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STUDY ON THE EFFECT OF ADMINISTRATION OF RECOMBINANT BOVINE SOMATOTROPIN TO DAIRY COWS DURING ONE LACTATION ON HEAT STABILITY AND MINERAL DISTRIBUTION OF MILK

BY

WOJCIECH PIKUS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND

RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN

DAIRY SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

SPRING 1990

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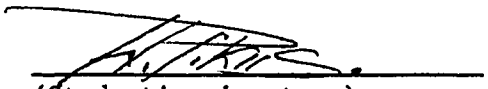

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FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED "STUDY ON THE EFFECT OF ADMINISTRATION OF RECOMBINANT BOVINE SOMATOTROPIN TO DAIRY COWS DURING ONE LACTATION ON MILK HEAT STABILITY AND MINERAL DISTRIBUTION " SUBMITTED BY WOJCIECH PIKUS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE IN DAIRY CHEMISTRY.

Dr. Lech Ozimek (Supervisor)

Dr. Michael E. Stiles

Dr. John Kennelly

ABSTRACT

Two separate studies under the same experimental conditions, experiment 1 and experiment 2, were designed to study the effect of injection of recombinant bovine somatotropin (rBST) to dairy cows on heat stability of milk and mineral distribution of milk during one lactation. Twenty dairy Holstein cows were selected for each experiment. They were combined according to parity (heifers and mature cows) based on their calving dates, and then randomly assigned to one of two experimental groups. The first group (ten animals) received an injection of 350 mg of rBST and the second group (ten animals) received injections of saline at the same time. The rBST sustained-release formula was administered at 14-day intervals by subcutaneous injection, starting between 28-35 days postpartum and continuing for a full lactation period. Milk samples were collected regularly (p.m. and a.m. milkings) for a whole treatment period, starting one week prior to the first injection and one week following injection. Milk samples obtained by this method were representative of two consecutive injections. Heat stability of milk has been determined as a function of heat coagulation time (HCT) and pH. The HCT-pH profiles of all milk samples were typical of "type A" milk with the HCT max. at pH 6.65 and the HCT min. at pH 6.80. Heat coagulation time of milk samples from cows treated with rBST was lower ($P< 0.05$) at HCT max. at pH 6.65 (16.3 ± 7.6 min.) compared with the control samples (18.2 ± 7.6 min.). There were no significant changes in the heat coagulation time of milk samples artificially adjusted to pH 6.80 (11.0 ± 6.5 min. vs. 11.1 ± 7.0 min.) and at the average natural pH 6.68 of milk (15.2 ± 7.7 min.

vs. 16.0 ± 8.6 min.) in the rBST and the control group, respectively. The concentration of total calcium was significantly ($P< 0.05$) higher (1136.2 ± 152.6 vs. 1083.0 ± 176.9 mg/l), but soluble calcium was significantly ($P< 0.05$) lower (415.8 ± 82.3 vs. 451.2 ± 83.6 mg/l) in rBST than in the control group. The higher concentration of total calcium in milk from rBST group might be related to the significantly ($P< 0.05$) higher concentration of proteins. Concentrations of total and soluble magnesium and total and soluble phosphorus were similar in milk from both groups.

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I. INTRODUCTION

The manufacturing sector of the dairy industry provides an important link in the chain starting with milk production at the farm and ending with a variety of wholesome dairy foods for the consumer. A variety of milk processing techniques, such as separation to remove fat and produce low fat products, pasteurization and sterilization to produce long shelf-life fluid products, fermentation to produce yogurt and cheeses, and freezing to produce ice cream and other frozen desserts are applied to convert milk into a diversity of products that satisfy a wide range of consumer needs.

Variability in milk composition and milk properties have a significant influence on manufacturing processes and final product quality. It is well established that changes in the chemical composition of milk result in changes in the manufacturing properties of milk. For example, variations in the protein content in milk cause significant changes in cheese yield, variations in fat content cause changes in milk flavour and in the melting properties of butter, and variations in the mineral salts cause changes in heat stability of milk.

The chemical composition and functional characteristics of milk vary due to factors such as diet, breed, genetics, mastitis, stage of lactation. Some changes in milk composition are of little importance to the farmer, and may be important to product manufacture. Recent introduction of bovine somatotropin as a farm management procedure

resulting in increased milk production could be another cause of changes in milk composition. There is not sufficient information available about the influence of bovine somatotropin administration to dairy cows on detailed composition of milk. Furthermore, there is not sufficient information available regarding changes in milk composition due to administration of bovine somatotropin to dairy cows during lactation.

Concern has been expressed by the dairy industry that changes in metabolic and digestive efficiencies may cause changes in milk components (fat, protein and mineral). Changes in milk components would also affect milk properties, such as the process of renneting, syneresis and heat stability of milk, and could have desirable or undesirable influences on characteristics of the milk for manufacturing. Therefore, it is important to determine the effect of bovine somatotropin administration to dairy cows on milk properties.

The object of this study is to evaluate the effect of administration of recombinant bovine somatotropin to dairy cows during one lactation on heat stabilty and mineral distribution of milk.

II. LITERATURE REVIEW

A. Effect of bovine somatotropin on milk production and composition

i. The role of hormones in milk biosynthesis and secretion

The importance of anterior-pituitary hormones in milk secretion was first demonstrated when it was shown that crude extracts of this gland could initiate milk secretion in rabbits with suitably developed mammary glands. Attempts to determine the specific hormone responsible for initiation of milk secretion led to the isolation of a specific protein which was given the name prolactin.

Subsequent studies by Denamur (1971), Banerjee (1976), and Forsyth & Hayden (1977) revealed that other anterior-pituitary hormones, progesterone and glucocorticoid, in addition to prolactin, were implicated in milk secretion (Fig. 1). The concept of a lactogenic hormone complex for the initiation of milk secretion was proposed by Folley and Young in 1941 (Folley, 1961).

Numerous experiments (Turkington & Kadohama, 1972; Denamur, 1971) led to a general acceptance of the concept that these hormones may activate specific genes and promote the transcription of a new species of mRNA. In recent research, Burton et al. (1987) fully substantiated this concept and it is now evident that bovine somatotropin (BST) may be a component of a lactogenic complex and that the components of this complex may vary somewhat in different species.

It has been customary to distinguish between the induction of milk secretion (lactogenesis) and the maintenance and enhancement

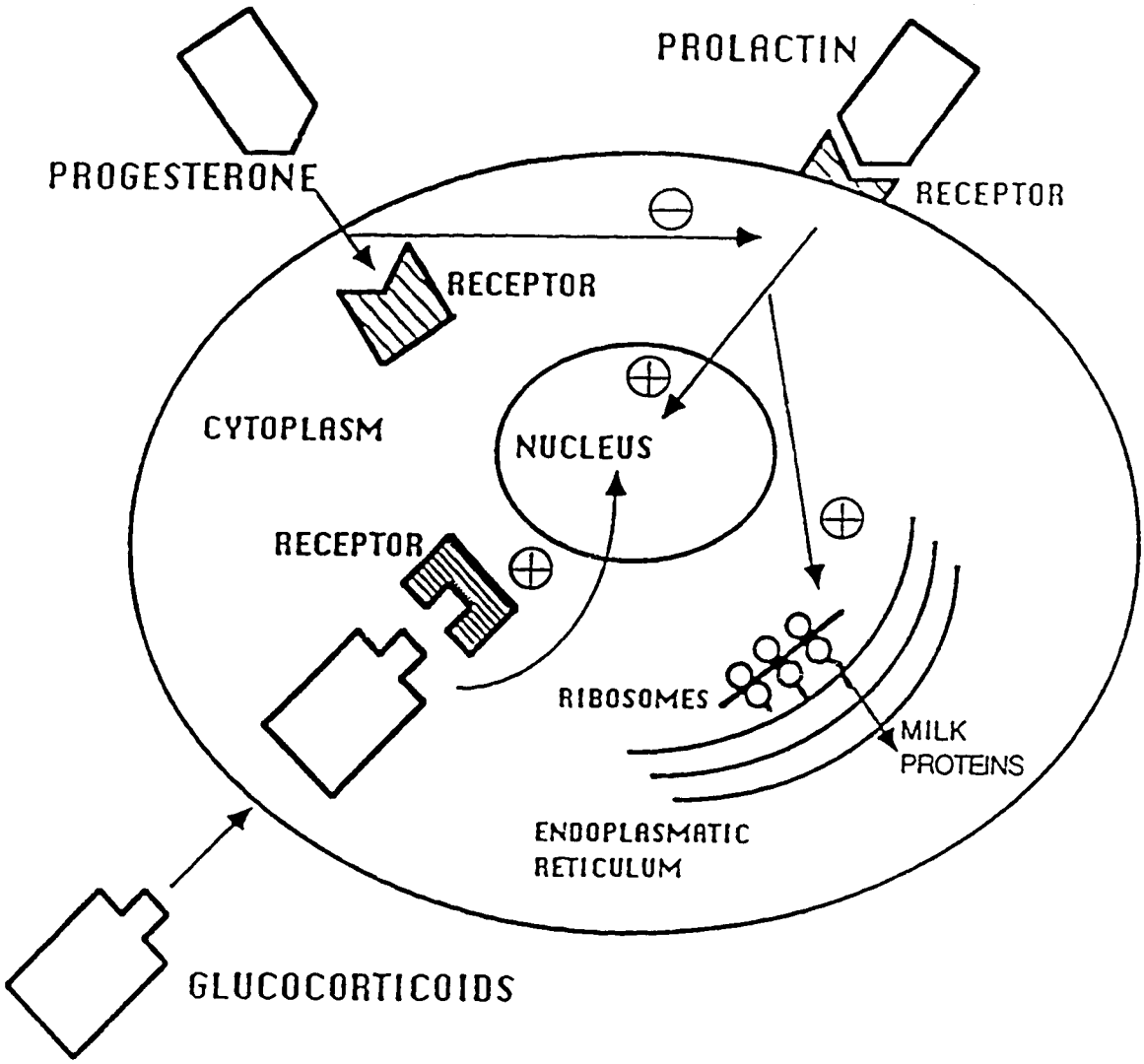

Figure 1. Schematic representation of hormonal stimulation of milk secretion in the epithelial cell, adapted from Mepham et al.(1986).

(galactopoesis) of an established secretion. Studies in rats from which the pituitary gland was removed indicated that prolactin, glucocorticoids and somatotropin are all important factors in both lactogenesis and galactopoesis. Injection of prolactin in a healthy lactating ruminant does not have a significant effect on milk yield, probably because of sufficient production of prolactin by the pituitary gland. On the other hand, injection of BST induces considerable increase in milk yield. These complex factors require further research.

ii. Bovine somatotropin

Recent advances in recombinant DNA technology have made possible the commercial availablity of bovine somatotropin (BST). The newly developed methods are based on gene "splicing". After identifying (through DNA "mapping" techniques) the gene or coding material responsible for a particular desirable characteristic, the gene is "spliced" and introduced into the DNA pool of bacteria. The genetic information incorporated into bacteria is expressed and under appropriate conditions, in combination with the fermentation and extraction process, results in high yields of pure hormone. BST obtained by this method has a similar biological activity to the natural hormone derived from the anterior pituitary gland (Bauman et al., 1985; Kronfeld, 1987; Eppard et al., 1987; Burton et al., 1987).

Bovine somatotropin is a large, complex peptide hormone containing 190-199 amino-acids. This hormone differs from steroid hormones such as oestrogen, progesterone or glucocorticoids. Its molecular weight is approximately 22,000 daltons, while the molecules of steroid hormones are much smaller, ranging from 200 to 300 daltons.

BST is not active when administered orally, while steroid hormones are active. Steroid hormones are not degraded in the digestive tract. A final difference between BST and steroid hormones is their different mode of action on the epithelial cell. BST probably binds to receptors on the cell surface and exerts its action from those sites, while steroids enter the cells and are transferred to the nuclei of cells where they exert their action (Baldwin & Middleton, 1987).

Peel & Bauman (1987) reported that the primary action of bovine somatotropin is to enhance the formation and secretion of insulin-like growth factor 1 (IGF-1) by the liver. They suggested that IGF-1 causes increases in biosynthetic capacity of epithelial cells in the mammary gland. These suggestions regarding the possible mechanism of IGF-1 mediation in increasing milk production were supported by Baumrucker (1986) who in his in vitro studies found that IGF-1 is active in stimulating metabolic capacity of the mammary tissue of cows. Gluckman et al. (1987) found that there was a threefold increase in blood plasma IGF-1 concentration following the administration of BST to dairy cows. They proposed a mechanism for increased milk biosynthesis in which the IGF-1 exerted biological effects like other hormones, by reacting with surface receptors of epithelial cells. Glim et al. (1988) in their in vitro studies provided evidence that IGF-1 interacts with the basal surface of epithelial cells and plays an important role in the enhancement of milk production in cows treated with BST.

iii. Effect of bovine somatotropin on milk production and composition

There have been numerous experiments that have specifically studied the response to BST at various stages of lactation. Annexstad (1987),

Eppard et al. (1987), Peel et al. (1983), Peel & Bauman (1987), Bonczek et al. (1988), Baer et al. (1989), Lough et al. (1989) and De Boer & Kennelly (1989) reported that long-term administration of BST was effective in enhancing milk yield when given throughout lactation. De Boer & Kennelly (1989) found that daily injections of BST significantly increased milk yield of dairy cows fed with low (11%) and high (16%) protein diets, but the effect was greater in cows fed the high protein diet.

In contrast, Bines & Hart (1982) reported that BST did not increase milk yield in early lactation, but the same cows did improve milk production when given BST between the fifth and six month of the same lactation. They postulated that exogenous BST is not effective in improving milk yield when endogenous BST is high, as would be the case in early lactation. Chalupa et al. (1987) examined the responses of cows to BST injections beginning at 28-35 days postpartum and continuing for 266 days. Cows receiving 12.5, 25.0 and 50 mg BST/day produced more fat than the controls, but feed intake was increased.

The effect obtained with BST is potentially important in dairy production but its acceptance depends on a number of factors, particularly:

- long term effects on animal and human health;
- economics of milk production
- effects on milk components in relation to milk processing; and
- the acceptability of the technique in relation to animal welfare.

Changes in the chemical composition of milk could results, in

changes in the manufacturing properties of milk. For example, changes in the fatty acid composition of milk fat will influence the flavour and texture of any dairy product that has a relatively high fat content (butter, ice cream and high fat cheeses). Changes in the milk proteins can influence moisture, texture, flavour and yield of various cheeses. Changes in the mineral balance of milk combined with changes in proteins, can influence heat stability of milk, which is important in the manufacture of sterilized and concentrated products.

Eppard et al. (1985), Bitman et al. (1984), Baumrucker (1986), Chalupa et al. (1987), Bauman (1987), Baer et al. (1989), Bonczek et al. (1989) and Lough et al. (1989) presented results of the fatty acid profiles of milk fat from BST treated cows. These authors indicated that there was an increase in the proportion of long chain fatty acids (particularly oleic acid) and a concomitant decrease in the percentage of short chain fatty acids. Baer et al. (1989) did not find a significant difference in flavour between milk samples from BST-treated and control cows.

Chalupa et al. (1987) did not observe an alteration in milk protein concentration during administration of BST to animals. On the other hand, Phipps (1988) reported a small but significant increase in milk protein (control 3.52% vs. treated 3.61%). Eppard et al. (1985) indicated that there was an increase in the concentration of α-lactalbumin up to 32% in milk from cows receiving an injection of 100 International Units (IU) of BST per day. Similarly, Baer et al. (1989) found during short term studies that significantly higher levels of α-lactalbumin were detected in milk from cows receiving daily

doses of BST. Baer et al. (1989) demonstrated that cows injected with BST in negative nutritional or energy balance produced milk with lower concentration of casein and higher concentration of serum proteins. The concentration of mineral elements (calcium, phosphorus, sodium, iron, copper and magnesium) in milk did not change during administration of BST to animals (Eppard et al., 1985; Peel & Bauman, 1987).

Administration of BST increased milk production up to 20-40 percent (Barbano et al. 1988; Phipps, 1988; Lough et al. 1989). This may cause changes in milk proteins and mineral concentration of milk. Variations in the concentration of certain inorganic salts or ions, particularly calcium, are known to affect the coagulation of casein in abnormal milk (Muir et al. 1978; Darling, 1980; Dalgleish et al. 1987)

Recent reports from studies on BST administration to dairy cows are contradictory and there is no clear picture if this hormone can influence the detailed composition of milk and its properties. Although, the galactopoetic effect of BST in dairy cows has been clearly demonstrated, there is lack of sufficient information concerning the physiology of somatotropin regulation and action in dairy cows. Futher successful commercial application of BST in the dairy industry will require research aimed at the development of more efficient production and delivery (method of administration of BST to cow) systems.

However, any changes in milk biosynthesis and secretion may lead to qualitative and quantitative changes in the distribution of macro- and micro-elements in milk. Furthermore, any potential changes may affect

the physico-chemical properties of milk that are important in milk processing.

One of the most important processes in the dairy industry is heat treatment. The most important property of milk related to this process is the heat stability of milk.

Knowing that BST may affect both the quantity and quality of milk, it is important to evaluate the effect of administration of BST on milk proteins, minerals and heat stability. Both proteins and minerals have a decisive effect on heat stability.

B. Heat stability of milk

i. Definition

The keeping quality of raw milk is comparatively poor. Therefore, for the preservation of milk and milk products different heat treatments are applied by dairies. When milk is heated, several important reactions take place. The pH is lowered by the breakdown of lactose to form formic acid (Sweetsur & White, 1975); the caseins are partly and wholly dephosphorylated (Howatt & Wright, 1934; Belec & Jenness, 1962; Fox, 1986); caseins dissociate from the micelles (Singh & Fox, 1985); calcium phosphate precipitates (Darling, 1980); and there are interactions between the serum proteins and the κ-casein on the micellar surfaces (Fox, 1986; Singh & Fox, 1987a).

Each of these reactions has the potential to accelerate milk coagulation when heated. Moreover, changes in milk fat, casein, serum protein and mineral composition due to seasonal, breed and nutritional factors, as well as management practices, may stimulate these reactions and affect the heat stability of milk.

Heat stability is one of the properties of milk that is important in the manufacture of dairy products. At present, most countries routinely heat treat their milk supplies. Knowledge of the major factors influencing heat stability of milk may prevent undesirable characteristics from developing in the final product, as well as optimizing the cost of the processing operation.

The heat stability of milk has been the subject of investigation by dairy scientists for a period of 60 years or more. Sommer & Hart (1922), Webb & Holm (1932), Pyne & McHenry (1955) and Cole & Tarassuk (1966) described the phenomenological property of milk to withstand high temperature treatments for long times.

White & Davies (1958) and later Fox (1986), defined heat stability of milk as the length of time that elapses between the placing of the milk sample in an oil bath at a defined temperature and coagulation indicated by flocculation, gelation and changes in protein sedimentability.

A subjective test for determination of the heat stability of milk was first described by Sommer & Hart (1922). Consequently, modified tests based on this principle have been used in studies of heat stability. However, all of these tests have same common drawbacks; they are empirical and subjective and they all require an experienced laboratory worker.

The method of determining heat stability, first described and used by White & Davies (1958), is the most popular. It measures time which elapses between immersion of milk sample in the hot oil bath and the moment when milk coagulate. A number ofexperimental factors, such as:

degree of tube fill, head space gas volume, rocking rate during heating, angle of tilt and assayed temperature are of importance when conducting the test.

The moment of milk coagulation (White & Davies, 1958; Rose, 1961a, b; Sweetsur & White, 1974; Fox & Morrissey, 1977; Fox, 1986) described as heat coagulation time (HCT), is of a specific nature, but the mechanisms involved have not been completely elucidated.

ii. Kinetics of heat induced changes in milk

There is no general agreement on the reason for milk coagulation at high temperatures, and there may be no single mechanism or set of reactions which causes milk to coagulate (Morrisey et al., 1981; Fox 1986). The remarkable stability of the caseinate system during heating is due to two constituents: κ-casein and colloidal calcium phosphate.

Thompson et al. (1969) reported that a high degree of hydration of casein micelles (approx. 2.0g H_2O/g protein) and a high negative zeta potential (approx. -18 mV) are both important for maintaining casein in the colloidal state. Schmidt (1982) found that caseins possess low levels of secondary and tertiary structures, a feature which contributes to their remarkable stability at high temperatures.

On the other hand, serum proteins maintain their stability and activity by a delicately balanced minimization of free energy in the "native" compact structure. However, long heat treatment at high temperatures results in changes in stability by disturbance of the native protein structure. The magnitude of such perturbations range from reversible changes in the spatial location of single amino acid

residues (low heat treatment), to total destruction of the secondary and tertiary structures maintaining the molecular structure, that is largely irreversible.

The best process design for the heat treatment of foods is one in which the chosen temperature/time combination of heating insures that the desirable effects are obtained but deleterious changes are kept to a minimum. By introducing a kinetic rate of reaction it is possible to describe the effect of temperature on the rate of decomposition of milk components.

The kinetic rate of reactions occurring during heat treatment of milk is dependent on temperature. The relationship between the rate constant (k) and temperature is frequently indicated (Walstra & Van Boekel, 1989) by the well known Arrhenius equation:

(1) $$k = A' \exp\left(-\frac{\Delta E^*}{RT}\right)$$

A' - is a so-called frequency factor, representing the frequency of encounters between reactants, and is the simplest case for a biomolecular reaction.

ΔE^* - is the activation energy, i.e. the kinetic energy of reactant molecules (expressed per mole) that is necessary to achieve sufficient contact between these molecules for the reaction to occur.

R - is the universal gas constant.

T - is the relevant temperature in °K.

The Arrhenius equation is an empirical equation that fits any reaction, and is therefore frequently used. However, the observed rate

constant should be interpreted with caution. In many cases, studying kinetic reactions in milk undergoing heat treatment, the observed rate constant will be an apparent rate constant. Therefore, determination of the order of a reaction in milk during heat treatment is necessary.

The order of reaction is determined experimentally. For example, denaturation of milk proteins is a first order kinetic reaction and is expressed as :

(2) $$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where k_1 and k_2 are rate constants.

The rate of reaction (k) is the change with time in concentration of the substance under investigation. For decomposition reactions it is -dA/dt and for the reactions leading to the formation of new compounds, +dB/dt. For the decomposition reactions of the "n" order of reaction the following equation is suitable:

(3) $$-\frac{dA}{dt} = k_n A^n$$

based on the reaction (2) following equations can be introduced:

(4) $$-\frac{dA}{dt} = k_1[A]$$

(5) $$-\frac{dB}{dt} = k_1[A] - k_2[B]$$

(6) $$\frac{d[C]}{dt} = k_2[B]$$

The integrated rate equation for equation (4) is a so-called first-order equation:

(7) $$\ln[A] = \ln[A] - k_A t$$

The rate equations for (5) and (6) are more complex. Walstra & Van Boekel (1989) found most of the chemical reactions occurring in milk to be second order. For the reaction of second order (8):

(8) $$aA + bB \ldots \dashrightarrow pP + qQ \ldots$$

is second order, the following possibilities exist:

(9) $$-\frac{d[A]}{dt} = k_A [A] [B]$$

(10) $$-\frac{d[B]}{dt} = k_B [A] [B]$$

However, it is often impossible to apply the kinetics described above to study the kinetics of reactions occurring in milk. This is due to the fact that many reactions occur simultaneously, each one with its own temperature dependence and products that may influence other reactions. This does not mean that kinetics are not useful in studying reactions in heated milk. In many cases the observed rate constant and order will be apparent and this can be helpful in determining the behaviour between particular milk components and establishing proper time-temperature conditions of heating (Walstra & Boekel, 1989).

iii. Effect of pH on heat stability of milk

A remarkable dependence between heat stability of milk and pH for bulk milks and individual cow's milk was first noticed by Rose (1961a, b). He indicated that heat coagulation time (HCT) of milk is

characterized by a particular curvelinear relationship when milk adjusted to pH 6.4-7.1 and heated at 140°C. Subsequent work (Rose, 1962) indicated that milks can be classified as " type A" and " type B" according to their HCT-pH profile. Most bovine milk has a "type A" HCT-pH profile, characterized by the presence of maximum heat stability at pH 6.7 and minimum heat stability at pH 6.9. Bovine milk classified as "type B" has an increasing HCT with an increasing pH. Rose (1961b), Fox & Hoynes (1975) and Fox & Hearn (1978) found that the addition of β-lactoglobulin or α-lactalbumin to artificial milk systems increases heat stability in the pH range 6.4-6.8, but decreases it at pH 6.8-7.1. The authors suggested that the effect of β-lactoglobulin on heat stability is due to the shift of HCT-pH profile to more acidic values.

Singh & Fox (1987a) studied the effect of heat treatment on isolated serum protein free colloidal micelles (SPFCM) and concluded that maximum heat stability in the HCT-pH profile is due to the presence of β-lactoglobulin and α-lactalbumin added during the experiment. Singh & Fox (1985) suggested that the presence of minimum heat stability in the HCT-pH profile curve of SPFCM is due to the dissociation of κ-casein from the casein micelles at pH > 6.9. Furthermore, Singh & Fox (1987b) concluded that dissociation occurs as a consequence of electrostatic repulsions which are promoted by β-lactoglobulin, and that β-lactoglobulin inhibits the dissociation of κ-casein at pH < 6.7.

Kudo (1980) presented a similar explanation for the HCT-pH profile for milk heated at 140°C and demonstrated that the occurrence of a

minimum heat stability in the HCT-pH curve is related to the presence of calcium ions. Their electric charge renders casein micelles unstable and reduces HCT of milk. Pyne & McHenry (1955) found that the pH of unadjusted milk decreases to 5.5-6.0 at coagulation, and indicated an inverse relationship between HCT and pH. They suggested that heat induced coagulation is a type of acid coagulation due to a decline in pH.

Fox (1986) claimed that the principal factors responsible for the decrease of milk pH during UHT treatment are: production of organic acids (formic acid) from lactose; the expulsion of CO_2; precipitation of calcium phosphate with concomitant release of hydrogen ions; and hydrolysis of organic (casein) phosphate with the release of hydrogen ions. Pyne & McHenry (1955) and Sweetsur & White (1975) concluded that both hydrogen and calcium ions promote heat coagulation.

iv. Mineral distribution in milk

The mineral components of milk are present mainly in the form of soluble salts (White & Davies, 1958). The major basic elements, such as calcium, magnesium, and sodium, form salts with acid elements: proteins, citric acid, phosphates, and chlorine. Some mineral elements are present in the form of colloids in association with caseins. This is the case particularly with calcium and magnesium phosphates and citrates (Schmidt 1982; Visser et al., 1979; Koop et al., 1979). All of the sulphur, part of the phosphorus, and many trace elements exist in organic combinations.

To a great extent, the heat stability of milk depends upon the ionic balance of milk (Sweetsur & White, 1975; Kiesker, 1977; Lyster,

1979). This effect is due to mainly polyvalent calcium, magnesium, phosphate and citrate elements. Calcium phosphates and citrates are particularly interesting from a technological point of view, because of their relative importance in the formation of casein micelles and the fact that their equilibrium is so variable (Kiesker, 1977).

It has been shown that calcium, magnesium, and phosphorus can be found in the form of proteinates, chlorides, phosphates and citrates (Schmidt, 1982). Phosphates and citrates can be mono-, di- and tri-calcium; mono-, di- and tri-magnesium or mono-, di- and tri-sodium salts. The equilibrium between these components is very difficult to predict, because it varies with many factors, such as pH, relative amounts of mineral salts, their dissociation constants, the solubility and degree of ionization of the salts, etc. (Kiesker, 1977). In the case of proteinates, the valency numbers of acid radicals, and their dissociation constants are not known so that it is impossible to establish the equlibrium reactions (Pijanowski, 1980). It is evident that an increase in hydrogen ions pushes the equilibrium towards the less dissociated state while an increase in calcium ions, pushes the equilibrium downwards (Darling, 1980). Because of its low solubility, tricalcium phosphate precipitates, producing a greater dissociation of phosphate ions. Thus the salt equlibrium of milk is affected by temperature. Contrary to most salts, the solubility of tricalcium phosphate decreases with an increase in temperature. It is important to note that the establishment of a new equilibrium is not instantenous, in fact it may take several hours. Therefore, control of mineral distribution in milk is necessary to avoid interruption of

manufacture pasteurized, sterilized and concentrated milks. Furthermore, knowledge about reactions occurring between mineral components (when appropriately applied in the process of heat treatment of milk) can cause an increase of heat stability of milk (Newstead et al., 1977a).

v. Effect of minerals on heat stability of milk

Early electron microscopic studies of heated milk indicated that high heat treatments caused an increase in the casein micelle size (Hostettler et al., 1965). It appears that the increase in size is related either to serum protein denaturation and aggregation with the casein micelles (Moor, 1975; Rüegg & Blanc, 1979); or to a shift in location of calcium phosphate (Sweetsur & White, 1974; Kudo, 1980).

Increases in both heating time and temperature cause, increases in the micellar size, which is also influenced by pH. Based on this information, Sweetsur & White (1974) described an alternative mechanism for milk HCT in the pH range 6.4-7.1 at 140°C. They suggested that the occurrence of minimum heat stability in the HCT-pH curve is a result of "premature coagulation", caused by a deposition of precipitated calcium phosphate on casein micelles.

Fox & Hoynes (1975) reported that destabilization of the β-lactoglobulin-casein micelle complex at pH > 6.8 and 140°C, caused precipitation of calcium phosphate from milk serum on the caseinate system. In support of this research, Darling (1980) indicated the importance of calcium concentration in heat stability of milk. He reported that the presence of the minimum in the HCT-pH curve is due to the combination of two effects, namely protein interaction

and calcium phosphate precipitation.

However, Dalgleish et al. (1987) indicated that heating does not necessarily cause the permanent deposition of calcium phosphate from the milk serum on the casein micelles. They concluded that the minimum in the HCT-pH profile can not be explained exclusively by the behaviour of the inorganic constituents of the milk. According to these authors, the major effect of heat is to reduce the progressive mineralization of the micelles as the serine phosphate of casein is converted into inorganic phosphorus. This will almost certainly have consequences for the weakening micellar structure by the rupture of serine-phosphate bonds.

Problems connected with the lack of adequate methods of measurement of this phenomenon arise when designing an experiment to measure the true concentration of soluble and colloidal minerals in milk subjected to high heat treatment (>90°C). Dalgleish et al. (1987) suggested that although calcium phoshate may be precipitated on casein micelles at high temperatures, some or all of the deposited minerals may be redissolved on cooling. This creates problems for quantitative estimation. Lack of precise observation inhibits the complete elucidation of the mechanisms of interactions between minerals and other milk components. Therefore, the importance of such reactions on heat stability of milk remains unclear.

Lyster (1979) suggested that the micellar calcium phosphate is in a form of apatite. Koop et al. (1979) indicated the importance of phosphate and citrate as well as calcium for the formation of the typical substructure of casein micelles. Results of their work

suggest that the structure and stability of casein micelles in milk is determined by a very sensitive equilibrium between caseins, calcium, phosphate and citrate ions.

Schmidt (1982), supporting this observation, was unable to define the state in which colloidal calcium phoshate (CCP) is present in the micelles and the way in which it is linked to casein. He suggested that CCP is not a separate phase in the micelles and that calcium and inorganic phosphate are bound to the casein at different sites. Various solutions have been proposed (Rose, 1965; Schmidt, 1982;) for the nature of the binding between CCP and casein. Schmidt (1982) concluded that the binding between CCP and casein is electrostatic in nature, the CCP being positively charged and the casein negatively charged. This assumption could explain the ion-exchangable properties of micelles when milk is heated.

Fox and Hoynes (1975) found that the removal of CCP from casein micelles results in an increase in heat stability of milk at or below the pH of maximum stability. The removal of up to 40% of CCP also increases stability at the minimum pH, but further depletion of CCP renders the caseinate system very unstable at pH above 7.1.

Studies by Holt (1985) have shown that the calcium phosphate in the casein micelles is a form of tricalcium phosphate. To reconcile physical measurements of this to the known composition of the micelles, it is necessary that the phosphate groups of the phosphoseryl residues of the caseins are incorporated into the calcium phosphate. If this is indeed the case then the consequence is twofold: the distinction between the inorganic and organic forms of phosphate in the micelles

become difficult to make, and any dephosphorylation of the caseins may have an important effect on the structure of the calcium phosphate.

Schmidt & Payens (1976) proposed a casein model based upon submicelles consisting of a hydrophobic core, surrounded by a hydrophilic coat of carboxylic and phosphate groups linked together by calcium, magnesium and CCP. They found phosphorylation of casein-serine groups to be very important for the stabilization of casein micelle complexes. Calcium binds to these phosphoserine residues and causes reduction in the electrostatic repulsions between the proteins and promotes interactions between the hydrophobic regions. In the presence of low concentrations of calcium ions, such interactions promote micelle formation.

Studies by Whikehart & Rafter (1970) showed that removal of the phosphate groups from α-casein (91-96% dephosphorylation) drastically reduce its ability to be stabilized in such complexes by κ-casein when 20 mmol Ca^{2+}/l was added to the mixture. Yoshikawa et al. (1981) observed that an increase in the number of phosphate groups in both α- and β-casein by phosphorylation did not alter their ability to form micelles with κ-casein. It did, however, alter their binding capacity for calcium, and consequently increase their stability in the presence of the cation. Therefore, higher concentrations of calcium are required to bring about their precipitation in comparison with normal untreated caseins. On the other hand, removal of phosphoserine residues from β-casein prevented such binding and left the β-casein unaffected even by an increased calcium concentration.

The stability of milk proteins declines rapidly when milk is concentrated (Fox, 1982). Dalgleish (1987), studying heat stability of ultrafiltered (UF) milk, found that the calcium and phosphate in the milk serum are close to precipitation. He observed that the gentle heating of milk ultrafiltrate, especially when the ultrafiltrate has been prepared from cooled milk, leads to mineral instability.

Brule et al. (1978) found that serum proteins, small casein fragments and especially the protease and peptone components of milk, important in exercising a stabilizing effect on the potentially unstable calcium and phosphate, thereby preventing their precipitation when milk is severly heated.

There is little information on the effect of monovalent and multivalent ions on heat stability of milk. It is known that in unheated milk the citrate ions are extensively complexed with calcium in the serum (Holt, 1985). The importance of citrate ion in maintaining the integrity of the casein micelle has been documented by Visser et al. (1979) and Koop et al. (1979). However, low concentrations of these ions in milk make it doubtful that change in citrate concentration can influence heat stability of milk (Holt, 1985).

vi. Effect of breed on heat stability of milk

White and Davies (1958), Rose (1961a, b) and Morrisey et al. (1981) found considerable variation in the heat stability of milk from different breeds at the natural pH of milk. McLean et al. (1982) observed large fluctuations in the heat stability of concentrated milks prepared from milk of Jersey and Friesian cows. Fox and Hoynes (1976)

found that milk from different species: bovine, ovine and caprine, has similar heat stability at their respective maximum heat stabilities. According to Ganguli (1979), buffalo milk is less stable than bovine milk and he suggested that calcium concentration in buffalo milk is related to lower heat stability.

Differences in milk composition between breeds and species is one of the factors affecting heat stability of milk (McLean et al., 1987). Milk of various breeds and species differs also in milk protein composition (Aschaffenburg and Drewry, 1955; McLean et al., 1987). Aschaffenburg and Drwery (1955) provided the first example of genetic variation in milk proteins. From their data it can be concluded that the average values for both casein and milk serum proteins arise higher in milk from Jersey and Guernsey cattle than in milk from Holstein and Ayrshire cattle. Rolleri et al. (1956) found that Holstein milk contains less of the major caseins and more γ-casein than milk from other breeds.

Marked differences also exist between breeds in the distribution of the genetic variants of some proteins. Aschaffenburg (1968) observed that the frequency of occurrence of κ-casein A and B genetic variants was in proportion five to one in Ayrshire cattle, compared with a proportion of one to nine in Jersey cattle.

Little is reported on the influence of the genetic variation of milk proteins on heat stability of milk. Fegan et al. (1972) were unable to demonstrate any differences between HCT of milk protein genetic variants at natural pH, but they found that HCT of milk at maximum heat stability was affected by genetic variants of κ-casein

and β-lactoglobulin. Lin (1977) found that milk at natural pH was more heatstable due to the presence of genetic variant κ-casein AB instead of genetic variant κ-casein A. McLean et al. (1987) found considerable variation in the heat stability of concentrated milk at its natural pH and its HCT-pH profile in the pH range 6.5-7.0 due to genetic variants of κ-casein and β-lactoglobulin in milk from Friesian and Jersey cows. Moreover, they observed that maximum heat stability was affected by genetic variants of κ-casein (B>AB>A) and β-lactoglobulin (B and AB>A), whereas natural heat stability was affected only by genetic variants of κ-casein (B>AB>A). McLean et al. (1987) concluded that the difference in heat stability of skim milk between breeds could be explained by the difference in concentrations of genetic variants of γ-casein in milk.

vii. Effect of stage of lactation on heat stability of milk

White & Davies (1958) and Rose (1961a, b) provided evidence that heat stability of milk changes during the lactation and that these changes are related to variations in milk composition. They reported that stages of lactation have a strong influence on composition of milk proteins and minerals, particularly at the beginning and near the end of lactation. Bovine colostrum milk is exceptionally rich in proteins containing a large amount of immunoglobulins, β-lactoglobulin and α-lactalbumin. During the transition from colostrum to mature milk, the total protein content falls rapidly and at ten weeks postpartum it slowly increases again. The changes in casein concentration in milk during lactation are simlilar to changes in milk serum proteins (Rook & Campling, 1965). White and Davies (1958)

reported that total and soluble calcium decline in the transition from colostrum to mature milk and slowly increase through mid-lactation. The total calcium content rises towards the end of lactation while soluble calcium concentration stays below average. These changes in concentration of total and soluble calcium affect the heat stability of milk.

Fegan et al. (1972), in Australian studies, found that seasonal pasture changes (summer vs. winter) affect heat stability of milk and that this is related to changes in gross composition of milk. A similar observation was made in England by Fox (1986), who found that the lowest heat stability of milk at natural pH is in the period December-May, whereas the highest heat stability of milk was found in the period between July and November. McLean et al. (1987) could not find a relationship between observed changes in natural pH of skim milk, concentration of γ-casein and HCT of milk.

viii. Effect of hormonal stimulation on heat stability of milk

Early investigations by Asdell (1932), Evans & Simpson (1931) and Lyons (1942) showed that injection of crude pituitary extracts into lactating animals (rats, pigs and cows) increased milk production during lactation. Hart et al. (1979) reported that the administation of thyroxine, bovine somatotropin and thyroprotein (made by iodination of casein) to dairy cows all caused a significant increase in milk yield.

From a survey of the literature (Barbano & Dellavale, 1985; Bauman & Eppard, 1985; Eppard et al., 1985), it can be concluded that studies using bovine somatotropin (BST) have very little impact on gross

composition of milk. An increase in percentage of total fat, protein and lactose was reported by Hutton (1957). Contradictory to this, Peel et al. (1982) found a significant decrease in the percentage of total protein, paralleled by an increase in the percentage of fat, and no change in the percentage of lactose. Peel et al. (1983) and Fronk et al. (1983) reported no influence on the percentage of fat or lactose, but a statistically significant decrease in the percentage of protein in milk from BST treated cows. At this point, very little has been reported on the detailed chemical composition of milk produced by cows treated with BST. There is no information in the literature which relates changes in milk composition from cows treated with BST to heat stability of milk.

ix. Modification of heat stability of milk by technological methods

Changes in milk composition with advancing lactation, variations in protein concentration due to the genetic differences between breeds, and changes caused by change in seasonal pasture, all influence heat stability of milk. Because of this and to avoid disturbances during milk sterilization, evaporation, and spray drying, standardization of raw milk composition is commonly required.

The standarization of spring and autumn milks with demineralized whey permeates is one of the methods of standarization. This can be economically desirable and used satisfactorily, as indicated by Newstead et al. (1977a, b). The work of Newstead and associates was directed towards modifying heat stability of milk by standardization of milk with ultrafiltrates or demineralized ultrafiltrates. Sweetsur & Muir (1980) indicated the possibility of direct ultrafiltration of skim

milk as a suitable method of increasing heat stability of milk.

The early work of Sommer & Hart (1922) indicated the importance of salt balance on heat stability concentrated milks. Since then, the addition of phosphate and citrate to concentrated milks has been a common practice. However, the use of stabilizing salts affects the pH and therefore it is necessary to control pH of milk and the amount of salts added. Another method promoting control of heat stability of milk is forewarming. Thisconsists of heating milk at high temperatures before final processing. Davies & White (1966) found that forewarming of milk at 80-90°C for 10 minutes had a considerable stabilizing influence on heat stability of milk when the concentration of solids not-fat (SNF) was higher than 13%. They concluded that the stabilization effect during forewarming was due to a shift of the HCT-pH curve tc more acidic values by 0.16 pH units.

Muir et al. (1978) found that the preheating of reconstituted milk at 85°C for 30 minutes extends the region of minimum heat stability of milk. Sweetsur & Muir (1981) reported that forewarming for 1.5 minutes at 150°C is a suitable method of increasing the stablity of unconcentrated milk throughout the pH range 6.6-7.3, particularly in the region of minimum heat stability. A similar effect was observed by Sweetsur & Muir (1981) when milk was preheated at 120°C for more than 20 minutes.

Newstead et al. (1975) found forewarming at ultra high temperatures (UHT) for short periods of time useful in the production of milk concentrates that were characterised by higher stability during subsequent processing. Presently, the application of the forewarming

process to increase heat stability of milk is very popular (Newstead et al., 1975; Kiesker, 1977; Griffith et al., 1977; Fox, 1986). Griffith et al. (1977) reported that adjustment of milk to lower pH values than the pH at which a maximum heat stability occurs after forewarming is an additional factor that can cause an increase in heat stability of milk during further processing. Newstead et al. (1975) found an increase in heat stablity by lowering of pH of milk by 0.15 units. They indicated a considerable variation between milk samples and suggested caution with pH manipulation when adding buffer salts such as NaH_2PO_4 or Na_2HPO_4. In certain circumstances a mixture of these two salts can be added. This increases the level of phosphate at a given pH value and often gives stability where the single salt fails to do so. While stabilizing salts are necessary to establish the required stability and viscosity, their use should be kept to a minimum because they have an adverse effect on the flavour of the product when used in high concentrations. Fox (1986) indicated that the temporary readjustment of milk pH at 140°C to its natural pH value during HCT test was cause for an increase of HCT of milk.

x. Effect of thermal processing on heat stability of milk

The data in table 1 shows the changes in milk constituents by heating. A comparison of these data makes it clear that "short time" pasteurization preserves the organoleptic quality of the milk better than "high" temperature treatment. Milk proteins are already beginning to be denatured at pasteurization temperatures. Therefore, application of higher temperatures and longer holding times for milk processing, produces a cooked flavour in the milk and leads to the destabilization

TABLE 1. Effects of thermal processing on milk *

Heat Treatment	Thermization	Pasteurization		Sterilization	
		Short-time	High	UHT	Autoclave
Temperature (°C)	62-65	71-74	≥ 85	135-150	≥109-115
Holding time (s)	15-20*10^2	>15-30	> 2	2-20	12-24*10^3
Effects	XXXXX 100% XXXX Very severe XXX Severe XX Weak X Virtually none -- Not detectable				
Denaturation/ destabilization					
β-Lactoglobulin	--		XX	XXX	XXXXX
Whey proteins	--	--	XX	XXX	XXXXX
Casein	--	--	X	XX	XX
Fat globule membrane	--	--	XX	X	X
Vitamin loss					
Thiamin (vit.B_1)	--	--	--	--	XX
Riboflavin (vit.B_2)	--	--	--	--	--
Ascorbic acid (vit. C)	X	X	X	X	X
Binding of amino acids					
Lysine	--	--	--	--	XX
Formation of reaction products	--	--	--	X	XXX
Cooked taste	--	--	X	X	XXXX

* Adapted from Kessler (1989).

of protein-mineral complexes in milk (Renner & Schmidt, 1981).

UHT treatment in comparison to sterilization in an autoclave presents an advantage. UHT processing is clearly superior to sterilization because the treatment times are only 1/1000 of that of sterilization. Moreover, the rate of chemical reactions occurring during UHT treatment is lower than during the sterilization process.

Milk heated for a few seconds (by direct or indirect UHT method) undergoes changes in its nutritive value, colour and flavour due to changes in the casein, whey proteins, milk sugar and amino acid composition (Renner & Schmidt, 1981). There is some reversible movement of calcium, magnesium, citrate and phosphate ions between casein micelles and milk serum during UHT processing. These reactions both influence changes in heat stability of milk and the outcome of further processing.

Casein is not denaturated during UHT heating, but unfolding of certain peptide chains occurs (Fox, 1986). Burton (1988) reported that higher heating temperatures cause changes in the degree of aggregation of casein micelles. Aggregation is probably accompanied or followed by an increase in the number of small casein micelles and the total amount of soluble casein. Harjinder & Fox (1985) reported sedimentation of the casein micelles together with whey proteins after heating milk at 140°C for one minute at pH < 6.9. On the other hand, Harjinder & Fox (1985) found that heating milk at pH > 6.9 resulted in the dissociation of whey proteins and κ-casein from the micelles. Rüegg & Blanc (1979) reported that UHT heated milk had a larger number of casein micelles, which was responsible for a 25% increase in the mean diameter

of casein micelles. Hansen & Melo (1977) observed a release of sialic acid upon unfolding of the protein molecules due to UHT heating (sialic acid is incorporated in the glycomacropeptide of κ-casein). However, the authors reported that casein micelles were more resistant to alteration of their structure, while whey proteins especially β-lactoglobulins, were very susceptible to structural changes and that these changes are related to the sedimentation problem.

Ashton (1970) repoted that 90% of the β-lactoglobulin in the whey protein fraction of milk was denaturated during UHT processing of milk. Lyster et al. (1971) found that whey proteins were only 68% denaturated by UHT processing compared with 82% when processed by indirect heating. The denaturation of whey proteins is accompanied by an aggregation of whey protein molecules linked by disulphide bridges with casein particles. Burton (1988) reported that heat coagulation of whey proteins is mainly responsible for the formation of a sediment, however, the soluble fraction of casein also contributes to a certain extent. Hong et al. (1984) found different amounts of sulphydryl reactive groups in heated whey depending on the kind of UHT system used. Kirchmeier et al. (1984) suggested that sulphydryl reactive groups are responsible for cooked flavour, decreases in heat stability of milk, and deposit formation in the heat exchanger during processing.

Burton (1973) and Metha (1980) found that lactose is covalently bound to the protein fractions due to reactions between amino acids and reducing sugars (Maillard reactions). The concentration of Maillard compounds formed in UHT milk depends strongly upon the kind of UHT treatment (direct or indirect). Burton (1988) found for eleven milk

samples processed by UHT direct system, the concentration of hydroxymethylfurfural (HMF) was in the range of 3.1-7.6umol/l, versus 5.1-16.8umol/l HMF in fifteen milk samples processed by UHT indirect system.

Finot et al. (1981) found that intermediates of the Maillard reaction involve the formation of ε-deoxylactulosyl-lysine which undergoes acid hydrolysis and degradation to two new amino acids: furosine and pyridosine. These newly formed compounds are responsible for a drop in milk pH, a change that could lead to destablization of milk.

Minerals are also affected by UHT treatment. Hansen & Melo (1977) reported that concentrations of soluble calcium and phosphorus decrease with increased temperature. Changes in mineral distribution combined with changes in the pH affect heat stability of milk. For this reason, before evaporation or spray drying of milk, the viscosity of the product is determined and the solids content is adjusted to the required level. The addition of phosphate and citrate is required to stabilize the proteins. Small variations in pH, which influence the solubility of certain minerals, should also be corrected to improve heat stability of milk.

xi. Effect of evaporation and spray drying on heat stability of milk

Heat stability is a particular problem in the manufacture of evaporated milk because of the concentrated nature of the product and the need for sterilization. Kruk et al. (1972), working with conventional evaporated milk, indicated a 90% loss of heat stability

during concentration. A further 37% drop in heat stability occurrs when milk is sterilized. On the other hand, factors such as preheating and the adjustment of mineral concentration improved the heat stability of milk. Kiesker (1982) reported the influence of factors such as heating, pH and the concentration ratio on heat stability of milk, is largely dependent on their effect on the salt balance. According to Newstead (1977a, b), changes in the mineral level (in particular calcium), pH, protein level, whey proteins, and lactose, affect heat stability of concentrated milk.

The effect of lactation on protein and mineral concentration is of utmost importance when milk is evaporated. Milk obtained during early and late lactation has an abnormal composition. Muir & Sweetsur (1976) found that changes in the concentration of urea in milk from lactating cows affects heat stability of evaporated milk. Pearce (1979) reported that increasing urea levels produced small decreases in heat stability of evaporated milk regardless of whether urea was added before or after the preheating.

In an effort to improve the overall efficiency of the spray drying process, the tendency has been to concentrate milk as much as possible prior to drying. In modern plants, skim and whole milks are concentrated to 48-50% total solids. However, higher concentrations influence the subsequent solubility of the milk powders. The stability of the casein is decisive for the solubility of the milk powder; poor solubility is usually due to coagulation of the casein. Casein stability is extremely sensitive to variations in pH and salt balance, and both are affected during the process of milk evaporation. The pH

of milk concentrated to 48-50% total solids is approximately 6.1. Therefore, only a slight increase in the acidity of milk is enough to decrease the heat stability of milk when it is concentrated.

C. Summary

The ability of bovine milk to withstand high processing temperatures is a very important characteristic of milk, without which the processing of milk (pasteurization, sterilization and dehydration) would not be possible. Although mineral distribution is only one of the determinants of heat stability of milk, it can be a dominant factor when other compositional factors are constant and may affect heat stability. The introduction of BST as a new method of increasing milk production may influence mineral distribution in milk, thereby causing changes in the heat stability. Therefore, it is important to evaluate the effect of administration of BST on mineral distribution and heat stability of milk.

III. STUDY ON THE EFFECT OF ADMINISTRATION OF RECOMBINANT BOVINE SOMATOTROPIN TO DAIRY COWS DURING ONE LACTATION ON HEAT STABILITY AND MINERAL DISTRIBUTION OF MILK[1]

A. Introduction

Injection of recombinant bovine somatotropin (rBST) into lactating cows may increase milk production up to 40% (Bauman et al., 1985; Eppard et al., 1987; Barbano et al., 1988). There is evidence from other studies on lactating animals (rats and goats) that the use of various stimulating hormones particularly oxytocin or prolactin, cause changes in mineral concentration of milk (Linzel & Peaker, 1971; Peaker, 1978), and protein concentration (Forsyth, 1986) of milk. However, there is no information whether higher milk production causes changes in the properties and detailed composition of milk, both of which would be of importantce to the dairy processing sector. Most research assumes that the use of rBST does not affect milk composition. Alterations in mineral concentration could influence heat stability of milk, which is an important property in the manufacture of sterilized or concentrated dairy products.

Heat stability of milk, frequently described as heat coagulation time (HCT), is defined as the time required to coagulate milk proteins at a given temperature. White and Davies (1958), Kudo (1980),

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and Fox (1986) reported that milk components such as calcium, magnesium and phosphorus participate in determining the HCT. Rose (1961a, b) studied the dependence between the HCT of milk at 140°C and the pH in the range of 6.4-7.0. He reported that the shape of the HCT-pH curve is characteristic of individual milks and can be classified as "type A" and "type B". Milk "type A" has its maximum HCT (HCT max.) at pH 6.7 and minimum (HCT min.) at pH 6.9, whereas milk "type B" does not have a maximum and minimum HCT but demonstrates an increasing HCT with increasing pH.

Rose (1962) demonstrated that the removal of colloidal calcium phosphate from milk "type A" by acidification and dialysis caused an increase in HCT and disappearance of the minimum HCT from the HCT-pH curve. He suggested that the minimum HCT-pH at pH 6.9, for milk "type A" heated at 140°C could be attributed to heat-induced precipitation of calcium phosphate on casein micelles, destabilized earlier by complexes created with heat denaturated whey proteins. Fox & Morrisey (1977) concluded that an increase in the value of HCT minimum at pH 6.7 in milk can be explained by a decrease in heat-precipitated calcium phosphate, while a decline of the value of HCT maximum at pH 6.9 is due to the slower diffusion of calcium from the casein complex compared with phosphate and citrate. Pyne & McHenry (1955) suggested that the subsequent increase of concentration of soluble calcium in milk serum and the calcium phospate in the casein complex is accompanied by a rapid decrease of the HCT of milk when it is acidified.

Sweetsur & White (1974) reported the dependence of HCT on added

serum salts. The authors observed that increased concentration of serum salts, such as phosphate and citrate causes an increase in the HCT at pH 6.7, decreases the minimum HCT at pH 6.9, introduces a second minimum at pH values above 7.1 and the HCT-pH profile becomes similar to milk "type B". This indicates that minerals present in the milk serum determine the milk type with regard to HCT-pH dependence.

Darling (1980) suggested that coagulation of milk at 140°C on the acid side of the HCT-pH curve depends upon the "complexation reaction" of calcium with casein. As the concentration of Ca^{2+} decreases, pH increases and the casein micelles become stable to heat but protein polymerization and dephosphorylation occurs. The dephosphorylation process reduces the stability of the casein to such a degree that the lower concentration of Ca^{2+} levels can induce protein precipitation. The precipitation of insoluble phosphates results in further dissociation of the casein micelles and precipitation by heat is then determined simply by protein polymerization. The heat precipitation of these insoluble salts of calcium and phosphate increases with an increase in pH, causing an increase in HCT.

Recent research on the use of rBST focuses on the yield of milk and its approximate composition. No attention has yet been paid to whether rBST influences mineral distribution or milk HCT. Therefore, the main objective of this research was to examine the effect of injection of exogenous rBST in dairy cows during an entire lactation on HCT and the distribution of calcium, magnesium and phosphorus in milk.

B. Materials and methods

This study is part of comprehensive and collaborative studies on the effect of recombinant bovine somatotropin (rBST) on milk production, health aspects of dairy cows, reproductive physiology and milk quality at the Dairy Research Centre, Department of Animals Science, University of Alberta.

Selection of animals, their distribution into experimental groups, formulation of diets to meet nutritional and energetical requirments of cows, milking, records, feeding, blood sampling and other measurable parameters were performed according to research protocols #159-01-DK and #159-03-OK, specific for these experiments. More specific information with regard to these research protocols is available from Department of Animal Science and the Alberta Dairyman's Association Research Unit, Department of Food Science, University of Alberta. Selection of animals, their distribution and nutrition was designated to assure the minimum possible variation in milk composition, except for rBST.

i. Design and protocol of the experiments

Twenty dairy Holstein cows were selected for each experiment. They were combined according to parity (heifers and mature cows), based on their calving dates and randomly assigned to one of the two experimental groups. The hormonal group (ten cows) in each experiment received subcutaneous injections of 350 mg of recombinant bovine somatotropin (rBST), supplied by Cyanamid Canada, starting 28-35 days

postpartum and biweekly for the full lactation. The control group (10 cows) in each experiment received equivalent subcutaneous injections of saline. The experimental treatment and observations were made during 40 weeks of lactation, or until 70 days prior to the next calving.

ii. Diets

Cows were fed four rations based on concentrate, alfalfa silage (first cut) and oatlage during the experiment. The rations were changed at 10, 20 and 30 weeks after the first injection. The composition of the four rations and the dietary regime shown in Table 3.1. All animals were fed ad libitum to minimize the impact of the diet on milk production and composition.

iii. Milk sampling

Milk samples were aseptically collected from all cows (p.m. and a.m. milkings) starting one week prior to the first hormone injection, one week after the first injection and then every 4 weeks, so that milk samples were always taken in weekly intervals, between biweekly injections. Milk from afternoon and morning milking was mixed and sodium azide was added to prevent any microbiological changes in milk.

iv. Milk composition

Fresh milk samples taken at four weeks intervals were analysed for fat and lactose using infrared Milko-Scan apparatus, model 605. Protein was determined using the macroKjeldahl method (Pearson, 1970). Protein was calculated by multiplying the nitrogen percentage by 6.38, which is

TABLE 3.1. Composition of the experimental diets (percent of dry matter)

	A_1	B_1	C_1	D_1
Concentrate	48	41	33	26
High-Moisture Barley	17	14	12	9
Alfalfa Silage	30	30	30	15
Oatlage	5	15	25	50

A_1- First ration was given to animals during the first 10 week treatment period.

B_1, C_1 and D_1- Subsequent rations were given to animals at ten week intervals, following the first ration interval.

customary in protein determinations of all dairy products (A.O.A.C., 1975).

v. Determination of pH of milk

Milk pH is one of the major factors influencing milk HCT. To study the HCT-pH profile milk samples were adjusted to various pH values in the range 6.4-7.0, using 0.1 N NaOH or 0.1 N HCl. Milk samples were held at 4°C to achieve equilibrium at 4°C. The adjustment and reading of milk pH was done potentiometrically at 20°C using a Fisher glass electrode, model 13-620-271. The Fisher standard borate buffer solutions (pH 4 and 7) were used to verify the performance of the pH meter and electrodes at assigned pH values. Electrode calibration was repeated when the observed reading differed from the expected by more than 0.005 pH units.

vi. Test for heat stability of milk

Heat coagulation time at 140°C was determined as a function of pH by adjusting pH with 0.1N NaOH or 0.1N HCl in the range 6.5 -7.1 (every 0.1 ± 0.05 pH unit) by the "subjective heat stability test" of Davies and White (1966). The test was repeated in triplicate.

The 2 ml milk sample was placed in a 5 ml glass tube made from Pyrex tubing. The tube was closed with a silicone-rubber stopper and clamped crosswise on a brass carriage. The carriage was held horizontally and immersed to a depth of about 4 cm in a bath of hot liquid paraffin. Timing was recorded from the moment the carriage was immersed in the bath. Physical changes occurring in milk were then

observed. As soon as moving particles or clots were noticed throughout the milk the time was recorded as the heat coagulation time (HCT).

From the plot of HCT against the pH, the pH of the maximum heat stability (HCT max.), and the pH of the minimum heat stability HCT min.) and the heat stability of unadjusted milk (HCT nat.) were determined. The HCT-pH profile was measured in the pH range 6.5-7.1 up to the fourth month of lactation, to determine milk type, the minimum, maximum and natural HCT. In as much as the pH values for the HCT max., HCT min., and HCT nat. were consistent, after four months HCT measurments were done for three pH values corresponding to HCT values.

Test for heat stability of milk was conducted in the paraffin bath which had the following internal dimensions: length 30 cm, breadth 20 cm, and depth 15 cm. The temperature of the liquid paraffin (140°C) was indicated by a mercury thermometer graduated in 0.1°C. The ends of the sample carriage were fitted into slots in brass bearings, and fixed on the front and rear walls of the bath. The rear bearing was linked by 2 brass connecting rods in a vertical plane to an eccentric wheel (3.2 cm effective diam.) driven by a variable speed geared electric motor (4-12 rev./min.). The movement of the rotating eccentric wheel caused the sample carriage to rock in the vertical plane about its centre through an arc of approximately 36° and the milk sample to flow gently from one end of the tube to the other. The tube was illuminated from the above by a 150 W clear lamp and viewed at its centre with a magnifying glass. The thermostat was housed in a fume cupboard and fumes removed by a strong flow of air over the top of the bath and away from the operator.

vii. Determination of total calcium and magnesium contents of milk

Total Ca [Ca_t] and Mg [Mg_t] contents were determined using a Perkin-Elmer atomic absorption spectrophotometer, model 4000. Samples were prepared according to the method of Brooks et al. (1970).

Stock standard solutions for calcium and magnesium, prepared and standardized by McGaw Supply Ltd. (Canlab), were used in the preparation sequential dilutions 1, 2, 3, 4, 5 p.p.m. for Ca and 0.1, 0.2, 0.3, 0.4 p p [illegible] respectively. To increase sensitivity of method and to eliminate differences between concentration of ions in standard and milk solution, 1.6 mg/l Na, 5.0 mg/l K, 500 mg/l La and 1.2% (w/v) TCA was added to all standard solutions. All standard determinations were compared with a reagent blank containing 500 mg/l La and 1.2% TCA.

The milk sample was prepared by transferring a 5 ml aliquot of milk to a 100 ml volumetric flask, dilution with 50 ml of 24% (w/v) TCA and dilution to volume with deionized water. The sample was shaken at 5 minute intervals for 30 minutes, filtered, and a 1 ml aliquot of the filtrate was transferred to a 25 ml volumetric flask. Lanthanum solution, 0.2 ml of 5% (w/v), and 0.1 ml 1.2% (w/v) TCA was added and diluted with deionized water. Operating parameters for calcium and magnesium analysis were chosen according to Brooks et al. (1970).

The standard curve for calcium (Figure 3.1) was prepared based on readings obtained from analysis of standard solutions at the linear range of 5 mg/l. To assure the linear relationship between the concentration of ions in solution and spectrophotometric readings,

measurments were done at absorbance range lower than 4.0 p.p.m.(= 0.2 AB units). Similarly, a standard curve for magnesium, (Figure 3.2) was prepared based on the readings obtained from analysis of standard solutions in the linear range below 0.4 p.p.m.(= 0.2 AB units).

viii. Determination of soluble calcium and magnesium contents of milk.

Raw milk samples were forewarmed to 25°C and centrifuged at 77,000xg (30,000 rev/min.) for 30 min. in a Beckman ultracentrifuge, model L2-65 (rotor type 40). Soluble calcium [Ca_s] and magnesium [Mg_s] contents were determined, as described by Aoki and Kakao (1983). A 10 ml volume of milk serum, after separation from casein by ultracentrifugation, was transferred to a 100 ml volumetric flask and prepared for analysis using the same method as described for total calcium and magnesium contents.

ix. Determination of total and soluble phosphorus contents of milk

Milk samples (2 g) used for the determination of total phosphorus contents and the milk serum samples (2 g) for the determination of soluble phosphorus were transferred individually into silica dishes and evaporated in the vacuum oven (GCA, model 9), dried and transferred to the furnace and heated at 525-550°C for 12 hours and cooled in a desycator.

Hydrochloric acid solution (3ml, 1N) was added to dishes containing the ash and the samples were quantitatively transferred to 100 ml volumetric flasks, filled with deionized water and shaken to ensure a thorough mixing. The solutions were filtered through a dry filter

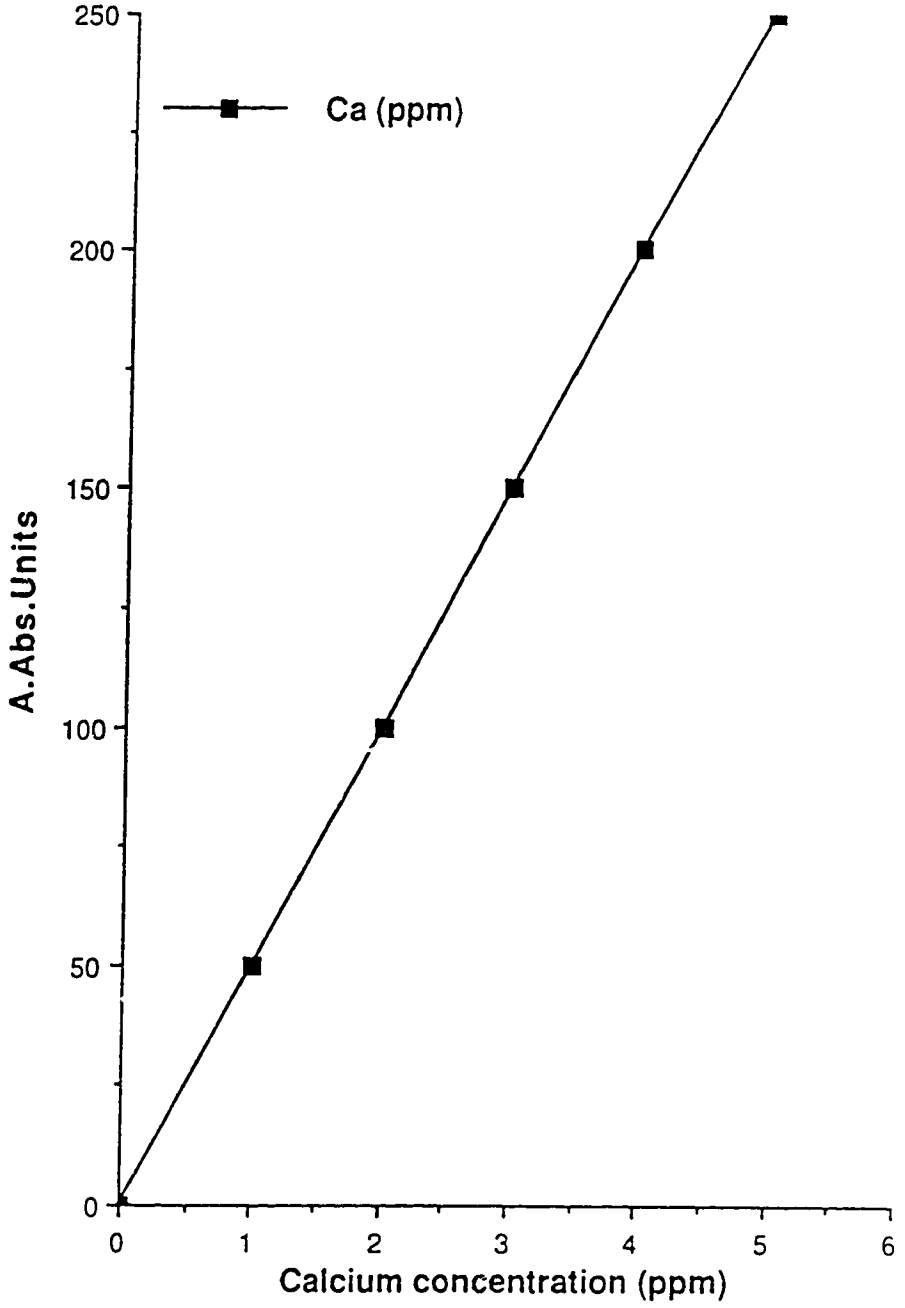

Fig. 3.1 Standard curve of calcium concentration versus atomic absorption spectrophotometer units.

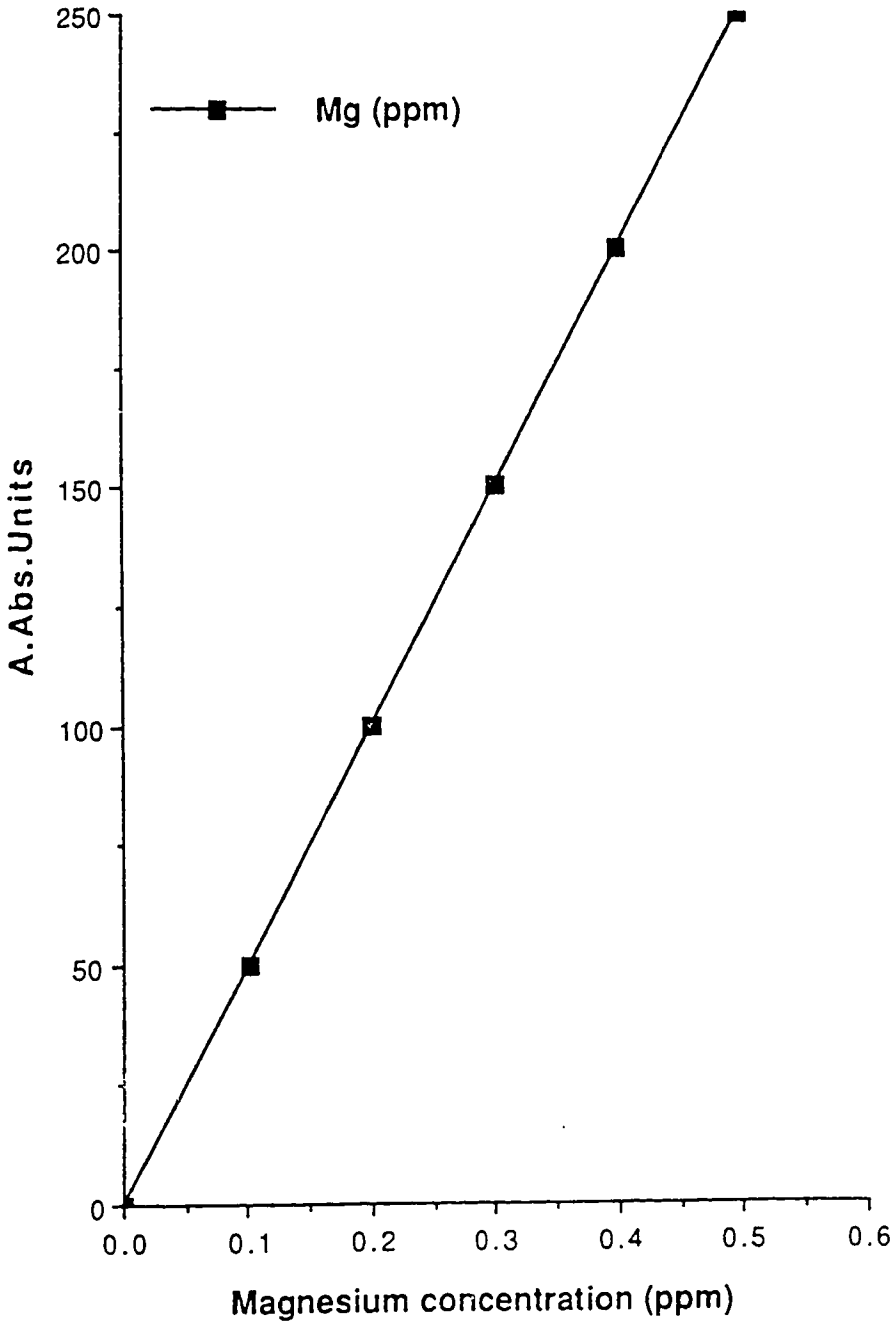

Fig. 3.2 Standard curve of magnesium concentration versus atomic absorption spectrophotometric units

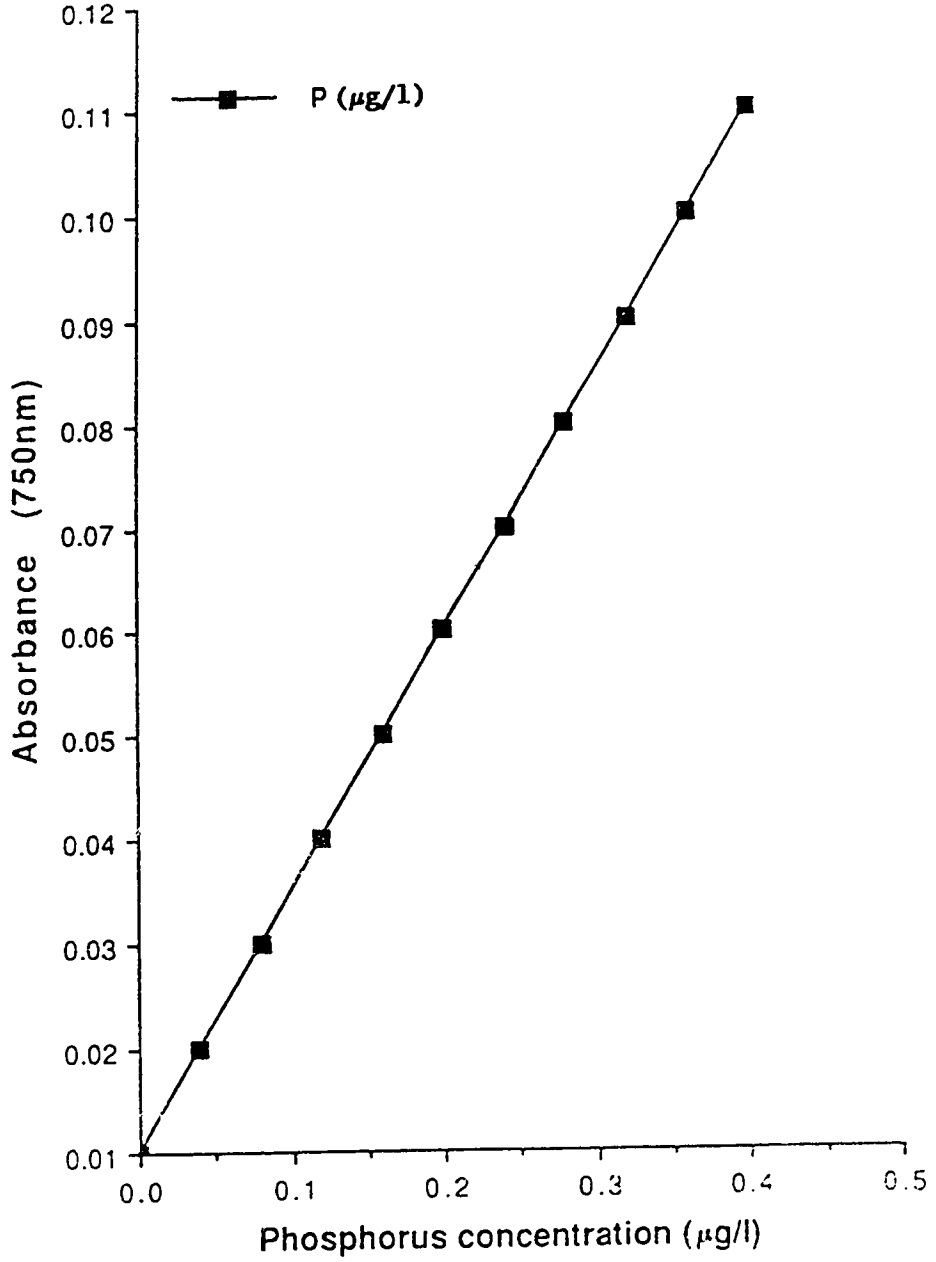

Fig. 3.3 Standard curve of phosphorus cocentration versus absorbance units at the wave length 750 nm

(Whatman # 42) and 10 ml of the filtrate was pipetted into a 100 ml volumetric flask, diluted to the mark with the deionized water and mixed thoroughly (Australian Standard, 1974).

Standard solutions for phosphorus were prepared by diluting 4.393g of potassium dihydrogen orthophosphate (dried at 105°C for 2 hours) to 1 liter with deionized water. The prepared solution (10 ml) was transferred into a one liter volumetric flask and diluted to the mark (1 ml of standard solution is equal to 10 ug phosphorus). Selected volumes of the standard solution were pipetted into a 25 ml volumetric flask for determination of the optical densities of the aliquots, and preparation of standard curve (Figure 3.3).

For determination of the phosphorous content, 5ml of ash solution, 2 ml of perchloric acid solution, 2 ml of diaminophenol and 1 ml of molybdate solution were pipetted into a 25 ml volumetric flask and filled to the mark with deionized water. After 5 minutes, absorbance was measured at 750 nm using a Baush & Lombe spectrophotomer, model 21.

x. Analysis of blood samples for bovine somatotropin by radioimmunoassay method

This part of experiment was done in the Department of Animal Science. Results are reported here to verify the administration of rBST to the to dairy cows by increased level of this hormone in the blood of experimental cows (De Boer & Kennelly, 1989). Blood samples were taken from each group of cows from the tail vein or artery by vacutainer, between 10.30 a.m. and 12.00 a.m. on the appropriate day relative to the first injection (-14, -7, 0, 7, 28, 84, 140, 196, 252

and 7 days prior to drying off). A new vacutainer needle was used for each cow. Blood was collected into stoppered vacutainer and centrifuged at room temperature for 10 minutes. Blood plasma samples were analyzed for BST.

xi. Statistical analysis

Statistical analysis of all data was conducted using SPSS-X package (SPSS-X User's Guide, 1988). The effect of treatment (saline vs. rBST) on HCT and the distribution of minerals in milk was analysed using the Student's t-test procedure. Simultaneously, the overall effect of treatment across the entire treatment period was tested using the repeated measures design procedure.

C. Results and Discussion

i. Comparison of replicate studies

Statistical analysis by t-test and repeated measure design procedure was done on the data from experiments 1 and 2 and did not show significant difference ($P > 0.05$) between experimental groups of cows. The only difference between the two experimental groups (replicates) were different animals. All other parameters were controlled to create the same experimental conditions of the two studies commenced in December, 1987 and June, 1988. Therefore, further statistical analysis was done on combined results from both experimental groups of cows, giving a total of 20 treated and 20 control animals in the study.

ii. Effect of recombinant bovine somatotropin (rBST) administration on the concentration of growth hormone in the blood of dairy cows

Treatment with rBST (350 mg/cow at 14 day intervals) increased concentrations of circulating BST in blood plasma of treated cows from 10 ng/ml at the beginning of the experiment to 19 ng/ml by the end of the experiment. The average concentration of circulating endogenous hormone in the control group was 9 ng/ml throughout the experimental period. The statistical analysis of blood samples from cows, conducted by the Department of Animal Science, showed that there was a significant increase in the concentration of rBST ($P < 0.05$) in blood of treated cows between the first and forty-first week of lactation (Figure 3.4).

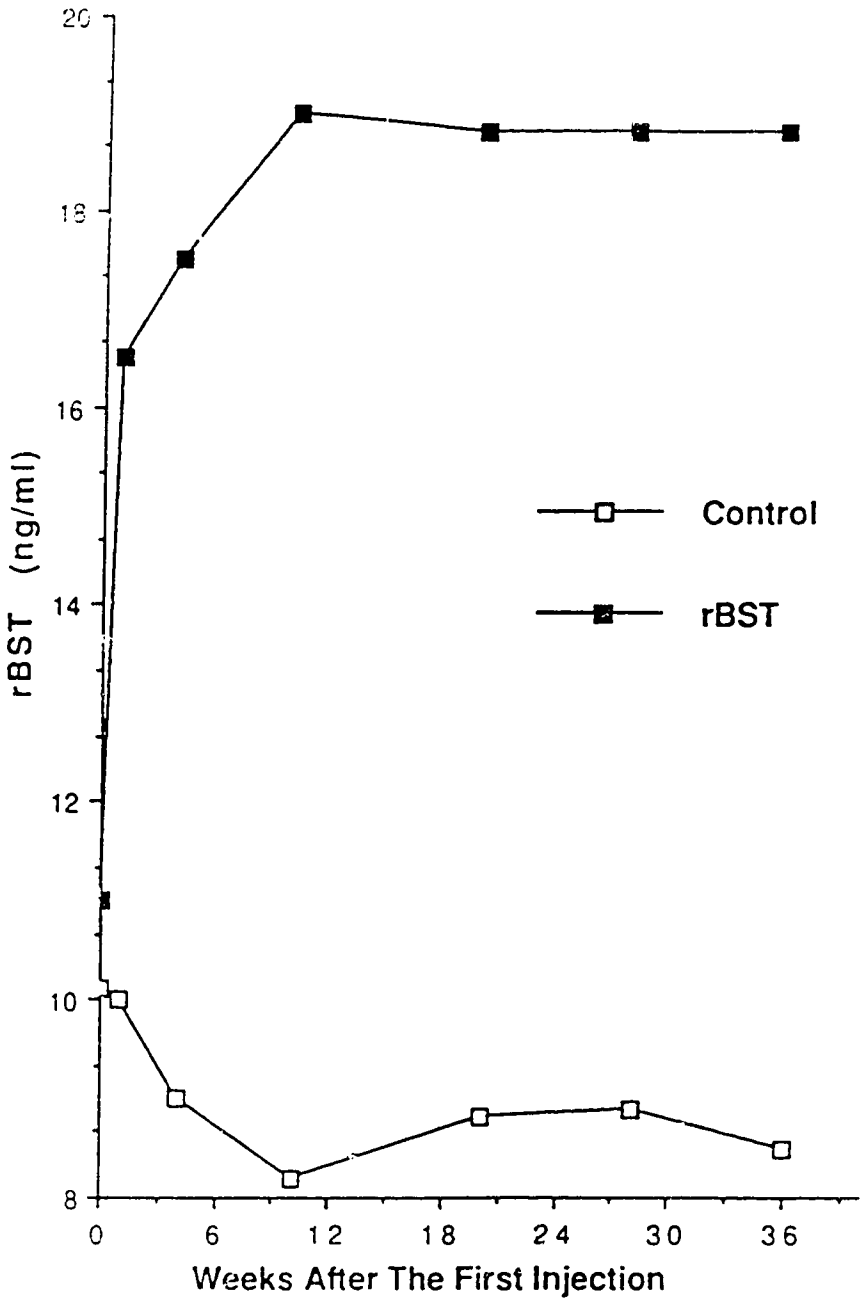

Fig. 3.4 Concentration of hormone in the blood from cows injected with saline or rBST during one lactation

The biggest difference in hormone concentration between rBST and the control groups was observed from the twelfth to the forty-first week of lactation. The higher content of BST in the blood of cows injected with rBST confirmed that the treated cowsd could be affected and that there could be an affect on milk composition.

The effect of growth hormone on milk yield has been reported in several studies (Eppard et al., 1985; Gluckman et al., 1987; Pocious & Herbein, 1986). Concentrations of hormone in blood after injection with exogenous hormone was increased from 5-9 ng/ml to 20-24 ng/ml. Similarly, Eppard et al. (1985, 1987) found higher concentrations of endogenous BST in the BST treated group of cows compared with the control group. Gluckman et al. (1987) found that an increased concentration of BST in the blood (due to supplementary BST) was accompanied by increased milk production.

iii. Effect of rBST administration to dairy cows on milk yield and milk composition

The mean values and standard deviations for milk yield, concentration of fat, protein and lactose in milk samples during one lactation for forty animals are given in Table 3.2. There was a significant effect of rBST treatment on the milk proteins ($P< 0.05$), but no treatment effects were observed on concentrations of fat and lactose during the entire lactation period. There was no significant increase in milk yield as a response to rBST treatment in the whole treatment group. This could be explained by the fact that selected

TABLE 3.2. Yield and composition of milk from cows injected with saline or rBST during one lactation

		Replicate 1	Replicate 2	Overall Mean	± SD	P[1]
Milk Yield	Control	27.3 kg/d	27.5 kg/d	25.9 kg/d	7.9	0.233
	rBST	27.6 kg/d	27.9 kg/d	27.4 kg/d	7.6	
Fat	Control	3.7%	3.6%	3.6%	0.7	0.253
	rBST	3.8%	3.7%	3.8%	0.8	
Proteins	Control	3.3%	3.5%	3.3%	0.3	0.003*
	rBST	3.5%	3.4%	3.5%	0.4	
Lactose	Control	4.8%	4.8%	4.8 %	0.2	0.141
	rBST	5.0%	4.9%	4.9 %	0.3	

[1] Significance of difference

* Significant difference in the concentration of proteins in milk between the rBST and the control group during one lactation.

cows were already high milk producers (Personal communication Dr. J.J. Kennelly, Department of Animal Science, University of Alberta).

To date, several long term studies using a sustained release vehicle for BST injections have been reported (Eppard et al., 1987; Bauman et al., 1985; McGuffey et al., 1987). A study reported by McGuffey et al. (1987) compared injection intervals of 14, 21, and 28 days and the BST injection of 360, 640, and 960 mg. Mean increase in milk yield over controls for the respective intervals of injection averaged 21, 18 and 17 percent. The authors noted that the magnitude of response to exogenous BST decreased with time after injection.

Ozimek et al. (1989) studied the protein concentration and distribution in milk samples from the same animals used in this study, and reported significant increases ($P< 0.05$) in total crude protein, true protein, caseins and whey proteins in the rBST treated group compared with the control group. Increase in proteins in milk from BST treated dairy cows were also reported by Eppard et al. (1985), Peel et al. (1983) and Bauman et al. (1985).

The impact of BST administration to dairy cows on the concentration of various components in milk is of great importance to manufacturers of dairy products and farmers. Changes in concentrations of milk components may affect the physical and chemical properties of milk. Davies & White (1966) and Fox (1982) found that heat stability of milk was significantly correlated ($P< 0.05$) with variations in the concentration of milk proteins and minerals, and that these components of milk were affected by the stage of lactation.

iv. Effect of rBST administration on heat stability of milk

The HCT-pH profile of milk is shown in Figure 3.5. In this study, milk samples for the treatment and the control groups showed HCT-pH profiles resembling "type A" milk with HCT max. at approximately pH 6.65 and HCT min. at pH 6.80. The average pH of fresh milk samples is 6.68 and is only slightly different from the pH at which the maximum HCT is observed (Rose, 1961a, b). In this study individual HCT values ranged from 2.8 minutes to 40.0 minutes with variations in the types of coagulation from very poor to very good. The standard error for the individual milk samples was acceptable when lower than 30 seconds. It has been suggested that variation in HCT between individual milk samples at 140°C are caused by changes in milk composition (Fox & Morrissey, 1977; Morrissey et al., 1981; Fox & Hearn, 1978).

Data for HCT of milk from treated and control milk samples is shown in Table 3.3. The values of HCT max. at pH 6.65 for the whole lactation was lower ($P< 0.05$) in the rBST group (16.3 ± 7.6 min.) than in the control group (18.2 ± 7.6 min.). There was a decrease in milk HCT max. at pH 6.65 for the rBST group vs. the control group in samples taken during mid-lactation, as shown in Figure 3.6. There were no differences in HCT min. at pH 6.8 (11.0 ± 6.5 min. vs. 11.1 ± 7.0 min.) and in HCT nat. at pH 6.68 (15.2 ± 7.7 min. vs. 16.0 ± 8.6 min.) for the whole lactation and during subsequent sampling weeks between the rBST and the control groups, respectively (see Table 3.3).

Results indicated that changes in milk HCT throughout the lactation period are not standard, as shown for the HCT max., HCT min. and

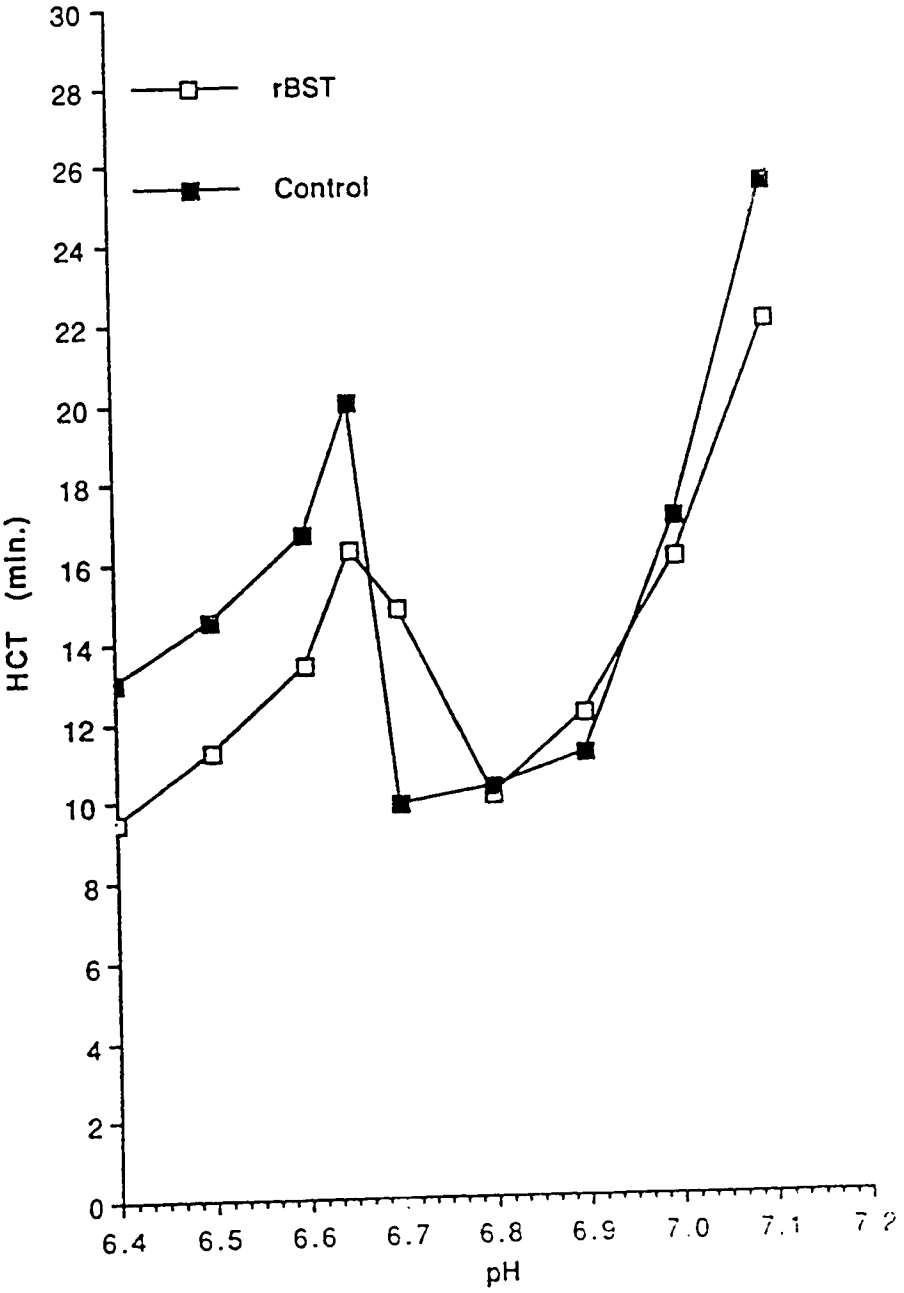

Fig. 3.5 Heat coagulation time (HCT)-pH profile of milk from cows injected with saline or rBST during one lactation

TABLE 3.3 Heat coagulation of milk (pH 6.65, 6.80 and 6.68 at 140°C) from cows injected with saline or rBST during one lactation

		Replicate 1 (min.)	Replicate 2 (min.)	Overall Mean (min.)	± SD	P[1]
HCT at pH 6.65	Control	18.1	18.3	18.2	7.6	0.001*
	rBST	16.0	16.5	16.3	7.6	
HCT at pH 6.80	Control	10.4	11.8	11.1	7.0	0.070
	rBST	9.8	12.2	11.0	6.5	
HCT in natural milk pH	Control	14.7	17.3	16.0	8.6	0.358
	rBST	14.0	16.4	15.2	7.7	

[1] Significance of difference.

* Significant difference in HCT at pH 6.65 for the rBST and the control group during one lactation.

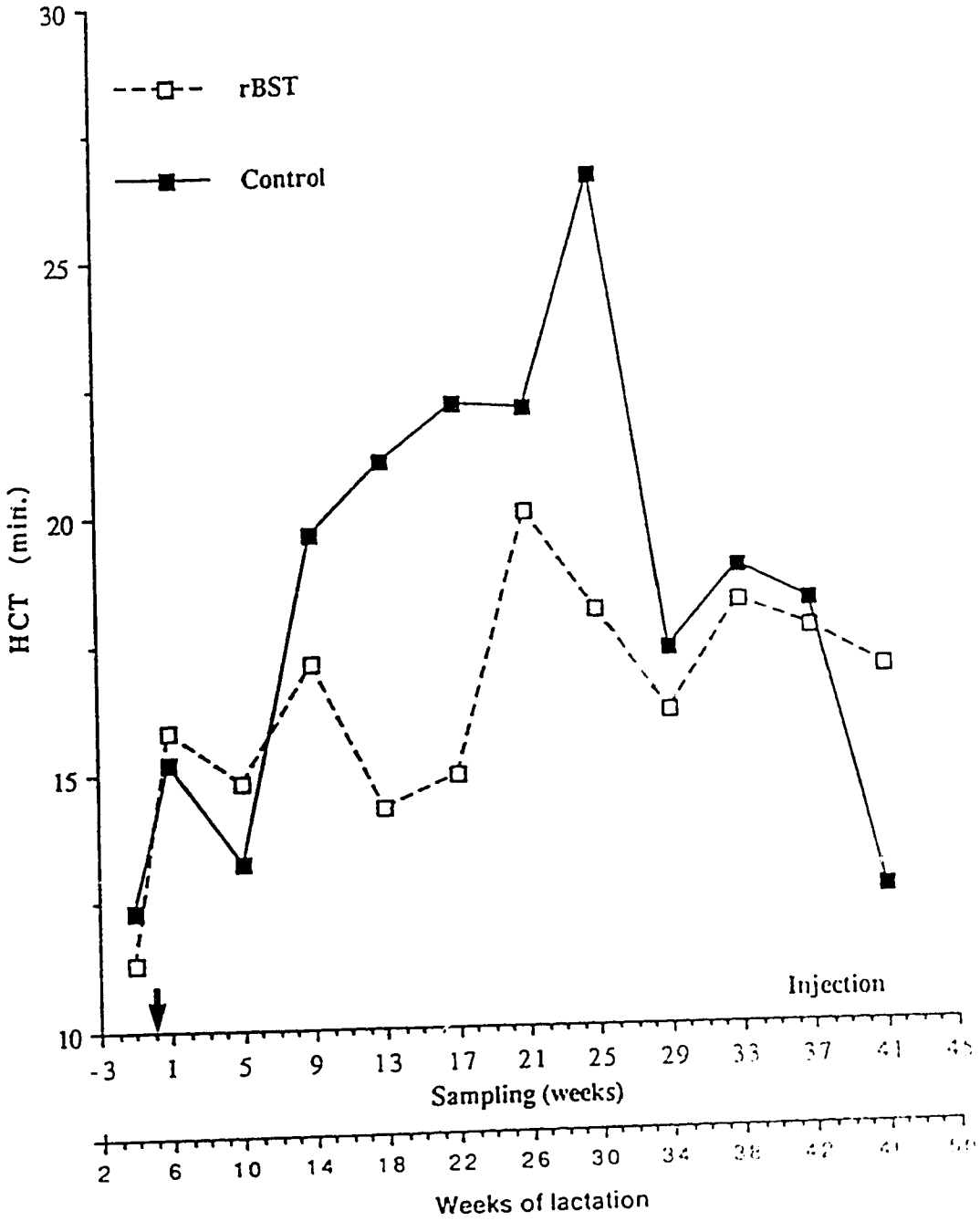

Fig. 3.6 Heat coagulation time of milk (pH 6.65 at 140°C) from cows injected with saline or rBST during one lactation

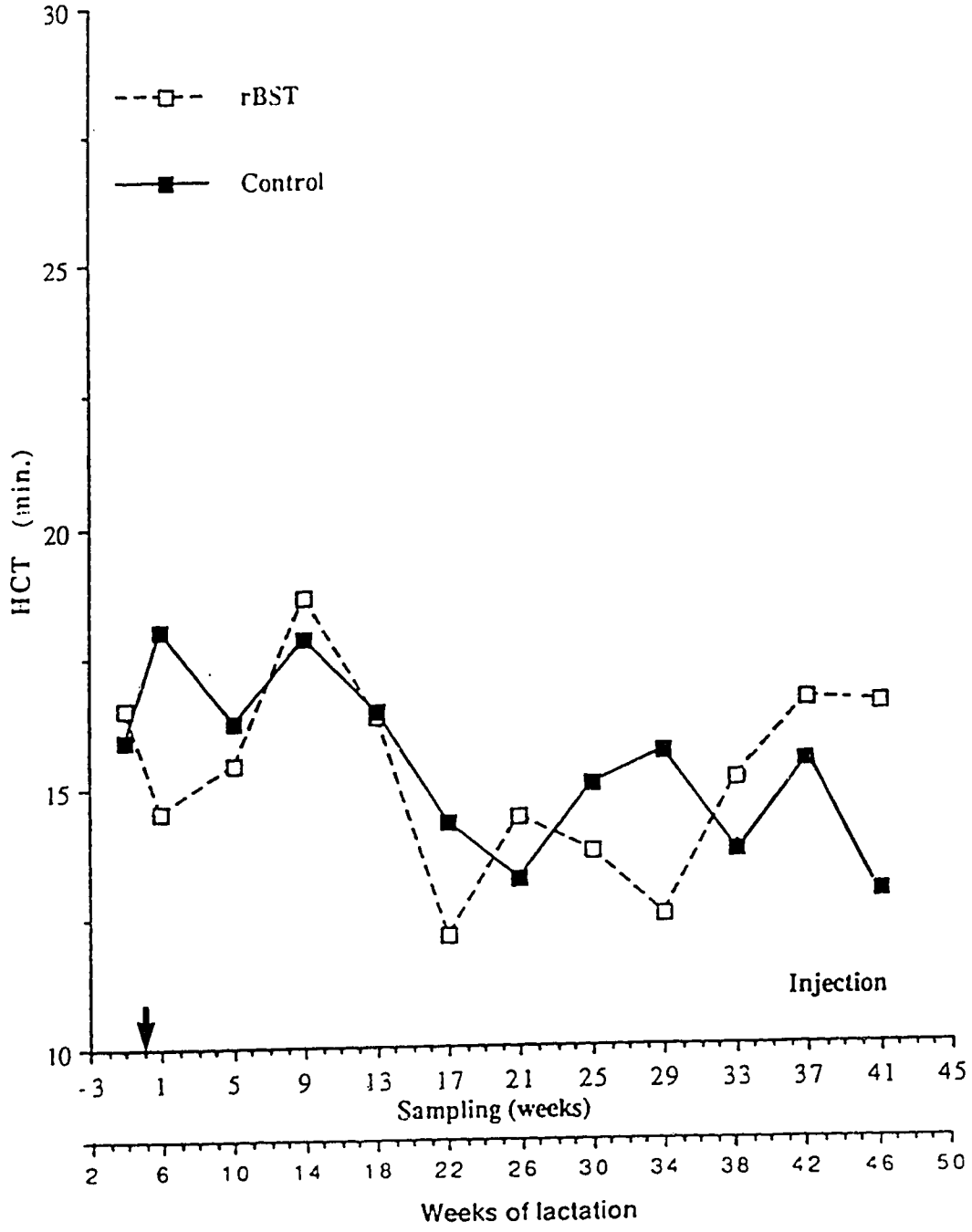

Fig. 3.7 Heat coagulation time of milk (pH 6.80 at 140°C) from cows injected with saline or rBST during one lactation

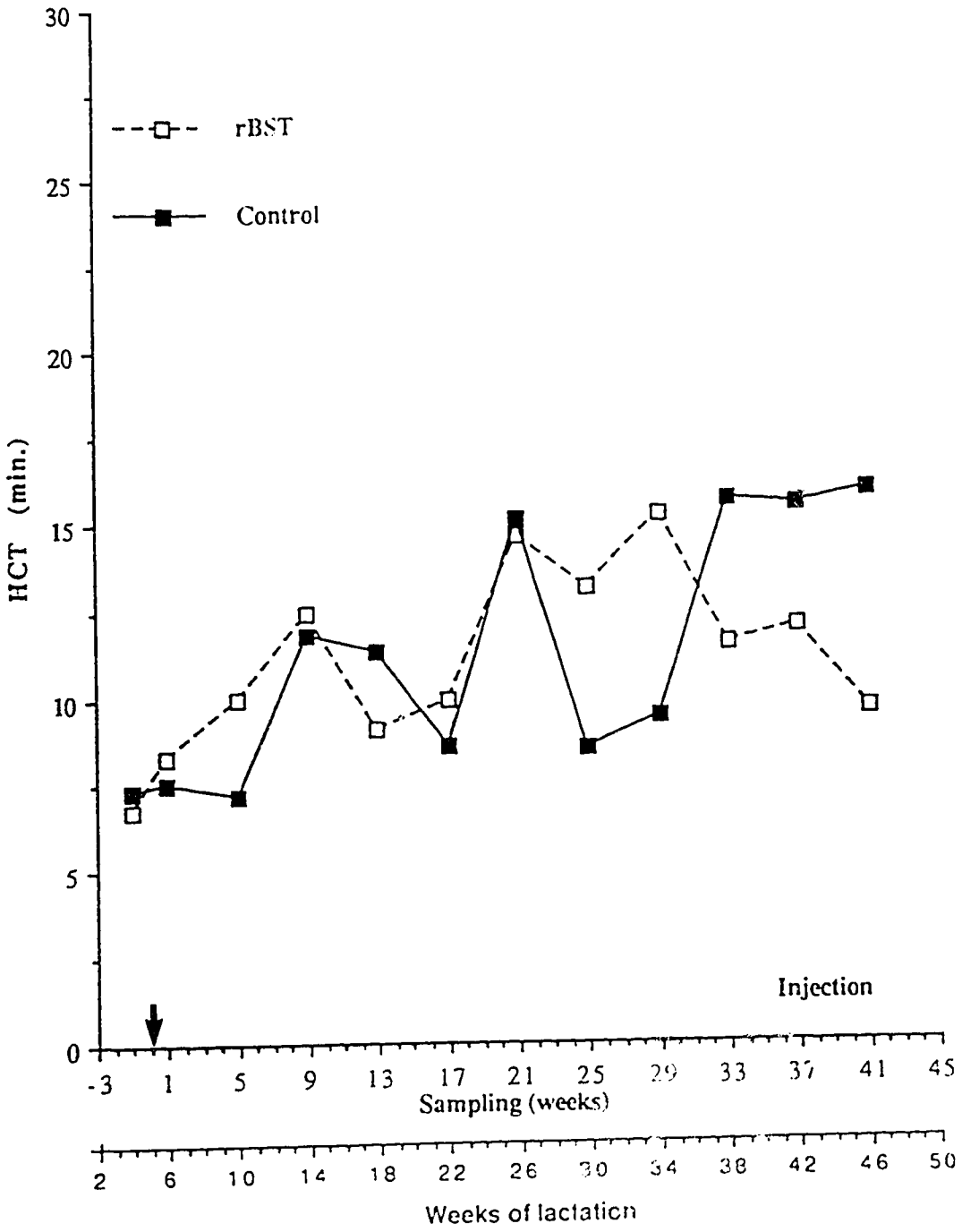

Fig. 3.8 Heat coagulation time of milk (pH 6.68 at 140°C) from cows injected with saline or rBST during one lactation.

HCT nat. data in Figures 3.6, 3.7, and 3.8, respectively. The milk HCT max. at pH 6.65 was the highest during mid-lactation and lowest during early and late lactation, especially in the control group (Figure 3.6). On the other hand, HCT min. (pH 6.80) had a decreasing tendency during the progress of lactation for both the treated and control groups (Figure 3.7) while, HCT nat. showed increasing HCT during the progress of lactation, except for a decrease in the rBST-treated group during the last ten weeks of lactation (Figure 3.8).

Statistical analysis confirmed the effect ($P< 0.01$) of rBST on milk HCT max. at pH 6.65 and the lack of difference in milk HCT min. at pH 6.80 and in milk HCT nat. at pH 6.68. There was a decrease in milk HCT min. at pH 6.65.

HCT-pH profile was first described by Rose (1961a, b). Rose found that milk described as "type A" had a profile curve with a maximum (pH 6.7) and a minimum (pH 6.9) heat stability at pH range 6.4-7.1 and at 140°C. Davis & White (1966) found that heat stability of milk from lactating cows increased with advancing lactation. They observed a rapid increase in milk coagulation time as colostrum was succeeded by less abnormal milk and that there was a tendency for the late lactation milks to have the longest coagulation times. Additionally, they emphasized that variations in coagulation times were related to changes in mineral distributuion of milk and advancing lactation, and that HCT times differed from cow to cow.

These results are in support of the hypothesis proposed by Sweetsur & White (1974) and Darling (1980), which suggest that the increase of colloidal calcium concentration in milk causes a decline in HCT max.

According to these authors, increased concentration of colloidal calcium may cause an increase of the hydrodynamic radious of casein micelles leading to their instability at elevated temperatures.

v. Effect of rBST administration on mineral distribution in milk

Concentrations of total and soluble mineral contents of rBST and control milk samples are shown in Tables 3.4 and 3.5, respectively. An increased concentration of [Ca_t] was found in the rBST group throughout the whole lactation period (P= 0.004). In contrast, the concentration of [Ca_s] throughout the whole lactation was lower (P< 0.05) in the rBST group as opposed to the concentration of [Ca_s] in the control group (Table 3.4 and 3.5). This is futher illustrated by the data in Figures 3.9 and 3.10.

Statistical analysis of results obtained throughout the whole lactation indicated that concentrations of [Mg_t], [P_t], [Mg_s], and [P_s] in milk were not signifficantly affected by rBST treatment but, as shown in Figures 3.11-3.14 the concentration of these minerals in milks from treated and control animals did not show a significant trend. The appreciable differences were found between the minimum and maximum concentrations of most mineral constituents for individual animals during the lactation. These variations could be one of the reasons for differences in milk stability.

Studies conducted by Davies & White (1966), Fox (1986), Sweetsur & White (1974) and Webb & Holm (1932) suggest that concentration of minerals in milk is one of the most important factors contributing to milk heat stablity. The concentration of calcium in milk is higher

TABLE 3.4 Concentration of total calcium, magnesium and phosphorus in milk from cows injected with saline or rBST during one lactation

		Replicate 1 (mg/L)	Replicate 2 (mg/L)	Overall Mean (mg/L)	± SD	P[1]
Total Calcium	Control	1082.4	1083.6	1083.0	176.9	0.004*
	rBST	1147.7	1124.7	1136.2	152.6	
Total Magnesium	Control	110.0	116	113.1	17.0	0.623
	rBST	113.3	116.2	114.8	17.7	
Total Phosphorus	Control	1211.4	1421.1	1316.3	194.0	0.050
	rBST	1203.8	1379.6	1291.7	157.5	

[1] Significance of difference

* Significant difference in total calcium [Ca_t] concentration in milk for the rBST and the control group during one lactation.

TABLE 3.5 Concentration of soluble calcium, magnesium and phosphorus in milk from cows injected with saline or rBST during one lactation

		Replicate 1 (mg/L)	Replicate 2 (mg/L)	Overall Mean (mg/L)	± SD	p[1]
Soluble Calcium	Control	458.3	444.1	451.2	83.6	0.005*
	rBST	431.9	399.7	415.8	82.3	
Soluble Magnesium	Control	88.1	93.4	90.8	11.0	0.210
	rBST	83.1	89.6	86.7	14.3	
Soluble Phosphorus	Control	615.7	679.9	647.8	73.8	0.100
	rBST	652.7	681.5	667.1	63.2	

[1] Significance of difference

* Significant difference in soluble calcium [Ca_s] concentration in milk for the rBST and the control group during one lactation.

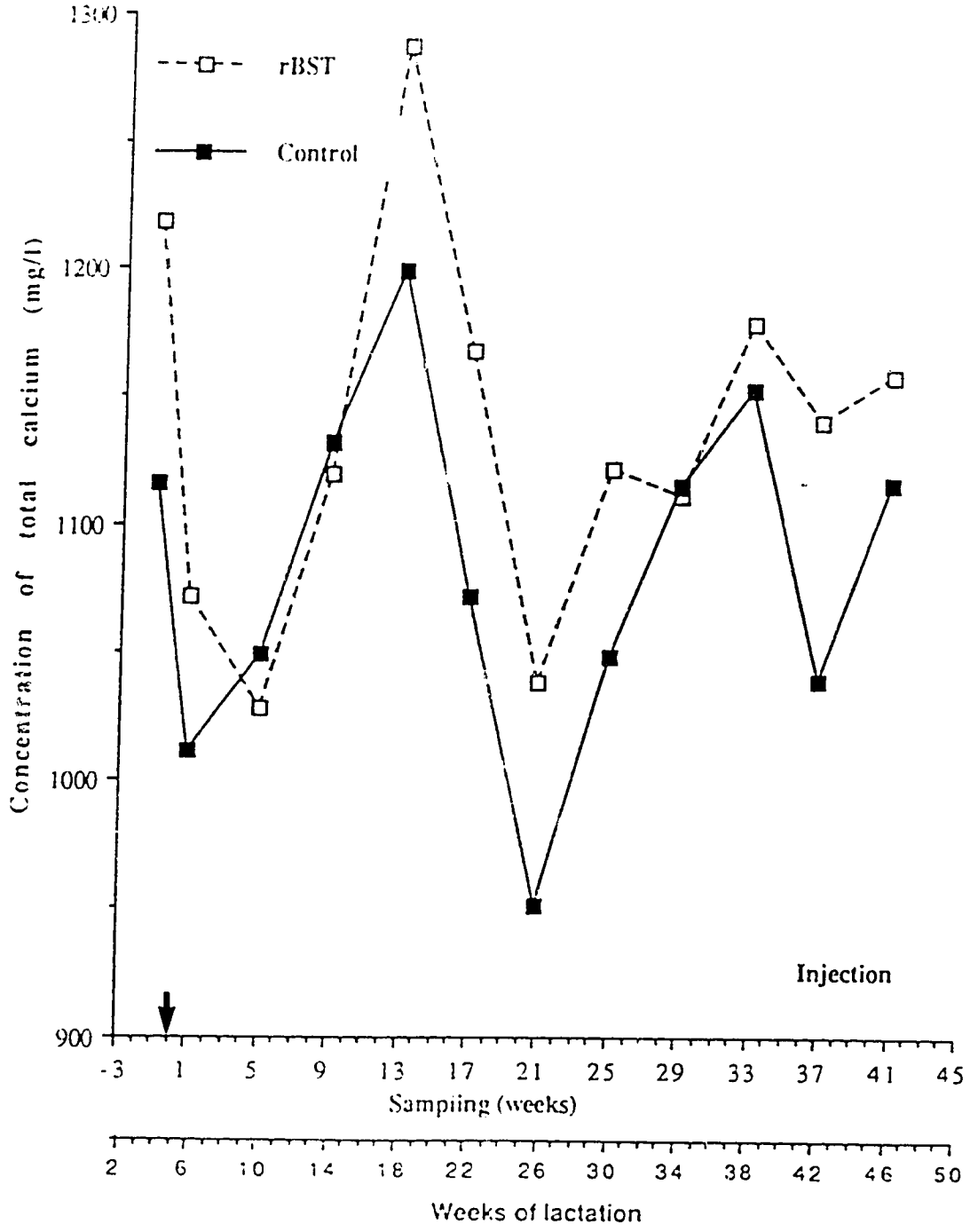

Fig. 3.9 Concentration of total calcium in milk from cows injected with saline or rBST during one lactation

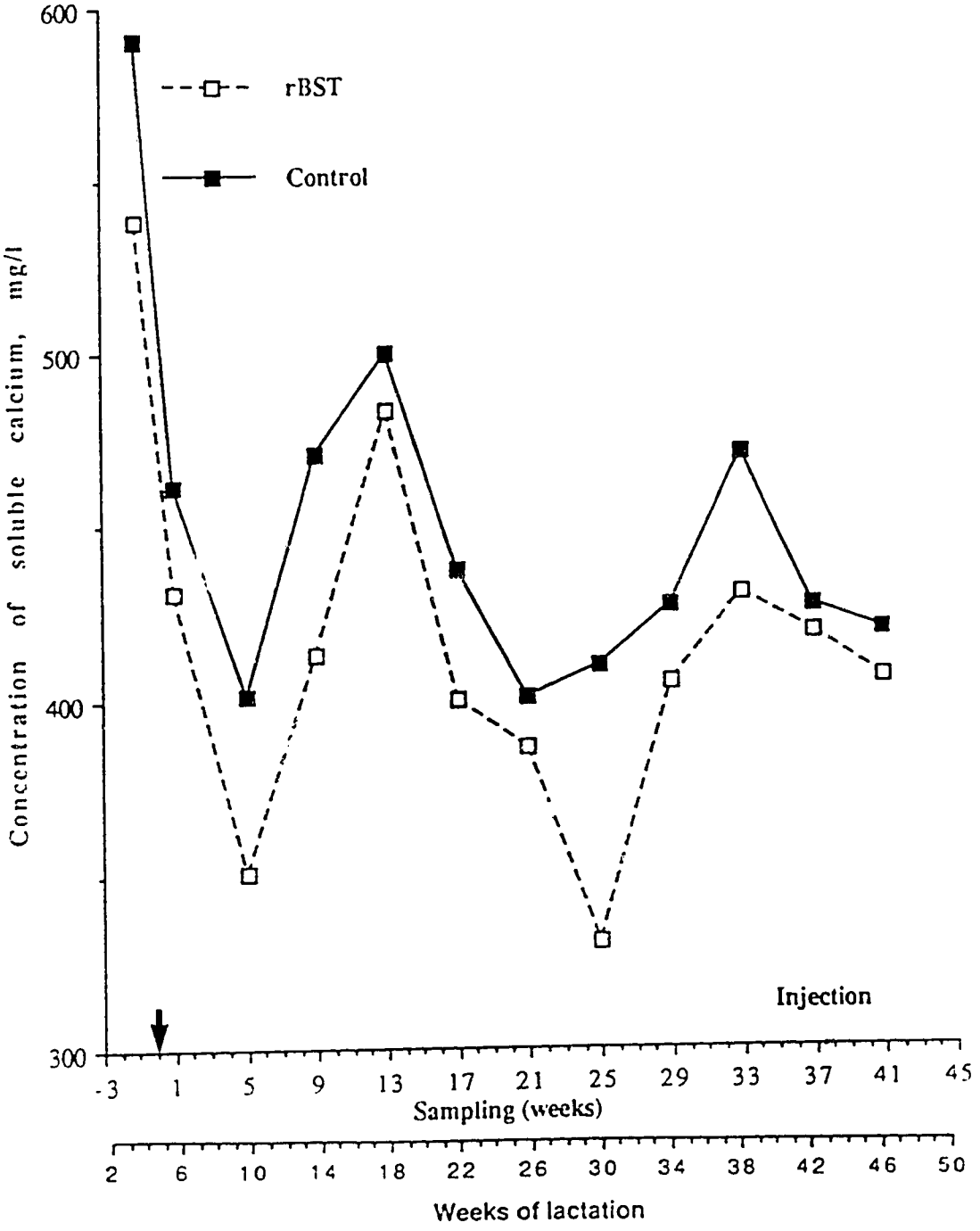

Fig. 3.10 Concentration of soluble calcium in milk from cows injected with saline or rBST during one lactation

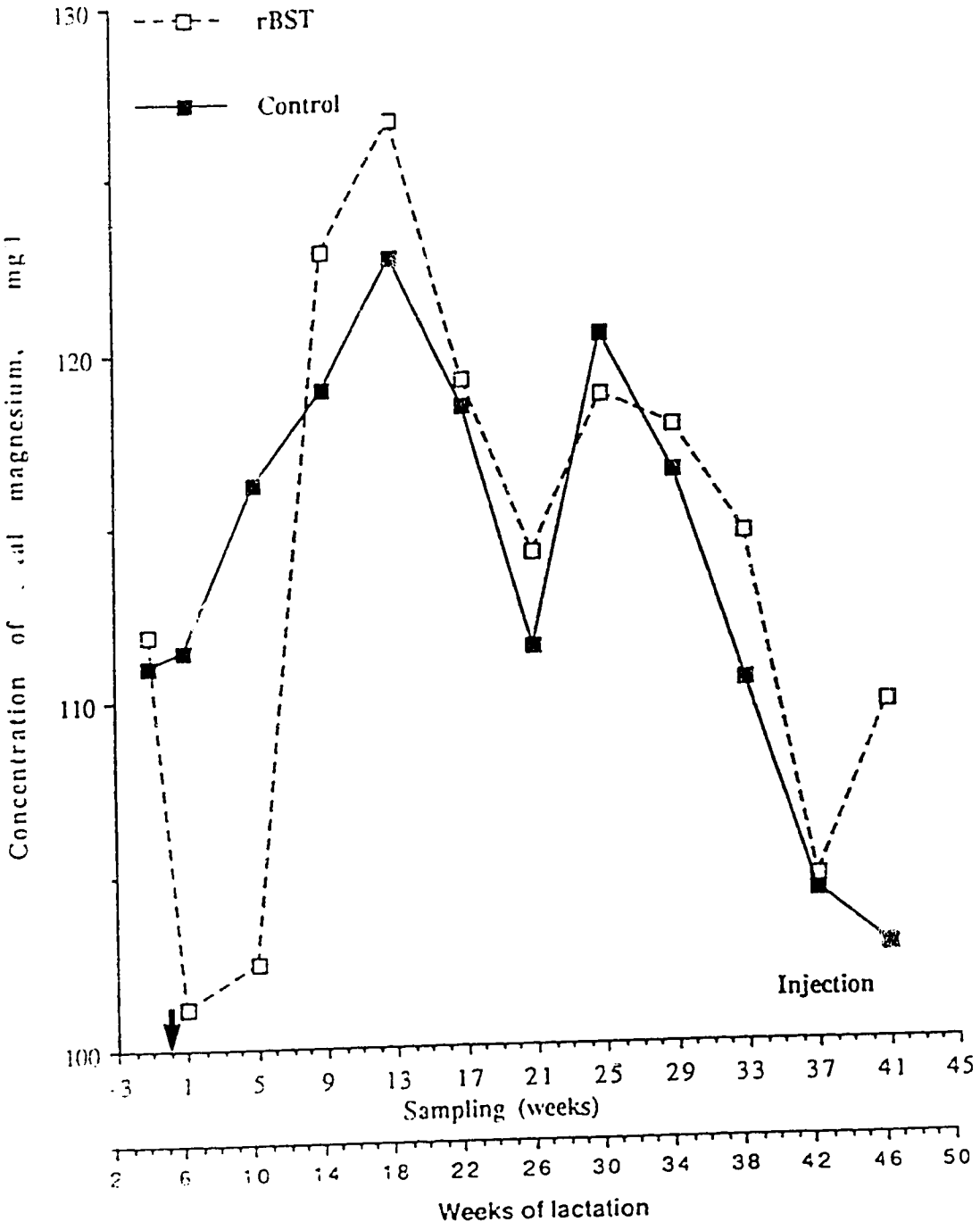

Fig. 3.11 Concentration of total magnesium in milk from cows injected with saline or rBST during one lactation

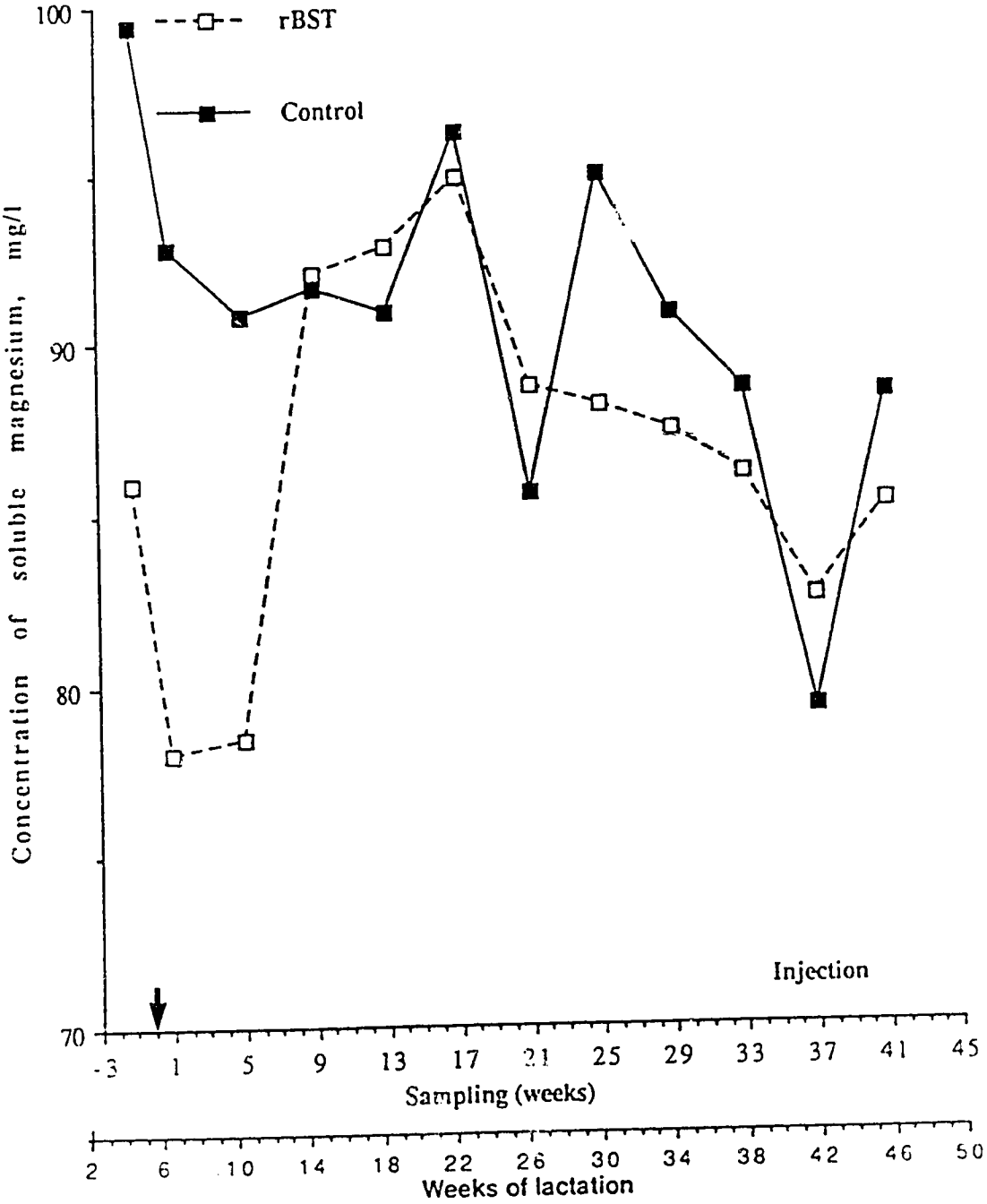

Fig. 3.12 Concentration of soluble magnesium in milk from cows injected with saline or rBST during one lactation

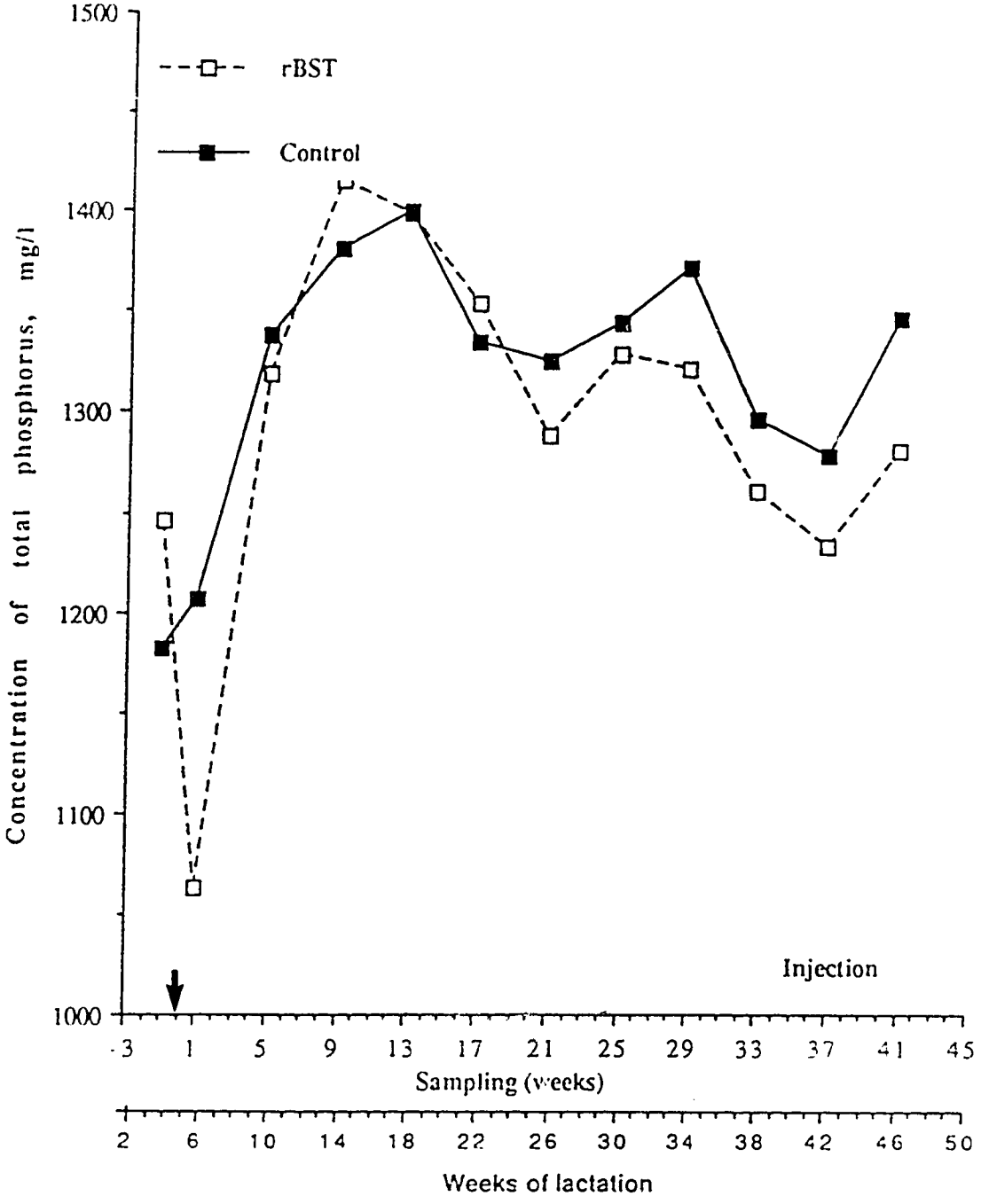

Fig. 3.13 Concentration of total phosphorus in milk from cows injected with saline or rBST during one lactation

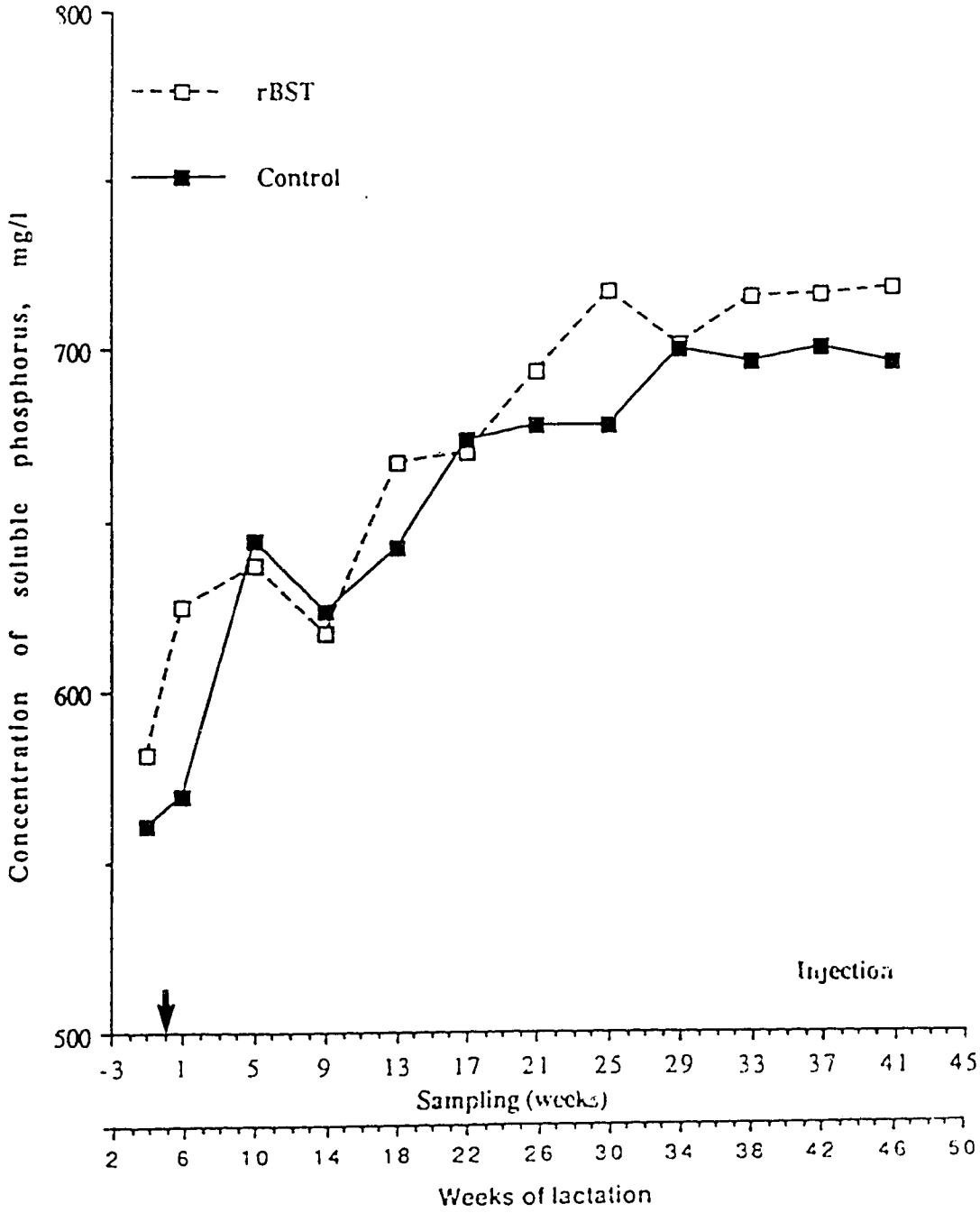

Fig. 3.14 Concentration of soluble phosphorus in milk from cows injected with saline or rBST during one lactation

than other mineral forms (ten times higher than magnesium). Therefore, the concentration of calcium is probably the most important single factor affecting heat stability of milk. Fox (1982) reported that relatively unstable milks contained more soluble calcium and magnesium but less soluble inorganic phosphorus.

Davies & White (1966), Pyne (1958), Rose (1961b) and Webb & Holm (1932) indicated the importance of soluble mineral forms on milk heat stability. They noted that unstable milks contained higher concentrations of soluble calcium and soluble magnesium. According to their observation, the lower concentrations of soluble calcium in milk from the rBST group indicated the possibility of increased heat stability of milk.

This dependence was not confirmed by the heat stability test in this study. Although concentration of soluble calcium in milk from the rBST group was lower than in milk from the control group, milk HCT for the rBST group was significantly lower than that for the control group. Observed changes in heat stability of milk suggest that other compositional factors then soluble calcium may affect milk HCT.

There are conflicting results on the effect of administration of rBST on the concentration of different protein fractions of milk (Bauman & Eppard, 1985; and Ozimek et al., 1989). Any changes in the concentration of milk proteins and particularly in casein, can be correlated to changes in concentration of minerals. Consequently, these may affect heat stability of milk.

Sweetsur & White (1974) showed that alterations in total mineral forms of calcium, magnesium and phosphorus can be a reason for milk

instability when heated. Further, Visser et al. (1979) suggested that colloidal calcium phosphate, inorganic phosphate, and citrate ions are also important factors which could affect milk HCT. White & Davies (1958) and Morrisey et al. (1981) did not find an obvious relationship between HCT and the composition of colloidal and soluble calcium. Fox (1986) suggested that although the HCT-pH curve can be readily altered by modifying the concentration of soluble and colloidal salts, there is no correlation between natural variations in HCT and concentrations of indigenous salts.

Presently, there are no results which would specify a tolerable range in natural mineral variation without affecting milk HCT. It is possible that the increased concentration of colloidal calcium with a subsequent decline of [Ca_s] concentration, increases the ratio of mineral exchange during heat treatment and therefore lowers its HCT. Moreover, the change in distribution of calcium could be reason for alterations in the distribution of protein fractions that are important in milk processing (Kudo, 1980; Aoki & Kako, 1983). The observed decrease in [Ca_s] concentration in milk from rBST treated cows may be caused by changes in conformation of whey proteins or caseins. α-lactalbumin present in milk serum is a calcium binding protein, and the calcium saturated form is more resistant to conformational changes which are induced by heat treatment or coagulants (Hiraoka et al., 1980, Permyakow et al., 1985). Increased stability of α-lactalbumin can result in lowering its shielding effect of casein fractions, exposing them to the denaturation temperature, and accelerating milk coagulation (Fox & Hearn, 1978; Hunziker & Tarassuk, 1965).

Singh & Fox (1987) found that, depending on pH, casein micelles dissociate on heating at or above 90°C. Dissociation of casein micelles, especially that of κ-casein, is affected by the concentration of $[Ca_s]$ and phosphate.

On the other hand, the observed increased concentration of $[Ca_t]$ in milk from rBST treated cows can be explained by simultaneous hormonal stimulation in the process of milk protein biosynthesis, along with an increase in calcium transport via the mammary cell. Presence of calcium is necessary for the assembly of casein micelles in the Golgi complex (Wooding & Morgan, 1978; Baumrucker, 1978). Subsequently, the increased rate of protein biosynthesis does not imply an identical rate of synthesis of the cognate proteins (Mepham, 1983). There is ample reason to believe that increased protein synthesis observed in this experiment is accompanied by the selective effect of rBST on their processes of transcription and translation. Simultaneously, this increase can result in possible changes in protein conformation, partition between fractions, and mineral distribution.

Lack of changes in the concentration of magnesium and phosphorus suggests that the administration of rBST to cows causes an increase in the secretion of the remaining minerals into milk alveoar lumina in a dose-responsive manner, similar to that of the increase in milk yield (Eppard et al., 1985). Present studies do not describe the relationship between rBST, HCT and the concentration of other minerals (Na, K, Co, etc.) in milk. Also, there is little evidence as to the influence of these minerals on the HCT. Therefore, additional research will have to be conducted in order to elucidate the relationship

between rBST treatment and milk properties.

D. Conclusions

1. Although there was no significant increase in milk yield in this studies, it was apparent that milk from the rBST-treated animals showed some changes in heat stability and distribution of calcium in the milk.
2. The analysis of the heat stability of milk from cows injected with BST or saline during one lactation gave HCT-pH profile curves ressembling "Type A" milk.
3. Long term rBST treatment decreased the milk HCT max. at the pH 6.65, but did not affect the milk HCT min. at the pH 6.80 or at the milk HCT nat. at the pH 6.68.
4. An increase in the concentration of [Ca_t] was paralleled by a decrease in concentrations of [Ca_s] for the rBST group for the whole lactation, suggesting a direct relationship between the decrease of the milk HCT max. of milk and an increase in calcium concentration.
5. The lack of change in the concentration of total and soluble forms of magnesium and phosphorus (both for the rBST and the control group) during a whole lactation is indicative of a diverse response of the mammary cell to hormonal treatment.

IV. SUMMARY

Dairy farmers and dairy breeders seek new methods to improve their production efficiency (milk produced/production cost) primarily by selecting superior animals as parents to generate offspring capable producing milk more efficiently.

Introduction of rBST on the dairy market as a new method of increasing milk production have not met those concerns of the consumer, those being milk quality and safety. However, there have been numerous tests done concerning the quality and composition of milk from rBST cows suggesting that nothing is wrong with such milk. To date, many findings are contradictory, and do not explain observed differences (ie. milk yield, milk composition) caused by the injection of rBST to animals in contrast to untreated animals.

Results and findings accumulated during this study, if confirmed by other studies, are of importance in the manufacture of fluid, concentrated and formulated dairy products. The observed decrease in heat stability of milk at the pH max. of milk (where the highest stability is expected), and change in mineral partition due to rBST treatment suggests that milk from rBST treated cows may undergo an earlier sedimentation or coagulation process causing a decrease in the efficiency of processes. Moreover, changes in the heat stability of milk may cause the necessity to change physical parameters, traditionally applied during processing, and therefore could be a reason for higher operation costs in the production of high heated and dried milks. However, higher concentrations of casein and calcium

observed in rBST treated milks could be in the favour of cheese manufacture. Higher concentrations of casein and calcium in milk accelerates the process of rennet coagulation and milk syneresis and is important for curd firmness. This can be advantageous to the manufacture of hard and semi-hard cheeses.

Further studies on animals receiving supplemental rBST are necessary. This could allow the determination of changes in other milk components resulting from the administration of rBST.

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CHAPTER VI. APPENDIX I.

TABLES AND FIGURES, EXPERIMENT 1

TABLE 1. Heat coagulation time of milk (pH 6.65 at 140°C) from cows injected with saline or rBST during one lactation, experiment 1

	milk HCT at pH 6.65						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	±SD	STAND. ERROR	$\bar{x}$ minutes	±SD	STAND. ERROR	2-Tail Prob.
-1	5.1	1.6	0.7	10.5	5.9	2.9	0.029
1	12.3	6.7	2.5	7.2	4.2	1.7	0.319
5	13.7	7.9	2.4	11.5	9.8	3.0	0.512
9	18.6	10.2	3.0	21.6	8.6	2.6	0.639
13	15.5	6.9	2.1	23.7	4.7	1.4	0.241
17	17.2	9.0	2.7	25.7	6.7	2.0	0.355
21	22.5	7.1	2.1	23.3	10.4	3.3	0.249
25	18.9	8.2	2.5	27.7	9.5	3.0	0.632
29	16.9	8.9	2.7	20.1	10.2	3.4	0.675
33	16.9	5.2	1.6	19.7	9.2	3.1	0.093
37	16.9	6.3	1.9	18.5	9.0	4.0	0.330
41	17.4	5.7	1.8	8.2	0.2	0.2	0.058

TABLE 2. Heat coagulation time of milk (pH 6.80 at 140°C) from cows injected with saline or rBST during one lactation, experiment 1

	milk HCT at pH 6.80						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	± SD	STAND. ERROR	$\bar{x}$ minutes	± SD	STAND. ERROR	2-Tail Prob.
-1	7.5	5.5	2.5	7.4	6.5	3.2	0.738
1	7.6	5.9	2.2	3.5	1.1	0.4	0.002
5	6.5	3.5	1.0	7.7	9.2	2.8	0.005
9	11.9	8.5	2.6	11.1	9.3	2.8	0.784
13	5.8	3.6	1.1	10.4	8.3	2.5	0.015
17	8.9	7.1	2.2	7.7	5.1	1.5	0.297
21	11.8	8.3	2.5	13.6	10.8	3.4	0.435
25	12.0	6.2	1.9	7.0	2.2	0.7	0.004
29	10.1	4.2	1.3	11.3	6.3	1.9	0.222
33	10.8	6.1	1.8	15.3	11.5	3.9	0.063
37	13.5	9.8	2.9	14.4	8.9	3.6	0.890
41	11.6	7.2	2.3	15.8	0.4	0.3	0.076

TABLE 3. Heat coagulation time of milk (pH 6.68 at 140°C) from cows injected with saline or rBST during one lactation, experiment 1

	milk HCT at natural milk pH						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	±SD	STAND. ERROR	$\bar{x}$ minutes	±SD	STAND. ERROR	2-Tail Prob.
-1	11.1	6.0	2.7	12.9	7.8	4.5	0.600
1	10.5	6.0	2.3	15.3	9.0	3.7	0.352
5	14.0	4.1	1.2	17.0	12.7	3.8	0.001
9	17.8	8.3	2.5	20.9	9.2	2.8	0.739
13	15.3	7.8	2.4	13.6	9.7	3.0	0.507
17	10.2	8.5	2.6	10.2	5.6	1.7	0.204
21	15.0	6.5	1.9	11.2	7.9	2.5	0.529
25	12.5	4.6	1.4	14.4	8.2	2.6	0.087
29	12.4	7.7	2.3	17.1	8.3	2.6	0.820
33	14.4	7.0	2.1	12.7	7.3	2.4	0.894
37	18.0	7.9	2.4	24.4	11.4	4.7	0.293
41	16.2	6.9	2.2	6.7	0.4	0.3	0.095

TABLE 4. Concentration of total calcium in milk from cows injected with saline or rBST during one lactation, experiment 1

	Total Calcium concentration in milk						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	1206.3	119.7	59.8	1105.0	100.4	57.9	0.877
1	1116.7	137.5	56.2	1007.0	103.9	46.5	0.607
5	1017.0	261.8	82.8	1000.5	159.0	50.3	0.154
9	1024.0	273.1	86.4	1003.5	233.0	73.7	0.644
13	1271.9	140.3	44.4	1185.0	189.5	59.9	0.383
17	1263.5	148.3	46.9	1144.5	234.0	74.0	0.190
21	1074.5	237.6	75.1	953.3	203.9	67.9	0.677
25	1205.0	171.7	54.3	1131.7	167.0	55.7	0.948
29	1217.0	154.7	48.9	1175.6	137.9	45.9	0.756
33	1166.0	147.2	46.6	1155.0	240.1	84.9	0.173
37	1137.0	149.7	47.4	1048.0	167.6	74.9	0.711
41	1073.3	189.5	63.2	1080.0	120.0	50.1	0.710

TABLE 5. Concentration of total magnesium in milk from cows injected with saline or rBST during one lactation, experiment 1

Total Magnesium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	85.7	17.2	9.9	86.0	4.2	3.0	0.345
1	87.5	13.4	5.5	97.8	25.4	11.4	0.190
5	87.6	22.0	6.9	104.8	30.6	9.7	0.336
9	115.2	22.8	7.2	120.2	28.5	9.0	0.518
13	128.7	18.6	5.9	118.0	19.0	5.9	0.958
17	136.7	15.6	4.9	137.3	18.4	5.8	0.632
21	124.0	15.6	4.9	120.8	18.7	6.2	0.602
25	129.3	13.0	4.1	128.2	22.8	7.6	0.115
29	129.4	9.7	3.1	123.6	20.1	7.1	0.046
33	118.9	18.4	5.8	109.5	21.7	7.7	0.632
37	108.1	16.9	5.3	95.2	5.1	2.3	0.034
41	108.5	17.3	5.8	91.0	6.0	3.0	0.044

TABLE 6. Concentration of total phosphorus in milk from cows injected with saline or rBST during one lactation, experiment 1

Total Phosphorus concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	1106.3	209.9	105.0	1050.0	494.9	350.0	0.199
1	1006.7	128.9	52.6	1044.0	292.3	130.7	0.102
5	1197.5	145.7	46.1	1244.0	238.9	75.6	0.157
9	1323.0	192.1	60.7	1249.0	190.4	60.2	0.979
13	1284.5	144.7	45.8	1302.5	178.3	56.4	0.545
17	1239.0	153.4	48.5	1191.0	193.6	61.2	0.499
21	1121.5	157.1	49.7	1171.1	175.6	58.5	0.742
25	1262.6	123.5	42.2	1221.7	238.6	79.5	0.103
29	1258.0	180.97	57.2	1297.8	242.8	80.9	0.399
33	1236.5	163.5	51.7	1271.3	233.2	82.5	0.318
37	1199.0	121.9	38.5	1204.3	207.6	78.5	0.147
41	1211.3	123.6	41.1	1290.0	178.4	54.2	0.158

TABLE 7. Concentration of soluble calcium in milk from cows injected with saline or rBST during one lactation, experiment 1

Soluble Calcium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	512.4	114.9	33.2	585.7	68.1	21.5	0.127
1	435.7	137.0	35.4	459.2	99.5	27.6	0.274
5	382.1	116.5	26.1	402.3	102.9	24.3	0.614
9	438.2	125.5	29.6	441.6	135.5	31.9	0.756
13	480.7	116.9	26.1	493.3	143.1	33.7	0.395
17	430.7	79.0	18.6	468.1	116.9	27.6	0.115
21	392.4	53.1	12.2	401.5	70.4	17.1	0.250
25	352.6	96.4	22.1	429.7	97.1	24.3	0.967
29	431.4	46.3	10.6	465.3	63.2	16.3	0.213
33	464.5	65.1	19.6	520.6	116.3	41.1	0.095
37	451.7	78.7	24.9	412.6	59.0	26.4	0.609
41	410.0	39.7	13.2	420.0	48.0	22.4	0.264

TABLE 8. Concentration of soluble magnesium in milk from cows injected with saline or rBST during one lactation, experiment 1

Soluble Magnesium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	60.7	5.0	2.9	79.0	13.7	7.9	0.236
1	60.7	12.7	5.2	77.5	27.5	11.2	0.114
5	64.7	9.4	2.9	85.1	20.8	6.6	0.028
9	76.9	21.8	6.9	82.4	24.3	7.7	0.753
13	90.0	29.9	9.5	84.8	26.8	8.5	0.750
17	112.9	10.4	3.3	107.0	14.5	4.6	0.331
21	99.9	14.9	4.7	94.6	13.8	4.6	0.838
25	94.3	13.3	4.2	102.9	8.1	2.6	0.173
29	94.1	11.5	3.6	97.3	17.4	5.8	0.237
33	85.8	13.5	4.3	88.0	19.6	6.9	0.295
37	80.4	8.2	2.6	71.4	9.8	4.4	0.598
41	83.9	5.4	1.8	88.0	7.9	5.3	0.728

TABLE 9. Concentration of soluble phosphorus in milk from cows injected with saline or rBST during one lactation, experiment 1

Soluble Phosphorus concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	506.7	81.4	47.0	586.7	80.8	46.7	0.294
1	578.0	73.6	32.9	510.0	74.2	33.2	0.184
5	643.0	64.1	20.3	621.0	87.4	27.6	0.529
9	602.0	119.4	37.8	545.0	84.8	26.8	0.234
13	655.0	51.5	16.3	562.0	95.4	30.2	0.014
17	666.0	102.3	32.4	591.0	85.6	27.1	0.092
21	689.0	115.9	36.7	646.7	48.9	16.3	0.324
25	729.0	63.7	20.1	648.9	45.4	15.1	0.006
29	712.0	99.8	31.5	641.1	54.6	18.2	0.076
33	721.0	62.3	19.7	667.5	56.8	20.1	0.078
37	715.5	45.5	14.4	688.0	69.1	30.9	0.368
41	716.1	45.8	15.3	680.0	84.9	60.0	0.394

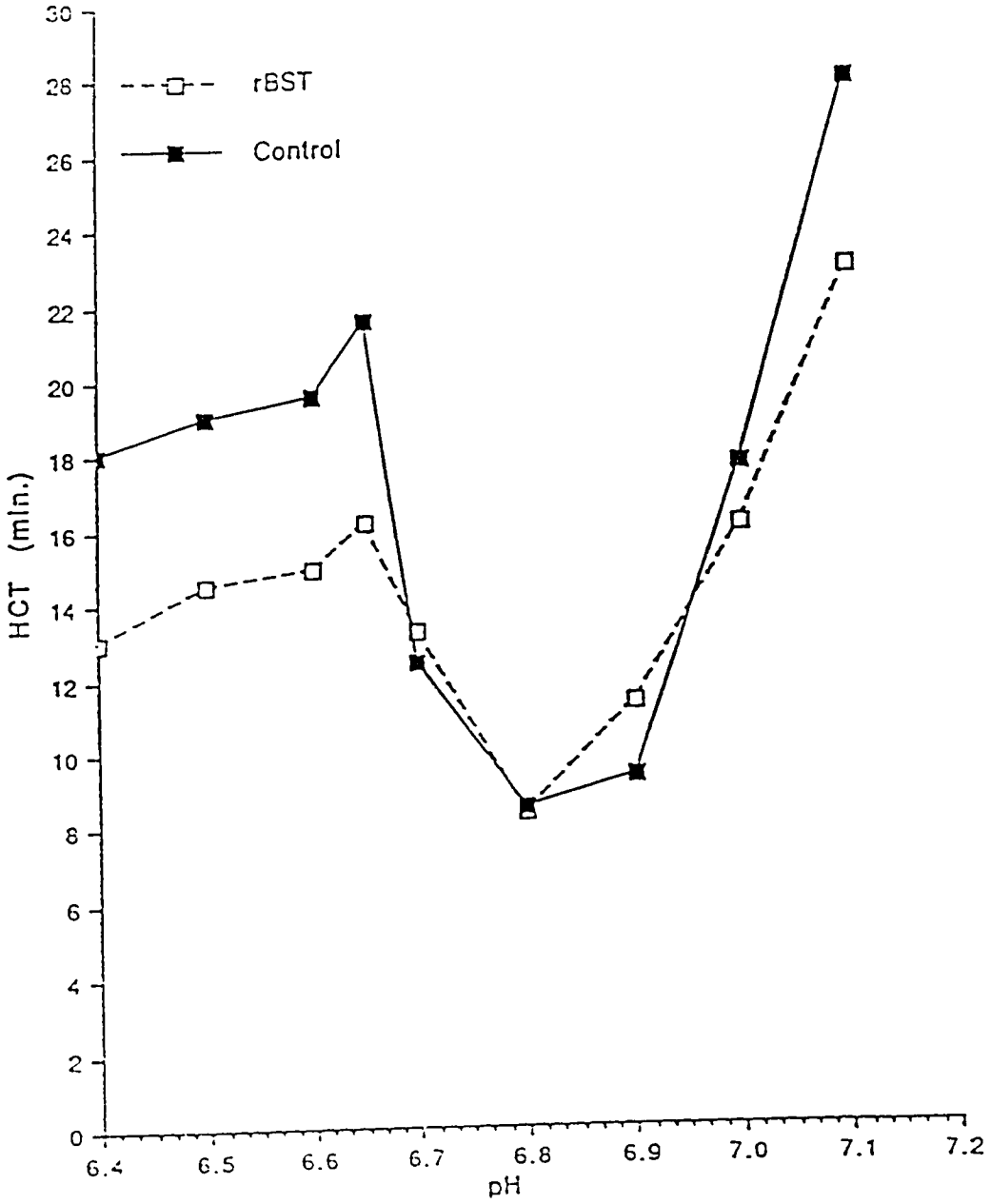

Fig. 1 Heat coagulation time (HCT-pH) profile of milk from cows, injected with saline or rBST, experiment 1.

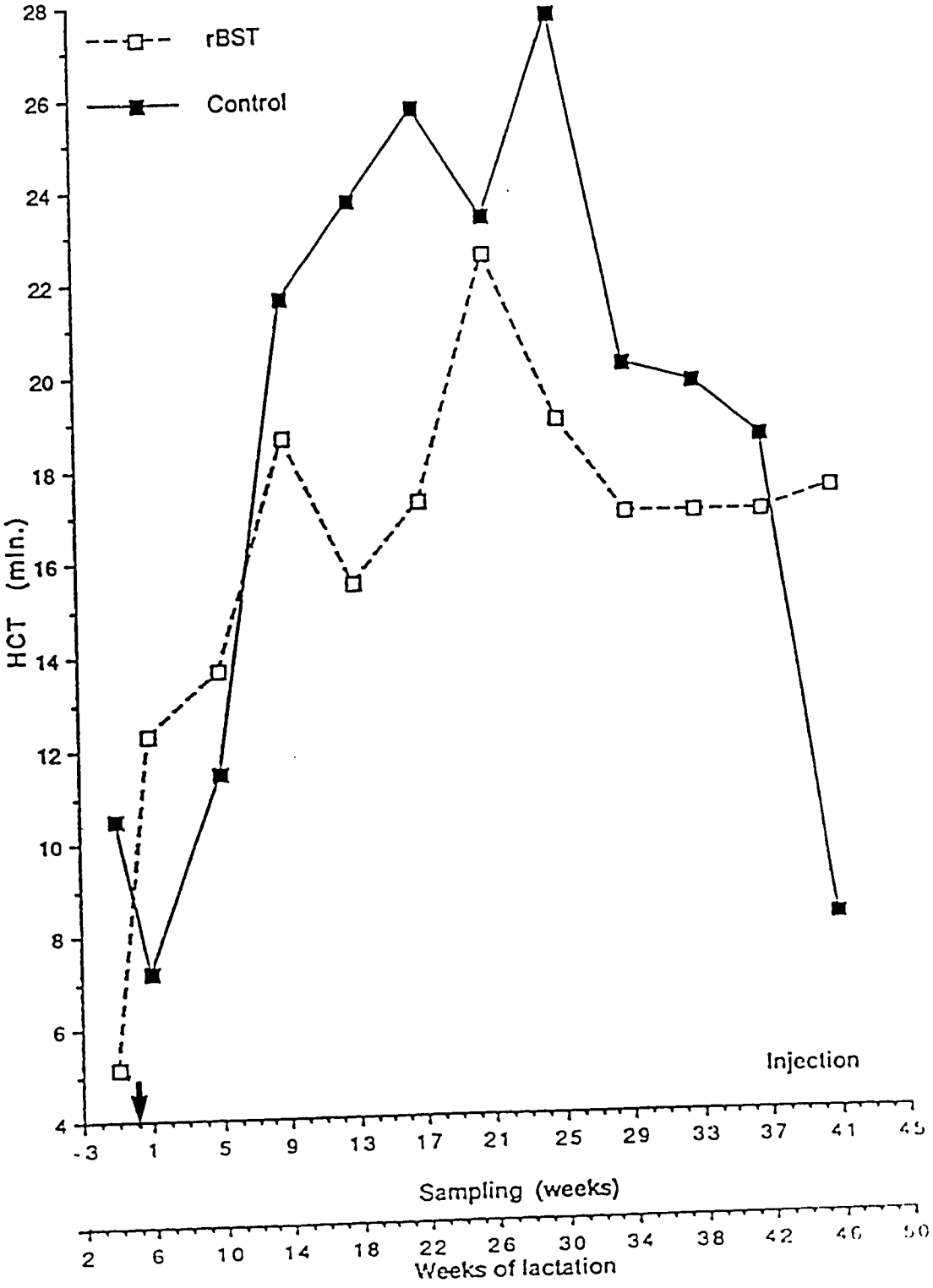

Fig. 2 Heat coagulation time of milk (pH 6.65 at 140°C) from cows injected with saline or rBST, experiment 1.

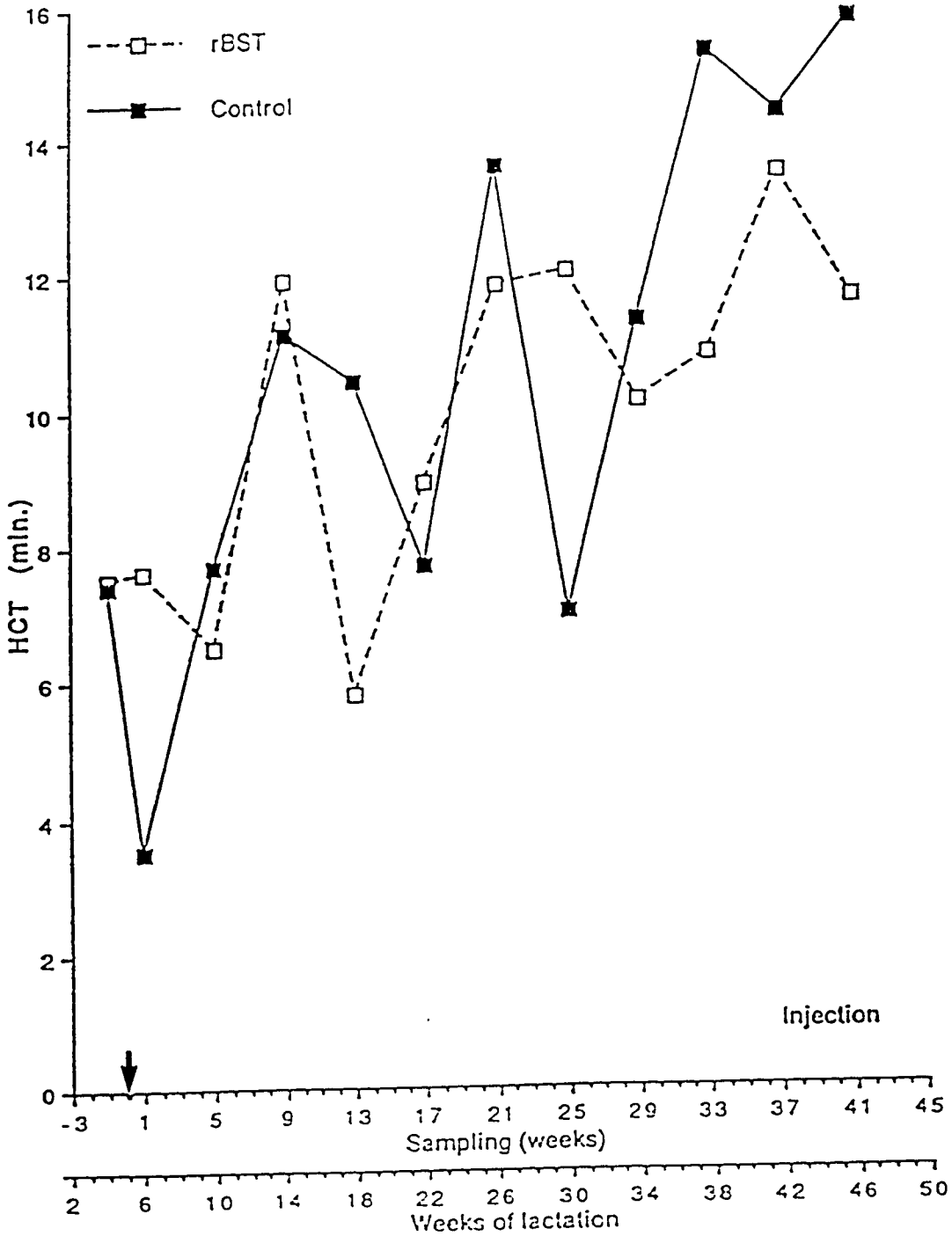

Fig. 3 Heat coagulation time of milk (pH 6.80 at 140°C) from cows injected with saline or rBST, expreriment 1.

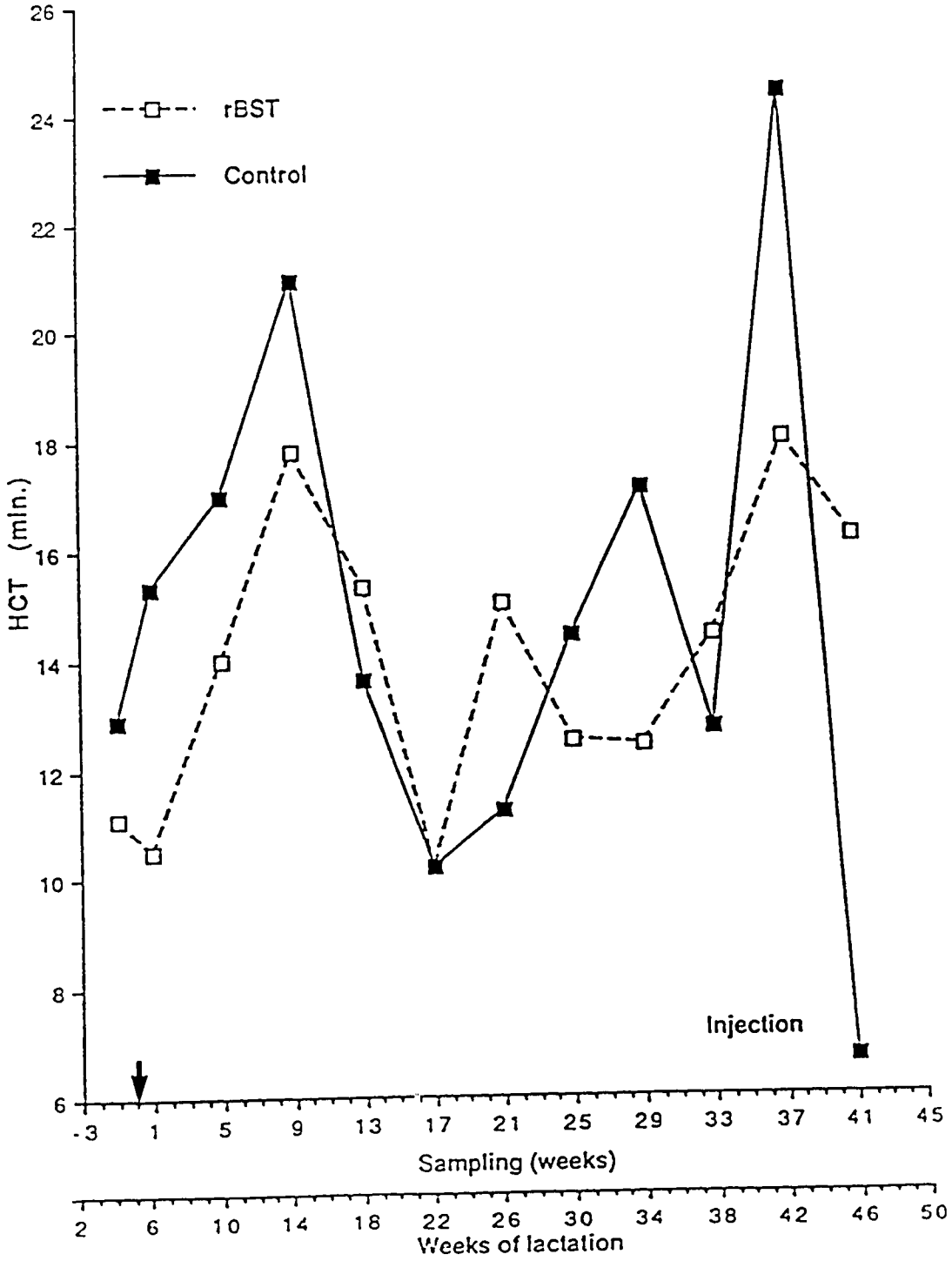

Fig. 4 Heat coagulation time of milk (pH 6.68 at 140°C) from cows injected with saline or rBST, experiment 1.

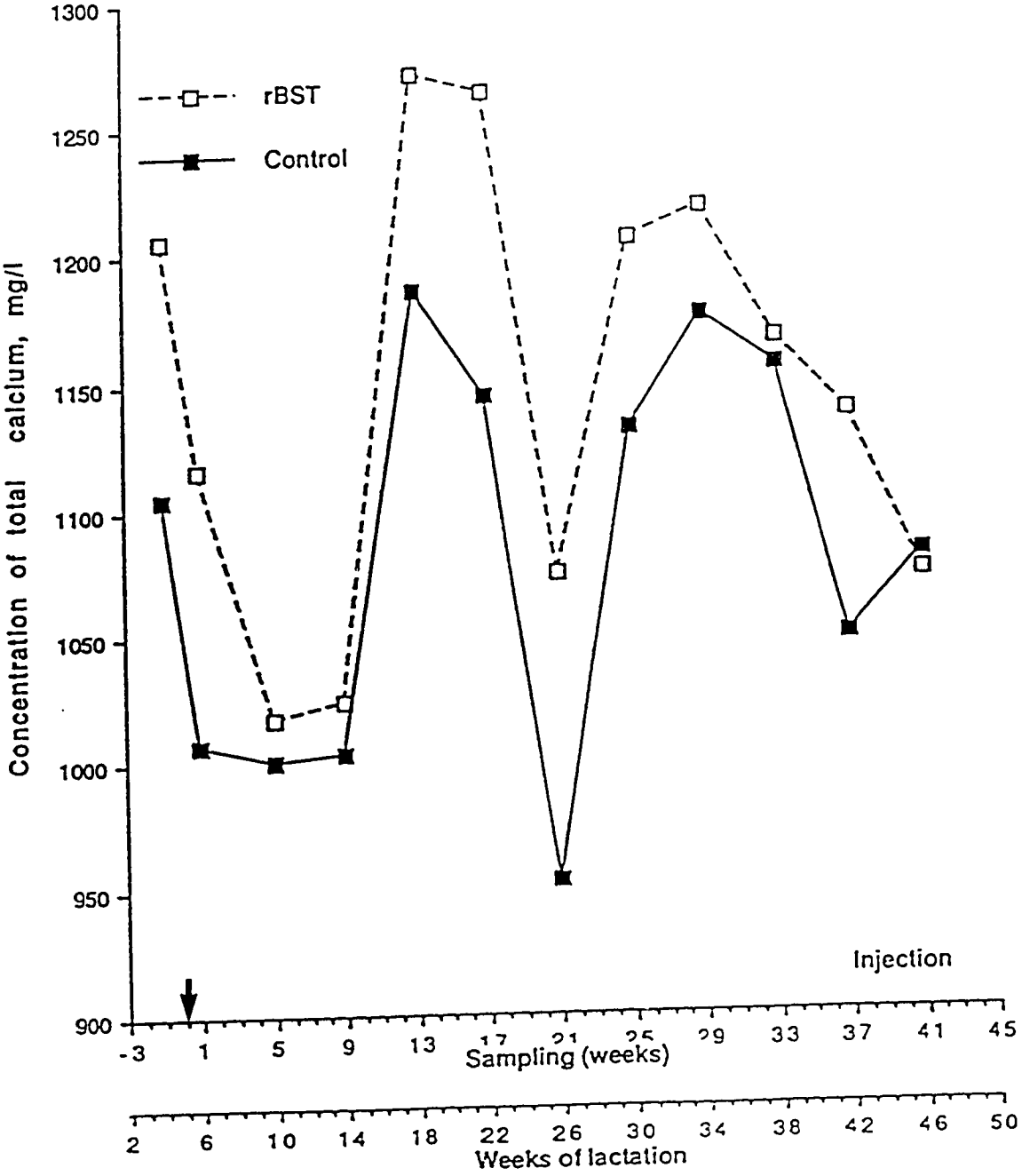

Fig. 5 Concentration of total calcium in milk from cows injected with saline or rBST, experiment 1.

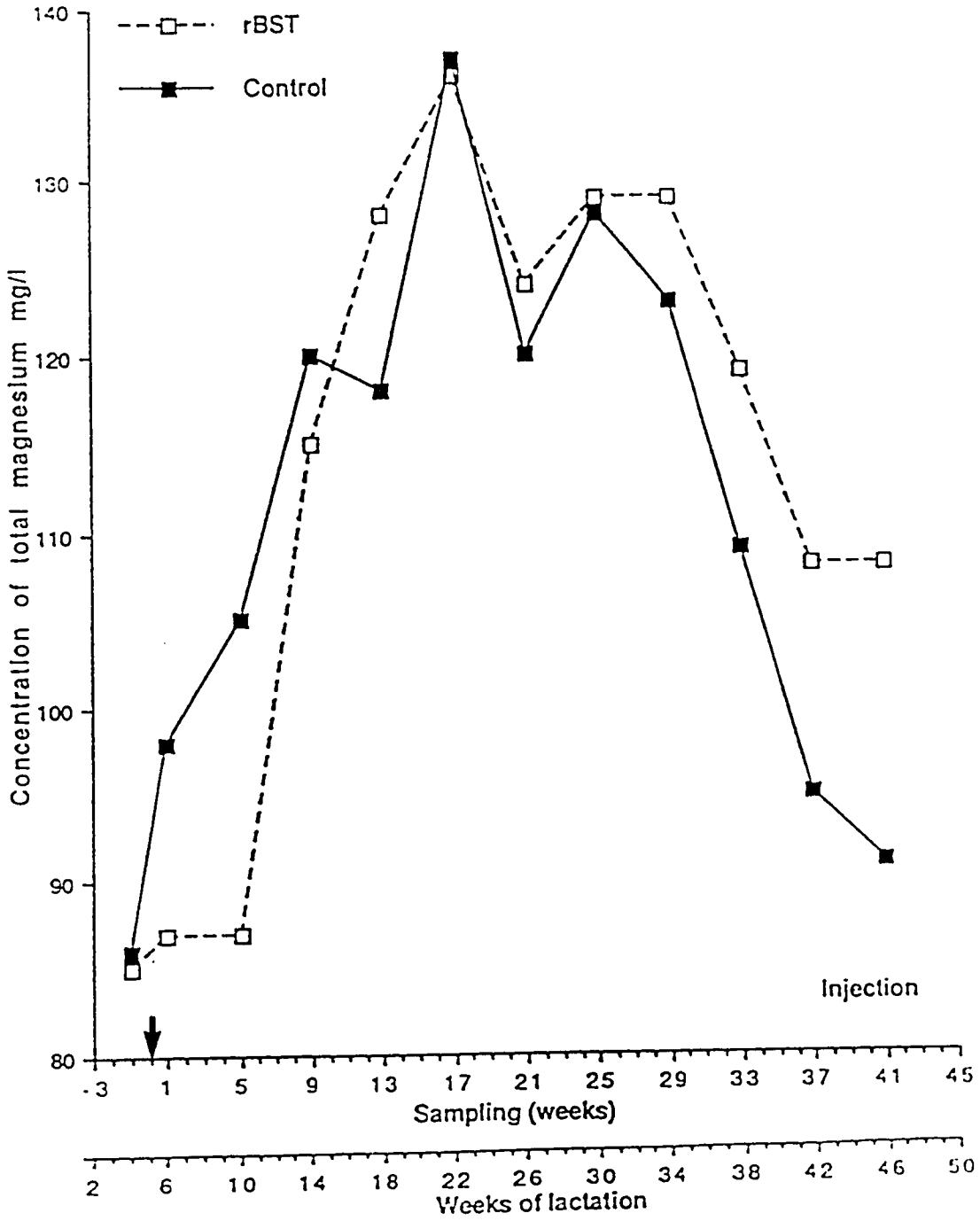

Fig. 6 Concentration of total magnesium in milk from cows injected with saline or rBST, experiment 1.

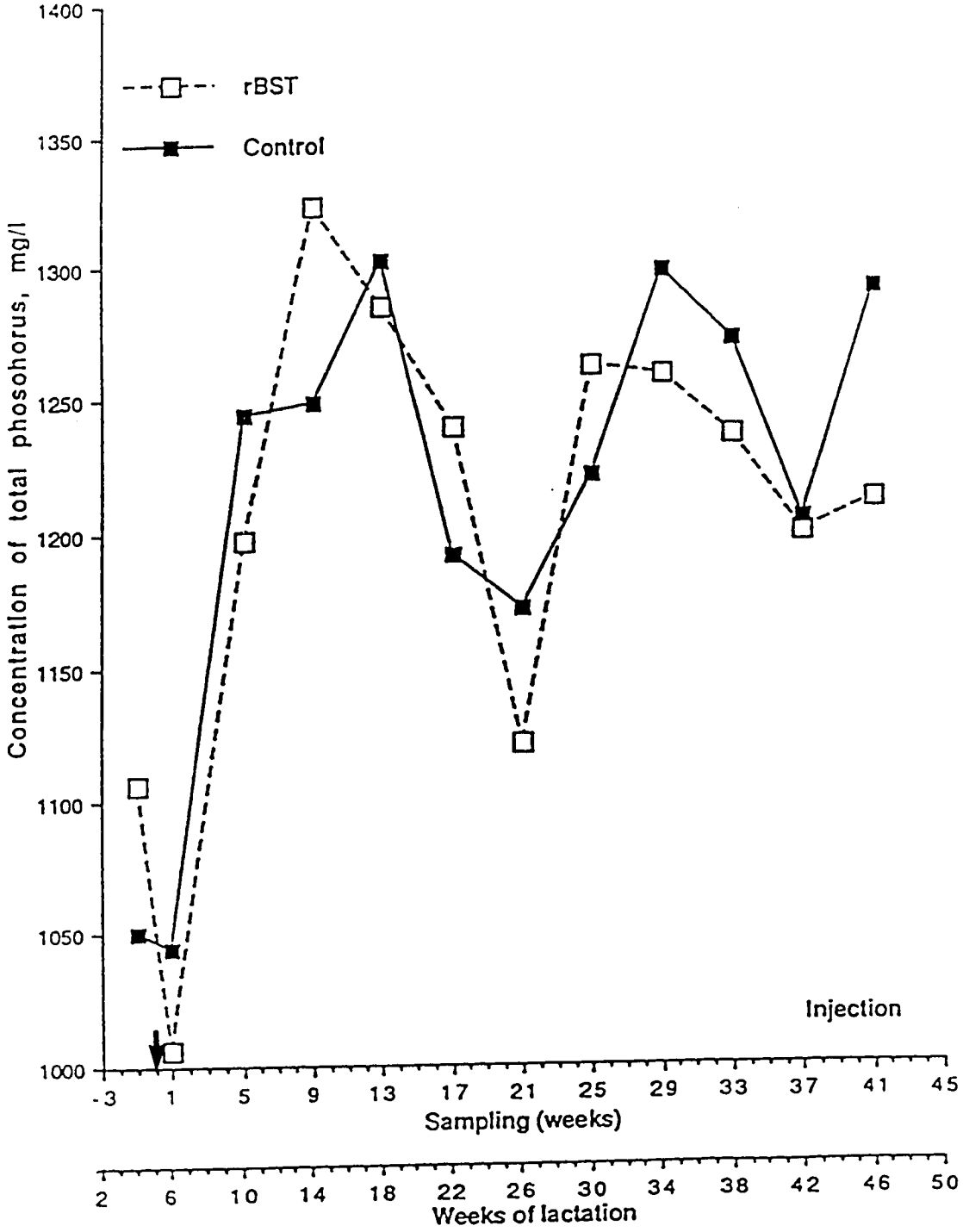

Fig. 7 Concentration of total phosphorus in milk from cows injected with saline or rBST, experiment 1.

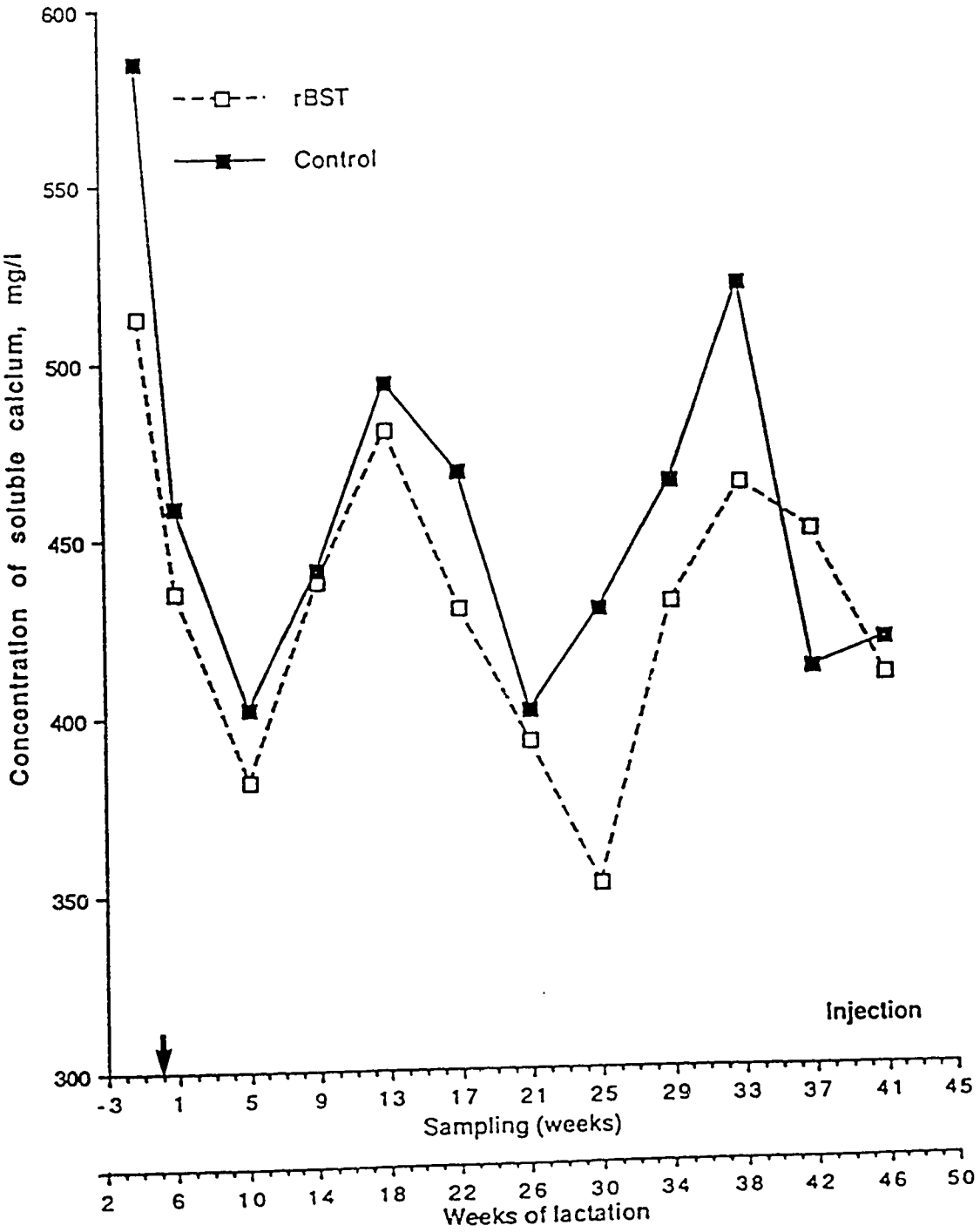

Fig. 8 Concentration of soluble calcium in milk from cows injected with saline or rBST, experiment 1.

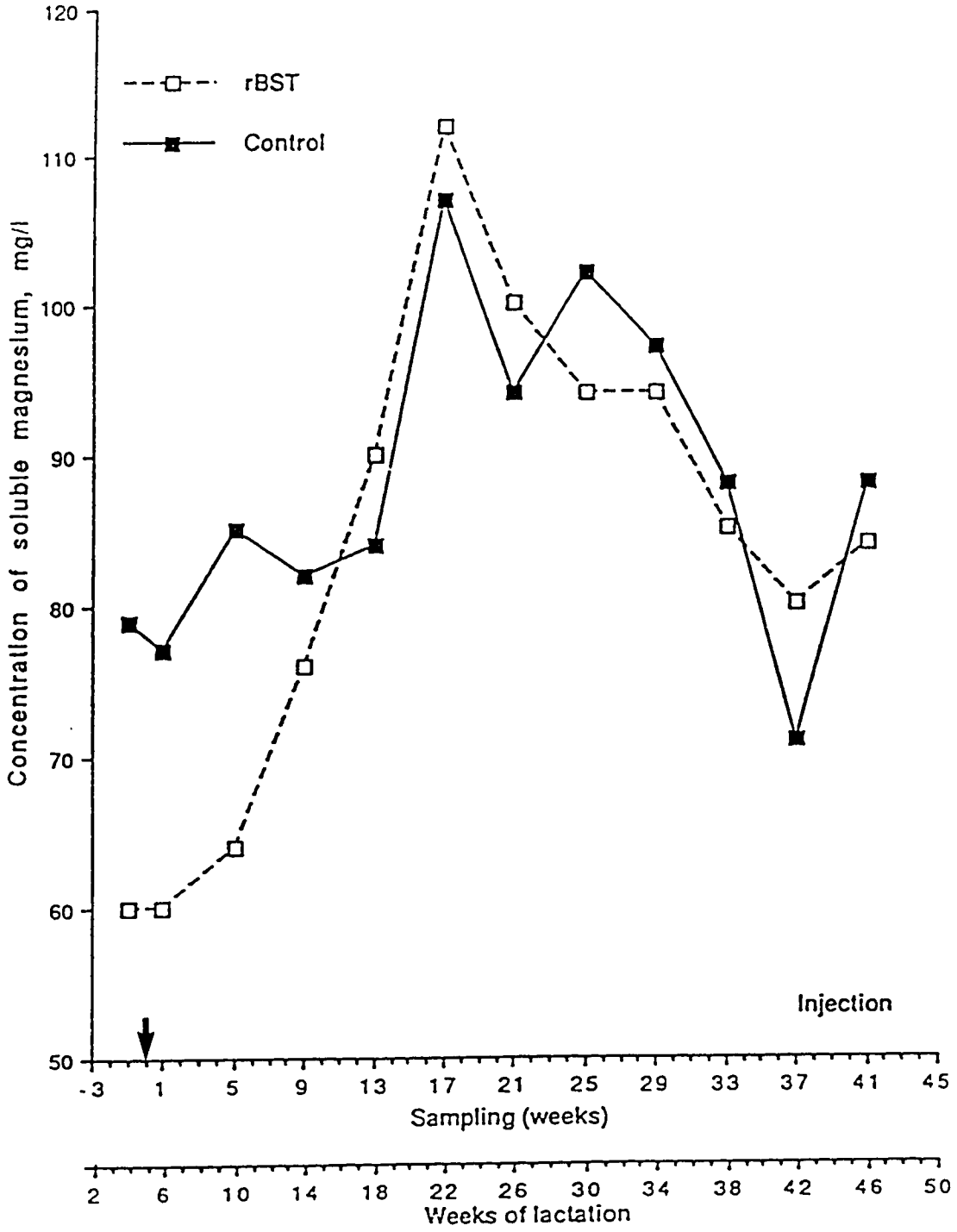

Fig. 9 Concentration of soluble magnesium in milk from cows injected with saline or rBST, experiment 1.

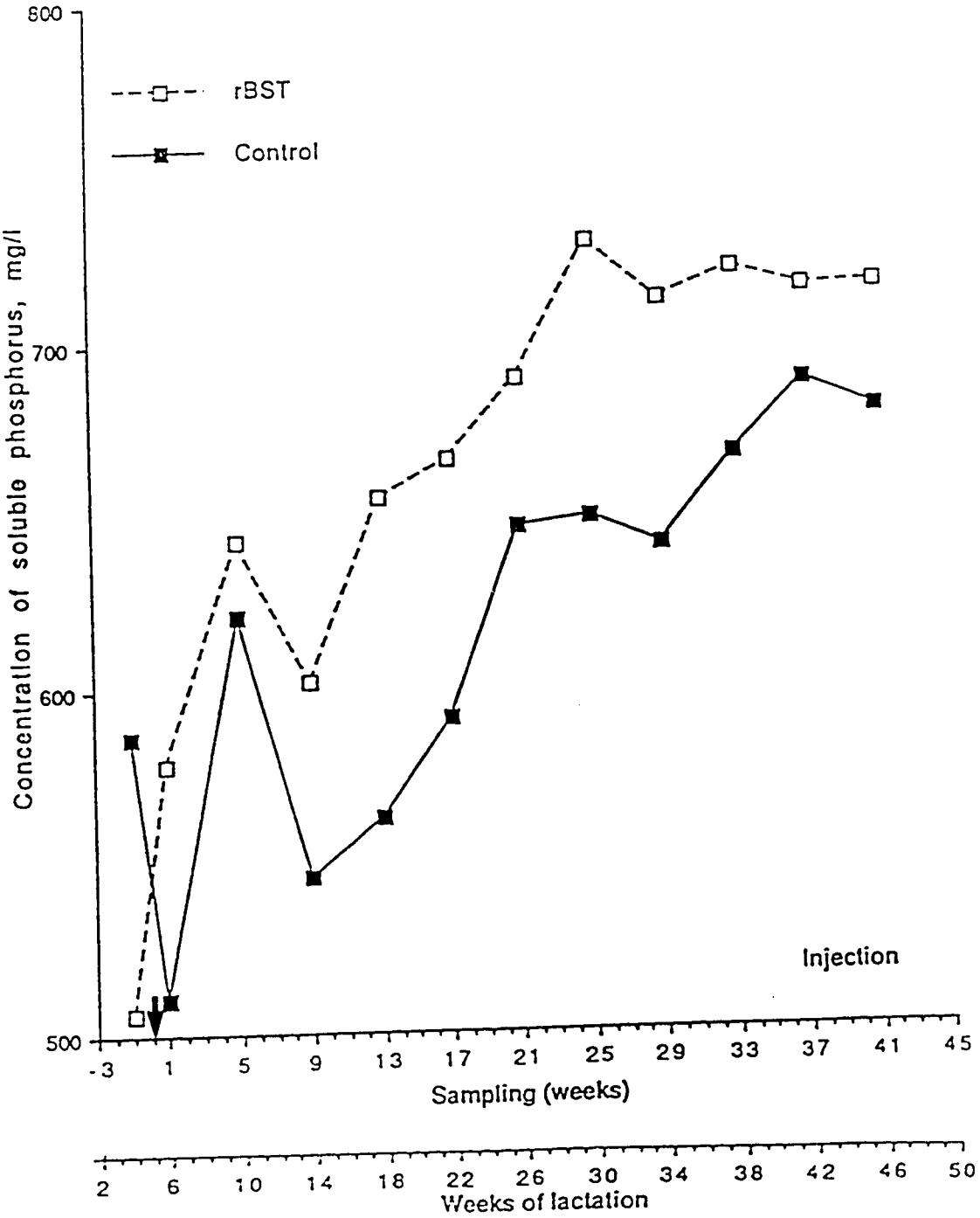

Fig. 10 Concentration of soluble phosphorus in milk from cows injected with saline or rBST, experiment 1.

CHAPTER VII. APPENDIX II.

TABLES AND FIGURES, EXPERIMENT 2

TABLE 1. Heat coagulation time of milk (pH 6.65 at 140°C) from cows injected with saline or rBST during one lactation, experiment 2

	milk HCT at pH 6.65						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	±SD	STAND. ERROR	$\bar{x}$ minutes	±SD	STAND. ERROR	2-Tail Prob.
-1	17.5	10.3	3.2	14.0	9.3	3.1	0.770
1	19.3	9.7	2.9	23.1	5.4	1.8	0.105
5	15.9	10.1	3.1	14.9	9.2	3.1	0.794
9	15.6	11.5	3.6	17.5	10.1	3.4	0.710
13	13.1	7.5	2.2	18.3	7.3	2.4	0.983
17	12.6	5.2	1.7	18.5	9.9	3.2	0.091
21	17.4	7.8	2.5	20.6	7.7	2.6	0.969
25	17.3	7.5	2.7	25.4	7.6	2.7	0.845
29	15.3	7.8	2.6	14.4	6.2	2.5	0.626
33	19.5	7.6	2.5	18.0	6.0	2.6	0.634
37	18.4	6.8	2.0	17.8	7.1	2.4	0.580
41	16.4	6.4	2.4	17.0	6.3	2.3	0.454

TABLE 2. Heat coagulation time of milk (pH 6.80 at 140°C) from cows injected with saline or rBST during one lactation, experiment 2

	milk HCT at pH 6.80						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	± SD	STAND. ERROR	$\bar{x}$ minutes	± SD	STAND. ERROR	2-Tail Prob.
-1	6.1	4.8	1.5	7.1	5.5	1.8	0.672
1	9.0	7.2	2.2	11.5	10.0	3.3	0.323
5	13.3	12.6	3.8	6.6	3.6	1.2	0.001
9	12.9	11.8	3.7	12.4	11.2	3.7	0.895
13	12.3	8.1	12.4	12.1	8.1	2.4	0.200
17	10.8	4.7	1.6	9.5	7.8	2.6	0.175
21	17.3	9.4	2.0	16.6	12.0	4.0	0.477
25	14.2	9.8	3.1	9.9	4.9	1.8	0.087
29	20.3	12.2	3.9	7.5	2.2	0.8	0.092
33	12.1	5.2	3.1	15.9	8.4	2.7	0.074
37	10.5	6.4	3.4	16.5	7.5	3.1	0.579
41	7.5	7.5	2.8	16.0	8.0	3.4	0.284

TABLE 3. Heat coagulation time of milk (pH 6.68 at 140°C) from cows injected with saline or rBST during one lactation, experiment 2

	milk HCT at natural milk pH						
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ minutes	±SD	STAND. ERROR	$\bar{x}$ minutes	±SD	STAND. ERROR	2-Tail Prob.
-1	21.8	10.6	3.3	18.8	5.9	2.0	0.116
1	18.9	10.1	3.0	20.7	8.6	2.9	0.669
5	16.8	10.8	3.2	15.3	10.0	3.3	0.842
9	19.4	9.4	3.0	14.7	9.8	3.3	0.905
13	17.3	8.1	2.4	19.1	11.5	3.8	0.302
17	13.9	6.6	2.2	18.3	9.5	3.2	0.319
21	13.8	7.9	2.5	15.1	6.0	2.0	0.446
25	14.8	9.6	3.0	15.5	8.6	3.0	0.787
29	12.5	8.4	2.6	14.0	5.9	2.2	0.409
33	15.8	7.6	2.8	14.7	6.2	2.9	0.451
37	15.2	6.6	2.5	22.6	10.8	2.8	0.284
41	16.8	7.0	2.4	19.0	9.6	3.1	0.348

TABLE 4. Concentration of total calcium in milk from cows injected with saline or rBST during one lactation, experiment 2

Total Calcium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	1228.9	183.6	61.2	1126.4	223.6	79.0	0.590
1	1026.5	237.2	75.0	1015.6	271.4	95.6	0.691
5	1038.9	210.4	66.5	1097.9	142.5	50.4	0.317
9	1214.4	100.6	33.5	1258.5	259.5	91.8	0.016
13	1301.3	155.7	49.2	1211.5	289.6	102.4	0.087
17	1070.0	149.9	53.0	999.0	116.4	41.1	0.519
21	1001.1	104.8	34.9	948.8	184.8	65.3	0.135
25	1037.8	132.4	44.1	964.3	157.4	59.5	0.635
29	1004.4	99.9	33.3	1053.3	80.7	32.9	0.662
33	1189.2	133.4	45.6	1148.2	178.1	56.5	0.328
37	1143.0	140.3	48.6	1030.0	160.4	40.9	0.402
41	1240.4	120.1	45.4	1150.3	125.8	38.2	0.433

TABLE 5. Concentration of total magnesium in milk from cows injected with saline or rBST during one lactation, experiment 2

Total Magnesium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	138.2	15.7	5.5	136.0	14.9	5.3	0.904
1	114.9	30.9	10.3	124.9	27.8	9.8	0.790
5	117.2	23.0	7.3	127.5	17.1	6.1	0.447
9	130.3	25.5	8.5	117.5	32.8	11.6	0.496
13	124.4	24.6	7.7	127.1	29.4	10.4	0.601
17	101.5	15.5	5.5	99.3	5.4	1.9	0.013
21	104.1	21.7	7.2	101.9	11.8	4.2	0.125
25	107.8	14.9	4.9	112.4	14.9	5.6	0.543
29	106.0	16.5	5.5	109.2	7.0	2.8	0.076
33	110.4	12.1	5.6	111.2	12.8	6.0	0.542
37	101.2	13.8	5.4	113.3	5.8	4.0	0.621
41	110.8	10.3	6.2	114.3	6.3	5.8	0.582

TABLE 6. Concentration of total phosphorus in milk from cows injected with saline or rBST during one lactation, experiment 2

Total Phosphorus concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	1386.3	208.4	73.7	1312.5	259.2	191.6	0.579
1	1118.9	242.7	80.9	1370.0	236.5	70.6	0.959
5	1438.0	164.8	52.1	1430.0	144.3	59.4	0.743
9	1505.6	154.6	58.2	1510.2	163.2	56.4	0.684
13	1511.0	153.8	77.0	1496.2	139.3	113.9	0.417
17	1466.3	150.4	67.8	1477.5	137.7	60.1	0.526
21	1453.3	138.1	72.7	1477.5	133.7	62.3	0.760
25	1393.3	141.5	83.8	1464.3	150.6	57.8	0.589
29	1384.4	149.2	66.4	1443.3	130.8	56.4	0.316
33	1284.5	150.3	55.6	1320.5	131.7	65.4	0.423
37	1265.6	145.4	34.4	1351.4	145.6	54.5	0.585
41	1348.3	135.5	66.7	1400.1	150.8	64.3	0.473

TABLE 7. Concentration of soluble calcium in milk from cows injected with saline or rBST during one lactation, experiment 2

Soluble Calcium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	561.0	79.9	26.6	594.6	51.5	18.2	0.264
1	425.6	165.4	35.1	463.0	124.9	24.2	0.475
5	319.0	165.4	32.6	400.3	108.9	38.5	0.765
9	387.4	53.5	17.8	498.6	93.3	32.9	0.142
13	484.2	85.1	28.4	505.6	120.6	42.6	0.349
17	369.6	29.3	10.4	406.9	29.9	10.6	0.960
21	379.4	48.5	17.2	399.3	77.1	29.1	0.252
25	307.5	58.9	20.9	390.0	57.3	25.6	0.543
29	378.4	60.3	10.2	389.1	68.3	24.4	0.463
33	395.6	45.4	10.4	420.5	48.4	18.1	0.324
37	386.6	60.6	11.6	440.4	55.4	20.4	0.058
41	402.6	52.4	10.2	420.3	49.4	24.5	0.354

TABLE 8. Concentration of soluble magnesium in milk from cows injected with saline or rBST during one lactation, experiment 2

Soluble Magnesium concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	111.1	11.7	4.1	119.8	16.7	5.8	0.391
1	95.3	20.9	6.9	108.0	19.9	7.0	0.901
5	92.1	17.9	5.7	96.5	20.5	7.3	0.688
9	107.1	13.2	4.4	100.8	25.9	9.2	0.079
13	95.6	26.6	8.4	97.0	28.8	10.2	0.801
17	76.6	16.0	5.7	85.3	19.1	6.8	0.655
21	77.5	9.9	3.3	76.6	12.2	4.3	0.574
25	81.8	12.3	4.1	86.9	5.9	2.2	0.092
29	80.6	14.5	4.8	84.2	7.5	3.0	0.160
33	86.4	15.5	3.5	89.2	10.6	4.6	0.563
37	84.6	13.6	4.2	87.2	11.4	3.8	0.664
41	86.7	14.7	3.8	89.0	10.3	4.1	0.478

TABLE 9. Concentration of soluble phosphorus in milk from cows injected with saline or rBST during one lactation, experiment 2

Soluble Phosphorus concentration in milk							
	rBST			Control			t-test
Sampling (Weeks)	$\bar{x}$ mg/L	± SD	STAND. ERROR	$\bar{x}$ mg/L	± SD	STAND. ERROR	2-Tail Prob.
-1	655.6	104.4	34.8	535.0	126.0	44.5	0.607
1	672.0	67.6	21.4	627.5	91.9	32.5	0.385
5	631.0	49.3	15.6	666.3	108.4	38.3	0.033
9	631.1	56.7	18.9	701.3	95.8	33.9	0.164
13	678.0	52.9	16.7	685.0	96.7	34.2	0.096
17	672.5	36.2	12.8	692.5	49.5	17.5	0.426
21	697.8	22.2	7.4	698.8	64.7	22.9	0.007
25	704.4	18.1	6.0	698.6	66.4	25.1	0.002
29	689.4	26.5	8.8	705.8	45.9	18.7	0.163
33	710.0	53.4	33.4	714.0	50.1	19.6	0.243
37	716.0	48.4	23.4	710.5	52.4	19.8	0.594
41	720.1	55.6	28.4	712.6	56.4	30.4	0.648

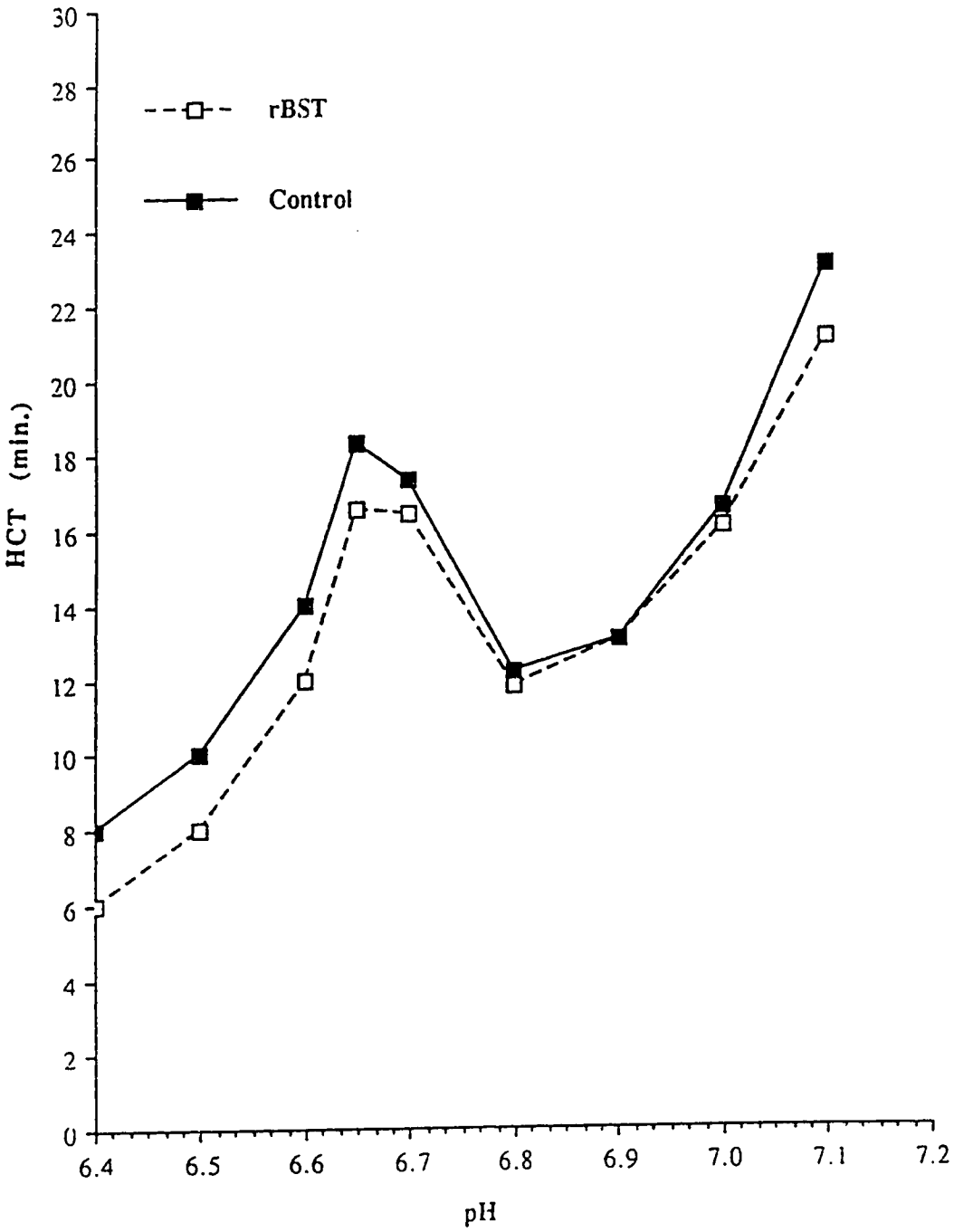

Fig. 1 Heat coagulation time (HCT)-pH profile of milk from cows, injected with saline or rBST, experiment 2.

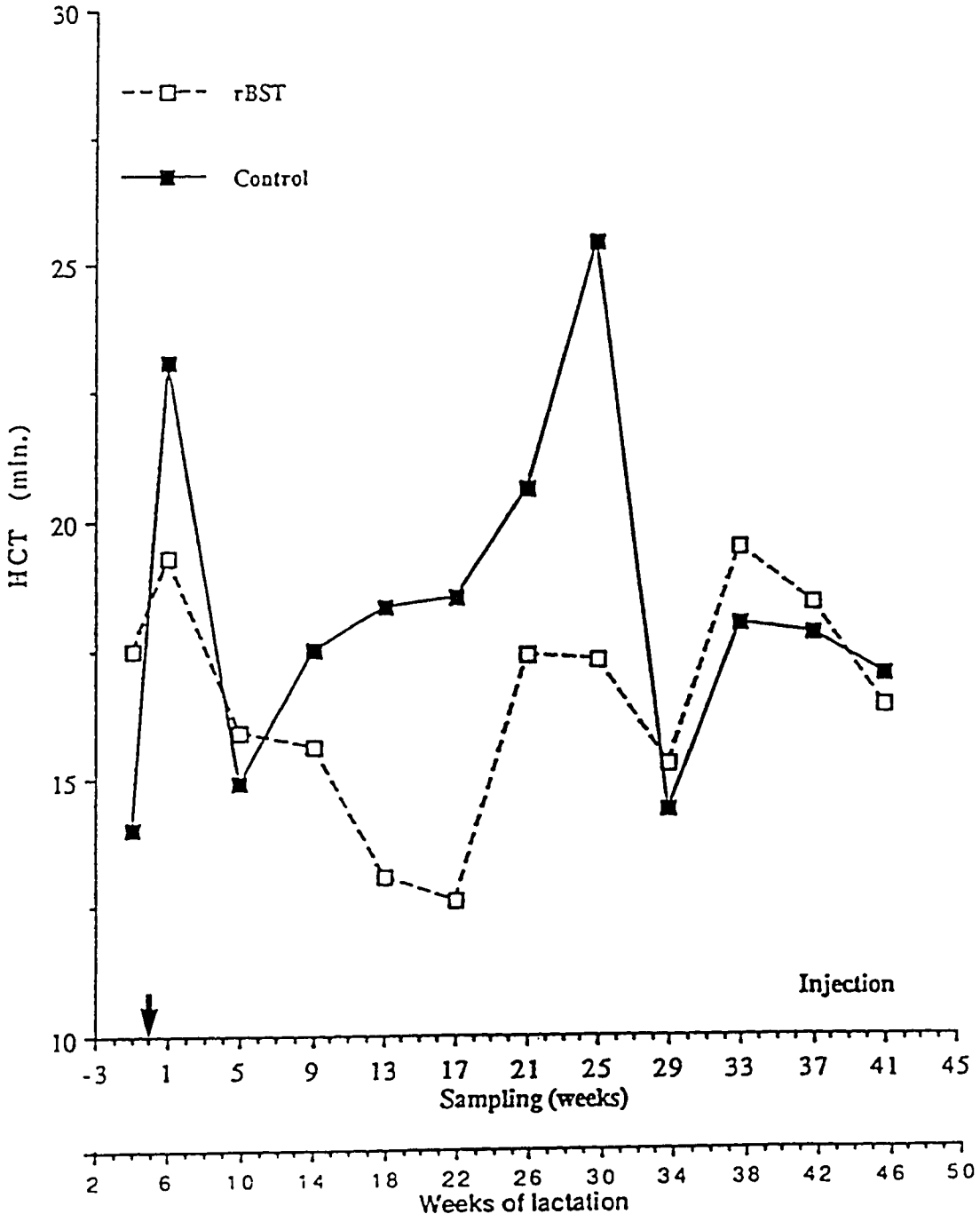

Fig. 2 Heat coagulation time of milk (pH 6.65 at 140°C) from cows injected with saline or rBST, experiment 2.

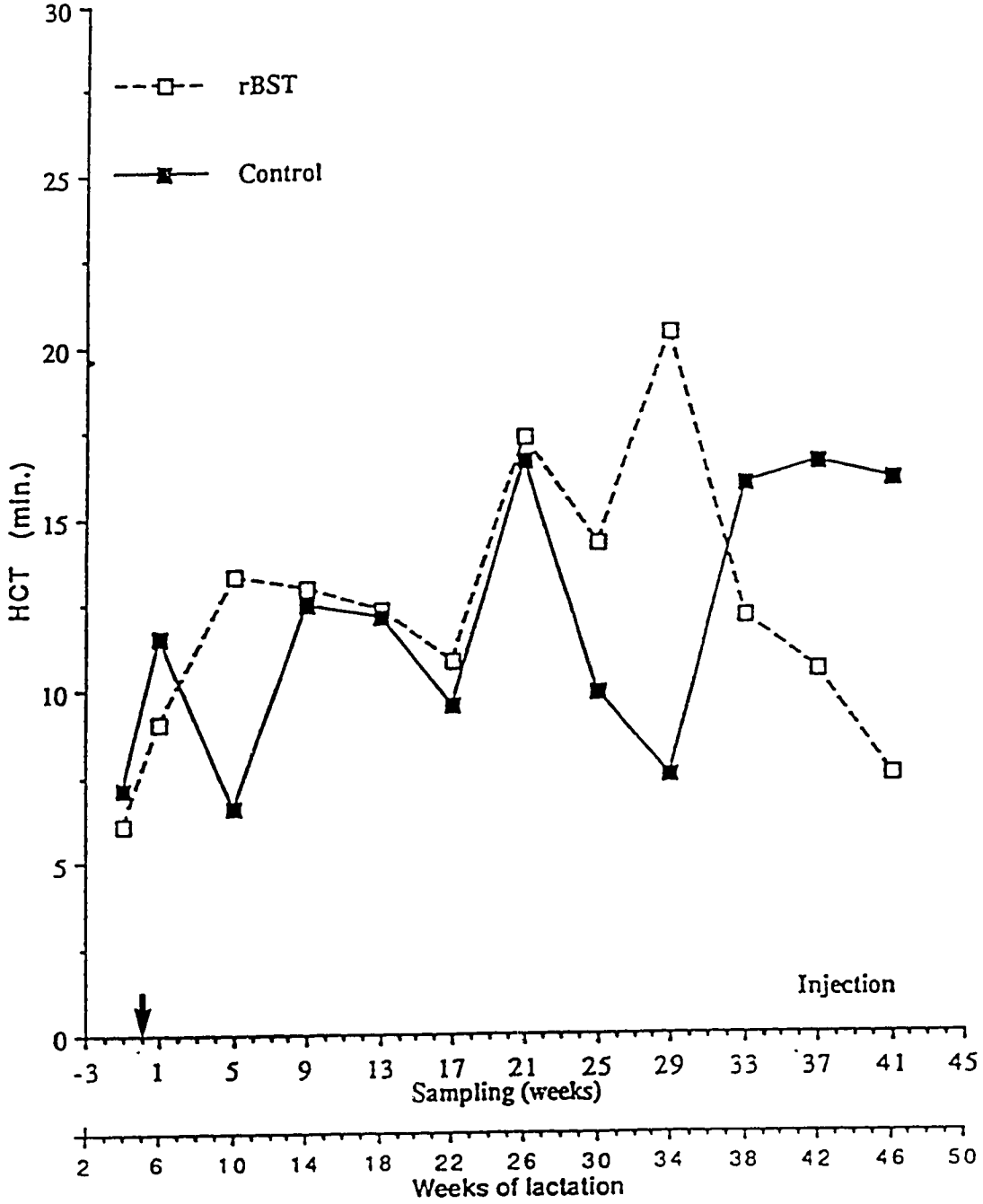

Fig. 3 Heat coagulation time of milk (pH 6.80 at 140°C) from cows injected with saline or rBST, expreriment 2.

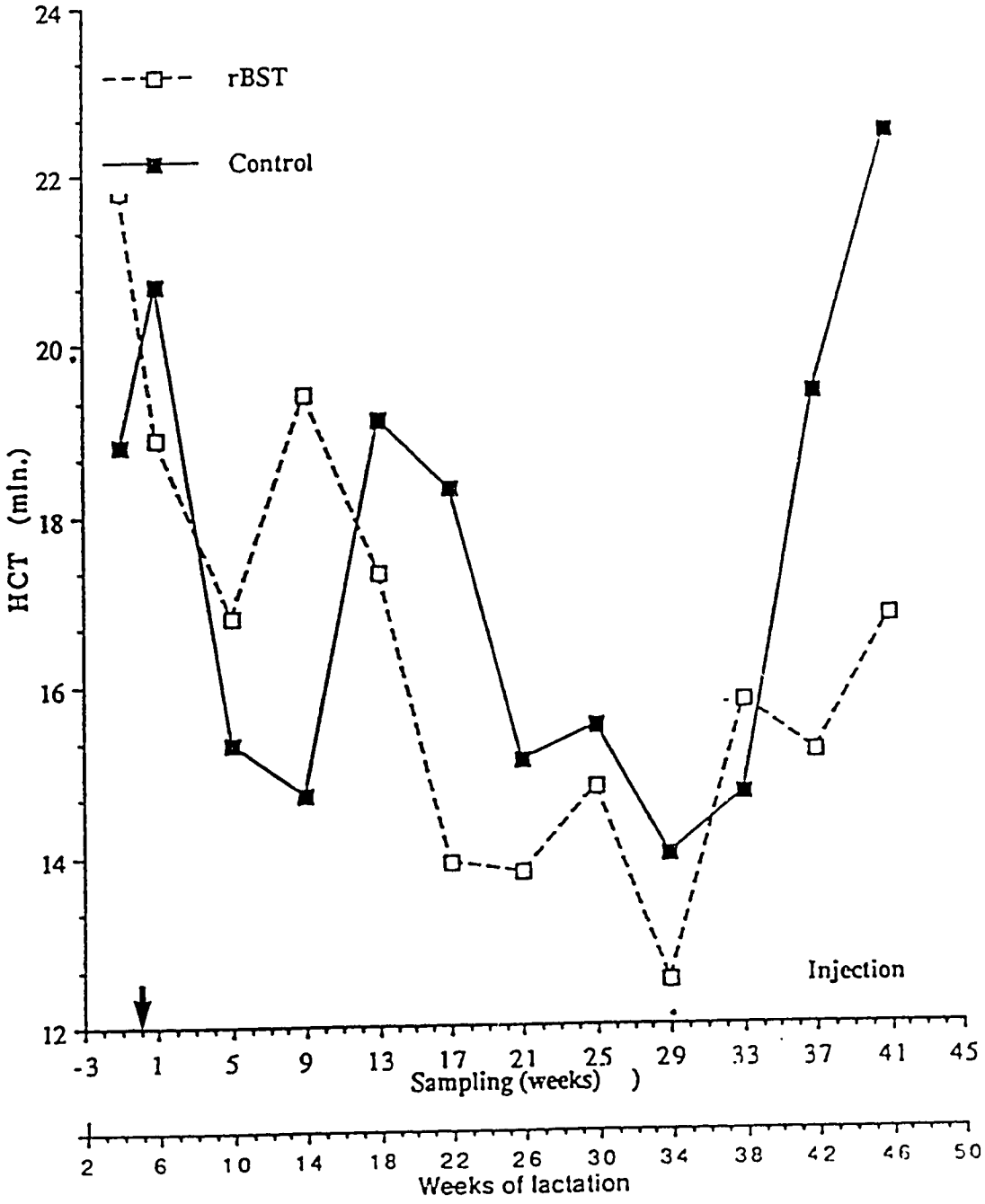

Fig. 4 Heat coagulation time of milk (pH 6.68 at 140°C) from cows injected with saline or rBST, experiment 2.

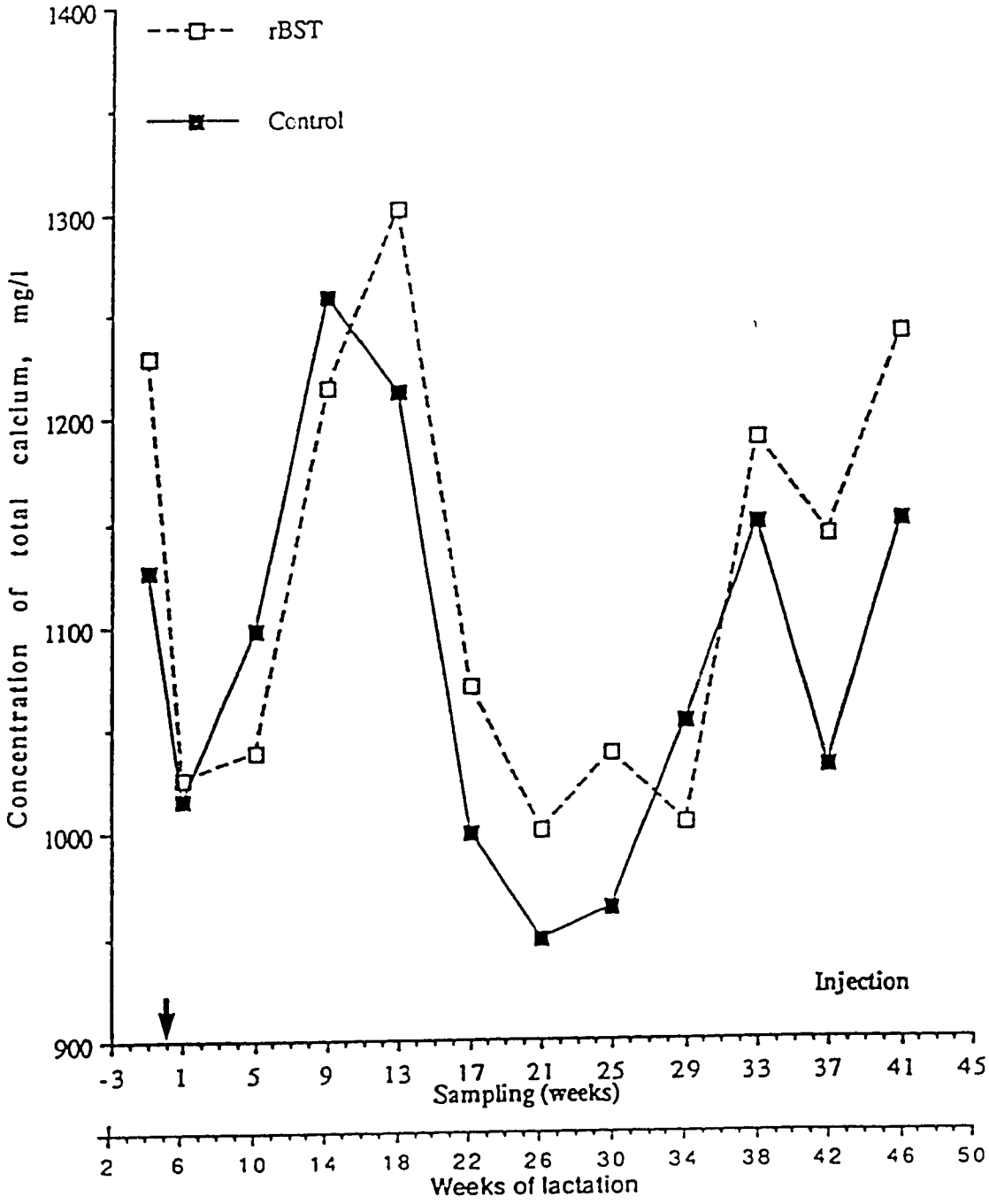

Fig. 5 Concentration of total calcium in milk from cows injected with saline or rBST, experiment 2.

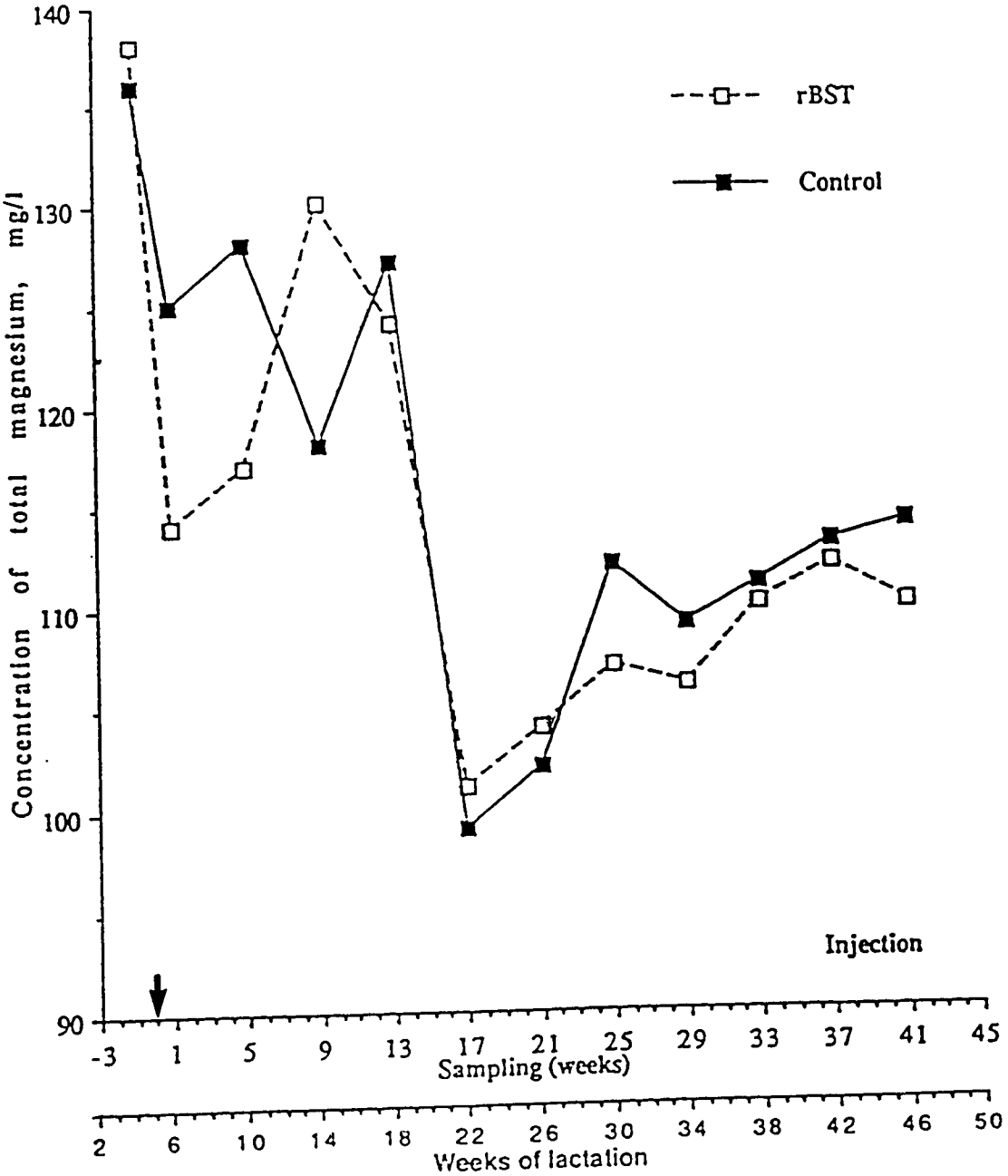

Fig. 6 Concentration of total magnesium in milk from cows injected with saline or rBST, experiment 2.

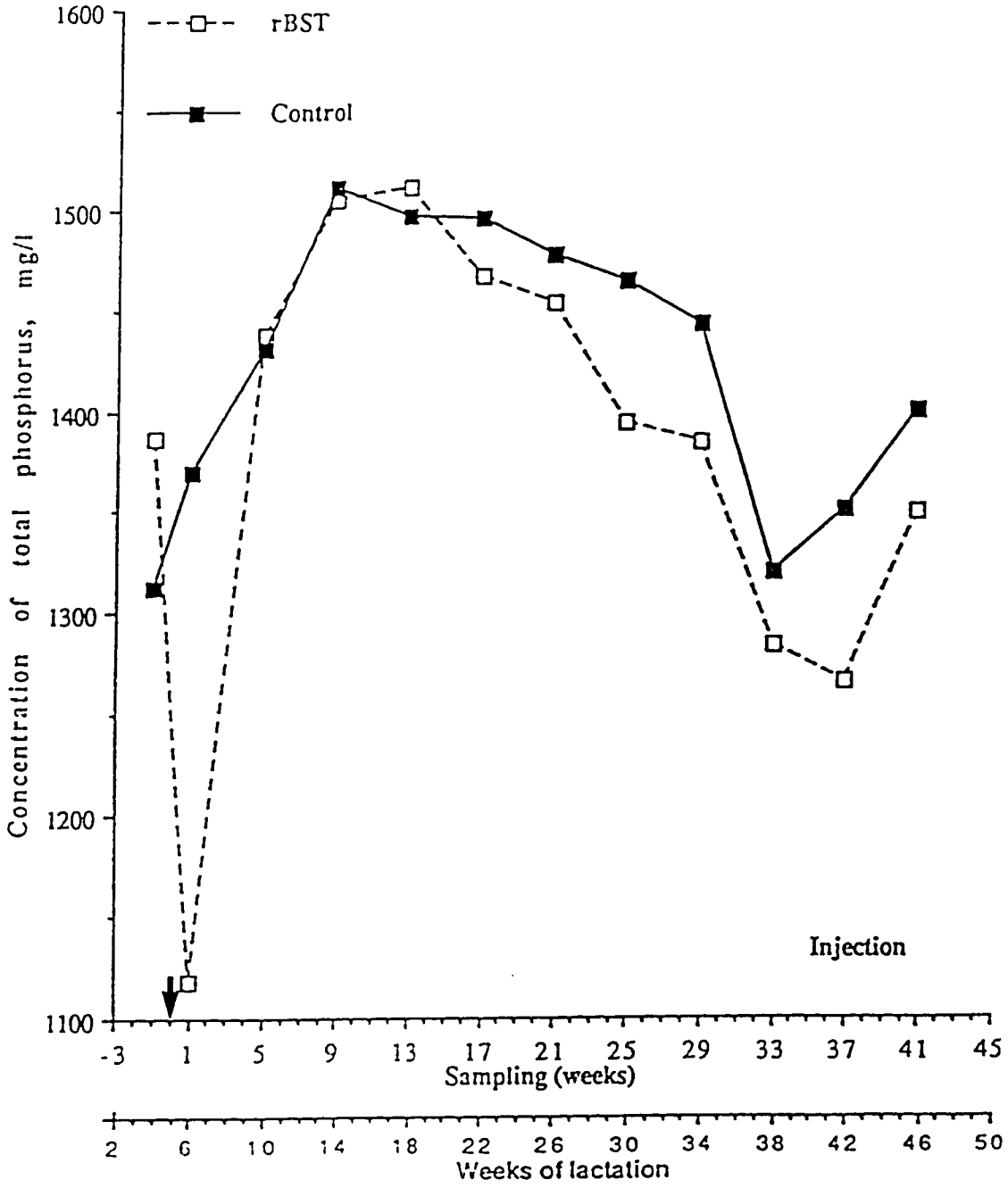

Fig. 7 Concentration of total phosphorus in milk from cows injected with saline or rBST, experiment 2.

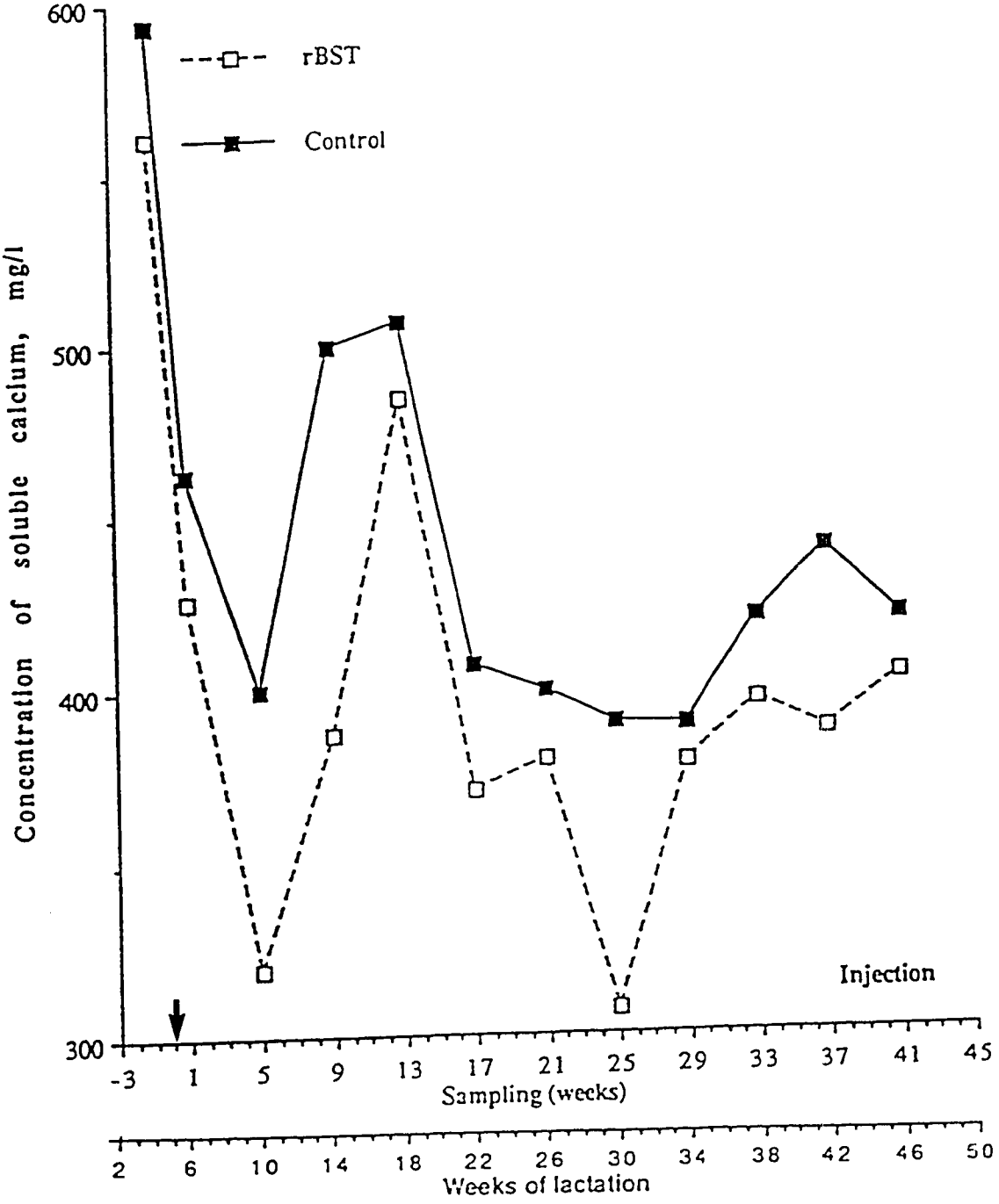

Fig. 8 Concentration of soluble calcium in milk from cows injected with saline or rBST, experiment 2.

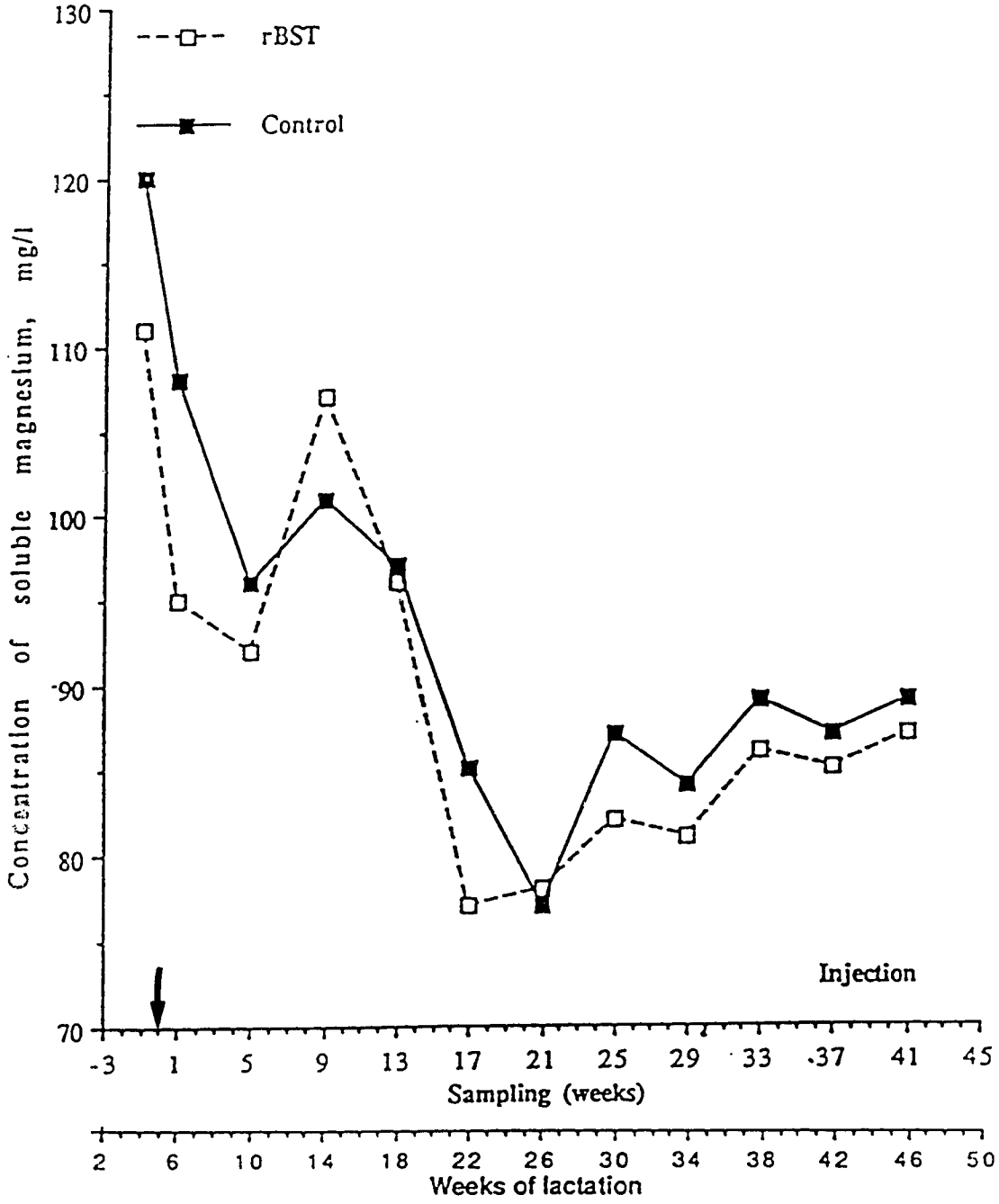

Fig. 9 Concentration of soluble magnesium in milk from cows injected with saline or rBST, experiment 2.

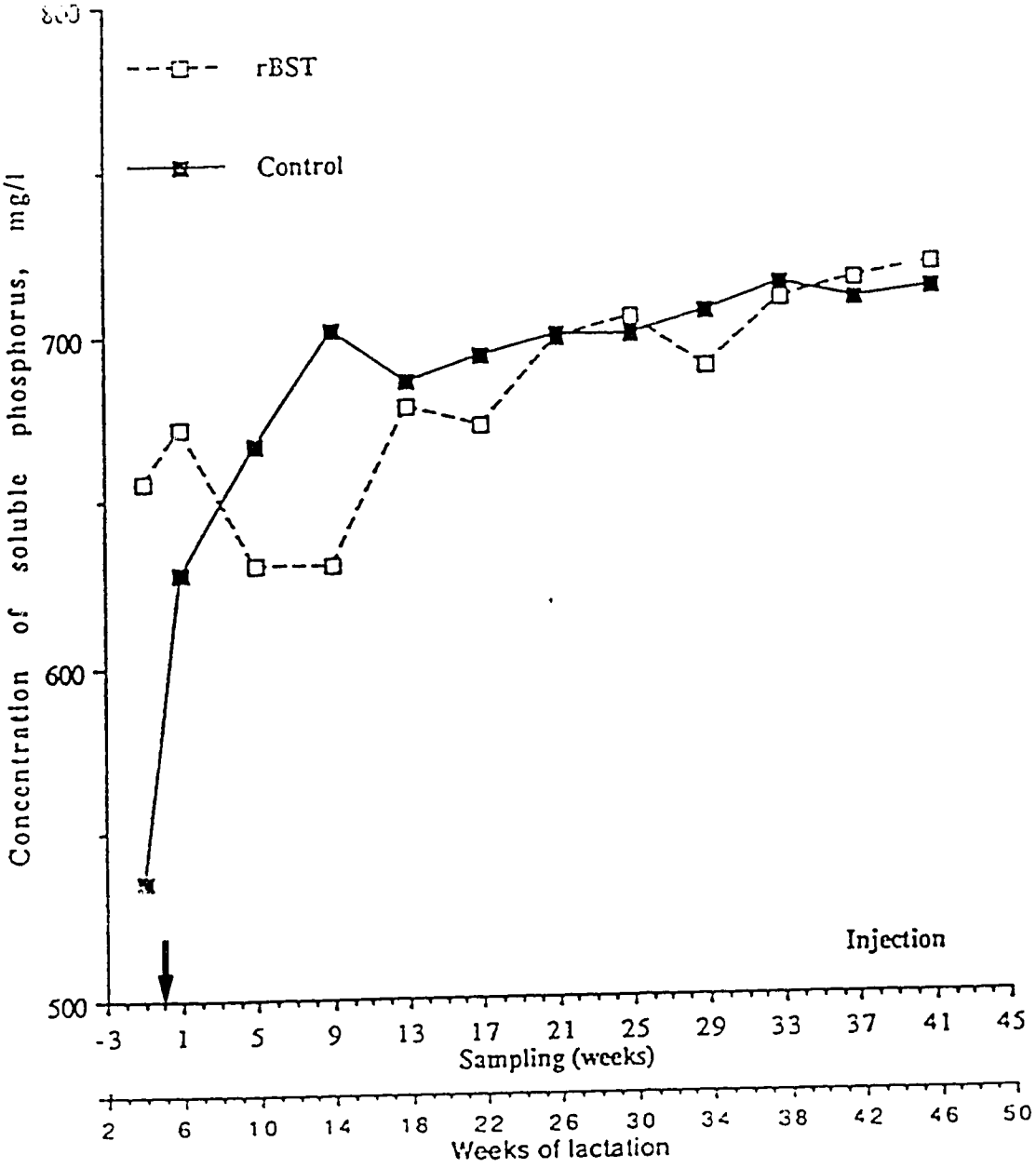

Fig. 10 Concentration of soluble phosphorus in milk from cows injected with saline or rBST, experiment 2.