affect INS concentration in the plasma, although there was a small increase in LT cows during the second bST injection. Reported effects of bST treatment on INS secretion in dairy cows are not consistent. Bines *et al*, (1980) have reported that plasma INS was increased by bST injection while another study reported only a small trend for increase (Davis *et al.*, 1989). Stimulation of INS release may be related

to the energy status of the cow, for INS rise was evident only in cows in positive energy balance i.e. low yielding cows or at late lactation (Bines *et al.*, 1980; Vicini *et al.*, 1991).

The administration of T4 resulted in a rapid and significant increase in milk yield over and above that of 4X plus bST in cows from both genetic lines. The galactopoietic effect of T4 in cattle has been known for a long time. Thyroprotein feeding (which contains low amount of T4) has been shown to stimulate an increase in milk yield of about 10-25% and several factors can affect the milk yield response such as dose, energy status, the availability and quality of feed, and environmental factors (Meites, 1961). More recent studies have reported that injection of 20mg/d of T4 for short periods to Jersey cows elicited an increase in milk yield of 15 or 25%, depending on the stage of lactation (lower response at peak lactation) (Davis *et al.*, 1987; Davis *et al.*, 1988a). Several studies have indicated that long term feeding of thyroactive material elicited an immediate increase in milk yield which was not sustained but lasted for about 2-3 months, after which a gradual decline in yield occurred which continued even after the cessation of treatment (Seath *et al.*, 1945; Hibbs & Krauss, 1947). The administration of thyroactive proteins proved not to be economical, since it increased metabolic rate in the whole body and as a consequence the maintenance cost was increased leading to a reduction in the efficiency with which feed was utilized for milk production (Thomas *et al.*, 1954). Therefore the use of thyroactive proteins as a means for milk yield stimulation in cattle which was proposed in the forties and fifties never came to fruition. It was used in this investigation as an experimental tool for increasing milk yield above that of frequent milking and bST so that a maximum milk yield capacity could be achieved.

The mechanism involved in relaying the effect of T4 on milk yield stimulation has not been fully elucidated. T4 treatment is associated with elevation in whole body

metabolism as indicated by the rapid changes in heart rate (increased by about 35% in this study). It has been estimated, indirectly through heat production, that a 30% increase in basal metabolism occur with a dose of 25gm/d of thyroactive protein in lactating cows, which is not as a high dose as that utilized in this study (Thorbek *et al.*, 1948). This increase in basal metabolic rate to T4 has been shown to be directly proportional to dosage in sheep (Blaxter, 1948a). The galactopoietic effect of T4, therefore, can be partially related to increase in the metabolic processes of the secretory tissues of the mammary gland as metabolism was generally elevated in the whole body. However, some evidence suggests that T4 causes specific effects in the mammary gland. Cardiac output which was increased by T4 was also associated with an increase in the proportion of cardiac output perfusing the udder (Davis *et al.*, 1988a). This will support the increase in milk synthesis by increasing the nutrient supply to the mammary gland. A significant increase in the uptake of one essential blood metabolite, glucose, by T4 administration was shown (Davis *et al.*, 1988b). However, there was also an increase in the ratio of glucose uptake to lactose output as a result of T4 treatment (Davis *et al.*, 1988b) which supports the contention of lower milk production efficiency in lactating cows receiving the thyroxine treatment. The increase in energy requirement to support greater milk production together with higher metabolic rate of the whole body tissues elicited by T4 would have challenged the cows for nutritional sources to satisfy their higher metabolic demand. Maintaining cows on thyroactive protein feeding for long times may be associated with increased in feed intake, but the losses in body weight seen in many studies with moderate doses of thyroprotein feeding were suggestive of the cows relying more on endogenous sources for meeting the extra energy requirement. Whether an increase in dry matter feed intake occurred during the T4 period cannot be determined in this study, but even if it had, this was most probably not enough to cover the elevation in nutrient requirement. The cows were losing body weight which was

continued during 1X/4X treatment period and the recovery period. A 10-15% loss in body weights during hyperthyroidism was also observed when moderate doses of thyroprotein were fed to lactating cows (Seath *et al.*, 1945; Hibbs & Krauss, 1947) which can be partially prevented by providing additional concentrate feeding, but this was not entirely effective in avoiding the dramatic loss in body weight (Thomas *et al.*, 1954). In sheep, hyperthyroidism induced by thyroprotein feeding depressed body weight by 8-20% in about 3 weeks and postmortem body composition analysis revealed that this was attributed to losses in muscle, bone and particularly body fat (Blaxter, 1948b) which suggest a rapid body catabolism, increase in protein deamination and adipose tissue lipolysis. In our cows, the severe depression in body weights together with decrease in body condition score values once T4 treatment started was strongly suggestive of high fat mobilization. There was also an acute and rapid increase in FFA and GRL levels, suggesting increased rate of lipolysis. However, the high rate of adipose tissues lipolysis seen in this study may not have been entirely elicited in response to a greater demand for energy, the lipolytic activity of T4 was most probably enhanced by bST, since it has been shown that bST enhances the lipolytic stimulus of epinephrine (Sechen *et al.*, 1990).

The increase in glucose consumption by the mammary gland as reflected by higher milk lactose synthesis together with elevation in plasma GLU levels seen in this study were indicative of increase in the rate of hepatic tissues gluconeogensis and maybe a reduction in oxidation of glucose by peripheral tissues. This increase in plasma levels of GLU was in accordance with other studies in lactating cows and sheep (Blaxter, 1948b; Davis *et al.*, 1988b) which might contribute in part to the galactopoietic effect of T4 in lactating cows. The gluconeogenic activity in the liver must have been activated, indeed it has been shown that treatment of sheep with high dose of thyroactive protein is associated with hypertrophy of the liver (Blaxter, 1948b). Also, T4 administration was associated with an increase in the

rate of glucose synthesis in liver tissues as indicated by the increase in activities of key enzymes involved in gluconeogensis (Heitzman *et al.*, 1971). The major and rapid changes in body weights and body condition score may have been of significance in providing precursors for glucose synthesis such as glycerol, lactate and probably amino acids which can be used as substrate for glucose synthesis (Vernon, 1988). T4 not only increases the availability of GLU but also increases the uptake of GLU by the mammary gland (Davis *et al.*, 1988b), thus supporting the increase in rate of milk production. Plasma GLU was elevated despite higher consumption by the mammary gland indicating that T4 enhanced the supply of GLU even more than the capability of the gland to utilize it. Higher plasma glucose concentration was associated with a fall in plasma level of INS. Similar findings were also reported in lactating cows (Davis *et al.*, 1987; 1988a). Presumably the anabolic activity of INS would have contradicted the catabolic effects of T4, thus a reduction in INS levels facilitated the generation of energy substrate needed to support higher metabolic rate. This fall in INS might be one of the pathways involved in the galactopoietic effects of T4 on milk yield.

The stimulation of milk yield by T4 was associated with a significant increase in milk fat percentage ($P=0.01$) but protein percentage was reduced significantly ($P=0.01$) in both treated groups when compared to that of controls. These changes in milk composition are in accordance with similar changes seen with thyroactive material feeding in lactating cows (Blaxter *et al.*, 1949).

The significant decline in period means of PRL when T4 treatment started suggests a possible interference between thyroxine and post-milking PRL release. However, basal PRL level determined from the frequent (hourly) samples were not suppressed, indeed they were elevated. This suggests that T4 might be responsible for the decline in PRL release after milking by acting via the negative feed back pathway on hypothalamic TRH release

which has been suggested to be involved in eliciting PRL surge after milking (De Greef *et al.*, 1987). Alternatively, the higher basal levels during T4 treatment may result in exhaustion of releasable PRL stores in the pituitary gland; Koprowski & Tucker (1973) reported a negative relationship between basal concentration of serum PRL and subsequent milking-induced release of PRL.

Average hourly PRL levels were about twice that in the controls during T4 treatment, and no assessment of basal PRL levels were made during 4X and bST treatment to be compared with that in T4 period. Evidence from the rat suggests that thyroid status exerts some influence on the secretion of PRL; T4 injection to euthyroid rats significantly increased PRL synthesis *in vivo* (Chen & Meites, 1969). Due to the spontaneous release of PRL and uncertainty of the presence of a definite PRL releasing factor, it is suggested that basal level is controlled by the PRL inhibiting factor, dopamine (Ben-Jonathan., 1985). So T4 might be affecting the release of dopamine into the portal system or it could act directly at the pituitary level to modulate the binding of dopamine to its receptors. The first explanation might be more acceptable since T4 has been demonstrated to greatly enhance the stimulatory effect of oestradiol on PRL secretion in thyroidectomized and ovariectomized rats by inhibiting the secretion of dopamine into hypophysial portal blood system (Wang *et al.*, 1994).

There was a tendency for T4 to result in low circulating GH in both lines. The high heart rate and the increase in cardiac output evoked by T4 which resulted in increase in blood flow in body tissues might have increased the clearance rate of GH. Furthermore, GH binding is enhanced by hyperthyroidism (Hochberg *et al.*, 1990) which in turn enhances the receptor mediated GH clearance from the circulation. Average GH concentration indicated that GH was increased in the first week of T4 injection, which coincided with the third bST injection in both genetic lines. The decline did not start until the second week of T4 in

which GH levels were declined in both lines with greater decline in LT cows. In the fourth bST injection, the cyclical GH pattern was not evident and this may be related to T4 treatment which prevented the initial rise in GH.

The decline in plasma IGF-I during T4 treatment was indicative of modulation in synthesis or clearance rate. Similar effects of T4 on IGF-I have also been shown in lactating Jersey cows injected with 20mg of T4 (Davis *et al.*, 1987). Experiments in rats (non-lactating) have demonstrated that circulating levels of IGF-I and IGF-I mRNA in the hepatic tissue were normal or increased in hyperthyroid rats (Miell *et al.*, 1993; Thomas *et al.*, 1993). Nonetheless, the bioactivity of the hormone was markedly inhibited. This suggests that T4 may not necessarily interfere with IGF-I synthesis and, therefore, the catabolic effects of T4 exhibited in the body tissues might be secondary to lower IGF-I activity. The biological activity of IGF-I is modulated by its binding proteins as the majority of circulating IGF-I is bound to IGFBPs with very low percentage found free in the blood stream. Thus, any changes in concentration and relative proportions of IGFBPs in extracellular fluids will modulate the localization of IGF-I in different tissues and hence their biological activity. Most circulating IGF-I is found bound to the 150 kd acid-labile-subunit of IGFBP3 (Barreca *et al.*, 1995) which is believed to be mainly responsible for maintenance of IGF-I pool in the circulation and for increasing the half life of the hormone. Much less IGF-I is bound to IGFBPs with lower molecular weights. If this proportion is reversed by decrease in IGFBP3 production or an increase in the production and binding of other IGFBPs (IGFBP1 or 2), this will result in decreasing the half life of IGF-I in the circulation and increasing its clearance from the circulation and significantly lowering its plasma levels. The modulation of IGF-I concentration in plasma by binding to IGFBPs has been proposed as a physiological adaptation in situations when growth is being compromised such as stress or severe malnutrition (Hossner *et al.*, 1997) which also might be true in the

case of hyperthyroidism. This is supported by finding that IGFBP1 increased with hyperthyroidism (Angervo *et al.*, 1993) and if the proportion of IGF-I bound to IGFBP1 is increased, this might participate in the inhibitory activity of T4 on IGF-I action by reducing the half-life of the hormone and also reducing its binding to type 1 IGF-I receptors (Jones & Clemmons, 1995). A possible depression on IGF-I gene expression by T4 cannot be excluded especially in this situation when T4 caused the cows to be in a state of low energy balance. It is accepted that T4 has affected IGF-I action by either affecting its synthesis or (and) inhibiting its bioactivity by modulating IGFBPs, both may participate in promotion of the catabolic activity of T4 on body tissues by inhibiting the anabolic activity of IGF-I.

The ability of lactating cows to respond to T4 treatment so that milk yield was increased above that of bST is indicative of the additive trend of these stimuli and also suggestive that bST and T4 were affecting milk yield by different mechanisms. Comparison studies between the effects of bST and T4 on milk production which were carried out by Davis *et al*, (1987; 1988a,b) have indicated that both of them gave a similar response in milk yield when injected for short term. However, a possible variation in their mode of action might exist; T4 caused a dramatic changes in homeostasis as indicated by higher GLU levels, and also substantial effect on body catabolism to support higher rate of whole body metabolism. On the other hand, bST resulted in increases in the metabolic activity of secretory mammary tissues while metabolism of other body tissues is altered so a greater proportion of nutrients can be utilized for milk synthesis. As a result, homeostasis was not altered by bST despite its effect on alterations in tissue response to homeostatic signals (Vernon, 1988).

Stimulation of milk secretory capacity elicited by the galactopoietic stimuli utilized in this study was accompanied by a significant stimulation of the growth of the udder. This represented a true udder growth which cannot be attributed to the presence of milk in the udder at the time of determination because the udder was emptied with oxytocin injection just before the measurement. The contribution of each galactopoietic stimuli on udder size during sequential treatments cannot be determined because measurement of udder size was not done during 4X or bST treatment, but it was measured during maximum treatment stimuli (T4 period). The increase in udder size might has been attributed to increase in epithelial cell number (hyperplasia) or cellular differentiation (hypertrophy). More frequent removal of milk from the goat udder increases secretory cell metabolic capacity but does not increase cell number until the treatment is applied for a long time (Wilde *et al.*, 1987a). Also, the metabolic activity of the secretory cells in the mammary gland was increased by four times milking in cows and histological analysis indicated an increase in cellular differentiation and hypertrophy (Hillerton *et al.*, 1990). Combined treatment of bST and frequent milking in goats increased parenchyma volume as assessed by magnetic resonance imaging, but without any increase in total cell number of the secretory epithelium suggesting that cellular hypertrophy was responsible (Knight *et al.*, 1990b). However, there was some evidence in the cows that frequent milking stimulated increase in cellular proliferation as indicated by an increase in DNA synthesis *in vitro* and in the number of epithelial cells per alveolus (Hillerton *et al.*, 1990). Therefore, the udder growth that has been observed in the treated groups can be attributed to a combined effects of frequent milking and bST; the former increased cellular differentiation and proliferation while the latter reduced the cell death and hence increased the longevity of the secretory cells.

Having established the upper metabolic capacity of cows from different genetic

merit, the next area that this study was designed to explore was if the restriction point determining maximum output was localized at the mammary gland level itself or elsewhere in the body. Maximum metabolic limit is determined by two factors, machinery associated with the supply of nutrients and machinery associated with the consumption of nutrients, which in the case of the lactating animal would be the mammary gland (Hammond & Diamond, 1997). The first factor is concerned with the availability of a well balanced ration at adequate levels, rate of feed intake, capacity of the alimentary tract for digestion and absorbtion, rate of processing of metabolites needed for milk synthesis and also the availability of body stores to cover for any energy deficit (body stores mobilization). Furthermore, another component which is related to the availability of nutrients is the direction of these nutrients to the mammary gland. At the mammary gland level, the synthetic capacity of secretory tissues, which is determined by the number and differentiative state of secretory cells, might be a limiting factor when considering the ability of secretory cells to increase their synthetic capacity. The method adopted to localize the restriction point was to reduce milking frequency in half of the udder to once daily while maintaining the other half on four times daily and at the same time maintaining the cows on the galactopoietic stimuli (bST+T4) and the same feeding system. At normal levels of output, reduced secretion in one half of the udder triggers a compensatory increase in the other half (goats: Henderson & Peaker, 1980. cows: Hamann & Reichmuth, 1990), showing that individual mammary gland yield had previously been submaximum. In other words, output was being regulated (restricted) at the level of the whole animal, not the mammary gland. The question that arises is would this still be the case when galactopoietic stimuli were applied to specifically increase mammary synthetic function, or would the point be reached where the udder was functioning to its maximum capacity such that it had become the restriction point?

Total milk yield declined by about 24% when milking frequency regime was changed to 1X/4X milking. RMYQ values which compare changes in the milk yields of 1X and 4X halves indicated less than unity value in the treated groups which was attributed to the decline in milk production in the half udder milked once daily. Reducing milking frequency from twice to once daily milking for one week resulted in a reduced milk yield by about 22% in cows (Knight & Dewhurst, 1994), and 26% reduction during 2 weeks of once daily milking was obtained in lactating goats (Wilde & Knight, 1990). The decrease in milk yield in response to once daily milking was the result of the inhibitory activity of FIL exerted on the secretory cells. Less frequent milking and infrequent removal of the inhibitor which increases its concentration as a result of accumulation, as well as increase in the time to which the secretory cells are exposed to the inhibitor ultimately reduces the rate of milk secretion (Wilde *et al.*, 1989). The reduction in rate of milk secretion was mostly related to a decline in the cellular differentiation as suggested by lower activities of some key enzymes during the transition from twice to once daily milking in goats (Wilde & Knight, 1990).

The milk yield of the half udder milked four times was not increased to cover the loss of yield caused by reducing the milking frequency in the other half, which indicates that the mammary gland had already been milking at its potential since it could not increase its rate of milk secretion, therefore, the maximum metabolic capacity was determined at the mammary gland level. However, this interpretation should be treated with caution since milk yield in the half udder milked four times actually decreased during this period which was probably related to the deleterious effects of thyroxine treatment for this length of time and dose. Major body catabolism had occurred to support higher demand for both milk yield and whole body metabolism. If this had reached a point where essential body functions were being compromised then non-essential processes, such as lactation, would have been shut

down. The fact that total milk output was decreasing and continued to decrease for some time after the treatment ended suggests that this was indeed the case. Different conclusion might has been obtained if the cows were not in a such severe body catabolism and substantial negative energy balance. A follow-up experiment has been done to test this, using the same sequential treatments of 4X, bST (by daily injection) and T4 but for a period of 5 days each. Milking frequency was then reduced to twice daily in one half of the udder while maintaining the other half on 4X (Sorensen A & Knight CH, personal communication). Reducing milking frequency resulted in a decline in milk yield in the less frequently milked half, but the total milk yield was not affected because there was an increase in the yield of the 4X half to compensate for the loss. This clearly shows that the restriction point in determining the maximum metabolic capacity was not localized in the udder as indicated by the ability of the mammary tissues to increase its rate of milk secretion so that the milk yield was sustained at the maximum level. Therefore, the maximum capacity is determined at the level of the whole body and not at the mammary gland level as was suggested above.

The behaviour of plasma glucose level during T4 treatment was inversely correlated with milk yield; glucose was greater in the 1X/4X period than during the T4 period, reflecting the dominance of the mammary gland in glucose clearance from the circulation. The tendency for elevated glucose does not necessarily reflect higher rates of gluconeogenesis, but rather the decline in mammary uptake and utilization of glucose as milk yield fall. In the lactating ruminant most of the glucose produced (60-85%) is utilized by the mammary gland (Annison & Linzell, 1964; Bickerstaffe *et al.*, 1974). Plasma concentrations of INS tended to recover from the depression that was seen during T4 period, probably as a response to the higher plasma glucose. Despite normal INS levels, GLU was maintained at high levels. This apparent INS resistance state may be accounted in part by

the antagonizing action of GH on INS action by peripheral tissues (Vernon, 1988). However, plasma GLU levels have been shown to be elevated independent of bST treatment (Blaxter, 1948b; Davis *et al.*, 1988b) suggesting the disruption of GLU homeostasis was likely to be evoked by T4 treatment. Furthermore, INS sensitivity is decreased with elevation of plasma T4 in humans (Ohguni *et al.*, 1995).

The drop in milk yields in the treated groups during the recovery period was so dramatic that it resulted in yields being significantly lower than that of the control cows. It is well known that thyroprotein feeding stimulates milk yield for some time but that milk yield declines following cessation of thyroprotein feeding and yields continue at subnormal levels thereafter (Swanson, 1954). Several attempts have been tried in the early studies to prevent the loss in milk yield after the cessation of treatment such as gradual withdrawal of the protein from the diet, transferring the cows into a good pasture or providing extra concentrate feeding, but these have not been successful in completely preventing the drop in milk yield (Swanson, 1954; Thomas *et al.*, 1954). The decline in milk yield can be related to continuation of T4 treatment and also to the cessation of its administration. It was obvious in this study that T4 treatment had pushed the cows into a state of energy deficit which was met by body fat mobilization to support high metabolic demands. The total milk yield declined during 1X/4X period which presumably resulted in a decline in the nutritional requirement of the mammary gland as a result of the decline in the metabolic activity of the less frequently milked udder half. Indeed, the milk yield continued to decline even after resumption of twice daily milking. This decline in milk yield can be viewed as an alleviation measure taken by the cows to reduce their energy expenditure. In this study a relationship between changes in milk yield and body reserves mobilization while the cows received T4 can be suggested; milk yield was high during the first few weeks of treatment because of higher mobilization but the yield then declined as a result of depletion of these body

reserves. It is known for bST that the energy requirement to support greater milk yield is supplied by body reserve mobilization in the first few weeks of treatment, but a gradual increase in feed intake will occur after some time so that the body reserves will be replenished. In the case of T4 the situation is different in a way that the cows tended to lose their dependence on energy from ingested feed so that their dependence on endogenous sources is not alleviated by long term treatment. The loss in body weight during T4 administration may not be entirely related to catabolism of body tissues, a decline in gastro-intestinal fill might be a contributing factor in changes in the body weight (Swanson, 1954). Furthermore, the digestibility of dry matter might be compromised (Blaxter, 1948a) and even the appetite may not be maintained especially with high doses (Blaxter, 1948b). Taken together, these possible alterations in the nutritional status might introduce a limiting factor to the supply of nutrients which are derived from feed intake particularly with high dose or treatment with T4 for long time. Therefore, it was possible that the reason that milk response to T4 was not sustained in this study was related to decrease in the nutrients availability from both exogenous as well as endogenous origins as treatment continued. At the first few weeks of T4 injection, the cow was capable of meeting the great demand of energy for maintenance as well as milk production from feed intake and body stores mobilization. However, continued T4 administration which resulted in depletion of body stores with concurrent decline in nutrients available from the ingested feed, imposed a restriction on the availability of nutrients resulting in shifting in the priority of partition of nutrients to support the high basal metabolic rate in the whole body tissues at the expense of the metabolic activity in tissues which are not vital, like the mammary gland.

The significant decrease in udder volume during the recovery period in the treated cows coupled with lower milk yield indicated that the mammary growth stimulation by the application of galactopoietic stimuli was abolished. The decline in yield was associated with

significantly reduction in secretion efficiency. This suggests that secretory activity of epithelial cells was reduced, which might be related to a decrease in the rate of secretory cell differentiation. However, an increase in mammary tissue involution cannot be excluded as a contributing factor to the decline in milk secretion. In fact, the observation of absence of recovery after long term thyroactive feeding is suggestive of loss of secretory cells. The mechanism by which T4 is causing such an effect at the level of the mammary gland, or whether this effect is related directly to the hormone or is a consequence of major alteration in body metabolism and severe body catabolism is not known. The decline in milk synthesis might be related to a decrease in the blood flow perfusing the udder or a mechanism by which the uptake of nutrients was attenuated. The accumulation of GLU in plasma during 1X/4X period and a similar trend in the recovery period is supporting this claim. The fact that the milk yield of the half that was milked once daily remained below that of the other half maintained at four times milking during the recovery period is somewhat surprising. Short term infrequent milking had no effect on lactation persistency when cows were returned to their normal milking (Knight & Dewhurst, 1994), which suggest that reducing milking frequency for a short time does not cause a detrimental effect on secretory cells. The maintenance of less frequent udder half yield below that of the other half was suggestive of a possible interaction between FIL action and deleterious action of T4 so that a detrimental effect on the secretory epithelium had occurred in that half. This might have arisen from alteration in response of the mammary tissues to systemic factors in the half milked once daily. Infrequent milking which increases the inhibitory action of FIL on secretory cells also lowers the number of PRL receptors (McKinnon *et al.*, 1988). Also, FIL can decrease the binding of IGF-I in mammary membranes (Bennett, 1993). Therefore, it was possible that the inhibitory effect of FIL on secretory cells was exacerbated by the proposed inhibitory action of T4.

The main objective of this study was to determine the maximum metabolic capacity of lactating dairy cows from different genetic merit and thereby assess how close these cows were functioning in relation to their maximum capacity. Numerous factors with different modes of action are involved in the control of milk secretion so that more than one galactopoietic stimuli was adopted to achieve the upper metabolic limit. A combination of a local factor, removal of FIL by the action of frequent milking, and treatment with GH and T4 were applied in stepwise fashion in addition to the availability of adequate well-balanced ration to fulfil the target of driving the cows to their maximum metabolic capacity.

The tendency for higher response to increase in milking frequency in HT cows might have been related to the relatively lower cisternal percentage compared to LT cows (20.77 vs 26.73% , $P>0.05$, ANOVA; for HT and LT groups, respectively). It has been proposed that the proportion of milk stored in the cistern will determine the effect of changing milking frequency, so cows with small cisternal capacity will benefit from increasing the frequency of milking more than cows with larger cistern (Dewhurst & Knight, 1994).

In agreement with what has been reported by other research groups, no interaction between bST treatment and genetic selection for milk yield was evident (McDaniel, 1988; Nytes *et al.*, 1988). However, the level of milk production might affect the bST response, cows with lower pre-treatment milk yields responded with greater increase in milk yield after bST compared to cows with higher milk yields (Leitch *et al.*, 1987). There was a non-significant trend for a better response in LT group. One of the key factors in provoking the stimulatory effect of bST on milk production is the availability of body reserves for mobilization (Bauman, 1992). LT cows had greater body condition score, reflecting higher fat stores that could be mobilized and this may explain this better response to bST. This was supported by a tendency for elevation in plasma levels of FFA during bST periods in LT cows, reflecting greater rates of lipolysis. Furthermore, body condition score declined

during the second period of bST in LT suggesting greater fat mobilization.

Although HT exhibited greater GH concentrations than LT cows after the administration of bST, the difference reached a significant level during the first bST injection only. This cannot be related to difference in dose:body weight because there was no differences in body weight between the two groups. This tendency for difference in GH concentrations was suggestive of variation in clearance rate between the two genetic lines; slower metabolic clearance rate in HT. In a situation when growth is compromised due to scarcity of nutrients available to be deposited in body tissues which inhibits anabolism in peripheral tissues, half-life of GH is increased and clearance rate is reduced in cattle (Trenkle, 1976; Lapierre *et al.*, 1992). Circulating GH is cleared in part by receptor-mediated cellular uptake and subsequent degradation within clearance tissues (Harvey, 1995) so it might have been related for example to poor receptor binding in these tissues.

Levels of IGF-I and GH in both lines during bST treatment indicated an inverse relationship between the two hormones; high GH levels in the high line cows were not associated with higher IGF-I levels. This paradox occurred in this study because GH is not the only factor that regulats IGF-I synthesis, nutritional status is also exerting an effect. In some situations when growth was temporarily retarded i.e., as a result of lower ingested nutrients, GH stimulated IGF-I synthesis was reduced in cattle (Breier *et al.*, 1986; Ronge & Blum, 1989). It is not only the basal levels of IGF-I which are affected by nutritional status, bST-stimulated IGF-I is also affected; in lactating cows the IGF-I response to bST was diminished by restricted energy intake (McGuire *et al.*, 1992) or reduced in early lactation when the cows were in negative energy balance compared to later in lactation (Vicini *et al.*, 1991). Generally, concentration of IGF-I is a reflection of anabolic status so that higher IGF-I is associated with higher planes of nutrition, and therefore, IGF-I could serve as an indicator of energy status modulating the stimulatory effect of bST on milk

production (McGuire *et al.*, 1995). A variation in energy status between HT and LT cows was anticipated, HT cows might have been in lower energy status than LT cows. This does not necessarily mean that HT was in negative energy balance and LT was in positive energy balance, but that HT cows had a higher demand for nutrients which resulted in a reduction in the availability of nutrients to be deposited in their body stores and hence in less anabolic status than LT cows. It is possible that changes in circulating concentration of IGF-I might signal the biological events and the magnitude of milk response that occur with bST treatment. Therefore, if stimulatory effect of bST on IGF-I synthesis is involved in stimulation of milk secretion, the smaller response of the HT cows may provide a protective measure to reduce the amount of nutrients directed to milk synthesis. This can be of significance in the case of lower availability of nutrients during lower feed intake or in high yielding dairy cows treated with bST during early stage of lactation, since by this adaptation the chance of collapse of metabolic control can be avoided. The cellular mechanism for this variation may be related to changes in hepatic GH receptors, as energy status can influence the GH binding sites. It has been reported that lower energy balance in heifers evoked by feed restriction decreased the mRNA for GH receptors and this was then associated with lower IGF-I mRNA (Vandehaar *et al.*, 1995). Also, in lactating cows, GH binding sites in the liver was related to nutritional status (Newbold *et al.*, 1997). Rose *et al*, (1992) grouped cows into high and low responders to short term bST treatment, and they observed that high responding cows were associated with lower GH peak after the injection but higher IGF-I plasma levels concentration. Therefore, it is conceivable that the extent of milk yield response to bST is greatly affected by the clearance rate of the hormone from the circulation which is dependent on the abundance of functioning GH receptors which is regulated by the nutritional status.

There was no difference in homeostatic balance between high and low genetic merit

cows, as measured by basal blood metabolite concentrations of glucose, free fatty acids, glycerol and β-hydroxybutyrate, during treatment periods, before the addition of T4 to treatment combination. However, there was a trend for a difference between them in plasma levels of glucose after T4 administration; low genetic merit cows tended to show higher plasma levels suggesting reduced glucose clearance from the circulation. The effect of T4-stimulated lipolysis was not influenced by genetic factor in this study since plasma levels of FFA and GLR were not significantly different between the two treated groups. This result agrees with another study (Michel *et al.*, 1991), in which epinephrine-stimulated acute lipolysis was not affected by genetic selection.

There was no evidence to support the contention that cows from high or low genetic merit were normally milking at different points relative to their capacity, so high genetic merit cows were not exposed to potential metabolic disturbance more than cows of low genetic merit. When they were pushed very hard to increase their output, cows showed similar responses. High genetic merit cows were milking at higher level than low genetic merit cows which means that their energy budget was also higher, but during maximum stimuli both groups exhibited a similar rate of body fat mobilization, as indicated by plasma levels of glycerol and free fatty acids. This might suggest that both groups were at a similar level of energy deficit despite the distinct difference in their energy expenditure and this might be related to a greater dependence of high genetic merit cows on feed intake to meet their nutritional requirements.

It can be concluded that high genetic merit cows were not operating close to their metabolic capacity as it was proposed and therefore they were not at more risk of collapse of metabolic control than low genetic merit cows. Cows from different genetic merit, under good management, are milking at a similar submaximum levels probably to maintain a

steady level of milk production and avoid any disturbances related to higher metabolic rate. Although there was no difference in risk between cows from different genetic potential when they were fed adequately, would the same conclusion be drawn if input (feed intake) was restricted while the cows were at their utmost output? This question is certainly deserving of further study.

CHAPTER SIX

Summary and conclusions

Milk yield is the function of the total number and activity of secretory cells, but several factors can be manipulated to influence milk yield. This thesis involves studies on manipulation of milk yield utilizing different galactopoietic stimuli. Of course each stimuli has its own mode of action and, therefore, it would be expected to observe some variations in response among these galactopoietic factors. Also, the responsiveness to such stimuli might vary between individuals.

First, an environmental factor, photoperiod, was manipulated to investigate its effect on milk production. Experiments were conducted with goats which utilized photoperiod as a potential stimulatory factor on milk yield and to examine if the response can be potentiated by short periods of conditioning with melatonin treatment (chemical short days). In this study, exposure to long light did not produce a clear stimulatory effect on milk production, but a small response was evident in autumn of the first year experiment and only in goats that were not primed by melatonin. This response was not observed when the experiment was repeated in the following year. The mechanism by which extended hours of lighting affect milk secretion is not fully elucidated, and several factors have been proposed, such as increase in feed intake or release of galactopoietic hormones from the anterior pituitary gland. Although PRL levels showed a parallelism with milk yield during the lactation cycle which might suggest a possible regulatory role of PRL in milk secretion, the absence of any association between stimulatory effect of long light on PRL and milk yield response weakens such conclusion. Also, there was no strong evidence to indicate that GH is involved in mediating the photoperiod effect. However, this does not exclude the possibility of alteration at the level of receptors of galactopoietic hormones which modulate the tissues

responsiveness to these hormones. It is possible that photoperiodic stimulation on milk yield is associated with stimulation of feed intake as has been suggested previously (Peters *et al.*, 1981). Indeed, there was indirect evidence from this study to support such contention; since there was a trend for an increase in body weight during the period of milk yield stimulation which imply that an increase in feed intake during that time did occur.

Short cycles of 2 or 4 weeks of melatonin treatment which were repeated at different times of the lactation cycle and even with different commencement times of year were not found to be effective in sensitizing the goats to subsequent response to long photoperiod on milk yield. Indeed, this treatment might produce detrimental effects on milk yield when started while the day length is still increasing. The reason for the lack of a conclusive and definite effect of photoperiod on milk yield was not clear. Studies of the effect of exposure to extended hours of lighting on lactation performance in goats are very limited and have not been always shown to be successful in producing a stimulatory milk yield response. This might be related, in part, to a possible existence of an innate rhythm for milk yield in goats so that seasonal factors like photoperiod have little effect on milk production (Linzell, 1971). Perhaps the photoperiodic stimulation is an indirect effect which can be elicited by other mechanism or sometimes may be blocked by other (uncontrolled) seasonal factors and there might be a difference in the sensitivity of such factors between goats and cows. The finding of this study suggests that goats are not a good model for cows as regard the utilization of photoperiod as a means of milk manipulation.

The objective of the second part of the project was to determine the maximum milk yield output of cows of different genetic merit, therefore, we adopted more than one galactopoietic stimuli applied in additive stepwise fashion. We showed that the lactating dairy cow was not milking at her maximum capacity, regardless of genetic merit, at peak lactation, since we were able to elicit a milk yield response to each of the stimuli. We utilized frequent milking which is associated with the relief of an inhibitor acting locally to control the rate of milk secretion, bST which is acting as a homeorhetic repartitioning of nutrients towards the mammary gland, together with T4 which increases the whole body metabolism. This combination has never been used before and it was applied in order to push the cows as far as possible to their maximum milk production. The fact that we observed an additive yield response at each stimuli indicates differences in the mode of action of each stimulus. One of the obvious aspects of increasing milk secretion capacity was the significant increase in mammary gland growth. The capability of the mammary gland to grow beyond its supposed maximum growth which occurs at peak lactation as a consequence of galactopoietic stimuli was clearly demonstrated. This indicates that milk secretion capacity of the mammary gland is not reached at peak yield, at which time the mammary gland normally contains a maximum number of highly differentiated secretory cells, nor is it achieved by applying only one galactopoietic stimulus. The galactopoietic stimuli utilized in this study, notably frequent milking and GH, have been shown to exhibit some stimulatory effects on mammary gland growth during lactation. More frequent removal of FIL, by reducing the interval between milking, stimulated not only the secretory activity of the epithelial tissues but also cellular proliferation in lactating cows milked four times daily (Hillerton *et al.*, 1990). Also, GH treatment can exert stimulatory effect on cellular hypertrophy and (or) increasing the longevity of secretory epithelium (Knight *et al.*, 1990), so the effects of these two factors were mostly responsible for the mammary gland

growth. The polyurethane foam casting technique used in this study for determination of changes in udder volume cannot give a precise picture of mammary growth because it only gives an estimate of gross udder volume. Nevertheless, mammary growth corresponded to a significant increase in alveolar milk volume which implies growth of the secretory tissue portion of the mammary gland.

Cows from high and low genetic merit showed similar responses when they were pushed towards their maximum capacity. This indicates that high genetic merit cows are not milking closer to their maximum capacity and therefore, are not at more risk of developing metabolic problems than cows from lower genetic merit. Would this conclusion still be the same if the cows underwent a situation of feed restriction while they are maintained at such strong galactopoietic stimuli? or when applied at different stages of the lactation cycle? Clearly these questions deserve further investigation.

The other question that was addressed in this study: was the metabolic capacity determined at the mammary gland level or elsewhere in the whole body? The finding suggests that it was the mammary gland which might limit the metabolic capacity and not the body. However, this should be interpreted with caution for the deleterious effects of thyroxine treatment on milk yield with such dose and length of time, in fact, milk yield tended to decline in the half milked at four times. A follow up study at which the duration of treatment phases was reduced to five days each indicated that the mammary gland is not a limiting factor to maximum milk yield capacity as indicated by the ability of the mammary tissue to increase its rate of milk secretion to compensate for the loss of yield in the half which was infrequently milked. This suggests that the maximum milk yield capacity determined by galactopoietic stimuli was regulated at the whole body level and not at the mammary gland level. The mammary gland was not milking at its potential because of its capability for further increase in the rate of milk secretion. At the level of the body, several

factors might be involved in the regulation of the maximum metabolic capacity. It is possible that such a limiting factor could reside in the machinery associated with the availability and supply of nutrients to the mammary gland or elsewhere in the body. This compensatory increase, in the follow up study, was evident at peak yield when the highest number of secretory cells are present. Could a similar response be achieved during later stages of lactation when the secretory cell number is declined? This question clearly needs to be addressed.

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APPENDIX

Effect of repeated cycles of melatonin and long light on milk production in the goat

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Introduction Exposure to long day photoperiod is known to stimulate milk secretion in dairy cattle, but the mechanism has not been determined. Melatonin (MEL), which is produced by the pineal gland, is stimulated during darkness and has been known to mimic the short day signal when administered during long day exposure. Preliminary evidence has suggested that long term MEL treatment sensitizes lactating goats to subsequent long light exposure (Knight, 1993). The present study was undertaken to test if repeated short cycles of MEL would prime goats to subsequent long light effect on milk yield.

Materials and methods Twelve Saanen goats, kidded in March, were used in their first lactation. Goats were housed in a non-light-proof building and milked twice daily at 08.00 and 16.00 h. They were fed 750gm concentrate at each milking and hay was available all time. First MEL cycle started in April (week 5 of lactation) when 3mg of MEL absorbed into small amount of concentrate was fed daily at 15.00 h to six goats while the other six were fed a similar prepared concentrate but without MEL. MEL feeding was continued for 2 weeks, after that MEL was stopped and the long light exposure started for 2 weeks. Light was switched on for 17h between 6.00-23.00 h. In the second cycle, MEL was fed for 4 weeks and followed by 4 weeks of long light. The same treatment procedure was applied in the autumn (September) when the goats were in late lactation. Milk yield was recorded at each milking. Average milk yields for each treatment period (MEL or long light) were tested for significance by ANCOVA and using the average weekly yield preceding each treatment period as a covariate.

Results Although milk yield in the MEL-fed goats tended to be lower than in controls, the difference was not significant either by ANCOVA or *t* test on individual periods (Figure 1). Prevailing day length (season) had no effect on milk yield response to MEL. Exposure to extended lighting produced a small but inconsistent effect on milk production. In both seasons, MEL treatment did not sensitize the goats to subsequent artificial long day exposure; no increase in milk yield for goats that were previously on MEL treatment was seen. Indeed, there was a tendency for a greater response to long light in controls during the final cycle (P=0.05).

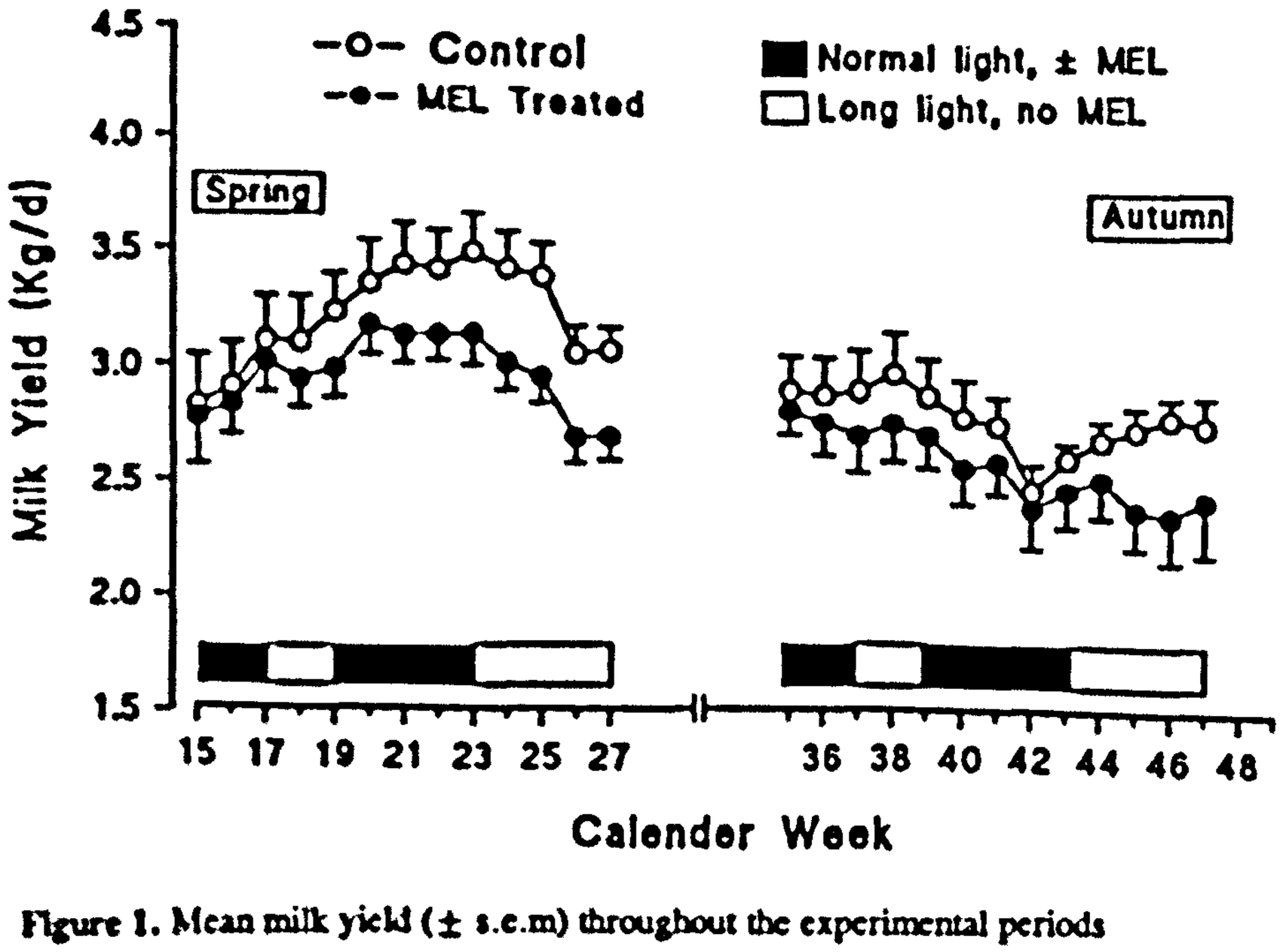

Figure 1. Mean milk yield (± s.e.m) throughout the experimental periods

Conclusions Repeated short cycles of MEL were not effective in sensitizing goats to subsequent artificial long light stimulation of milk yield, regardless of time of year. Exposure to long light did not produce a clear stimulatory effect on milk production, but may have had some stimulatory effect when applied in the Autumn.

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Physiological characteristics of high genetic merit and low genetic merit dairy cows: a comparison

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Introduction Genetic selection has greatly improved individual cow productivity. A high genetic merit Holstein-Friesian cow will produce 10,000 of litres of milk in a 305 day lactation, those of lower genetic merit half this amount. Despite major research effort in generating these differences, quantitative biological description of what has been achieved is lacking. The aim of this study was to compare biological variables of relevance to milk synthesis in well defined high genetic merit and lower genetic merit dairy cows.

Material and methods 12 cows of high genetic merit (HGM: top 5% of UK national herd) and 12 of lower genetic merit (LGM: close to UK average) were purchased from the Blythbank and Langhill herds; the ontogeny of these selection lines has been described elsewhere (Woolliams *et al.*, 1993). Management of the two groups was identical. Measurements were made over a period of two weeks close to peak lactation. Milk yield was recorded at each milking. Plasma samples were collected and analysed for a variety of hormones by radioimmunoassay and metabolites by established spectrophotometric assays. Udder size was determined by a casting technique (Dewhurst et al., 1993) and mammary biopsies for key enzyme activities were obtained as described by Knight et. al. (1992). Differences between lines were analysed by analysis of variance (ANOVA).

Results HGM and LGM had similar body weight. Milk yield and udder volume were higher in HGM than LGM; calculated secretion efficiency (ml of milk per ml of udder tissue) was similar in the two lines. Mammary enzymes did not differ between HGM and LGM (data not shown). HGM had higher GH but lower IGF1 and insulin than LGM, prolactin, thyroxine and cortisol did not differ. Body condition score (BCS) was lower in HGM than LGM. None of the plasma metabolites measured differed between HGM and LGM. Heart rate was similar in the two lines.

Table. *Comparison of high (HGM) and low genetic merit (LGM) cows. Values are mean±s.e, n=12. Line tests for difference between HGM and LGM, ANOVA.*

	HGM	LGM	H:L ratio	Line
Milk yield (kg/d)	37.04±2.2	28.9±2.1	1.3	P=0.01
Udder volume (l)	18.1±1.2	13.6±1.3	1.3	P=0.02
GH (ng/ml)	2.80±0.41	1.23±0.26	2.3	P=0.005
IGF-1 (ng/ml)	27.81±3.87	45.73±7.6	0.6	P=0.02
Insulin (ng/ml)	0.11±0.009	0.17±0.02	0.6	P=0.05
Body condition score	1.8±0.16	2.6±0.27	0.7	P=0.02
Body weight (kg)	590±12	589±15	1.0	n.s.
Heart rate (beat/min)	90±3	87±2	1.0	n.s.
Glucose (μmol/ml	5.78±0.36	5.61±0.29	1.0	n.s.
βOH-butyrate (nmol/μl)	1.50±0.40	1.35±0.17	1.1	n.s.

Conclusions The greater milk yield of HGM was a function of greater secretory tissue mass, not secretion efficiency. HGM mobilised a greater amount of body tissue than LGM, as indicated by their lower BCS and their tendency not to gain weight for longer after peak lactation (data not shown). This difference was probably a consequence of GH being higher in HGM, and the lower energy status was then reflected in decreased IGF1 and insulin. In identification of quantitative trait loci for future selection programmes, attention should be paid to factors regulating mammary development as well as function.

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