A WINDOW OF BOVINE SOMATOTROPIN AS A SCREENING METHOD FOR SELECTING DAIRY SIRES

A Thesis

presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

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In partial fulfilment of requirements

for the degree of

Master of Science

October, 1995

ABSTRACT

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University of Guelph, 1995

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Canadian Holstein bull calves (38) were bled monthly (3-5 mo of age) at 30 min intervals for 2.5 h in the morning and evening. Of the 38, four were selected, bled once per month on the same schedule as previously indicated and at hourly intervals for the hours remaining in a 24 h day until they were 13 mo of age. Bst parameters that were studied included baseline, mean, and peak concentrations, number of peaks, bst index and the area under the curve. Baseline ($r=0.34$, $P<0.03$) and mean ($r=0.35$, $P<0.03$) bst levels in the evening were related to the calves pedigree index of for milk production at the age of 5 mo. In ranking the 4 calves in the 24 h samplings, bst index overall was indicative of the genetic merit except for the switching of the 2 middle pairs. This work indicates that baseline and mean bst levels could be related to the genetic merit of young dairy sires at 5 mo of age and the importance of longer sampling time than 5 h to correctly characterize the bst secretion pattern in its use for prediction of genetic merit for production traits.

Acknowledgements

I wish to express my gratitude to Drs. G. J. King and J. H. Burton who served as members of my advisory committee. I am very much indebted to my supervisor Dr.R. R. Hacker for his frequent and close supervision and guidance. Special thanks to Dr. Emeka Okere for his help throughout the experiment and for his editorial assistance. Special thanks are due to the staff of Elora Dairy Research Centre for the use and care of the experimental animals. A hearty thank you to George Werchola for his great help in carrying out the RIA. I also wish to express appreciation for the cooperative work with Vanida Khumnirdpetch and for her help through out the experiment. The financial support from the International Development Research Centre for my program is also highly appreciated.

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Chapter 1.0 Introduction and Objectives

1.1 Introduction

Obtaining the most accurate estimate of genetic merit for animals that are used for breeding is the primary step in a genetic improvement program. Improving methods of dairy sire and cow evaluation may increase the rate of genetic gain for milk yield. Progress in dairy cattle breeding is hampered in that milk yield is only expressed in mature females and the reproductive capacity of females is low compared with that of males. Indicator traits for milk production measurable in young animals of both sexes could improve genetic progress. To date, the most common method of selecting dairy cattle is based on pedigree index. Progeny testing programs are useful in identifying genetically superior individuals and today are still the most accurate and timely method available. However, the process of identifying superior animals has a drawback in that the length of time required to conduct these testing programs slows the rate of genetic improvement. Young sire selection today is based almost totally on pedigree information which is an estimate of their ability to produce animals with superior production traits. However, it has been indicated that most young sires may not produce desirable daughters due to gene segregation during gametic meiosis (Kazmer et al., 1990). This means that many young sires must be tested which represents substantial cost. So as dairy cattle become more productive it is important to study other traits to determine how they respond to

selection for milk yield. Many factors can influence the level of milk production; bovine somatotropin (bst) is one. If physiological traits that can be used as indirect selection criteria for milk production are determined, progress in genetic selection could be made more rapidly and with less expense than with the current method of sire evaluations.

For these reasons bst parameters were studied. It has been reported that they are correlated with genetic potential for milk yield in dairy cows (Kazmer et al., 1986), calves (Lovendahl and Sejrsen, 1993; Woolliams et al., 1993; Mackenzie et al., 1988) and young bulls (Kazmer, 1990). Furthermore it has been shown that bst secretory peak height and frequency after deprivation of feed and water for 24 h are positively correlated with the pedigree index for milk production in young Holstein sires (Kazmer 1990). Thus plasma concentration of bst at certain specific periods of the day might be related to genetic potential for milk production, which would suggest that its concentration in plasma could be used as a rapid method for screening dairy sires.

1.2 Objectives

- To find the relationship of bst to genetic merit of young dairy sires for milk production using two windows (am & pm).
- To evaluate the possibility of using bst to predict the genetic potential of young sires for milk production.
- To determine the age at which bst can best indicate the genetic potential of sires for milk production.

Chapter 2.0 Review of Literature

Chapter 2.0 Review of Literature

2.1 Secretion of somatotropin

Somatotropin is a protein hormone secreted episodically by the anterior pituitary gland and transported by blood to various body organs and tissues where it has biological effects. The secretion of somatotropin is predominantly controlled by two reciprocally acting hypothalamic hormones, somatostatin, an inhibitory regulatory factor, and growth hormone releasing hormone. The quantity and pattern of somatotropin secretion is regulated in concert by the secretion of both regulatory factors from the hypothalamus through the hypothalamo hypophyseal portal system where they exert their actions at unique pituitary receptors (Baile and Buonomo, 1987). Furthermore, Guillaume et al. (1994) reported that growth hormone releasing peptide-induced somatotropin stimulation involved an activation of neurons in addition to the previously demonstrated direct effect on the pituitary cells. Tannenbaum (1980) demonstrated that somatotropin regulated its own secretion via a negative feed back system in rats. In this study injection of somatotropin resulted in a significant suppression in amplitude of somatotropin secretory pulses after an interval of 1 h and plasma somatotropin levels remained markedly depressed for up to 6 h after injection. Similarly Clark et al. (1988) showed that somatotropin inhibited its own release when given by i.v. infusion; the work indicated that since somatotropin responses to growth hormone releasing hormone were maintained during somatotropin

infusion, the feedback effect of somatotropin was unlikely to be exerted directly on the pituitary or by increasing somatostatin release. Rather somatotropin feed back involved an inhibition of growth hormone releasing hormone release. Somatotropin is species specific (Bauman, 1992). i.e there are differences in the ability of somatotropin in one species to elicit biological effects in other species.

2.2 Factors affecting the concentration of bovine somatotropin

2.2.1 Temperature

Mitra et al. (1972) reported that prolonged exposure to elevated temperature of 18°C to 35°C, decreased bst concentrations in plasma from 18.2 to 13.5 ng/ml; secretion rate also declined from 16.04 mg to 9.09 mg/cow/day. However, Olsen and Trenkle (1973) demonstrated an opposing temperature effect in that bst concentrations of cows increased during subzero cold exposure, with the highest daily mean occurring on day 3 of the exposure. On the other hand no changes were reported in bst concentration in relation to variation in temperature or day length during the year in cattle (Koprowski et al., 1973). Similarly it was shown that photoperiod [Peters et al. (1981); Kay et al. (1980)] and cold ambient temperature (Peters et al., 1981) did not affect concentrations of bst in prepubertal bulls and cows.

2.2.2 Restraint & stress

Prolonged exposure to stress such as noise, handling and restraint can modify concentrations of bst. It was demonstrated that increased bst concentration was found in steers immediately

following venous catherization (Eaton et al., 1968). Shirely et al. (1973) found that in cows' serum bst concentrations increased before surgery and returned to presurgery levels within 2 to 4 days; the increase in bst concentrations was attributed to stress associated with transport of animals or preparation for surgery. However Reynaert et al. (1976) showed that stress caused by transportation and further reinforced by a stay of several hours in unfamiliar surroundings, induced a significant decrease in serum bst in calves of both sexes, bulls, oxen, pregnant heifers and cows. Munksgaard and Lovendahl (1993) showed a very unique finding in that housing conditions which resulted in long term deprivation of lying reduced concentrations of bst in dairy cows.

2.2.3 Feeding/fasting

Nutritional status has a marked effect on circulating concentrations of bst. Breier et al. (1986) found that pulsatile release of somatotropin occurred episodically with a diurnal increase during night and morning hours only in steers on high nutritional intakes (3% of live weight in dry matter intake per day). In this work reduced feeding at both medium (1.8% of live weight in dry matter intake per day) and the low intakes (1% of live weight in dry matter intake per day) abolished the diurnal rhythm and significantly increased mean plasma somatotropin concentrations, the amplitude of pulses and the area under somatotropin profiles. Baseline concentrations and pulse frequency did not change through nutritional manipulation. In dairy heifers, it was shown by Lapierre et al. (1992) that the secretion of bst

by the pituitary was not affected by feed restriction. However, the findings of Parchuri et al. (1993) indicated that feed and water deprivation for 24 h significantly decreased overall plasma bst concentrations from 20.51 to 18.52 ng/ml in Holstein bull calves. Similarly imposition of a 24 h fast decreased mean plasma bst peak concentrations and maximum peak concentrations (Kazmer, 1990). Concentration of hormones in plasma could vary as a result of differences in rates of clearance as well as differences in rates of secretion. Trenkle (1976) and Lapierre et al. (1992) reported that fasting resulted in lower clearance rates of bst which may have been the result of lower metabolism of this hormone by tissues of the body involved in removing it from circulation; rather than having an effect on the secretion of the hormone. Mears (1993) demonstrated that plasma bst concentrations were lower after feeding than before feeding in new born calves.

2.2.4 Other hormones

Evidence from studies on cultured rat anterior pituitary indicated that oestrogens increase plasma somatotropin hormone through a direct stimulatory effect on somatotropin hormone synthesis and release at the pituitary level (Simard et al., 1986). It was demonstrated that testosterone or its metabolites enhanced bst secretion in bulls (Plouzek and Trenkle, 1991b). Hassan et al. (1992) reported that estradiol-17β or 3α-diol did not affect somatotropin release induced by growth hormone releasing hormone (GHRH); however, testosterone and dihydrotestosterone increased GHRH-induced somatotropin release above control concentrations.

Davis et al. (1977) demonstrated that treatment with androgen or estrogen increased overall and baseline somatotropin concentration and non significantly increased the amplitude of somatotropin secretory episodes in sheep. In a study on rats it was found that testosterone stimulates expression of GHRH mRNA in neurons of the hypothalamus, exerting its effect on GHRH gene expression predominantly through direct activation of androgen receptors (Zeitler et al., 1990).

2.2.5 Age

Blood concentration of somatotropin decreases with age in a variety of species, including cattle; however there are marked differences in the pattern of decline. Reynaert et al. (1976) suggested that calves of both sexes had significantly higher bst levels than older animals. In Holstein bull calves it was found that average concentration of bst significantly decreased from <1 to 42 weeks of age (McAndrews et al., 1993). In intact and castrated male and female cattle at 5,8,12 and 15 mo of age, it was shown that as the animals aged overall bst levels in plasma decreased, baseline concentration declined, secretion rates declined and half-life of bst decreased significantly (Plouzek and Trenkle 1991a). Plasma volume of bst was increased significantly by aging in dairy heifers, but when expressed as percent of body weight, it decreased with aging (Lapierre et al., 1992). Basal somatotropin levels were either significantly lower or tended to be lower in older compared with younger pigs; metabolic clearance, secretion rate and total serum volume were all significantly

greater in 30 mo compared with 3 mo old pigs (Farmer et al., 1993). In a study of Holstein bull calves of two genetic groups (selection for milk production and control) conducted from one to nine months of age, the mean bst concentration in selection calves was higher during the first month and then dropped gradually, but not significantly, until 5 mo of age. In the control calves the mean bst concentration remained nearly constant during the first 5 mo. After 5 mo of age, plasma bst concentration increased in both groups and reached a combined mean of 24.0 ng/ml by 8 months of age (Parchuri et al., 1993).

2.3.6 Sex

Plouzek & Trenkle (1991a) reported that in nearly all comparisons, bulls had a higher bst status than did steers or heifers. Bulls had greater plasma concentrations of bst as a result of more secretory periods of higher amplitude with no change in metabolic clearance rate; all males had more frequent bst spikes and secretory periods of higher amplitude, greater secretion rates and greater bst responses to GHRH than females. On the contrary no differences were observed between bulls and steers in mean bst concentrations, or pulse frequency and amplitude (Lee et al., 1991). Similarly, it was shown that males had higher plasma bst levels in four breeds at three ages (Irvin & Trenkle, 1971), bulls generally had higher bst levels than heifers (Keller et al., 1979) and males had higher serum bst values than females (Reynaert et al., 1976). The gender-associated dimorphic pattern of bst secretion implies that gonadal steroids play a role in bst

secretion. Plouzek and Trenkle (1991a) indicated that measurable levels of testosterone were present only in bulls and plasma concentrations of total estrogens were also greater in bulls. In another study Plouzek and Trenkle (1991b) demonstrated that gender and castration affected plasma concentration of total estrogens; bulls had greater mean concentration of total estrogens than heifers and steers. The level of total estrogens were 9.6 & 7.6 pg/ml in males and females respectively, and 9.4 & 6.5 pg/ml in intact & castrated animals, respectively.

2.3 Exogenous bst

2.3.1 Effects on milk production

Evidence from various sources shows that bst plays an important role in lactation; when administered exogenously, bst significantly improves milk production and efficiency. Many studies indicated that supplemental administration of somatotropin to lactating cows increased milk yield by as much as 10-40% (Erdman et al., 1990; Zwickl et al., 1990; Peel and Bauman, 1987; Bauman et al., 1985; Peel et al., 1983). Increasing amounts of recombinant bst (rbst) resulted in a linear increase in milk yield, the magnitude of the response being 2.9-5.2 kg/day. Fat and protein yields also increased up to 0.28 and 0.23 kg/day, respectively (Erdman et al., 1990). The magnitude of response in milk yield is related to bst dose. The maximum response in milk yield was achieved at a daily bst dose of about 30-40 mg/d ; no further increase occurred even at doses much higher (Peel and Bauman, 1987). Quality of management will be the major factor affecting the

magnitude of response in milk yield to bst (Bauman, 1992). Prepartum bst treatment of primigravid ewes was reported to have significantly increased subsequent milk production during the first eight weeks of lactation; milk production was 42% higher in ewes treated prepartum with bst than in those treated with saline (Stelwagen et al., 1993). In agreement with this finding Fernandez et al. (1995) demonstrated that in lactating dairy ewes injected with recombinant bst (sometribove) in sustained release formulation, milk yield increased by up to 53.2%. In a commercial dairy herd and over a full lactation bst may be expected to increase feed efficiency from 10-20% and milk production by 10-25% (Ensminger, 1993). Peel and Bauman (1987) indicated that exogenous bst mediates changes in metabolism resembling those brought about by selection for increased milk yield; which implies that variation in endogenous bst secretion may mediate genetic differences in milk yield.

2.4 Endogenous bst

2.4.1 Effects on milk production

The concentration of bst was reported to be significantly higher in a high yielding group of cows (1.89 ng/ml) than the average yielding group (1.49 ng/ml) (Beerepoot et al., 1991). Similarly Bonczek et al. (1988) reported increased circulating somatotropin concentrations with selection for milk yield in Holsteins. Pooled values, for peak and mid lactation were 4.46 and 3.73 ng/ml for the selection and control group, respectively. In 50 Holstein cows which had blood samples taken for three hours

prior to and 4 h following thyrotropin releasing hormone administration at 30, 90 and 200 days postpartum, Kazmer et al.(1986) found that mean plasma bst concentrations were greater in daughters of selected sires (treatment group) for milk production than in daughters of unselected sires (control group). Before thyrotropin administration mean bst concentrations were 6.7 and 5.0 ng/ml in the treatment and control group, respectively. After thyrotropin administration mean bst concentrations increased to 8.3 and 6.6 ng/ml in the treatment and control group respectively and decreased with advancing lactation. This indicates that greater plasma bst concentrations are characteristic of daughters of genetically superior sires. In another study designed to investigate the effect of genetic selection for increased milk yield in Holstein cattle on concentrations of endogenous hormones and metabolites, it was reported that plasma bst differed overall with age. It was greater in 6 and 24 mo old animals and lowest in 18 mo old heifers. Selection group animals had significantly higher mean bst concentrations overall. This increased bst concentration was consistent after feeding and insulin injection, however increases relative to basal bst concentration were similar between genetic groups. (Barnes et al., 1985). The results indicate that calves and milking cows had greater amounts of circulating bst than did yearling or bred heifers.

2.4.2 Mechanism of action

Genetically superior animals differ from inferior animals mainly in their regulation of nutrient utilization; to date it is

widely accepted that somatotropin indirectly increases milk synthesis through the partitioning of nutrients to the mammary gland (Peel and Bauman, 1987; Kazmer et al., 1986). This involves coordinating the metabolism of various body organs and tissues. These changes in tissue metabolism involve both direct effects on some tissues, like adipose & liver, and indirect effects mediated by somatomedin (IGF-I) for other tissues like the mammary gland. Lipid mobilization is increased, while glucose uptake by peripheral tissues, whole body oxidation of glucose and amino acids and lipid accretion are reduced. The net effect of these changes is that a limited supply of glucose and amino acids are spared for synthesis of milk components, whereas lipids are used as an energy source by the cow (Oldenbroek and Garssen, 1993). Cows injected with bst consumed more feed on a body weight basis than the control group, but their relative feed energy efficiency was higher. The increased feed efficiency could be accounted for by the smaller proportion of total intake required to meet the cows'maintenance requirements. The work of Tilakarante et al. (1980) suggested that calves with different potentials for milk production vary in aspects of their energy and nitrogen metabolism.

2.4.3 Relationship with genetic merit

With a view to identifying possible predictors of genetic merit for milk fat production, responses to metabolic challenges were studied. Results of a study on 8 mo old Friesian bull calves revealed that, relative to low breeding-index bulls, those in a high breeding-index group exhibited elevated plasma bst

concentrations during a fasting period (Mackenzie et al., 1988). Similarly red Danish dairy calves of both sexes and of two lines (high and low milk fat) were tested at 4 mo and 10 mo of age for their bst release following intravenous administration of either thyrotropin releasing hormone (TRH) or arginine hydrochloride. The bst response was measured in serial blood samples for 0.5 h prior to and for 2 h following the intravenous injections. It was found that the response peak following TRH was greater in the line selected for high yield at 10 mo (High yield, 42.4 μg/l; Low yield, 20.6 μg/l) but not at 4 mo of age (High yield, 25.4 μg/l; Low yield, 18.6 μg/l); the response peak following arginine was smaller than the peak following TRH and did not differ between selection lines. After puberty (10 months) male calves responded more to both secretagogues than females while there was no difference before puberty (4 mo) (Lovendahl and Sejrsen, 1993). The work by Lovendahl et al., (1991) on 80 Friesian calves of both sexes and of 2 selected lines indicated that there was a positive association with the release of bst following TRH and GHRH administration. The predicted breeding value for milk yield, and individual responses obtained depended critically on concentration prior to secretagogue administration. Similarly it was reported that the correlation coefficient between estimated breeding value for milk production and average 9 mo plasma bst concentration of 24 Holstein calves was 0.45 ($p < 0.05$). For each 453 kg increase in estimated breeding value for milk production, plasma bst concentration increased 0.58 ng/ml when calculated as the average of all bleedings over the 9 mo

period (Parchuri et al., 1993). Woolliams et al., (1993) studied 55 British-Friesian dairy calves of both sexes from low and high genetic groups and found, similarity to previous study of Lovendhal et al., (1991), that bst release following GHRH administration was positively related to predicted breeding value and the response was moderately repeatable. In bull calves of two divergent genetic groups, calves selected for higher estimated breeding value had a greater average, but similar stimulated plasma bst concentrations. Age of calves, but not short term feed and water deprivation influenced average plasma bst concentrations both before and after stimulation with GHRH (Parchuri et al., 1993). In this study feed and water deprivation for 24 h decreased overall plasma bst concentrations from 20.51 ng/ml to 18.52 ng/ml, this decrease was influenced more by the 2.5 ng/ml decrease by the control calves than by the 1.5 ng/ml decrease by the selection calves. In another study, imposition of a 24 h fast decreased mean plasma bst peak concentrations, maximum peak concentrations and pulsatile frequency; mean basal bst concentrations were not related to any measures of genetic merit or altered by fasting in young Holstein calves (Kazmer et al., 1990). Mears et al. (1993) reported that body weight had a large effect on basal plasma bst concentration and plasma bst kinetics. As body weight increased, basal plasma bst concentration and bst half-life, bst steady state volume of distribution, metabolic clearance rate and secretion rate per kg of body weight all decreased.

2.4.4 The bst profile

Bst is secreted from the pituitary in a pulsatile manner in cattle (Plouzek & Trenkle 1991b; Kazmer et al.,1990). It was reported that serum bst concentrations of 9 mo old bulls and steers fluctuated in a pulsatile manner during a 6 h bleeding window (Lee et al., 1991). In characterizing plasma bst patterns in steers, Wheaton et al. (1986) found that bst surges occurred at an average frequency of 0.7/h, the rate of which did not differ markedly among steers nor hour of the day. The magnitude of bst secretory surges varied significantly among steers and during the 24-h period. Bst peaks averaged 47.0 and 27.2 ng/ml in steers having the highest and the lowest bst surges respectively. When steers were fed at 1400 h, all bst levels fell from 1400 to 1600 h and then rebounded with two to four high amplitude surges. Peak and mean bst levels were associated positively (r=0.93). Both were associated negatively with rates of gain (r=-0.82 and -0.74, respectively). Woolliams et al.(1993) noted that the timing of pulses was not random and that some common stimuli were present. The rhythmic nature of the bst secretory profile and its persistence in constant conditions of lighting and temperature suggest that neural mechanisms play a crucial role in bst regulation (Tannebaum & Martin 1976). The bst profile for each calf (Wheaton et al., 1986) and each lactating cow (Vasilatos and Wangsness, 1981) was reported to be unique. It was also demonstrated that certain characteristics of the patterns of bst secretion may be heritable (Klindt, 1988). Davis et al. (1979) noted that each individual animal has a characteristic

secretory pattern of somatotropin that may be inherently or genetically predetermined. The exact nature of the hormone secretory pattern that is ultimately expressed (phenotype) would then be determined in part by the inherent ability of the animal to respond to changes in the internal and external stimuli. Thus, the pattern of secretion and levels of endogenous bst represents the physiological status and the potential of animals in response to their environments.

Chapter 3.0 A Window of Bst as a Screening Method for selecting Dairy Sires

3.1 Abstract

To establish the relationship between circulating bovine somatotropin (bst) and genetic merit, and to evaluate the possibility of using bst to predict the genetic potential of young sires for milk production, 38 Canadian Holstein bull calves were studied. Bull calves, 3 mo of age and from 6 proven sires and 38 dams were bled via the jugular vein once monthly at 30 min intervals for 2.5 h in the morning and evening for a period of 3 mo. Of the 38, four calves were kept beyond 5 mo and, were bled once per month for the same 2.5 h window morning and evening as well as once an hour for the remainder of a 24 h day until the age of 13 mo. All calves were kept under environmentally regulated conditions. Bst concentrations were measured by double antibody radioimmunoassay. The maximum and non specific binding was 41.8 and 2.9% respectively. Average recovery of bst over 10 different concentrations representing the standard curve between 18 and 90% binding was 93%. The assay was also validated for parallelism. The intra assay coefficient of variation was 10.8% and was determined using a pool of plasma with bst content of 50% binding compared to the standard curve. The data were analyzed by repeated measures analysis using the general linear model from SAS. The mean minimum and maximum bst concentrations ranged from 0.78 to 24.08 ng/ml in the morning and 0.15 to 48.21 ng/ml in the evening. There were no significant differences ($P>0.05$) in the baseline, mean and peak bst concentrations between the morning and evening bleedings at any

ages except at 4 mo for peak concentrations ($P<0.05$) and at 11 mo for mean and peak concentrations ($P<0.05$). After 6 mo of age most bst parameter values were higher ($P>0.05$) for the evening than the morning samples except the area under the curve ($P<0.05$) above lines of 3.5 and 5 ng/ml. Mean bst concentrations differed ($P<0.05$) among age groups after 6 mo of age. Of all bst parameters only the baseline and mean concentrations for the evening bleeding were related ($r=0.35$, $P<0.03$) to the calves' pedigree index at 5 mo of age. From the 24 h bleedings it was observed that bst index and pedigree index of calves were in agreement only at the age of 3 mo. This work demonstrates that using the above sampling windows, baseline and mean bst concentrations can be related to the pedigree index of calves at 5 mo age; and suggest the importance of a longer sampling time to accurately characterize the bst profile.

3.2 Introduction

Improvement of efficiency and economic return is an important goal in dairy farming. Efficient breeding schemes and proper management are necessary to achieve this goal. Producing offspring that possess the necessary genes to produce the maximum amount of milk of the desired composition and to develop the desired genotype remain important objectives in dairy cattle breeding. The breeding value of sires can be more accurately determined than cows because sires will have more offspring; thus it is through the selection of sires that the major portion of progress in genetic improvement is currently made. The most desirable method of

selection is based on a progeny testing program, however this method is both time- and resource-consuming. Therefore, identifying and incorporating indicator traits such as hormone concentrations which could be related to the genetic merit of cattle for milk or milk components would benefit the dairy industry. Furthermore, the use of suitable indicators which are strongly related to genetic merit and relatively easy to measure could result in higher rates of genetic improvement. Some of the potential indicators which appear to be related with genetic merit include blood concentrations of glucose, urea, non-esterified fatty acids and insulin (Mackenzie et al., 1988). Additionally several different studies have shown that there is an association between bovine somatotropin (bst) concentrations and genetic potential for milk yield in dairy cows (Beerepoot et al., 1991; Kazmer et al., 1986.) and dairy sires (Parchuri et al., 1993; Kazmer et al., 1990). These results indicate that bst concentrations in dairy animals are correlated with genetic merit for milk production traits. Such profile characteristics may be economical in terms of time and actual cost in selecting young sires for progeny testing programs. Thus, this study was designed to use a sampling window of circulating bst as a method of screening young dairy sires.

3.3 Material and Methods

3.3.1 Animals

Thirty-eight Canadian Holstein bull calves from 6 proven sires and 38 dams at Elora Dairy Cattle Research Centre of the Ontario Min. of Agric. and Food were used in this study. Calves were born

over a 7 mo period beginning in August, 1993 and entered the study at 3 mo of age. At 5 mo of age 4 of the 38 calves were selected on the basis of age to form a uniform group and maintained to 13 mo of age. These 4 calves were born from 2 sires and 4 dams. The mean dam index for milk production was 4.74, with a range from -32 to 34. The mean sire index for milk production was 17.53, with a range from 8 to 23. The mean pedigree index of calves for milk production was 11.14, with a range of -7 to 28.5.

3.3.2 Housing

All calves were kept under regulated environmental conditions where they were subjected to a constant light-dark cycle, 5:00 h to 19:00 h light and 19:00 h to 5:00 h dark each day. Calves of similar age were housed in a common pen with a maximum of 9 calves/pen. The 4 calves which continued on in the study were housed separately after they were 10 mo of age.

3.3.3 Feeding

Calves were provided with free choice good quality hay (grass alfalfa mixture) and an average of 2 kg of 18% protein calf starter per day. The concentrate was given once a day, at about 5:30 h. This feeding regime was maintained until calves were 5 mo of age. The four calves from the group of 38, which were kept on trial, were provided with an average of 3 kg of 16 % dairy ration/head/day and free choice good quality hay (grass alfalfa mixture). Additionally they received about 8-10 kg of corn silage once a day around 14:00 h, after they reached 10 mo of age. They were group fed until 10 mo of age, and individually thereafter. A continuous

supply of clean water was available for all calves.

3.4 Sample collection

At the age of three months and monthly thereafter up to the age of 5 mo, calves were bled for 2.5 h in the morning (A.M.) from 10:00 to 12:30 and evening (P.M.) from 19:00 to 21:30. Four of the 38 calves were bled on the same schedule until they were 13 mo of age. These 4 calves were also bled at hourly intervals for 24 h once per month. Calves were restrained in chutes and cannulated via the jugular vein one day prior to blood sample collection. The neck area was cleaned with ethanol, a 14 gage 5.08 centimetres long hypodermic needle was inserted into the jugular vein after which microbore tubing with internal and external diameters of 0.86 mm and 1.27 mm, respectively, and of 70 cm length was introduced approximately 25 cm into the jugular vein through the needle. The tube was soaked in zepharin hydrochloride (10 ml/l) one day prior to cannulation. An 18 gage needle with stopper was fitted at the external end of the tubing. The tubing was filled with about 1 ml diluted heparin (dilution: 5 ml heparin in 1000 ml of saline) to avoid blood coagulation. The cannula was kept immobile by an adhesive bandage which was wrapped around the calf's neck. Cannulated calves were provided with feed and water as outlined above. The following day beginning at 10:00 h and 19:00 h, 5 ml of blood was drawn at 30 min intervals for 2.5 h from each calf by a 10 c.c syringe. Each time a blood sample was taken the tubing was filled with 1 ml heparin. The blood was transferred to a test tube with 6 mg disodium ethylene diamine tetracetate (EDTA) in 100 μl of

distilled water and kept in a refrigerator at about 4°C to avoid metabolism by the red blood cells until all samples were collected. At the end of each monthly sampling, each calf received 7 c.c./day of penicillin for 3 days.

3.5 Bst assay

Blood was centrifuged at 2201 x g, 4°C for 30 min. Plasma was harvested, placed in plastic vials and stored at about -20°C until assayed for bst. The plasma was assayed for somatotropin hormone using double antibody radioimmunoassay of Schalla et al. (1994).

3.5.1 Standard and quality control

Rbst was used as a standard at concentrations of 8, 4, 2, 1, 0, 0.8, 0.6, 0.4, 0.2, and 0.1 ng/ml (rbst, American Cynamide, USA) in assay buffer. The quality control was made from a pool of samples with medium binding of bst and stored at about -20°C until used. It was placed at intervals in the assay to check for consistency.

3.5.2 Antibodies

Rhesus monkey antibovine growth hormone antiserum (AFP 55) donated by Pituitary Hormones and Antisera Centre, USA, was used as the first antibody. Goat antimonkey gammaglobulin (Calbiochem, USA) and normal monkey serum (Sigma, USA) were used as the second antibody and a carrier, respectively. The first antibody (1 ml), the second antibody (5 ml) and normal monkey serum (1 ml) were all reconstituted with 1 ml, 5 ml and 1 ml distilled water, respectively before they were diluted to their final concentration.

3.5.3 Iodination

The iodination procedure described by Smith et el., (1977) was used with modifications. Five μg recombinant bst in 20 μl 0.01M Na HCO_3 was used for preparing ^{125}I-bst. The iodination was carried out in 1.5 ml micro cap at room temperature in the hot lab, to which 50 μl of 0.05 phosphate buffer with sodium azide (pH=7) was added and then transferred to 1.5 ml micro cap coated with 2 μg iodogen in chloroform (80 μg/ml) and blown dry with nitrogen. Immediately 5 μl of ^{125}I carrier free was added to the cap. The reagents were vortexed for 30 seconds and swirled vigorously on an orbital shaker. Reaction time was 10 min. Finally 300 μl of buffer was added and vortexed for about 30 sec. The reaction mixtures were then transferred by pipet to the top of Degas Sephadex G 75 column (1x20 cm). The tracer was completely transferred into gel, followed by the addition of iodination buffer (0.05M po_4,0.01% NaN_3 PH 7). Before the iodination, the column was run by 100 ml of the iodination buffer and then eluted with 2 ml of 1% Bovine Serum Albumin (BSA, A-7906, Sigma, USA) using sealed gravity feed management. In a single iodination a total of 35 fractions of 35 drips were collected using a Gilson fractionator (model FC-80k) into 12x75 glass tubes containing 100 μl of assay buffer. The chromatography profile of each fraction was made by transferring 5 μl of each fraction into 10x75 glass tubes with 100 μl 0.1M sodium thiosulphate and counting on a 1274 RIA gamma counter. Fractions with the highest peaks were selected and tracer tests were set up to inspect percent binding. The tracer test was set up using a

similar method to the assay. A pool of fractions which gave 54% and 56% binding was used in the assay.

The stored plasma samples were thawed by keeping them under room temperature. Each of the thawed plasma sample was shaken for about 30 sec by a shaker. From each thawed and shaken plasma sample, 50 μl were taken in duplicate and diluted with 350 μl phosphate buffer of pH 7 in 12x75 glass tubes. Following this, 50 μl of the first antibody diluted to 1:41666.66 using the standard assay buffer, was added. The reaction mixture was incubated at about 4°C for 24 h, followed by addition of 50 μl of radioiodinated bst as a tracer. The tracer was added at a concentration of about 15000 cpm/tube. After 24 h of incubation 50 μl of the second antibody was added at a dilution of 1:20 and 50 μl of normal monkey serum was added at a dilution of 1:200. In both cases the standard assay buffer was used in diluting to their respective final concentrations. After 48 h of incubation at about 4°C the samples were centrifuged at 2201 x g, 4°C for 30 min. The supernatant was removed and the pellets formed were counted for radioactivity on a gamma counter.

The maximum and non specific binding was 41.8 and 2.9% respectively. Average recovery of bst over 10 different concentrations representing the standard curve between 18 and 90% binding was 93% (n=10). The assay was also validated for parallelism. The intra-assay coefficient of variation was 10.8% and was determined using a pool of plasma with bst content of 50% binding compared to the standard curve. Concentrations of bst in

all samples were determined in a single assay.

3.6 Statistical analysis

Contrast of n=11 was first used to compare bst concentrations at different sampling times through ages 3-13 months. Bst profiles were analyzed by repeated measures analysis using the general linear model from SAS (Statistical Analysis System Inc. 1985). The overall model used for the analysis of the data was:

$Y_{ij} = \mu + C_i + A_j + \epsilon_{ijk}$

Where Y= bst concentration

C= calf, i= 1....39

A= age, j=3,4...13

ϵ= error term

Because of over parameterization, the effect of sire, dam and season couldn't be assessed using the above model. Therefore the mixed model was used in investigating the influences of these effects. Since dam and calf are virtually confounded, dam was excluded from the model. The model included time of sampling, age and season. Calf and sire were considered random. Estimates of repeatability between measurements on the same calf from month to month were calculated as $\sigma^2_t/\sigma^2_t + \sigma^2_m$. The intra class correlation within a month between morning and evening was obtained using $\sigma^2_{m/}$ $\sigma^2_m + \sigma^2_t$ formula; where σ^2_m=means square age and σ^2_t=mean square time.

Bst parameters were examined for differences among ages and between morning and evening bleedings. Bst parameters, i.e baseline, mean and peak bst concentrations and area under the curve were analyzed by repeated measures analysis using the general

linear models from SAS, with a similar model used in the analysis of the bst profile. In order to do the analysis the parameters were calculated for each calf for the morning and evening bleedings as follows:

- Mean bst= the mean of bst concentrations for the specified bleeding time
- Initial peak of bst= mean bst + 1 standard error (se)
- Baseline (basal) bst= mean bst after omitting the initial peaks
- Bst peak = baseline bst + 2 standard deviation
- Areas under the curve

  above the line of 3.5 ng/ml

  above the line of 5 ng/ml

  above the line of 10 ng/ml, were manually calculated from figures with the x-axis length of 7.29 cm and a width 12.85 cm. The lines indicated were chosen to include very low, intermediate and higher peak values.

The bst index was calculated as the sum of peak bst concentrations less the baseline value multiplied by the number of identified peaks in the respective sampling periods. To estimate the extent to which bst parameters i.e. baseline, mean and peak bst concentrations at specific ages differ from bst parameters at other ages, age correction factors were computed as final values less the initial value of the parameter divided by the difference of final and initial age. Age 3 mo was a reference (initial) age against which all other ages are compared and as a result with each other. The pedigree indices of calves were obtained from their dams and

sires, and were calculated as the sum of 1/2 sire index and 1/2 dam index for milk production. The bst index for the morning and evening samples, and the areas under the curves were computed for the 4 calves bled intensively and for 24 hours to see the association of bst parameters with the pedigree index for milk production of the calves. Correlation coefficient (r) was used to estimate the relationship of bst parameters with the pedigree index. Bst parameters for which the relationships with pedigree index for milk production were investigated include baseline, mean, peak bst concentrations, area under the curve above the line of 3.5, 5 and 10 ng/ml, bst index, maximum concentrations and the number of peaks.

3.7 Results

3.7.1 The bst profile

Bovine somatotropin hormone concentrations varied ($P<0.01$) between individual calves and age groups. Within calf, there were effects of sampling time ($P<0.05$) and age*time ($P<0.05$) on bst concentration. Age was insignificant ($P>0.05$) in influencing somatotropin concentration under 6 mo of age. Maximum concentration was affected by calf ($P<0.01$) and age ($P<0.01$). Mean maximum concentrations $\pm$ se at the ages of 8, 9 and 11 mo were 31.1 $\pm$ 9.22, 37.9 $\pm$ 8.96 and 31.2 $\pm$ 9.19 ng/ml respectively.These concentrations were similar ($P>0.05$), but they differed from the bst concentration recorded at the ages of 3, 4, 5, 6, 7, 10, 12, and 13 mo (13.2 $\pm$ 0.97, 13.3 $\pm$ 0.78, 13.4 $\pm$ 0.92, 13.1 $\pm$ 1.42, 16.2 $\pm$ 2.76, 19.3 $\pm$ 4.59, 17.9 $\pm$ 3.53, and 13.4 $\pm$ 1.66 ng/ml respectively). Maximum

concentration did not vary (P>0.05) between the morning and evening samples (14.5 ± 0.98 vs 16.21 ± 0.94 ng/ml respectively). Age of calf affected (P<0.05) the number of peaks. Mean peak number per calf appeared nearly constant between the ages of 3-5 mo for both the morning and evening samples. There was no definite pattern in the increase or decrease of peak number with advancing age for either sampling periods. Mean peak number ± se for evening samples were 0.79 ± 0.07, 0.79 ± 0.07 , 0.79 ± 0.07, 0.5 ± 0.5, 0.5 ± 0.5, 1.25 ± 0.43, 0.75 ± 0.43, 0.75 ± 0.43, 0.75 ± 0.43, 0.75 ± 0.43 and 0.75 ± 0.43 at ages 3-13 mo, respectively. The mean number of peaks for the morning samples at ages 3-13 mo were 0.9 ± 0.06, 0.84 ± 0.06, 0.87 ± 0.05, 0.75 ± 0.43, 1.0 ± 0.00, 0.75 ± 0.43, 1.0 ± 0.00, 1.0 ± 0.00, 1.0 ± 0.00, 0.5 ± 0.5, 0.5 ± 0.5, respectively. The sum of peak numbers for the morning was higher than for the evening, 127 vs 117, respectively. The repeatability of bst profile from month to month was 0.28 and the intraclass correlation within age between the morning and evening was 0.72. Season and sire were not significant (P>0.05) sources of variation in bst concentration.

3.7.2 Bst profile characteristics

Mean somatotropin concentrations at different ages for the morning and evening samples are shown in Figures 1 and 2. Mean concentrations did not differ (P>0.05) between the two periods of bleeding, except at the age of 11 mo where it was 7.2 vs 23.7 ng/ml (P<0.05). At the ages of 4, 5, 7, 8, 10, 11, 12 and 13 mo somatotropin concentrations tended to be higher for the evening samples than that for the morning. The effect of age on mean

concentration is shown in Table 1. Before 6 mo, age of calf had no significant effect on mean bst concentrations, but on the whole, age affected ($P<0.01$) mean bst concentrations. Within calf, period of bleeding (morning vs evening) ($P<0.01$) and the interaction of period of bleeding and age ($P<0.01$) influenced mean bst concentrations. Mean somatotropin hormone profile characteristics for the morning and evening bleedings are shown in Table 2 and Figure 3. No significant differences were observed in the mean baseline concentrations between morning and evening samples at different ages. But mean baseline concentrations (shown in Table 1) varied ($P<0.01$) between age groups. Baseline concentrations were not different ($P>0.05$) among calves. Within calf, period of bleeding and age interacted ($P<0.01$) to influence the baseline concentration. Similarly, peak somatotropin showed no difference between the morning and evening samples except at the ages of 4 (11.71 vs 15.05 ng/ml) ($P<0.05$) and 11 (15.3 vs 41.86 ng/ml) ($P<0.05$) mo. Peak somatotropin was affected by age ($P<0.05$). Within calf, period of bleeding was not significant ($P>0.05$) in influencing peak bst concentration but bleeding period and age interacted ($P<0.05$) in affecting this characteristic. The mean maximum peak occurred at the age of 9 mo (49.2 ng/ml) whereas the mean minimum peak occurred at the age of 13 mo (10.5 ng/ml) in the morning samples. Calves with higher peak somatotropin concentrations also had higher mean ($r=0.84$, $P<0.002$) and baseline ($r=0.56$, $P<0.002$) concentrations and vice versa.

Mean areas under the curve above lines 3.5, 5, and 10 ng/ml

for the morning and evening bleedings at different ages are given in Tables 3 and 4. Mean areas under the curve did not vary between morning and evening ($P>0.05$), except at the ages of 4 mo above line 10 ng/ml, and at the age of 11 mo above lines 3.5 and 5 ng/ml. Taken in general the mean areas above 3.5, 5 and 10 ng/ml were all higher ($P<0.05$) for the evening than for the morning i.e 2.04 vs 1.6, 1.86 vs 1.35 and 1.12 vs 0.64, respectively. Areas under the curve were not affected ($P>0.05$) by calf above the lines for all concentrations. But age ($P<0.001$) and its interaction with the period of the day ($P<0.01$) affected the areas under the curve. The sum of areas under the curve were 541, 477 and 262 for the lines 3.5, 5 and 10 ng/ml, respectively. The effect of age on the areas under the curve is given in Table 5. No significant changes in the area under the curve were observed until calves were over 7 mo of age. The areas under the curve were all significantly ($P<0.01$) related ($0.41 \leq r \leq 0.78$) to the baseline, mean and peak somatotropin concentrations. The highest relationship was observed with the peak and the lowest relationship was with the baseline concentration.

Age correction factors for the baseline, mean and peak concentrations are given in Tables 6, 7 and 8. These factors clearly reflect the somatotropin profile characteristics. The combined age correction factor for am and pm for the baseline, mean and peak concentrations tended to be lower before 6 mo of age. Between 6 and 7 mo of age the values seemed transitional. At the ages of 8, 9, 10 and 11 mo correction factors had higher values

followed by lower values for ages 12 and 13 mo.

3.7.3 Relationship of bst parameters and genetic merit

Most bst parameters and pedigree indices of calves for milk production were not related ($P>0.05$); but the baseline ($r=0.34$, $P<0.03$) and the mean ($r=0.35$, $P<0.03$) were related to the pedigree index at the age of 5 mo for the evening bleeding. The relationship of bst parameters and pedigree index for milk production is given in Table 9.

Rank of the 4 calves bled intensively in the morning and evening from 3 to 13 mo age is given in Table 10. There was a direct positive association between the pedigree index and the area under the curves above 3.5 (pm), 5 (pm) and 10 ng/ml (am & pm).

The mean bst profiles for the 4 calves from the 24 h bleedings, adjusted for age are shown in Figure 4. The profile of each calf was distinct from each of the others. These profiles reflect the bst index calculated for each of the calves. Bst index, by age, from the 24 h sampling for the 4 calves is given in Table 11. At 3 mo of age, the ranking of calves by pedigree index was similar to the ranking by bst index. But when all the indices for all age groups were summarized the index was indicative of the rank of calves according to pedigree index for the calves with the highest and lowest pedigree index, as was the case for the area under the curve above 10 ng/ml (Table 12). Rank of calves by pedigree index, bst index and area under the curve is given in Table 13.

Table 1. Mean bst profile characteristics by age

Age (mo)	n	Base-line conc (ng/ml)	se	n	Mean conc (ng/ml)	se	n	Peak conc (ng/ml)	se
3	316	3.38[d]	0.17	444	5.69[e]	0.26	64	13.84[d]	1.07
4	317	3.67[d]	0.18	454	5.86[e]	0.25	62	13.0[d]	0.84
5	311	3.54[d]	0.19	448	5.71[e]	0.27	63	13.9[d]	0.98
6	35	3.87[d]	0.42	48	6.04[de]	0.66	5	13.68[d]	2.08
7	32	5.69[c]	0.71	48	8.37[cd]	0.93	6	16.75[c]	3.24
8	35	8.69[a]	1.2	48	11.61[b]	1.39	8	31.1[ab]	2.93
9	33	9.3[a]	1.2	48	15.87[a]	2.3	7	37.03[a]	10.28
10	29	7.89[ab]	2.0	48	11.36[b]	1.35	7	20.27[bc]	4.95
11	31	8.58[a]	1.0	48	15.45[a]	2.72	7	28.33[b]	8.53
12	35	6.71[bc]	1.58	48	9.74[bc]	1.21	5	16.95[c]	5.31
13	29	5.56[c]	0.59	45	7.71[cde]	0.73	5	13.8[d]	1.86

Within column, means with different superscripts are different ($P<0.05$).

Measurements are based on 38 calves until 5 months age and on 4 calves thereafter.

Table 2. Mean endogenous bst profile characteristics at different ages

Age (mo)	Bst (ng/ml)	n	A.M	se	n	P.M	se
3	Basal	155	3.37	0.23	161	3.39	0.24
	Mean	219	5.68	0.38	225	5.65	0.41
	Peak	34	13.63	1.23	30	14.01	1.78
	No.peaks	34	0.9	0.06	30	0.79	0.07
4	Basal	152	3.43	0.23	165	3.90	0.26
	Mean	226	5.43	0.32	228	6.26	0.40
	Peak	32	11.71[a]	0.86	30	15.05[b]	1.26
	No.peaks	32	0.84	0.06	30	0.79	0.07
5	Basal	165	3.19	0.19	146	3.94	0.34
	Mean	227	5.42	0.36	221	6.08	0.39
	Peak	33	14.49	1.17	30	13.38	1.53
	No.peaks	33	0.87	0.05	30	0.79	0.07

Within row, means with no superscripts are not significantly different ($P>0.05$).

Table 3. Mean area under the curve (cm^2) at different ages

	>3.5 ng/ml		>5 ng/ml		>10 ng/ml	
Age (mo)	am	pm	am	pm	am	pm
3	1.52	1.49	1.21	1.32	0.40	0.7
4	1.18	1.93	1.12	1.71	0.25[a]	0.79[b]
5	1.46	1.68	1.15	1.45	0.60	0.74

se (>3.5 ng/ml)=0.25, se (>5 ng/ml)=0.24, se (>10 ng/ml)=0.2

* Within row, means with no superscript are not significantly different (P>0.05).

Table 4. Mean area under the curve (cm^2) at different ages

	Bst level*					
	> 3.5 ng/ml		> 5 ng/ml		> 10 ng/ml	
Age (mo)	am	pm	am	pm	am	pm
6	2.25	0.79	2.06	0.79	0.79	0.11
7	0.52	3.10	0.43	2.73	0.12	1.33
8	5.79	2.08	5.79	2.10	4.46	1.60
9	5.64	3.39	5.12	3.06	4.84	3.00
10	1.63	2.36	1.63	2.19	0.70	1.85
11	1.06[a]	9.94[b]	0.92[a]	9.83[b]	0.12	9.01
12	1.12	3.05	1.12	3.05	0.65	2.82
13	1.10	1.85	1.11	1.69	0.16	0.69

se (>3.5 ng/ml)= 0.9, se (>5 ng/ml)=0.86, se (>10 ng/ml)= 0.73

* Within row, means with no superscript are not significantly different (P>0.05).

Table 5. Mean area under the curve (cm^2) by age

Age (mo)	N	>3.5 ng/ml	se	>5.0 ng/ml	se	>10 ng/ml	se
3	38	1.51[b]	0.20	1.27[b]	0.18	0.55[d]	0.12
4	38	1.55[b]	0.21	1.35[b]	0.20	0.52[d]	0.09
5	38	1.57[b]	0.19	1.30[b]	0.19	0.66[d]	0.15
6	4	1.51[b]	0.29	1.42[b]	0.55	0.42[d]	0.57
7	4	1.81[b]	0.37	1.58[b]	0.54	0.68[d]	0.65
8	4	3.93[a]	1.78	3.93[a]	2.06	3.02[bc]	2.06
9	4	4.57[a]	1.67	4.41[a]	1.63	3.93[ab]	1.59
10	4	1.76[b]	0.66	1.68[b]	0.64	1.28[d]	0.61
11	4	5.51[a]	2.23	5.4[a]	2.13	4.58[a]	2.13
12	4	2.09[b]	0.65	2.09[b]	0.63	1.74[cd]	0.63
13	4	1.48[b]	0.25	1.4[b]	0.49	0.43[d]	0.49

Within column, means with different superscripts are significantly different ($P<0.05$).

Table 6. Age correction factors for baseline bst concentrations

Age (mo)	am	pm	pooled
3	0	0	0
4	0.06	0.51	0.29
5	-0.09	0.27	0.09
6	0.46	-0.2	0.13
7	0.15	0.96	0.56
8	0.4	1.76	1.08
9	1.23	0.62	0.93
10	0.8	0.53	0.66
11	0.14	1.67	0.9
12	0.39	0.3	0.34
13	0.22	0.1	0.16

Table 7. Age correction factors for mean bst concentrations

Age (mo)	am	pm	pooled
3	0	0	0
4	-0.25	0.61	0.18
5	-0.13	0.22	0.04
6	0.36	-0.11	0.12
7	0.14	1.21	0.67
8	0.95	2.2	1.57
9	1.94	1.44	1.69
10	0.78	0.85	0.81
11	0.19	2.25	1.22
12	0.33	0.57	0.45
13	0.11	0.18	0.14

Table 8. Age correction factors for peak bst concentrations

Age (mo)	am	pm	pooled
3	0	0	0
4	-1.92	1.04	-0.44
5	0.43	-0.31	0.05
6	0.62	-0.71	-0.04
7	-0.51	1.97	0.73
8	4.30	2.61	3.45
9	5.93	1.81	3.87
10	0.20	1.64	0.92
11	0.21	3.42	1.82
12	0.14	0.56	0.35
13	-0.31	0.31	0.00

Table 9. Pearson Correlation coefficient for bst parameters and pedigree index for milk production at different ages

Age (mo)	Time am/pm	Area >3.5	Area >5	Area >10	Np	Bc	Mc	Pc	Bst ind.
3	am	.05	.06	.11	.06	.12	.20	.04	.04
3	pm	-.16	.16	-.02	.01	.03	-.12	-.15	-.14
4	am	.17	.13	.17	.12	.02	.11	-.04	-.07
4	pm	-.05	-.05	-.17	-.02	-.27	-.18	-.31	-.13
5	am	-.17	-.19	-.06	.04	.15	.12	-.04	-.05
5	pm	.24	.29	.21	.07	.34*	.35*	.27	.14

Area >3.5, Area >5, and Area >10 represent the area under the curve above line 3.5, 5 & 10 ng/ml respectively.

Np, Bc, Mc, Pc & bst ind. represent the number of peaks, the baseline, the mean and the peak bst concentrations and bst index.

* $P<0.03$

Table 10. Rank of young sires bled intensively in windows of time

Criteria	Calf number			
	7976	7973	7974	7975
Pedigree index: milk	1	2	3	4
A.M bst index	1	2	4	3
P.M bst index	1	2	4	3
Area under the curve:				
>3.5 ng/ml, am	1	2	4	3
>3.5 ng/ml, pm	1	2	3	4
>5 ng/ml, am	2	1	4	3
>5 ng/ml, pm	1	2	3	4
>10 ng/ml, am	1	2	3	4
>10 ng/ml, pm	1	2	3	4

Table 11. Bst index for 24 h blood samplings of young sires by age

	Calf number			
Age (mo)	7973	7974	7975	7976
3	83	67	31	453
4	103	40	63	212
5	17	425	15	77
6	86	62	65	121
7	100	37	289	16
8	196	261	54	202
9	177	124	78	59
10	313	140	21	40
11	39	56	16	195
12	172	130	79	135
13	41	129	76	217

Table 12. Rank of young sires by pedigree index, bst index and area under the curve for the 24 h sampling.

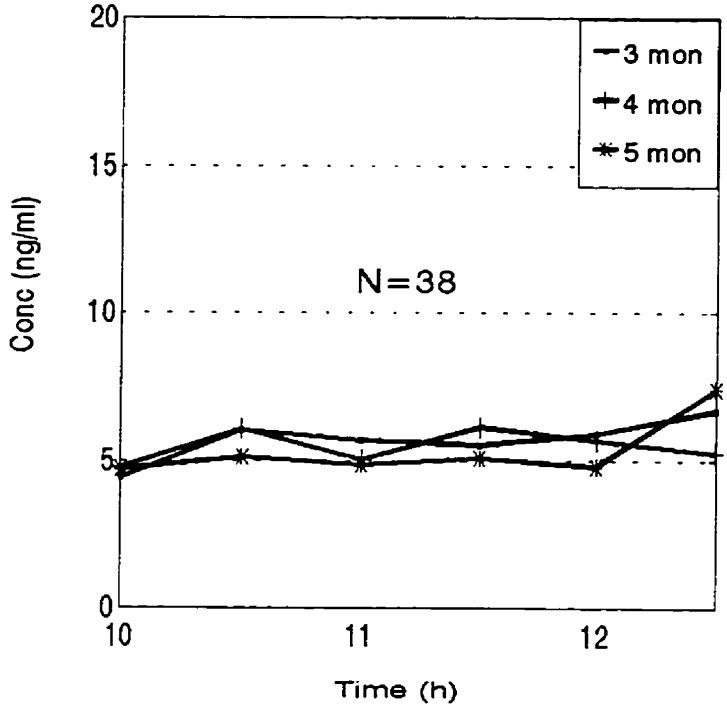
Criteria	Calf number			
	7976	7973	7974	7975
Pedigree index: milk	1	2	3	4
Bst index	1	3	2	4
Area under the curve:				
>5 ng/ml	1	2	4	3
>10 ng/ml	1	3	2	4

Table 13. Pedigree index, bst index and total area under the curve for the 4 calves bled for 24 h at 3-13 mo of age

Calf	Pedigree index	Bst index	Area >5 ng/ml	Area >10 ng/ml
7976	28.5	23801	116.85	82.95
7973	14	13196	90.6	39.02
7974	8	13649	54.57	44.84
7975	-3	8297	64.4	23.55

Bst index is calculated as indicated on page 28.

Figure 1. Mean endogenous bst concentrations at ages 3-5 mo (N=38).


20
15
10
5
0
10
11
12
Time (h)
Conc (ng/ml)
N=38
3 mon
4 mon
5 mon

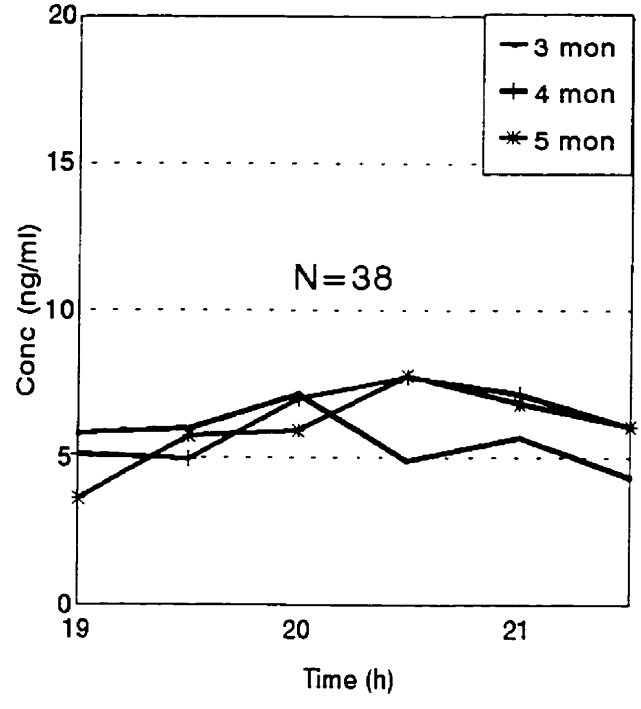
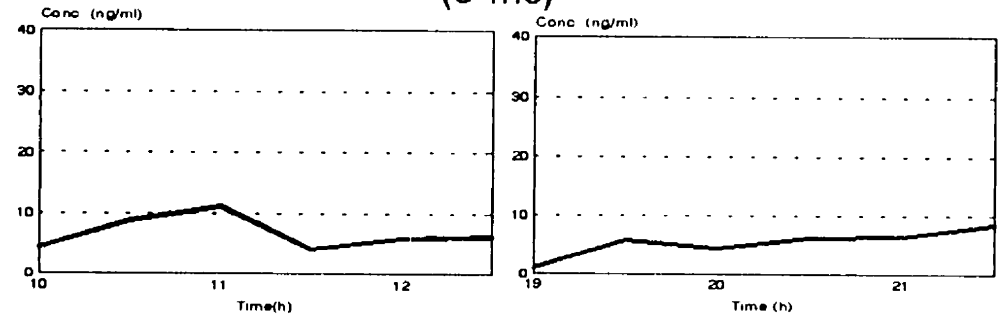
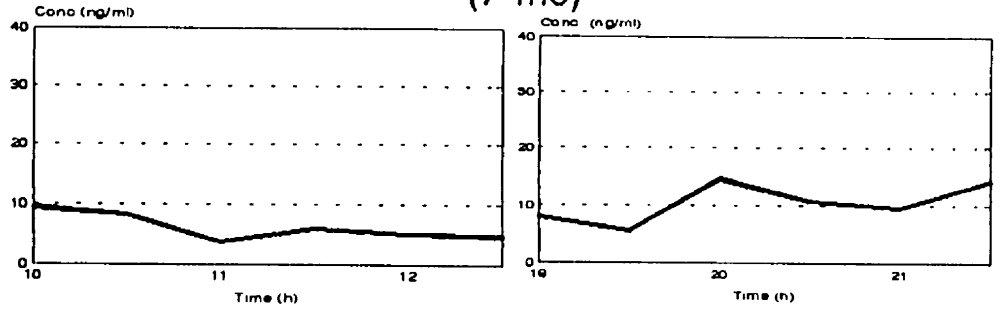

20
15
10
5
0
19
20
21
Time (h)
Conc (ng/ml)
N=38
3 mon
4 mon
5 mon

Figure 2. Mean endogenous bst concentrations at ages 6-13 mo (N=4).

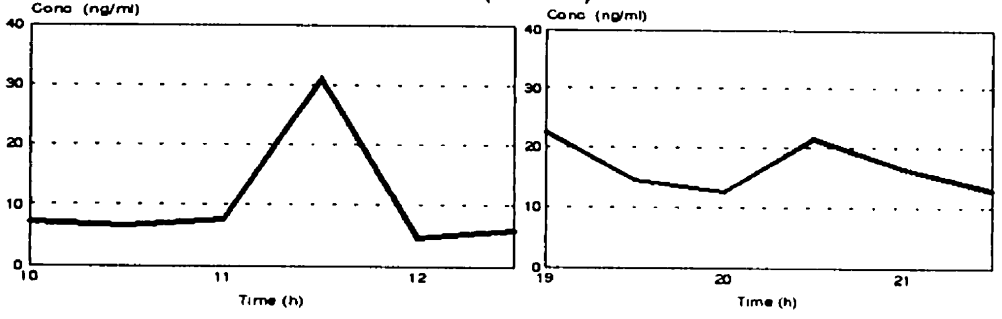
(6 mo)



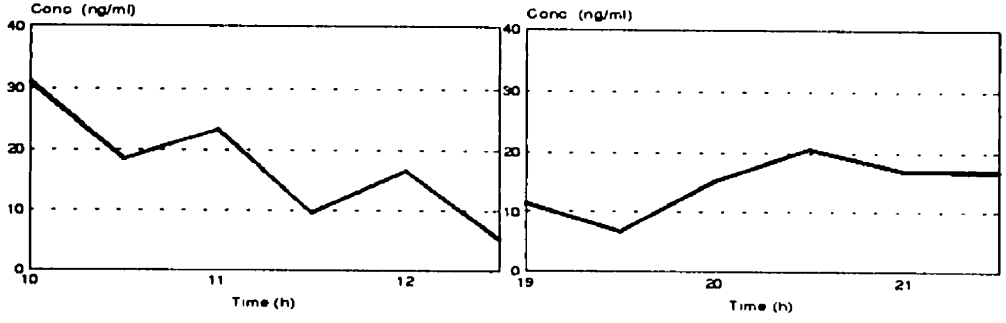
(7 mo)



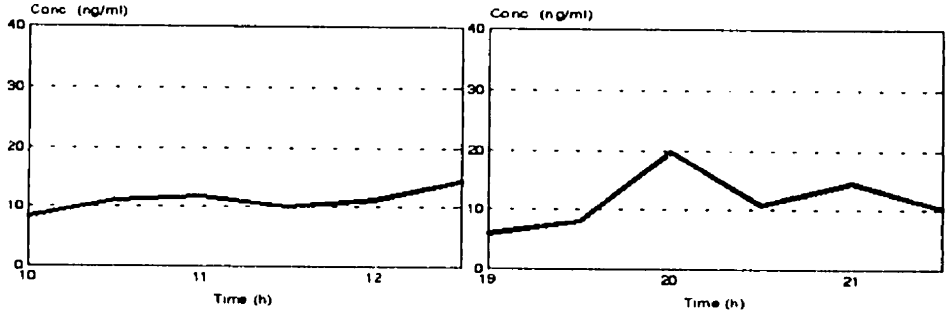
(8 mo)



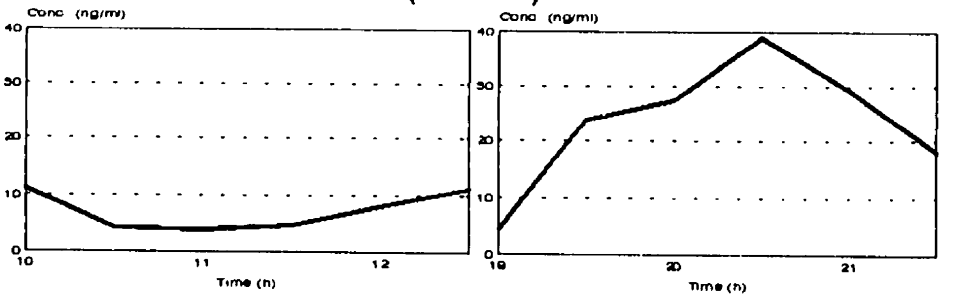
(9 mo)



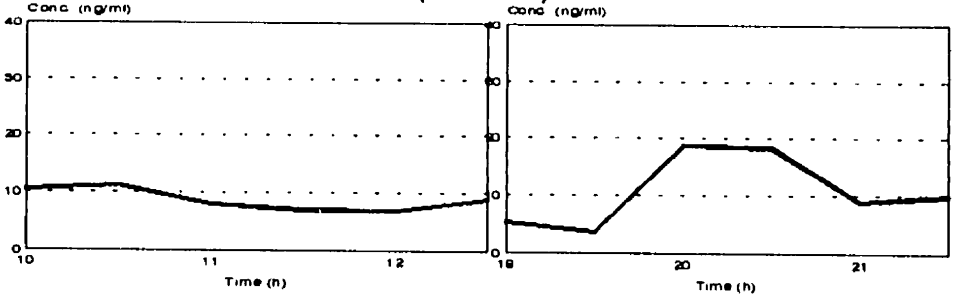
(10 mo)



(11 mo)



(12 mo)



(13 mo)

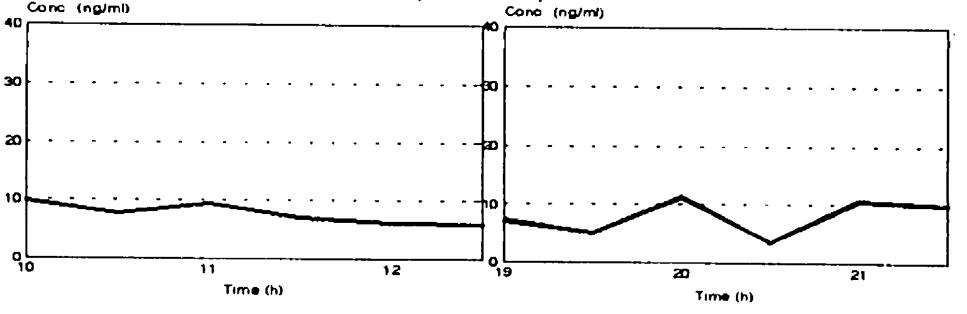
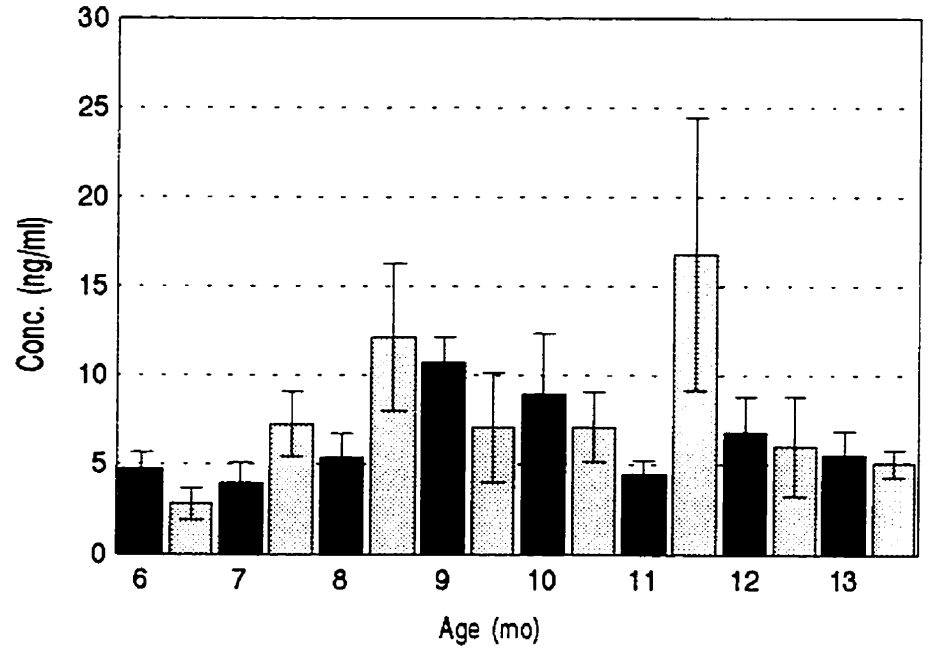


Figure 3. Mean and standard error (se) of baseline (A) and peak (B) bst concentrations at ages 6-13 mo (N=4) .

A


30
25
20
15
10
5
0
Conc. (ng/ml)
6
7
8
9
10
11
12
13
Age (mo)

A.M

P.M

B

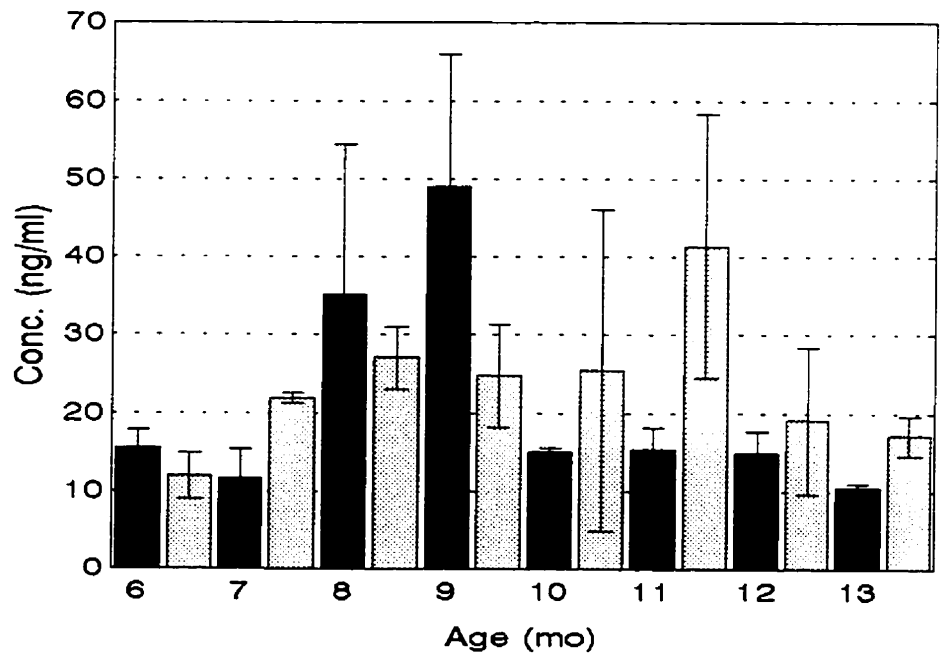
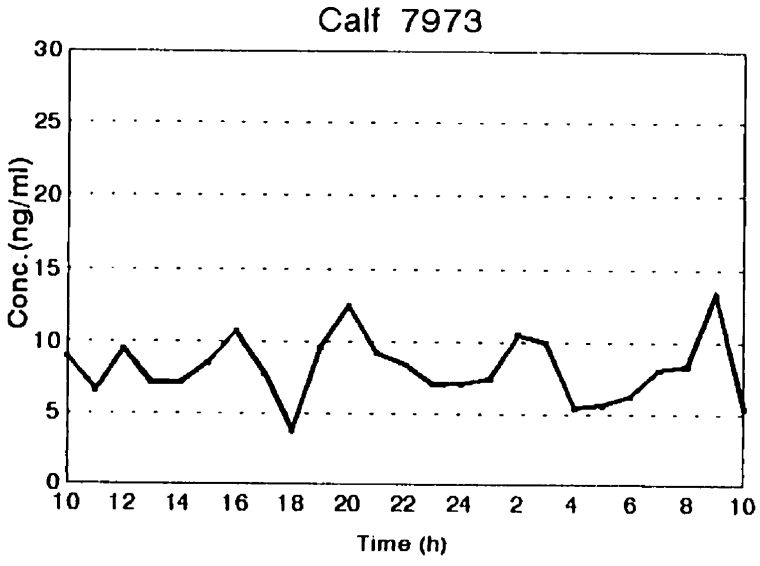
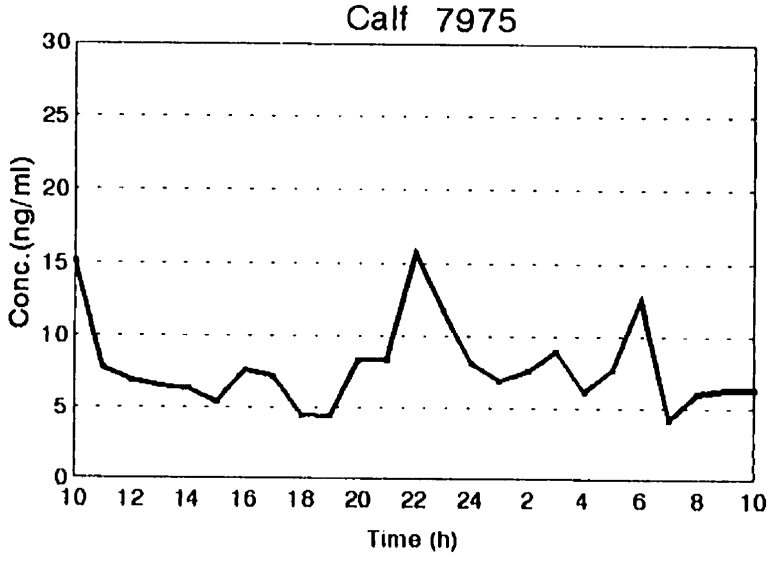
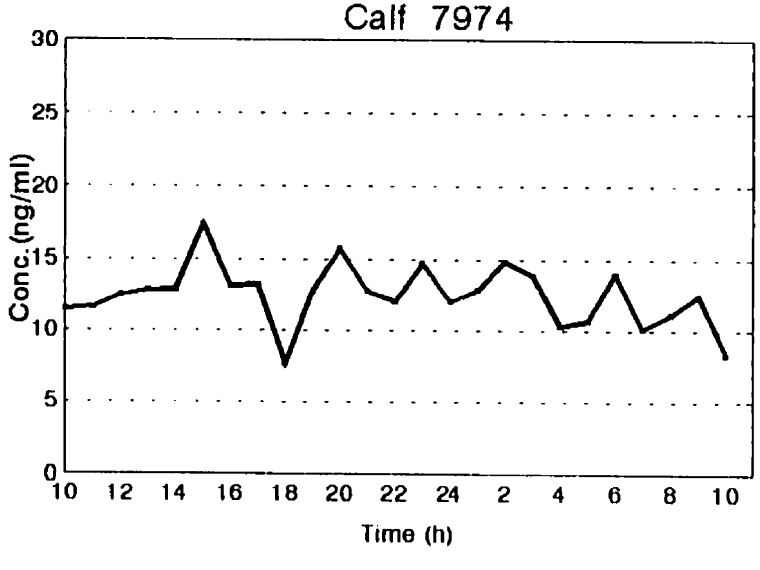
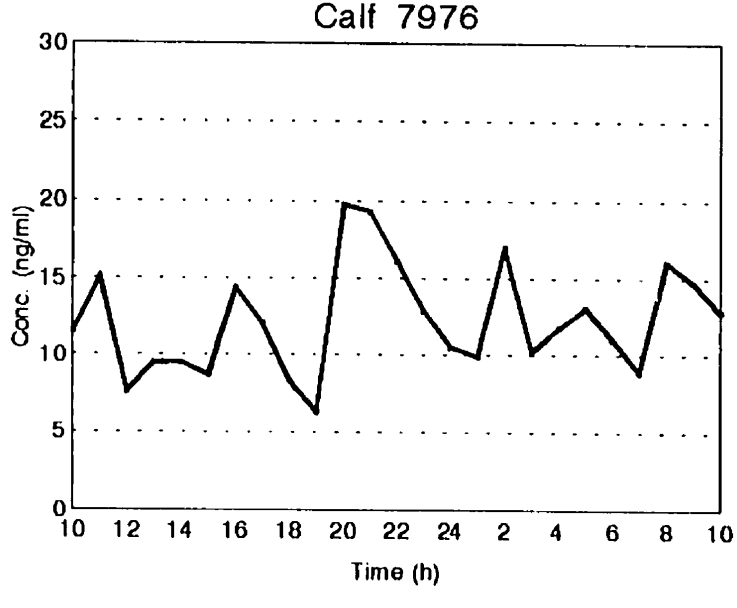

70
60
50
40
30
20
10
0
Conc. (ng/ml)
6
7
8
9
10
11
12
13
Age (mo)

Figure 4. Mean endogenous bst concentrations adjusted for age for the 4 calves bled for 24 h at 3-13 mo of age (N=4).


Calf 7973
Conc.(ng/ml)
30
25
20
15
10
5
0
10 12 14 16 18 20 22 24 2 4 6 8 10
Time (h)


Calf 7975
Conc.(ng/ml)
30
25
20
15
10
5
0
10 12 14 16 18 20 22 24 2 4 6 8 10
Time (h)


Calf 7974
Conc.(ng/ml)
30
25
20
15
10
5
0
10 12 14 16 18 20 22 24 2 4 6 8 10
Time (h)


Calf 7976
Conc. (ng/ml)
30
25
20
15
10
5
0
10 12 14 16 18 20 22 24 2 4 6 8 10
Time (h)

3.8 Discussion and Conclusion

3.8.1 The bst profile and its characteristics

Time affected bst concentration within calf significantly ($P<0.05$) which is in agreement with the reports of Beerepoot et al. (1993) and Bonczek et al. (1988). In most cases, most bst parameters measured, including mean bst concentrations, tended to be higher in the evening than in the morning. Woolliams et al. (1993) demonstrated that bst concentrations were higher (1.43 fold) in the afternoon than in the morning. Age contributed to the differences in the baseline, mean, peak bst concentrations and area under the curve. The effect of age on mean bst concentration was greatest beyond 6 mo of age. Mean bst concentrations overall tended to increase until 11 mo of age after which it declined. The increase in mean bst after the age of 6 mo and especially between 8-11 mo could be accounted for by stage of pubertal development. In contrast, in Holstein bulls in which samples were taken from birth to 12 mo of age, plasma levels were higher at birth than any other age and bst concentrations was relatively constant after falling from high levels at birth (Purchas et al., 1970). The work of Trenkle and Topel (1978) also in contrast, showed that plasma bst decreased with increasing age or body weight. But Yelich et al. (1994), in their work on heifers found that mean bst concentration were greater (12.9 ng/ml) at 1 week than 3 weeks before puberty (10.4 ng/ml); bst secretion increased prior to puberty. Similarly Keller et al. (1979) reported that bst concentration appeared to

increase in bulls reaching pubertal development. Peak bst concentrations, like the mean and the baseline concentrations, were higher between the ages of 8-11 mo. Baseline concentration tended to increase until 11 mo age. A decline in the mean baseline, mean and peak bst was observed after 11 mo age. The findings of Plouzek and Trenkle (1991a) showed that most bst parameters measured changed with age, especially as animals aged beyond 5 mo of age. They found that baseline concentrations decreased with age and total volume of secretion rate of bst increased with age but overall bst concentration in plasma significantly declined. But Lovendhal and Sejrsen (1993) reported an age related increase in basal and peak bst concentrations. The effect of age on area under the curve is uniform in that age had a significant ($P<0.05$) overall effect on area under the curve after 7 mo age.

The estimate of repeatability from age to age within calf was 0.28. These could probably be a result of secretory variation in bst and the shorter sampling windows. Within cows repeatability of bst concentration based on hourly samples collected for 2 weeks was 0.49 (Beerepoot et al., 1991). Similarly on the basis of hourly samples collected for 48 h a high repeatability of 0.92 was reported (Vasilatos and Wangsness. 1981). However Herbein et al. (1985) reported only a 0.21 day to day repeatability for bst concentrations.

In this study, although variation existed among sires of calves in their indices for milk production, sire didn't affect ($P>0.05$) bst concentration. Beerepoot et al. (1991) reported no

effect of sire on plasma bst concentrations of lactating Holstein cows. But Kazmer et al. (1986) showed that greater plasma bst concentrations were characteristic of daughters of superior sires. Similarly Mackenzie et al. (1988) indicated that young Friesian bulls from higher parental index values exhibited elevated plasma bst.

There are not many reports on the effect of season on bst concentration. Season was a large and significant source of variation with higher bst concentrations recorded in the summer (Beerepoot et al., 1991). In contrast Herbein et al. (1985) reported low bst concentrations in summer. The confusion is even greater since Bonczek et al. (1988) reported that season was insignificant in affecting bst concentration. The current results support the latter observation.

3.8.2 Relationship of bst with genetic merit

Numerous studies found that certain bst profile parameters have a potential predictive role in estimating production traits. Compared to the group with low genetic merit for milk production, those with high genetic merit exhibited high circulating bst concentrations (Lovendhal and Sejrsen, 1993; Parchuri et al., 1993; Lovendhal et al., 1991; Ohlson et al., 1987). With 7 and 8 h samplings at intervals of 15 and 20 min respectively, Kazmer et al. (1990, 1991) found low and insignificant relationships for basal, mean, maximum bst concentrations and area under the peak with the genetic merit of calves for milk production. In the work of Woolliams et al. (1993), it was the baseline bst concentrations

that were positively associated with the predicted breeding value.

In this study most bst parameters measured have low and insignificant correlations (r) with the pedigree index of calves for milk production. When the overall relationship was computed, it was only the baseline (r=0.15, P=0.007) and the mean (r=0.16, P=0.005) concentrations that were related to the calves' pedigree index. Further analysis by age and time of the day revealed that the baseline (r=0.34, P=0.0296) and the mean (r=0.35, P=0.0286) concentrations are related to the pedigree index of calves for milk production at 5 mo age, in evening samples.

For the 4 calves bled for 24 h at an hourly interval the bst index and the area under the curve above line 10 ng/ml are in agreement in ranking calves. But in both cases there is a switching of the ranks of the two middle calves when compared with the ranking by pedigree index. The switching may not be surprising because of the smaller variation between the indices of the 2 calves (14 vs 8) and the accuracy of pedigree index in predicting performances. The bst index for 24 h sampling is best indicative of the ranks of the calves at the age of 3 mo when the ranking of pedigree index is reconciled with ranking by bst index (Table 11).

3.8.3 Conclusion

Bst concentrations/secretion varied with the time of sampling and age. The effect of age on concentration was after 6 mo of age. Sire and season were not significant sources of variation in influencing bst concentration. Baseline and mean bst were related to the calves pedigree index at the age of 5 mo in evening sampling. Because long intervals of time separated the two successive sampling period, it is possible that major episodes of bst secretion were missed which may lead to misinterpretations about daily bst secretory patterns, and as a result its predictive role for production traits.

With 24 h sampling the bst index, even with small number of animals, was able to rank calves according to the pedigree index of calves for milk production at 3 mo of age.

Previous research works demonstrated that certain bst parameters could be related to the genetic merit of dairy calves and that these relationships can be exploited to save time and money in screening sires for progeny testing programs. However,this study has demonstrated that care must be taken in accurately characterizing the bst profile in order to obtain a high correlation with genetic merit. This dictates that the sampling time should be at least 24 h in duration at 60 min intervals.

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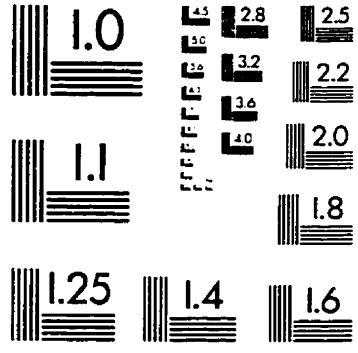

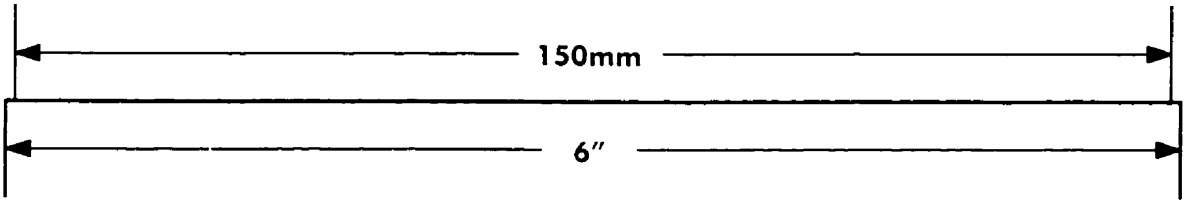

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