The Control Points of Lactose Synthesis in the Lactating Ruminant.

by

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In the words of Albert Einstein, *"He who probes the natural sciences is conscious of a spirit that reveals itself in the universe, a spirit more exalted than mankind. We with our modest capabilities, must bow down in humility".*

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CONTENTS

GENERAL SUMMARY

It has been established that lactose is the major osmole in the milk of many mammalian species, and hence that the amount of water secreted is approximately proportional to the amount of lactose secreted. It is envisaged that reduction of the amount of lactose synthesised would allow a reduction of the amount of water in milk.

This thesis studied the regulation of lactose synthesis in the mammary glands of goats by altering the flux of glucose and other intermediates of lactose synthesis. The consequent effects were determined by measuring the levels of various metabolites in milk.

The mechanism of the reduction of milk yield brought about by twenty-four hour fasting in goats was explored by looking for perturbations in some of these parameters measured. Also, known differences between certain bovine genetic groups in water secretion were investigated by measurements in milk of trace metabolites involved in lactose synthesis.

It was intended to further substantiate the paradigm that availability of glucose within mammary secretory cells of ruminants normally regulates lactose synthesis during mid-lactation. This widely held belief has not been supported by this research.

Chapter 1. INTRODUCTION

1. Research overview

1.1 The economic significance of water in milk in relation to industrial processing and in relation to breeding programs

In New Zealand in 1992 an advanced milk payment rate was as follows: protein was approximately $4.15 per kg, fat was approximately $2.30 per kg and there was a milk volume charge of approximately 4.4 cents per litre. The volume charge reflected the cost of the transport of milk in tankers, of evaporating water in milk powder production and other bulk related costs. On this basis one litre of typical Jersey milk would contain about 16.6 cents worth of protein, 13.7 cents worth of fat and -4.35 cents worth of water resulting in about 26 cents nett and similarly one litre of typical Friesian milk would contain about 14.1 cents worth of protein, 10.9 cents worth of fat and -4.35 cents worth of water resulting in about 20.7 cents nett. This is a component weighting of about 0.48 protein (Kg) : 0.39 fat (Kg) : 0.13 volume (*l*). The other major component of milk, lactose, is not mentioned in this formula because it currently has little economic value in its own right and is often disposed of, as a by-product, by spraying onto farmland.

On average Jerseys incur less of a volume penalty for their milk, but the protein to fat ratio (measuring yield in Kg or content in g/100g) of about 0.74:1 exhibited by a typical Friesian creates a balancing influence between the breeds, when compared with the approximate 0.66:1 of a typical Jersey. Furthermore the genetic correlations of milk protein with milk fat of 0.8 and 0.67 for Jerseys and Friesians respectively (Wickham, 1985) suggest that a higher rate of differential gain of protein over fat is likely to come from the Friesian breed.

This 'A+B-C' formulation was incorporated into the breeding index system operating in New Zealand (Bryant *et al.*, 1988), but extra weight was placed on protein yield because of a prediction of increasing economic importance of protein in relation to fat and also a high genetic correlation between protein and fat yields (Wickham, 1985). For 1991/1992 this weighting for payment breeding index, based on typical milk composition, was about 0.62 protein (Kg) : 0.24 fat (Kg) : 0.14 volume (*l*). The economic breeding index also includes a lightly weighted component for 'traits other than production'.

Since the genetic correlations of milk protein with milk volume were measured to be 0.6 and 0.83 for Jerseys and Friesians respectively (Wickham, 1985), it follows that this 14% selection pressure is applied oppositely to some of the same loci or to closely linked loci involved with protein yield. This markedly reduces the effectiveness of the applied selection

pressure for reduced milk volume, although steady but slower progress is still expected. These correlations suggest that a higher rate of gain of protein content is likely to come from the Jersey breed.

The loci available for selection are necessarily polymorphic. Some of these loci may still be rather unresponsive to selection in cases where beneficial recessive alleles occur at low frequencies, or if heterozygote inferiority or epistasis *etc.* occur. These genetic correlations suggest that the Jersey breed may have a greater number of, and perhaps more influential, polymorphic loci relevant to water secretion which are not relevant to protein yield, than the Friesian breed.

It follows that a more knowledgeable or information-rich approach to selection would be helpful for improving allele distribution at these polymorphic loci and that some form of intervention might be justified at identifiably important or relevant monomorphic loci. It might be that intervention would take the form of marker-assisted introgression of suitable alleles into breeds that are currently monomorphic at that locus or lacking favourable alternative alleles. Alternatively it might theoretically involve recombinant DNA procedures in order to make a direct modification of some form.

Thrice daily milking is often performed in northern hemisphere countries, in order to increase milk output by about ten percent and give a higher return on large capital investment. The discrepancy between this situation and the stated maximum tolerable milking interval before production loss of about 16 hours (Elliot, 1959; Davis *et al.*, 1987) probably results from a combination of the differing nutritional regimes of the countries and the substantial genetic progress on the world dairy scene since 1959. The latter is the likely explanation since the genetic correlation between fat yield and milk yield in some common breeds is approximately 0.5 (Wickham, 1985): fat yield was long the major selection criterion (in New Zealand) and volume yield of little consequence to the farmer.

Cattle suitable for once daily milking are a possible outcome of applied knowledge concerning the regulation of water secretion. Among present high milk-yielding bovine breeds, udder capacity (Davis *et al.*, 1987) is usually in the range of 20-30 hours whereas studies indicate that greater than thirty hours capacity would be required for reasonably successful once a day milking (Carruthers, 1989; L'Huillier *et al.*, 1989). Udder capacity is defined as the maximum volume that can be milked out of the udder in one milking divided by the normal twice daily milk synthesis rate. This information implies that udder filling slows down before the udder is fully distended

1.2 The potential for the economic utilisation of biotechnology through application of a mechanistic understanding of the regulation of water secretion

A greater understanding of how water secretion is controlled may help to identify specific associated gene products and hence result in the identification and perhaps cloning of the associated genes. Such understanding might be applied in areas such as reducing milk volume and reducing the cost of dairying by such means as improved selective breeding strategies through the discovery of appropriate physiological, biochemical (Shannon, 1989) or DNA-level genetic markers, immunological or hormonal modifications, or production of transgenic or syngenic animals.

As mentioned above a volume penalty component is included in the payment breeding index, thus a small proportion of the selection pressure applied through this system is directed against water secretion. Genetic markers correlating with breeding indices (Shannon, 1989) or components thereof can partially obviate the need for progeny test programs and provide information useful for selection purposes at an earlier stage and with a lower operating cost.

Some standard approaches to the elucidation and use of genetic markers follow:

DNA finger-printing is used as a fairly cheap alternative to genomic linkage mapping of monogenic and polygenic traits, relying on some luck to give pieces of the fuller picture: probes with multiple site specificity are used to increase the probability of detecting correlatable genetic variability, over that of using gene probes.

Even relatively exhaustive methods such as quantitative trait loci (QTL) mapping, given the availability of a suitable bovine linkage map, still rely heavily on assumptions of additive genetic factors and the distribution of positive alleles into only the high genetic group in order to detect effective factors with a known confidence level (Lander *et al.*, 1989). Reliable genetic markers for water secretion operating at the physiological or DNA level, with present technology and statistical models, are more likely to be discovered with the aid of mechanistic understanding than by an empirical approach alone. Discovery of such could reduce the need for genetic proofs resulting from the herd testing of lactating offspring, by allowing direct analysis of the young or lactating individual. A method for predicting future milk yield in bovines based on pre-partum blood α-lactalbumin and beta-lactoglobulin concentrations (Mao *et al.*, 1991) is a step in this direction.

Considering the DNA amplification power of the polymerase chain reaction (PCR) and its routine use in medical laboratories for the pre-natal analysis of human genetic defects (Kazazian, 1988), current schemes could be short circuited even more through the analysis of blood samples from young stock or analysis of cells derived from cultured blastocysts

(King, 1990; First, 1990; de Boer *et al.*, 1992), in order to detect the presence of favorable traits without waiting for lactation or lactating offspring.

Treatment of bovine Friesians with exogenous bovine somatotropin (bST) to increase milk yield under some circumstances is an example of a technically successful hormonal modification (Davis *et al.*, 1984). This concept has also been applied through genetic manipulation to produce transgenic pigs expressing high endogenous levels of bST (Pursel *et al.*, 1989). On this occasion increased growth was stimulated, but this was accompanied by side effects detrimental to the general health of the pigs.

A 10-30 Kd fraction of goat milk, shown to inhibit milk synthesis when injected intraductally into the mammary lumen of lactating goats and rabbits (Wilde *et al.*, 1984; Wilde *et al.*, 1988), has suggested the potential of an immunological route to accrue some of the benefits observed at higher milking frequencies in goats, since immunoglobulins derived from the bloodstream occur in ungulate milks (Butler, 1978; Lascelles, 1977). In bovines it is not known whether production loss due to extended milking intervals is due to lack of udder capacity or chemical inhibition (Davis *et al.*, 1987).

1.3 Ethical considerations

Some ethical considerations of these potential application procedures are as follows. The domestication of various species of use to humans has been precedented for a very long time *e.g.* In the middle east goats and sheep were being milked and under going domestication long before Christ. This process results in altered genetic composition of a population in response to the influence of man. Selective breeding procedures of any form, in this context, are easy to justify (Campbell, 1990), except perhaps for large scale inbreeding resulting in excessive loss of genetic variability from a species.

Immunological manipulations raise the issue of immunisation against self and of taking some responsibility for resulting action of the defeated or altered mechanism *e.g.* What happens to a full udder that keeps filling?

Hormonal manipulations fall into two major categories: that of applying hormones native or foreign to the species. bST treatment of dairy cows to improve milking performance, under certain conditions, falls into the former category.

Direct genetic manipulation falls once again into two categories resulting from the introduction of DNA (Broad, 1989) native or foreign to the species in question, giving rise to syngenic or transgenic animals respectively. This type of process may perhaps be seen as

an extension and acceleration of previous domestication procedures (Campbell, 1990). This is perhaps a useful but simplistic view, considering the modular nature of many DNA constructs and the often low degree to which site and frequency of integration into the genome is controlled (Pursel *et al.,* 1989).

Many well supplied markets require that animal products have only 'natural' components and that these products are produced according to a growing list of procedures and specifications. A political and legal debate over the use of bST of exogenous origin as a growth promotant in beef cattle is presently occurring in countries including the U.S.A. and Britain.

1.4 Theological considerations

Christian and Judaic religions refer to a divine mandate to subdue and to rule the earth and all its living creatures in the books of Moses. The biblical requirement of man concerning biological life on earth appears to be responsible stewardship. This has been applied to biotechnology by describing the knowledge as licit, but not without limits for its use (Strobel, 1987). Such limits might be defined through the above requirement for global responsibility, and as such include the anticipation of social and ecological consequences.

2. Lactose content determines water content of milk

It has been established that lactose is the major osmole in the milks of many mammalian taxa (Jenness, 1982) and consequently the usual major cause of water secretion in milk synthesis (Linzell *et al.,* 1971). Even the low yield of lactose observed in many marine mammals (Jenness, 1982) is somewhat in keeping with this principal, resulting in their highly concentrated milks, although in some of these cases lactose may not actually be detectable (L'Huillier *et al.,* 1989). It has been suggested that much interspecies variation in the ratios of lactose to protein and fat secretion and hence variation in water secretion may be related to the structure of α-lactalbumin (Brew, 1970).

The order artiodactyla within the mammalian infraclass eutheria contains the suborders ruminantia, suiformes and tylopoda. The ruminantia includes families: bovidae comprised of subfamilies: bovinae *e.g.* Bos taurus, caprinae *e.g.* goat, sheep; antilopinae; giraffidae; cervidae (deer); antilocapridae *e.g.* pronghorn. These are multigastric herbivores which chew the cud. Lactose concentration in ruminant milks is usually within the interval of two to six percent (Jenness *et al.,* 1970).

3. Lactose synthesis

3.1 Mammary gland anatomy

The secretory or epithelial cells of the developed mammary gland are arranged in single layers lining the the central cavities (lumina) of spherical structures called alveoli or acini. Each acinus is surrounded by a web of myoepithelial cells, which contract in response to oxytocin resulting in milk ejection through the duct system, a tree-like branching arrangement within the gland. A group of acini all draining into the same major milk duct is called a lobule. Depending on species, major milk ducts may empty directly through a multiple ducted teat or into a cavity called a cistern which empties through a single teat canal (Mepham, 1983).

3.2 The lactose synthetic pathway in the mammary epithelial cell

Figure 1 is a representation of the generalised lactose synthetic pathway for mammals which produce lactose (Kuhn, 1983; Jones 1974). Exocytosis of lactose and some other milk components involves the fusion of transport vesicles with the apical membrane of the secretory cell.

Glucose transported passively into the mammary epithelial cell, by a specific facilitative transporter in the basolateral membrane, is phosphorylated by hexokinase to give glucose-6-phosphate.

Glucose-6-phosphate is converted to glucose-1-phosphate by phosphoglucomutase, with a slight change in free energy and a fairly central equilibrium position.

Glucose-1-phosphate reacts with uridine triphosphate to produce UDP-glucose in the presence of UDP-glucose pyrophosphorylase with a large release of free energy including coupled hydrolysis of pyrophosphate. This reaction hence can potentially drive UDP-glucose to a high concentration *i.e.* the equilibrium is strongly to the right.

UDP-glucose is reversibly converted to its epimer UDP-galactose by UDP-glucose-4-epimerase, with a slight change in free energy and a fairly central equilibrium position.

UDP-galactose and glucose move across the Golgi membrane by processes believed to be passive in nature (Kuhn 1983).

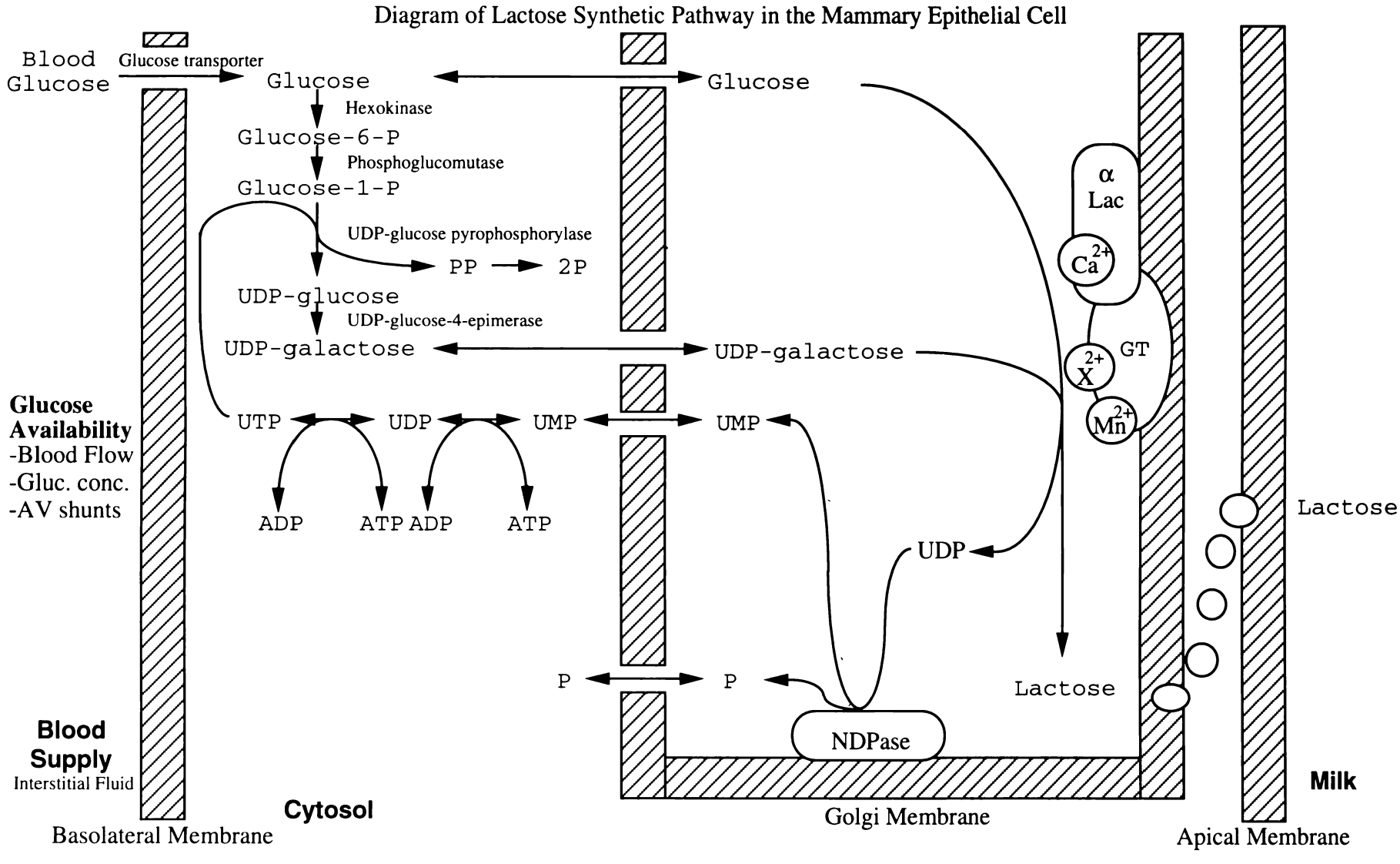


Figure 1. Lactose synthetic pathway.

Lactose synthesis occurs in the presence of lactose synthetase which is comprised of galactosyl transferase and α-lactalbumin accompanied by a large release of free energy including coupled hydrolysis of uridine diphosphate to uridine monophosphate. This reaction is capable thus of driving lactose to a high concentration from relatively low substrate concentrations. Lactose synthesis is potentially modulated by intracellular ATP status as are many other cellular processes.

4. Regulation of lactose synthesis

4.1 Blood glucose availability to the mammary epithelial cell

Glucose is the major blood precursor of milk lactose in those mammals which synthesise lactose. Glucose is at a premium in the grazing ruminant because little, if any, is absorbed from the alimentary tract. Hence almost all of the glucose supply is derived from gluconeogenesis and the major factor influencing the rate of gluconeogenesis is the availability of substrates. Gluconeogenesis is maximal after feed ingestion and decreases during feed restriction. In fed ruminants the principal precursors are propionate and amino acids, with lactate and glycerol making minor contributions to glucose production (McDowell, 1983). It is therefore vital that the animal has fine control over the utilisation of glucose in individual tissues. In particular in the lactating ruminant careful control must be maintained over glucose utilisation by the mammary gland, as the gland utilises approximately eighty percent of glucose entering the circulation.

A graph showing the rate of lactose secretion versus the concentration of milk glucose in goats exhibited normal Michaelis-Menten type kinetics, when conditions ranged from short term starvation to supra-normal mammary substrate levels (Faulkner, 1985a). However lack of correlation of milk glucose concentration with lactose synthesis under various nutritional regimens has been observed in rats (Grigor *et al.*, 1989).

The product of blood glucose arterio-venous (AV) difference and blood flow rate is the rate of glucose uptake. Mammary blood flow rate has been shown to decrease in the starved lactating goat (Davis *et al.*, 1985a).

Adenosine and adrenaline may be used to modify mammary blood flow when infused into an external pudic artery of the lactating goat (Linzell, 1974).

4.2 Baso-lateral membrane and glucose transport

The main paradigm (Kuhn T.S., 1970; Mepham, 1983) explaining lactose restriction under short term starvation (Faulkner, 1985a) is causation by reduced intracellular glucose concentration. Clearly factors affecting glucose transport including glucose transporter

proteins, in addition to those mentioned above, are of interest in testing this contention. Very little information has been published about the characteristics of glucose transporter proteins in ruminant mammary glands, such as V_{max} and requirements for metal ions and responsiveness to allosteric modifiers. These facilitative transporters are believed to be energetically passive (Wheeler *et al.*, 1985), with asymmetric K_m and V_{max} values implying faster equilibration in one direction as opposed to the other. Concentration gradients implied by milk and blood glucose measurements (Faulkner *et al.*, 1985b) suggest that they have at very least a damping effect on cytosolic glucose concentration.

The decrease in mammary glucose uptake brought about by starvation is accompanied by a decrease in estimated intracellular levels of glucose in the mammary epithelial cells of starved lactating goats (Faulkner, 1980).

Rate limiting to the supply of glucose for lactose synthesis could be the rate of transport of glucose across the basolateral membrane of the alveolar epithelial cell of the mammary gland of the lactating goat (Davis & Collier, 1985a; Faulkner 1985a).

4.3 Lactose Synthesis

Lactose synthetase is a bimolecular complex of galactosyl transferase (GT) bound to the Golgi membrane and α-lactalbumin, which spontaneously associates with GT. α-Lactalbumin confers lactose synthesising capability upon GT and as such is an enzyme modifier protein (Kuhn, 1983). It has been estimated from *in vitro* studies that GT in bovine mammary tissue has a turnover time of forty minutes in the lactating bovine mammary gland (Jones, 1977). It has also been calculated that the synthesising capacity of GT is stretched to the limit in this situation (Jones, 1977). Moreover, *in vivo* studies in fed lactating goats indicate that the enzyme is working at fifty percent of maximum capacity (Faulkner, 1985a).

It has also been proposed that α-lactalbumin may have a role in the regulation of lactose secretion (Kuhn, 1983; L'Huillier *et al.*, 1989). The concentration of α-lactalbumin has been observed to loosely correlate with lactose secretion over a wide range of mammalian species (Ley, 1970). A further potential regulatory feature is that α-lactalbumin possesses a looped domain which binds calcium, which in turn transmits a major conformational change through the molecule (Stuart *et al.*, 1986; Musci *et al.*, 1985) altering its lactose synthesising activity when measured in solution, and greatly decreasing its ability to adsorb to hydrophobic surfaces.

4.4 Neural and hormonal involvement in lactose regulation

Experiments have been carried out which have involved the sympathectomy of mammary glands in goats resulting in no observed effects on milk ejection and milk production (Peeters *et al.*, 1952). It has been suggested that adrenaline may hormonally mediate the milk reduction response under short term starvation (Linzell, 1974) since the mammary glands of goats respond to slight elevations of adrenaline from basal concentrations.

The actions of hormones may be ignored, in the first instance, as one focuses on finding points of regulation of lactose synthesis (see below). Discovery of these may eventually lead to the implication of an intracellular signalling mechanism and in turn to the method of control of signalling through the elucidation of hormone reception, intracellular feed-back and intracellular control mechanisms. Such an approach might loosely be conceptualised as reversed endocrinology.

5. Analysing lactose synthesis

5.1 The search for metabolite profiles

Estimation of enzyme activities operating *in vivo* by analysing cell extracts is fraught with such difficulties as providing a realistic biochemical environment and appropriate modifying factors.

The basic approach to the study of metabolic pathway dynamics used in this type of research is to obtain a metabolite profile, be it in reality just the measurement of a single parameter, under conditions of normal and modified lactose synthesis (*e.g.* short term starvation). If a transport process or an enzymatic conversion were a control point, one might expect to see metabolic changes focused around that point, particularly if the processes concerned are large in comparison with connected or interfering processes, *e.g.* cellular housekeeping metabolism. Typically one might see a bottleneck phenomenon where there is increased or unchanged upstream supply but downstream there is a decreased supply. Feedback and hormonal control mechanisms may be safely ignored at this stage if flux into the beginning of the pathway is confirmed to be constant, under normal and modified function, because these influences must themselves act through the elements of the process under scrutiny. It is difficult to include all the appropriate measures (*e.g.* factors influencing golgi vesicle budding rates are largely unknown), although a measurement of supply near the end of a process implies that there is no earlier control so long as alternative supply sources can be ruled out.

The idea of being able to use a single analytical procedure to measure many cellular intermediates from micro amounts of cell extracts with very high sensitivity and specificity

with a high throughput and good reproducibility is an ideal which has yet to be achieved. Analytical procedures such as two dimensional paper chromatography using cultures fed with high specific radioactivity compounds (Emerman *et al.,* 1979) are a step in the direction of obtaining such metabolite profiles. Such an accumulation of information would make it far easier to say that 'all other things are equal' or 'normal' and hence would create a more concisely defined cell experiment.

Capillary electrophoresis seems to exhibit separation resolution of similar resolution to gas chromatography, loading is similarly of a micro-sample nature but limits of detection often preclude detection of trace levels of compounds with low absorbance in the useful regions of the ultraviolet-visible spectrum. Inorganic ions are however detected at ppm levels using an intensely coloured ionic species in the running buffer, which is repelled by the presence of other ions of like charge thus creating a negative absorbance peak as ions from the sample migrate past the detector. This approach may be usefully applicable to some organic ions also.

A series of enzyme assays based on the measurement of NAD(P)H using highly sensitive bioluminescent detection (Arthur *et al.,* 1989) may be seen as a workable partial solution to this question, exhibiting good sensitivity, repeatability and specificity. High throughput technology using modified 96-well microplates is available for this, but at a somewhat prohibitive cost. The requirement of observing many metabolites is somewhat unfulfilled, although the possibility of developing methods to run a multiplicity of these assays on modern high throughput medical laboratory analysers also exists.

5.2 The nature of the apical membrane and trace analysis of lactose precursors in milk

The measurement of milk concentrations of metabolites is a convenient and non-invasive method to estimate intracellular levels of metabolites involved in the lactose synthesising pathway, particularly intracellular glucose in the mammary epithelium (Faulkner, 1980; Faulkner *et al.,* 1985b; Grigor, 1989). Validation of this procedure has relied upon the measurement of metabolites in tissue, and the calculation of milk to tissue ratios. Ratios in the vicinity of one are interpreted to mean that there is usually equilibrium of that metabolite across the apical membrane. Further confirmation is desirable.

Study of the transport of labelled 3-O-methyl-glucose, glucose and some other hexoses inferred that glucose can diffuse passively into the mammary secretory cell via the apical membrane (Faulkner, 1981; Faulkner *et al.,* 1985b).

Intraductal injection of the disaccharide sucrose has been used in a number of lactational experiments as an inert substance analogous to lactose and suitable for the control of milk osmolarity (Threadgold, 1984; Henderson *et al.*, 1984; Peaker 1977; Linzell *et al.*, 1976).

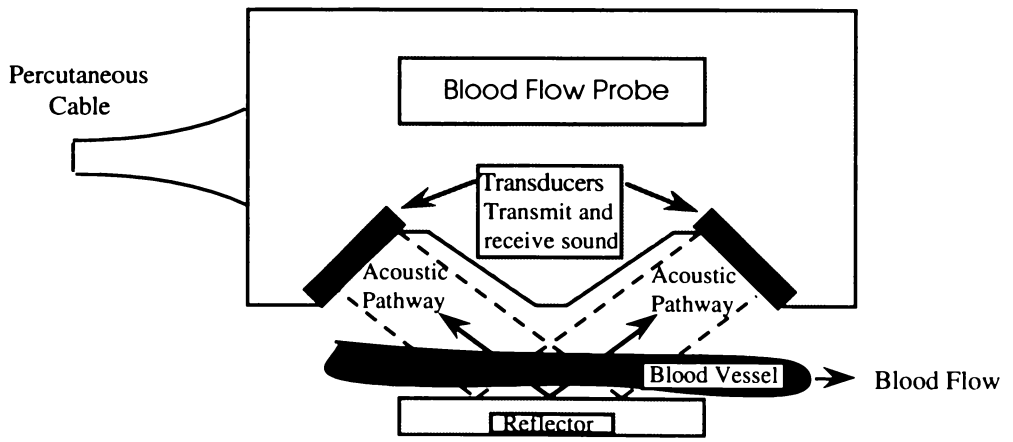
5.3 Arteriovenous differences and the ultrasonic blood flow probe

The measurement of mammary extraction or uptake of nutrients *etc.* from blood involves the measurement of arteriovenous differences of concentration, and blood flow. A T-101 blood flow meter package, by Transonic Systems Inc.*, can be used to measure blood flow in external pudic arteries of goats and cows *etc.*

The principle of blood flow measurements (net volume/time) involves the measurement of forward and reverse ultrasonic transit times (Figure 2). Measurements are independent of vessel size, vessel alignment and flow profile and, neither vessel constriction nor mechanical contact with the vessel are required.

The probes are factory calibrated and the measuring instrument validates the probes precision after implantation by measuring the received acoustic signal strength thus confirming that the probe cavity has been filled with 'liquid'. The probes are very precise but stable bias may be up to about 20% (Gorewit *et al.*, 1989).

Figure. 2. Diagram of a blood flow probe.



* Transonic Systems Inc.; Cornell Research Park,
138 Langmuir Labs.,; Ithaca, N.Y. 14850.
Fax: 607-257-7256

6. Suitable experimental systems exhibiting variation in lactose output

6.1 The starved lactating goat

Under short term starvation (24h) lactating Saanen goats exhibit an approximately thirty to fifty percent reduction in lactose synthesis and milk volume (Linzell, 1974; Chaibutr *et al.*, 1980a, 1980b, 1981, 1983). Short term starvation also results in: a decrease in blood flow from the approximate interval of about [300 - 600] to about [100 - 300] ml/min; a decrease in blood glucose from the approximate interval of [3.0 - 4.0] to about [2.0 - 2.5] mM and; milk glucose from the approximate interval of [0.2 - 0.4] to about [0.05 - 0.15] mM.

6.2 Some bovine genetic groups and their differences with regard to milk composition and water secretion

Holstein-Friesians and Jerseys are two bovine breeds. Furthermore within a breed it may be of interest to consider groups of individuals selected for extreme values of water secretion, *viz.* content of solids. They exhibit a difference in their levels of water secretion *i.e.* jerseys secrete less water in relation to milk solids, or a more concentrated milk. The metabolic differences between the lactating mammary epithelial cells of these breeds is thus of interest. The non-invasive method of comparing the levels of key metabolites, including lactose precursors, in milk to give a simple initial prediction of an intracellular metabolite profile may yield some explanation of the breed differences.

Since lactose is the major osmotic component of bovine milk, it is of interest to know what the physiological differences are between Friesians and Jerseys that result in the different rates of lactose secretion. The detection of such differences between breeds which differ in a character of interest could be the first step towards the elucidation of the genetic differences which clearly exist.

7. Scientific philosophy

7.1 Experimental design and confirmations of normalcy

A nice feature of the goat udder for experimental design is the possibility of within-animal comparison between glands using the animal as an experimental block in subsequent statistical analysis. Clearly the environments of the glands are virtually identical as they are connected to the same blood supply and are in close physical juxtaposition.

Circadian rhythms are a consideration particularly with multi-factorial designs with two levels of a factor represented by the two glands and another factor longitudinally oriented, *i.e.* with time. With this second factor there is usually the expectation of attaining a suitable treatment level followed by a return to initial conditions when conditions are

returned from treatment to control levels. Observation of this return to basal levels for each experimental block can serve as a means to help validate each individual animal's performance, or conversely observation of non-return can perhaps exclude it from a combined statistical analysis. This idea has been referred to as confirmation of normalcy, treatment and recovery, and the ideal form may be pictured graphically as a looper with four right angles or as 1.5 cycles of a square-wave. Cases in point are blood flow and plasma glucose concentration.

When there is more than one non-control level, the proper randomisation of the order of longitudinal factor levels is sometimes foregone, when it necessary to carry out all treatments in one day and one treatment is known to be the only one with effects which wear off slowly. In this circumstance, evidence, perhaps of a mechanistic nature, must be produced to indicate that the earlier treatments do not interfere with the later treatments. In the case of infusion of vaso-active substances into the mammary artery blood flow it is believed that all the effects of adenosine wear off quickly as does its blood flow effect but adrenaline is known to have a more lingering blood flow effect (Linzell, 1974). These compounds are then diluted in the systemic circulation to levels which are physiologically insignificant for the purposes of these experiments.

Confirmations of normalcy may be carried out in order to show the on-going validity of the experimental system being utilised:

It is thought that the integrity of the barrier between interstitial fluid and milk provided by the mammary epithelium may be confirmed through measurement of the concentrations of sodium and potassium in milk. In goats, sodium and potassium concentrations are usually in the vicinity of 430 mg% and 14 mg% respectively in blood (Altman, 1961a) and in the ranges 20-60 mg% and 110-240 mg% respectively in milk (Altman, 1961b).

Milk synthesis is quite a stable process and therefore it can be helpful, where appropriate, to monitor lactational records over the weeks preceding a mid lactation trial and during the trial, in order to compare these measures of milk composition and yield along with control measurements in the trial with similar measures carried out under treatment conditions as confirmations of normalcy.

Milk composition varies slightly from the beginning to the end of milking. Fat content is highest at the end of milking (Giesecke *et al.*, 1984).

Hourly milking with oxytocin injection allows frequent estimation of the rates of synthesis of milk components thus profiling lactational responses to treatments as they occur. There is

however some concern about epithelial integrity associated with the use of oxytocin and hence an added requirement for confirmation of epithelial integrity.

Constant nutrition rates are often employed as a means of reducing the contribution of nutritional effects to total variation in milk parameters.

Experiments involving attempts at the restoration of lactose synthesis rate to control levels by the application of treatments to starved goats derived from hypotheses about the mechanism of operation of the effects of starvation in the lactating goat are a major aspect of this research.

7.2 Explanatory Models

Explanatory models may be defined as logical structures which explain experimental observations to some extent. A model is useful in that it invokes specific questions and it may usually be tested in some way. A model or explanation which cannot be tested because certain appropriate measurements cannot be made may indicate a specific need for technological development, or that the model is simply not experimentally useful.

Conditions existing in complex systems must be well defined because different explanations may arise from very similar circumstances.

One of the experiments in this research investigates whether blood flow information increases our ability to explain decreased lactose output from goats under conditions of short term starvation. A typical difficulty arises in that one must ask oneself if lactose reduction and blood flow reduction are both caused by some 'starvation factor' and hence only correlate with each other because of a common cause or if reduced blood flow-rate is in some way causal in the reduction of lactose output under the conditions of the specific situation being investigated.

Some models which have withstood the test of time and gained recognition have been called paradigms (Kuhn, 1970). Though the word paradigm was used by Kuhn in many similar but distinct ways it is a popular and useful term in these practical contexts.

7.3 Hypothesis Testing

If one uses the philosophical basis of statistical hypothesis testing in a generalised sense the explanatory model, or more strongly the paradigm, may be presented as a null hypothesis. The null hypothesis may be accepted when the null hypothesis is tested rigorously and no contrary evidence is found. The validity of such a test requires that the assumptions of the

experiment be substantiated under a well defined set of circumstantial parameters. The discovery of contrary evidence yields a stronger negative deduction *c.f.* innocent until proven guilty, where the guilty verdict has greater certainty than innocent.

Chapter 2. INVESTIGATION OF THE EFFECT OF MAMMARY BLOOD FLOW ON MAMMARY GLUCOSE UPTAKE IN THE LACTATING GOAT

Summary

This experiment involved five non-pregnant fed goats in mid-lactation. Blood flow was measured in the right external pudic artery and vaso-active compounds were infused into this artery. Samples of blood from the right heart and mammary superficial epigastric vein were collected simultaneously for each blood flow treatment.

Increased blood flow, resulting from adenosine infusion, coincided with an approximate doubling of estimated mammary glucose uptake in two of the animals, coincided with a significant increase in one animal and two animals exhibited no significant increase in estimated mammary glucose uptake. For adrenaline treatments resulting in at least a fifty percent reduction in blood flow, mammary glucose uptake dropped by 75±7 percent (mean±S.E.M., n=5) on average.

Introduction

The availability of glucose, the precursor of lactose, to the lactating mammary gland from blood is a function of blood glucose concentration and blood flow rate. Increasing either parameter results in greater interstitial glucose availability. Mammary glucose arterio-venous difference increased for cows with increasing arterial glucose concentration (Davis & Collier, 1985) when comparing between studies. Mammary blood flow is observed to increase enormously at parturition (Linzell 1974). However there are no data from ruminants on what happens to mammary glucose uptake in mid-lactation if blood flow is varied other than as a consequence of the galactopoietic effect of the administration of exogenous somatotropin (Davis et al., 1988).

The milk veins carry mammary venous blood in standing goats after the first half of first pregnancy (Linzell 1974), when the valves of the caudal superficial epigastric vein become incompetent and blood flows in reverse through this vein and into the cranial superficial epigastric vein, thus arises the 'milk vein'. Occasionally, valves of the external pudic vein

also become incompetent, so that the milk vein may also obtain a proportion of its flow from the abdomen. This means that potentially there could be some inaccuracy in the measurement of mammary A-V differences, particularly in certain individuals, unless steps are taken to ensure sampling of pure mammary venous blood.

This experiment was designed to find out if altering blood glucose availability to the mammary glands of fed lactating goats, by modifying blood flow via the infusion of vaso-active substances, resulted in changes in the rates of glucose uptake.

A pilot experiment was carried out in order to examine the appropriateness of using right atrium venous (RAV) differences.

Confirmation of appropriately increased and decreased blood flow rates, and confirmations of normalcy of individual goat milk production status by routine milk fat, protein and lactose analysis were used to help validate data sets for individual goats.

This experiment was preliminary to the experiment in chapter 4 were the same animals were milked frequently under conditions of increased glucose availability. With greater resources aspects of these two experiments could have been combined.

Hypothesis

Modifying blood flow results in changes in the rate of glucose uptake by the mammary gland.

Methods

Animals and surgical preparation

Ethics approval for this experiment was obtained from the Ruakura Animal Ethics Committee. The experiment involved five fed goats in mid-lactation. During surgery several weeks before the experiments an ultrasonic blood flow probe (Transonics Systems Inc., Ithaca, New York) was implanted on the right external pudic artery and a polyvinyl chloride catheter was inserted for infusion of vaso-active compounds. On the day before each experiment the right milk vein was cannulated for sampling mammary venous effluent. Early on the day of the experiment a catheter was inserted into the right ventricle of the heart via a jugular vein, to enable the sampling of mixed venous blood.

Sampling and analyses

Pairs of right heart and external pudic artery samples were collected, in a pilot experiment which involved blood collections on four separate occasions. Blood samples were collected, into fluoride oxalate vacutainer tubes, at five minute intervals and plasma derived from these was later analysed with a manual Beckman glucose analyser.

Fifteen minutes after the beginning of each treatment in the main experiments, blood was collected at five minute intervals, into fluoride oxalate tubes, for twenty minutes. External pudic artery blood flow measurement, right heart and mammary venous blood collections were carried out simultaneously, for each glucose uptake measurement.

This collection procedure was repeated for normal, stimulated (nominally double), normal (stimulation removed) and restricted flow (nominally one third normal). The blood flow was monitored and interpreted with a minimal delay in order to stabilise the blood flow rate by manually modifying the pump speed. The order of these treatments was not randomised in the usual way. Variation in flow was achieved using infusion of the vasodilator adenosine (3 mg/min, 3mg/ml) or the vasoconstrictor adrenaline (5 μg/min, 2.5 μg/ml and 0.1 g/l ascorbate in saline). This resulted in forty plasma samples per goat.

Milk samples were collected from the night before and the morning of blood collection, as confirmations of normalcy, and combined to produce one composite sample.

Plasma was assayed for glucose with a manual Beckman glucose analyser. Milk was assayed for fat, protein and lactose by infra-red reflectance.

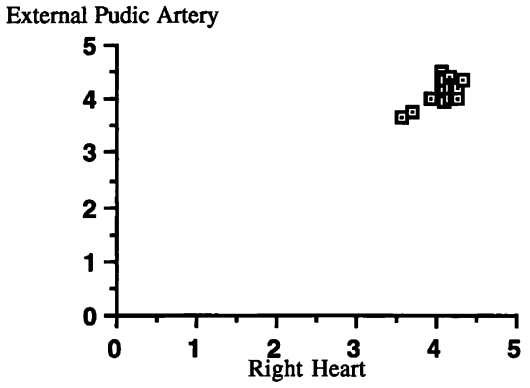
Results

The raw data have been tabulated in Appendix 2.

Comparison of external pudic and right heart plasma glucose concentration

Figure 3 is a scatterplot summarising the plasma glucose concentration measurements of blood collected from right heart and the right external pudic artery, from four preliminary experiments.

Figure 3. Graph of external pudic artery vs right heart plasma glucose concentrations (mM).



Regression analysis of external pudic artery on right heart glucose concentrations though significant only explained 30% of the variation. This was because the data were so tightly grouped that most of the variation was noise. The glucose assay itself was rated between days to distinguish between samples different by 0.1 mM at this concentration range. The plasma glucose concentrations of the right heart and external pudic artery were 4.106±0.029 (mean±S.E.M., n=16) and 4.166±0.035 respectively. A paired t-test showed no significant difference ($P>0.05$) in glucose concentration between these two types of blood samples *i.e.* the mean difference between the pairs was not significantly different from zero.

Difficulty experienced in obtaining effluent from the external pudic artery through the fine catheters which had to be utilised appears to be reflected in the increased range of concentrations exhibited by these samples.

Glucose uptake measurement

Milk samples volume and compositions were checked informally and no marked departures were observed in the recent individual records for any of the five goats (see Figure 4 and Appendix 2).

The haematocrit values were used to convert blood flow values to plasma flow. Glucose right atrium venous (RAV) difference and uptake values were calculated on the basis of a plasma pool of available glucose. This is usual practice because red blood cell glucose transporter activity in goats is believed to be quite low *c.f.* human red cells (Wheeler *et al.*, 1985)

The validity of each each measured RAV difference value for inclusion in an analysis of variance was checked by informally comparing the associated plasma flow values with the initial plasma flow measurements to confirm that the desired blood flow rate changes were actually achieved.

Figure 4.

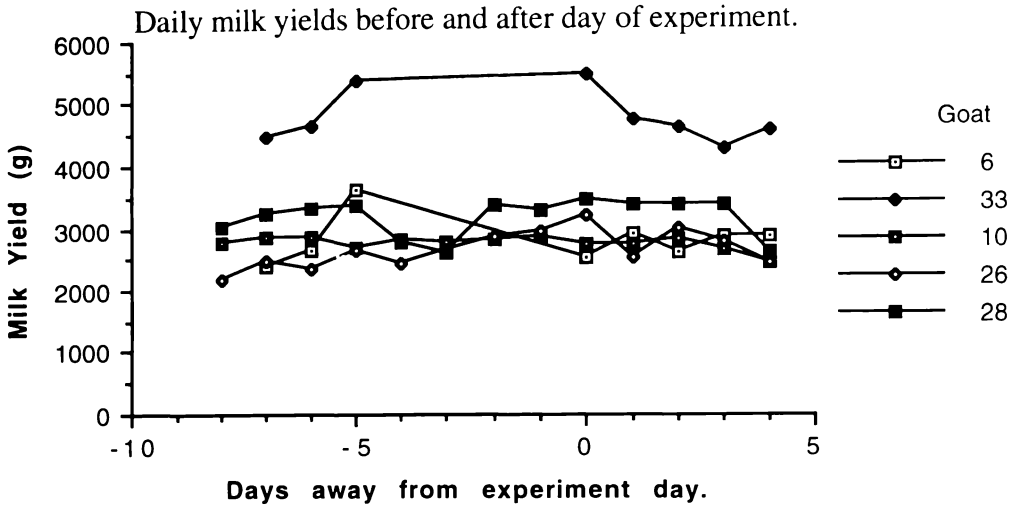
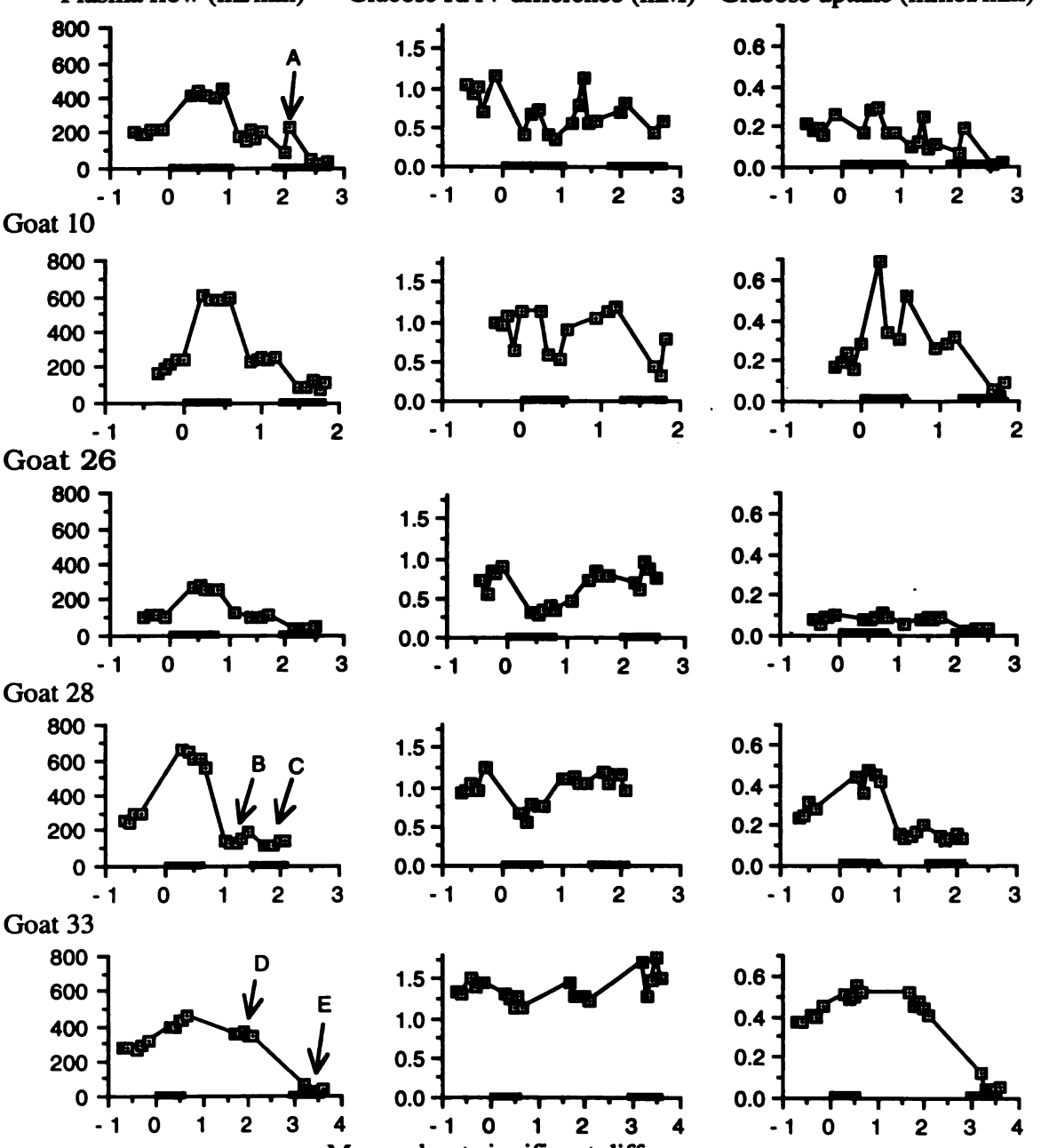


Figure 5. shows the results from this experiment in graphical form. The high blood flow exhibited in case A of Figure 5 from goat 6 resulted from a failure to correctly control the infusion pump speed whilst infusing adrenaline, and hence the glucose RAV difference and uptake values for this case were labelled as missing.

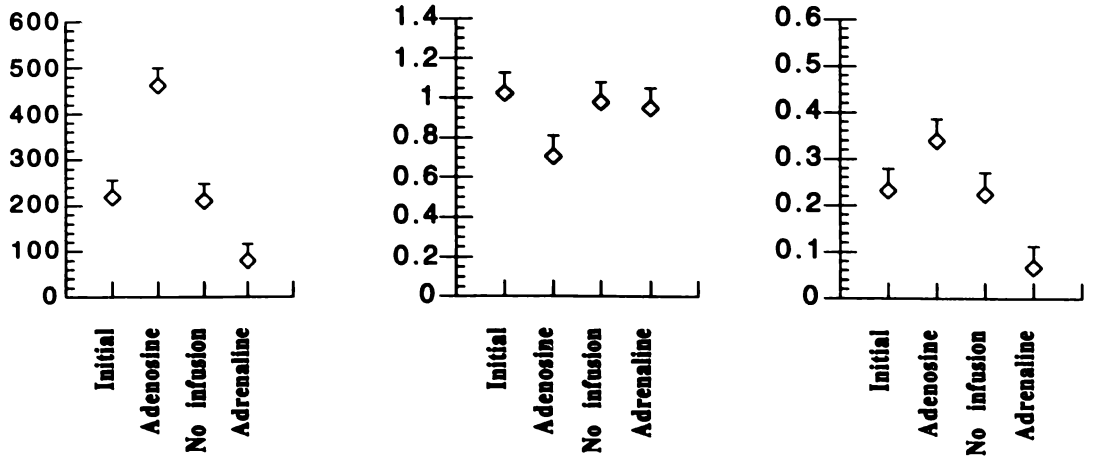
The groups of control blood flow measurements at B and D of Figure 5 were different from the initial blood flow measurements. These data were still included in analysis initially ignoring the question of what physiological significance these indications of perturbation to the experimental system might have.

A rough estimate of the ratio of glucose uptake to lactose output for a day was calculated on a weight basis, using a measurement of glucose uptake under initial conditions at several points close in time and the milk yield from the previous 24 hours.

Figure 5. Plasma Flow, Glucose RAV Difference and Uptake vs Time (h).
Goat 6 (The bars signify the adenosine and adrenaline infusion periods respectively.)



Mean + least significant difference.



This was a type of book–keeping check to show that the numbers obtained were realistic. The values may be expected to be greater than unity assuming negligible mammary gluconeogenesis, and about 1.3-2.0 according to Linzell (1974). In Table 1 it may be seen that all of the numbers are a little smaller than expected, and goat 26 appears to be lower than the others.

Table 1. Calculating the Ratio of Glucose Uptake to Lactose Output.

	Glucose g/day	4:00 PM Milk (g)	8:00 AM Milk (g)	Half Udder Milk (g)	Lactose %	Lactose g/day	Ratio G/L
Goat 6	52.1	780	1727	1254	4.50	56.4	0.92
Goat 10	53.6	870	1860	1365	4.77	65.1	0.82
Goat 26	21.6	1040	2170	1605	4.53	72.7	0.30
Goat 28	69.7	1170	2260	1715	4.26	73.6	0.95
Goat 33	103.5	2100	3346	2723	4.50	122.5	0.84

In these fed lactating goats doubling blood flow and reducing blood flow to about one third of normal resulted in correlated changes in glucose uptake. Doubled blood flow coincided with a 30% drop in mean glucose RAV difference and with about a 40% increase in mean glucose uptake. Reduced blood flow coincided with no significant change in glucose RAV difference and a 70% decrease in glucose uptake. These measurements of significance were based on close longitudinal comparison within animal and a 95% confidence level. Furthermore, for glucose uptake, plasma flow and glucose RAV difference, the infusion treatments were shown to be strongly associated with these responses on the basis of the observed inter-animal variation.

Discussion

The pilot experiment was carried out to confirm that reliable placement of catheters in the right heart via a jugular vein could be achieved by observing atrial or ventricular pumping reflected by the motion of an air bubble in the catheter, and also to confirm that plasma glucose concentration measured in blood collected from the right heart was an acceptable predictor of plasma glucose concentration in the mammary artery. Consider at this point that the bulk of blood low in glucose, which is derived from the main glucose sink in lactating goats, the mammary gland, returns to the heart via the anterior vena cava as does the blood from the main glucose source the liver, draining through the hepatic vein eventually also to

the anterior vena cava. The measurement of arterio-venous differences does however require high accuracy and therefore fully mixed returning blood.

The difference measurements were referred to as RAV differences because the passage of blood through lung and blood vessels to arrive at the external pudic artery offers some opportunity for modification. These data confirmed that lung and blood vessels were not impacting on blood glucose concentration in the pilot experiments. It would perhaps be slightly better in future to collect some pairs of right heart and external pudic artery blood samples at the beginning of each infusion day. It would appear that the difference in spread of the two data sets is more likely to result from haemolysis caused by excessive suction applied to the catheter, and small sample size resulting in much more concentrated anticoagulant in some blood samples from the external pudic artery, than from real short term variations in blood in the artery as opposed to the right heart.

A test mode, which allows the ultrasonic probes to be tested *in vivo,* checks that the probe cavity is filled with 'liquid', and thus can conduct the ultrasonic waves efficiently. Milk yields of the glands with and without a probe were compared within an animal in order to confirm that blood supply was not constricted by the implanted probe. The probes exhibit high precision within a day, and are able to easily confirm the successful modifications of blood flow, but the accuracy *in vivo* is somewhat unknown (Gorewit *et al.*, 1989). Measurements from the blood flow probes were not compared to a reference method as this was not feasible at the time. Inaccuracy in blood flow measurement is probably the major cause of discrepancy between glucose uptake and lactose yield data (see Table 1).

The goats were handled, as far as possible, according to their usual daily routine of feeding and milking. They were not allowed to lie down during the experiment as this behaviour usually changes mammary blood flow. Care was taken to minimise and to control the nature of any human contact before and during the experiment since any excitement resulting in release of endogenous adrenaline would superimpose a blood flow reduction.

Goat 28 exhibited a stress phenomenon, where its distress resulted from getting some food lodged in the trachea. Its second group of control plasma flow measurements were considerably lower than the first. Goat 33 also failed to return to the initial blood flow rate, notably it was impossible to increase its blood flow rate to about the 600 ml/min required.

One might argue that the results from these goats do not pass desirable high standards of rigour, although they and goat 26 the other candidate for removal, on the grounds of low glucose uptake to lactose ratio, exhibit the most well grouped RAV measurements, another possible criterion for validity.

Since no gross departures from their usual milk production levels were observed, further confidence was placed in the lactational performances as being normal for that goat, and that of a normal healthy goat since the health of the goats was monitored and well maintained.

The infusion of adrenaline presented an unstable positive feedback situation where a constant rate of infusion of adrenaline decreased blood flow resulting in increased blood adrenaline concentration and a further decreased blood flow rate *etc.*

Glucose RAV difference measurement in the reduced flow situation is the most susceptible to being compromised, by venous drainage from the abdomen via the external pudic vein and from a small flow from the perineal vein to the milk vein, since the contributions of these may remain constant and thus assume a much higher proportion of the total flow. Conceivably a reverse facing artificial valve could be inserted into the milk vein just below the entry point of the perineal vein returning valvular competence to this point, or perhaps finger compression of the milk vein at this point at the time of sampling would suffice (Linzell 1974). Linzell also used X-ray technology to check the blood flow characteristics of individual goats.

The small amount of adrenaline used for close mammary arterial infusion in order to cause a flow reduction has no appreciable effect on systemic blood pressure (Linzell 1974).

True values of the ratio of glucose uptake to lactose yield must be greater than unity if gluconeogenesis is a minor pathway in the lactating ruminant mammary gland (Emmanuel *et al.*, 1985). On this basis the glucose uptake to lactose output ratios suggest that goat 26 may have a large amount of non-mammary venous blood flowing through the milk vein. The surgical removal of minor vascular interconnections between the mammary glands would also increase confidence in these measurements.

According to the principles of simple diffusion a 70% reduction in flow should result in a large increase in RAV difference unless there is a reduction of available surface area, there is a change in the nature of the diffusion surface *e.g.* reduced glucose transporter activity, or

there is a high order relationship of uptake activity to glucose concentration by the glucose uptake surface. Conversely one would expect to see decreased RAV difference but increased glucose uptake at high flow if an explanatory model analogous to simple diffusion in a heat exchanger were qualitatively applicable *i.e.* where diffusion rate is positively correlated with the coolant flow rate (blood flow) and with the temperature difference across the exchanger (RAV), and the available amount of nutritive surface area (controlled by arteriovenular shunts) and the degree of surface insulation (uptake activity) are constant . Alterations to the diffusion surface characteristics would need to happen within 15 minutes or so to explain these observations. The possibility of regulatory sub-units to facilitative glucose transporters has not been ruled out (Wheeler *et al.*,1989).

Adenosine increases mammary blood flow (Linzell 1974). The observation that RAV differences go down is qualitatively consistent with a simple diffusion explanatory model *i.e.* no change in available surface area or surface characteristics is inferred. It should be noted that two of the adenosine treated goats showed no coincident significant increase whilst three did. If this inconsistency is real then it may reflect a qualitative difference in the responsiveness of the mammary epithelium to adenosine in terms of its effect on glucose uptake. Further experiments would be required to confirm this.

The infusion treatments were not randomised in the usual way because the effects of adrenaline were known to wear off only slowly (Linzell 1974). The blood flow effects of adenosine treatment were known to wear off quickly and furthermore the second normal period had been introduced to confirm this and to maximise the possibility that any lingering effects would wear off without waiting a length of time such that close longitudinal comparison could be lost.

The results indicate that an adrenaline-mediated decrease in mammary blood flow results in little alteration to glucose arteriovenous difference. Consistently, Linzell (1974) postulated that mammary blood flow reduction caused by insulin hypoglycemia is mediated by adrenaline, wherein glucose arteriovenous difference showed no change. This would suggest that either the measurements of arteriovenous difference are incorrect or that the explanatory model of simple diffusion does not even qualitatively explain this situation, and

thus that the amount of available diffusion surface area and/or the nature of the surface might have changed.

Arteriovenular shunts may offer non-nutritive channels in which the surface area available to participate in glucose uptake could be reduced whilst still maintaining flow through the secretory tissue. The higher diffusion rates of gases as opposed to comparatively larger molecules could still supply a basal respiratory requirement whilst limiting utilisation of many other substrates.

Conclusions

Correlations between blood flow and glucose uptake in the fed lactating goat suggest that modifying blood flow can cause a change in the rate of mammary glucose uptake, however it would be helpful to be more confident about the reliability of the RAV difference measurements.

There is no strong implication that adenosine modifies the mammary epithelium or that it modifies populations of open and restricted blood vessels although there is a possibility of a variable response with regard to the induced change in glucose uptake contemporary with the predictable response in blood flow rate. Adrenaline may however be altering the accessibility of mammary epithelium to nutritive blood flow, or/and altering the glucose uptake activity of the mammary epithelium.

Chapter 3. DOES INTRADUCTAL GLUCOSE INJECTION CHANGE LACTOSE SYNTHESIS IN THE STARVED LACTATING GOAT ?

Summary

This experiment involved three non-pregnant goats in mid-lactation on two separate days. Sterile solutions containing glucose or sucrose (control) were injected, via the teat canals, into the lumena of the mammary glands. Milk was collected after each injection. The experiment was designed to examine whether it was possible to alter lactose synthesis and secretion by intraductal injection of glucose into the mammary lumen making use of the permeability of the apical membrane of the secretory cell to glucose.

The right (glucose injected) and left (sucrose injected) lactose secretion rates were measured for control and treatment periods. No significant changes in the differences in lactose secretion between right and left glands coinciding with the injection period in comparison to the initial control period were observed.

Introduction

The argument that under starvation conditions in the lactating goat mammary glucose uptake and lactose synthesis are limited by the control of the passage of glucose across the basolateral membrane of the mammary epithelial cell (Faulkner, 1985a) would be strengthened if cytosolic glucose availability were increased by intraductal injection of glucose into the mammary lumen, and this resulted in an increase in lactose yield.

The following experiment was designed to examine whether it was possible to alter lactose synthesis and secretion by intraductal injection of glucose into the mammary lumen making use of the permeability of the apical membrane of the secretory cell to glucose (Faulkner *et al.*, 1985b). Sucrose was injected into the contra-lateral gland as a control because of its chemical analogy with glucose, its similar osmotic behaviour and its biochemical inertness in this situation (Henderson *et al.*, 1984; Threadgold *et al.*, 1984).

Confirmations of normalcy by measurement of milk sodium:potassium ratio and levels of milk fat, protein and lactose were used to help validate data sets for individual goats.

Hypothesis

Intraductally injected glucose increases lactose synthesis in the goat mammary gland.

Methods

Ethics approval for this experiment was obtained from the Ruakura Animal Ethics Committee. This experiment involved three non-pregnant Saanen goats in mid-lactation on two occasions. They were utilised firstly under their normal conditions of nutrition and, secondly one week later after 24 hours of fasting. The goats were milked four times in the twenty four hour period leading up to the beginning of hourly milking.

The goats were milked hourly, 55 min after previous milking, allowing 5 minutes to milk. They were milked initially for a four hour control period, after which sterile solutions (100ml), containing 300mM glucose or sucrose, were injected hourly for four hours aseptically into the right and left teat canals respectively via sterile cannulae into the lumena of the mammary glands. The glands were massaged after injection in order to maximise the contact of these solutions with the mammary epithelium. This interval was immediately followed by a further four hours of hourly milking without intraductal injection. At the end of the experiment each animal received a prophylactic dose of antibiotics via the teat canal.

Hourly right and left milk samples were collected and the yields of milk (including sugar solutions) were recorded. This resulted in a total of 144 samples. These samples were each split giving one sample for fat, protein and lactose by infra-red reflectance using bromopol as a preservative, and another sample was frozen to assay for glucose, sucrose and lactose by HPLC, and sodium and potassium by flame photometry.

Results

The data have been tabulated in Appendix 3.

Clearly sodium and potassium levels were disturbed by the injection of iso-osmotic sugar solutions (See Fig. 6). On initiating intra-ductal injections, sodium concentration dropped proportionately more than potassium did. This helps to confirm that there was no sudden influx of sodium rich interstitial fluid transcending the mammary epithelium.

The average milk fat content was observed to drop consistently for the injection treatment period (See Fig. 7). No unilateral changes in milk fat content were observed.

The consistent decline seen in fat content during the control period is likely to be related to the onset of frequent milking because of the hydrophobicity of milk fat and its greater association with hind milk. Though some trends were observable in fat content in the individual goats, it is fair to say that the mean rates of fat output during injection treatment appear to be normal.

The average milk protein content was observed to consistently drop for the injection treatment period (See Fig. 8). No unilateral changes in milk protein content were observed.

The milk protein secretion rate was observed to be stable within the experiments.

Lactose synthesis in each gland appeared to be stable throughout the experiments (See Fig. 9).

Values of 30 mmol were expected for glucose and sucrose yield if the injected material for that hour was recovered in the sample of milk-sugar solution. In all cases, most of the glucose or sucrose injected was removed at the subsequent milking.

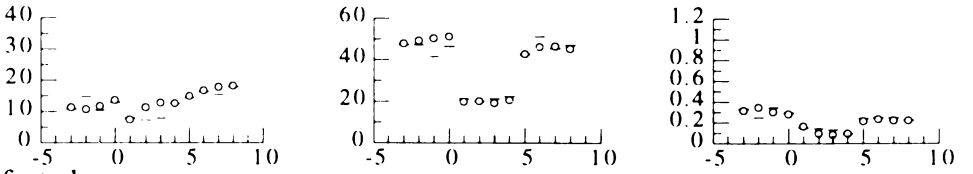
No significant increases in lactose yield associated with glucose injections were observed.

Figure 6. Graphs of sodium, potassium and their ratio versus time (h) from the commencement of intraductal injections of sugar solutions into the lumen of the mammary gland

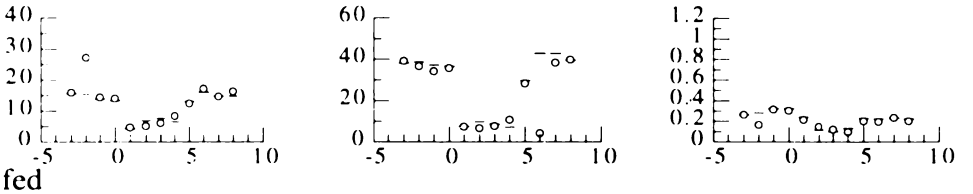
Milkings following injections occurred from t=1h to t=4h.

Sodium (mM) Potassium (mM) Ratio (Na:K)

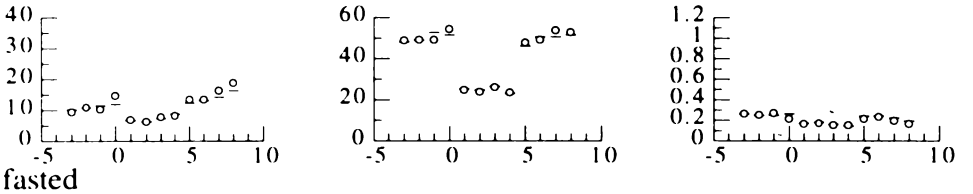
Goat 20 fed



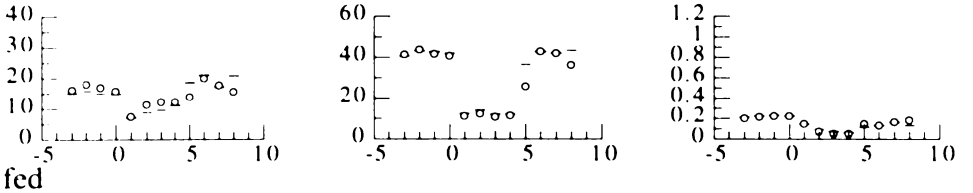
Goat 20 fasted



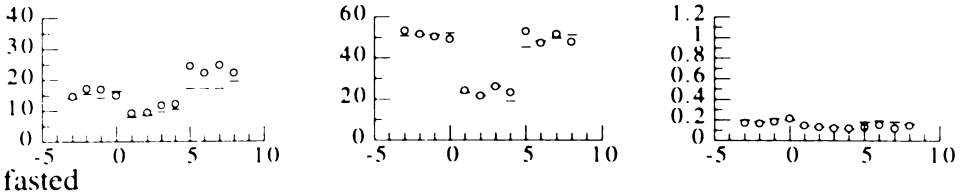
Goat 26 fed



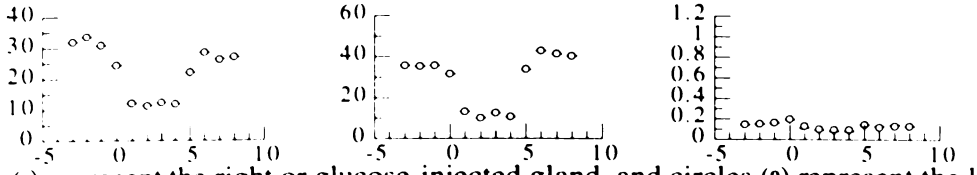
Goat 26 fasted



Goat 28 fed



Goat 28 fasted



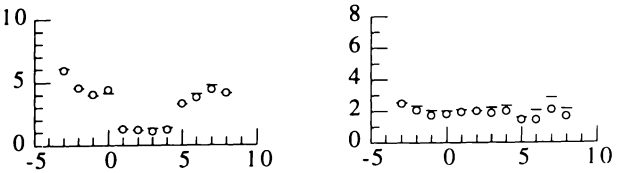
Dashes (·) represent the right or glucose-injected gland, and circles (°) represent the left or sucrose-injected gland.

Figure 7. Graphs of fat % and fat yield versus time (h) from the commencement of intraductal injections of sugar solutions into the lumen of the mammary gland

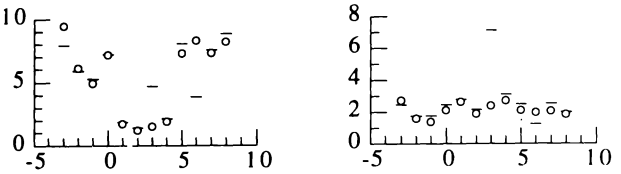
Milkings following injections occurred from t=1h to t=4h.

Fat (g/100g) Fat yield (g/h)

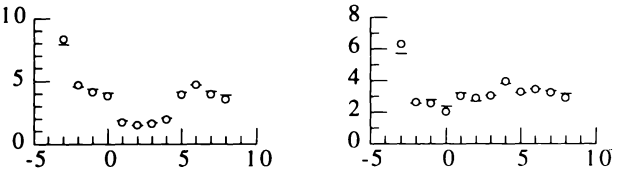
Goat 20 fed



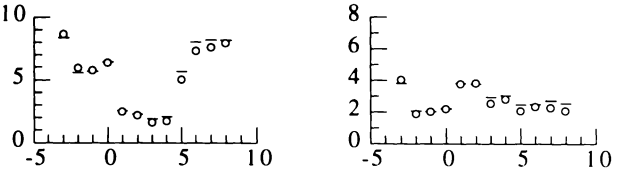
Goat 20 fasted



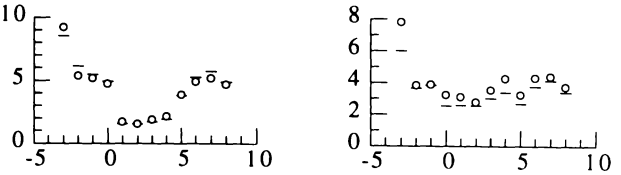
Goat 26 fed



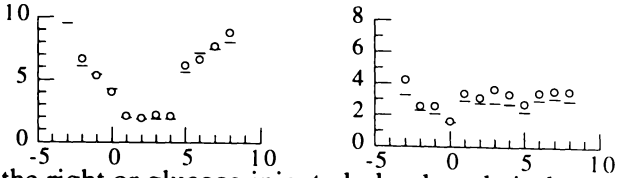
Goat 26 fasted



Goat 28 fed



Goat 28 fasted



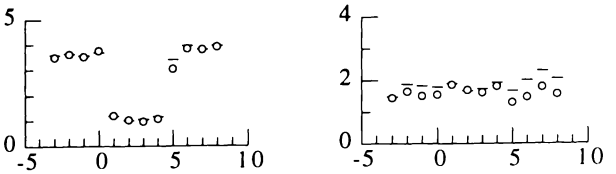
Dashes (-) represent the right or glucose injected gland, and circles (°) represent the left or sucrose injected gland.

Figure 8. Graphs of protein (%) and protein yield (g/h) versus time (h) from the commencement of intraductal injections of sugar solutions into the lumen of the mammary gland

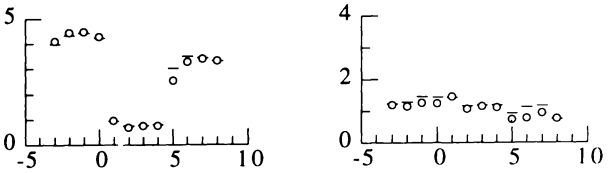
Milkings following injections occurred from t=1h to t=4h.

Protein(g/100g) Protein yield (g/h)

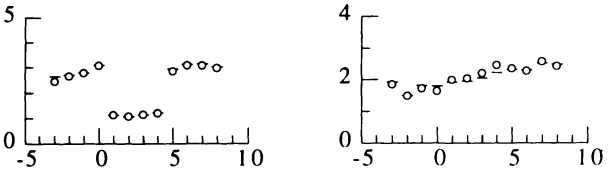
Goat 20 fed



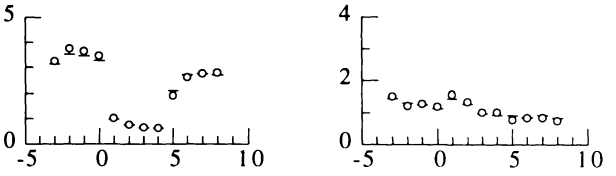
Goat 20 fasted



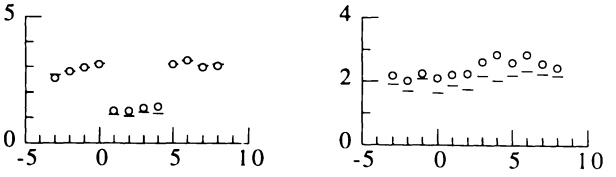
Goat 26 fed



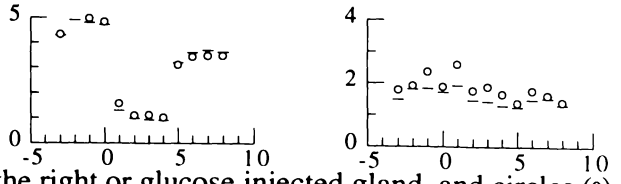
Goat 26 fasted



Goat 28 fed



Goat 28 fasted



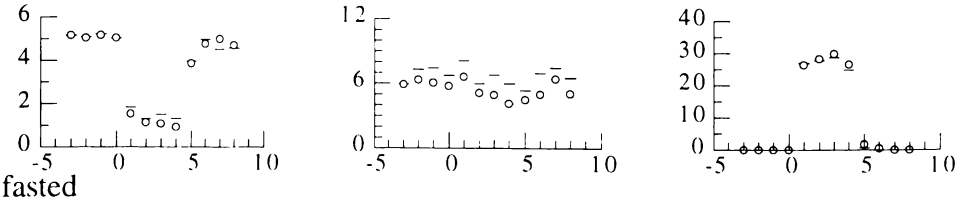
Dashes (-) represent the right or glucose injected gland, and circles (o) represent the left or sucrose injected gland.

Figure 9. Graphs of lactose (%), lactose yield and recovery of sugars [glucose or sucrose] (mmol/h) versus time (h) from the commencement of intraductal injections of sugar solutions into the lumen of the mammary gland

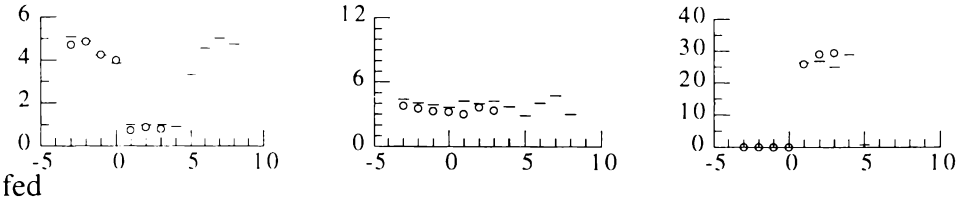
Milkings following injections occurred from t=1h to t=4h.

Lactose (g/100g) Lactose yield (mmol/h) Sugar (mmol/h)

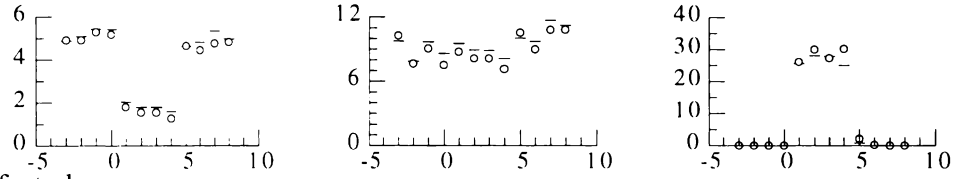
Goat 20 fed



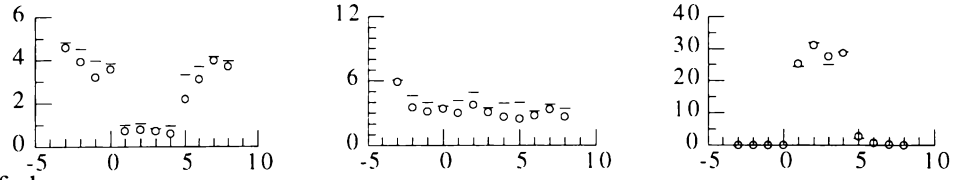
Goat 20 fasted



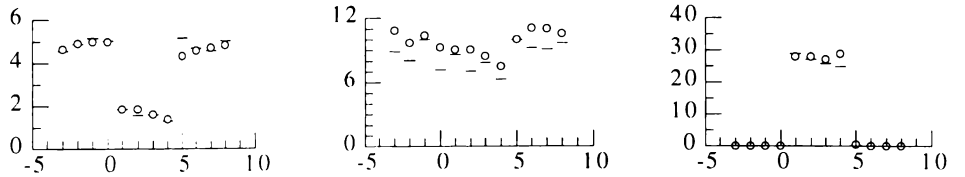
Goat 26 fed



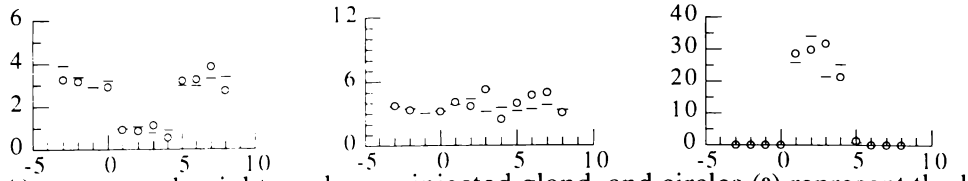
Goat 26 fasted



Goat 28 fed

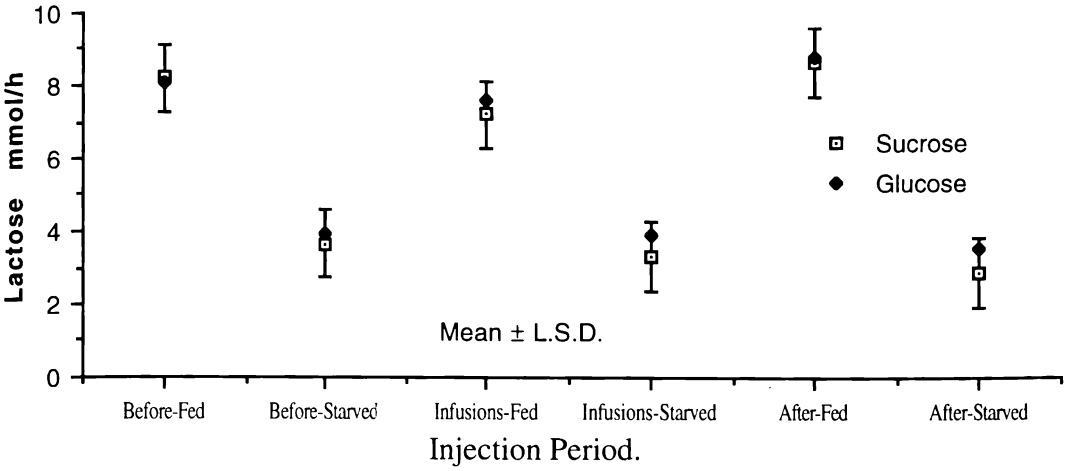


Goat 28 fasted



Dashes (-) represent the right or glucose injected gland, and circles (°) represent the left or sucrose injected gland.

Figure 10. Graph showing comparisons of lactose output (mean of 3 goats) for glucose injections and sucrose control injections, before during and after the injection interval and under normal and low levels of nutrition.



The usual large differences in lactose yield between normal and reduced levels of nutrition were observed (See Fig. 10).

Least significant differences were based on a comparison between right and left glands for each goat at the five percent level of significance. This analysis assumes that the right and left glands do not influence each other in any way which might have important consequences on the interpretation of these results.

As seen in Figure 10, there were no significant differences coincident with any injection period between lactose output of glucose injected and sucrose injected glands. More concisely interaction between injection period and injectate and interaction between injection period, injectate and nutrition were not significant.

Discussion

The animals were handled quietly and as much in accordance to their usual routine as possible. At no stage did any of them show any sign of distress or excitement. The animals were in good health, were milking consistently and were initially producing normal milk.

The sodium to potassium ratio indicated that tight junctions had integrity *i.e.* no para-cellular pathways were operating. The failure of tight junctions in this experiment would not indicate that any of the measurements were severely compromised *c.f.* milk glucose when used to represent intracellular glucose, but would give some indication of abnormality induced by the experimental conditions. The sodium and potassium levels lay somewhere

between their usual concentration and their usual amount as would result from a constant rate of secretion of these ions. The volumes of expressed material from the injection periods also helped to confirm that the 300mM sugar solutions recommended in the literature were iso-osmotic with the mammary epithelium *in vivo*.

In contrast, the amounts of fat and protein secretion were fairly constant throughout the experiments. This can be interpreted as a confirmation that no major perturbation of milk production had occurred in any of these individuals.

Intraductal sucrose injection does not affect metabolism biochemically since the mammary gland contains no sucrose-degrading enzyme activity. Furthermore because the apical membrane is impermeable to disaccharides, sucrose only occupies extracellular space and does not permeate the mammary epithelium (Threadgold 1984). This principal is applied as a generality in the preparation of cell culture media where sucrose is often used as an inert cellularly excluded modifier of osmolarity.

The biochemical inertness of sucrose in this context may also be used to argue that this control injection has little effect beyond the milk space of the gland into which it is injected. Injection of glucose into the systemic circulations of goats fasted for 24 hours resulting in a doubling of glucose concentration (Davis & Collier, 1985a; Ch.4) results in little or no impact on lactose synthesis over a period of hours, therefore glucose injected intraductally is unlikely to have a systemic impact. Consistently there is no obvious hormonal mechanism whereby major systemic impacts might arise. The pre-injection periods also help to confirm this, though one must bear in mind the longitudinal aspect of this and hence the inherently lower precision.

Knowledge of the bi-directional permeability of apical membrane to glucose (Faulkner *et al.*, 1985b) implies that intraductal glucose injection combined with effective massaging of the mammary gland should increase intracellular glucose concentration in the mammary epithelium.

Increased intracellular glucose concentration should result in increased lactose output if decreased basolateral glucose transport is the only cause of decreased lactose output due to starvation in the lactating goat.

Conclusions

There is no evidence to indicate that increased lactose output has occurred in response to putatively increased intracellular glucose availability in the mammary epithelial cell in starved or fed lactating goats.

The (null) hypothesis that intracellular glucose concentration regulates lactose synthesis has been rejected in fed and starved non-pregnant lactating goats under the circumstantial parameters of this experiment and based on the assumption that intra-cellular glucose concentration in the mammary epithelium has been increased by intra-ductal injection of glucose solutions.

Chapter 4. INVESTIGATION OF THE EFFECT OF SIMULTANEOUS MODIFICATION OF MAMMARY BLOOD FLOW RATE AND SYSTEMIC BLOOD GLUCOSE CONCENTRATION ON THE RATE OF LACTOSE SYNTHESIS IN FED AND STARVED GOATS IN MID LACTATION.

Summary

This experiment investigated whether mammary glucose uptake and lactose yield could be increased by the increase of blood glucose availability to the mammary gland. Five starved and three fed non-pregnant lactating goats were used. Blood flow was measured in the right external pudic artery and adenosine was infused into this artery. The goats were milked hourly, blood flow was measured and jugular plasma samples were collected.

Glucose infusion alone coincided with increases in milk glucose concentration from an average of 1.1 to an average of 2.6 mg/100ml in starved goats and from an average of 6.1 to an average of 12.8 mg/100ml in fed goats. Combined glucose infusion and increased mammary blood flow in the contra-lateral glands, in the same experiment and at the same time, coincided with concentrations of milk glucose increasing from an average of 1.4 to an average of 5.2 mg/100ml in starved goats and from an average of 5.5 to an average of 12.5 mg/100ml and fed goats. Increased blood flow coincided with a greater effect on the predicted intra-cellular glucose concentration in the mammary epithelium of the starved than of the fed glucose-infused lactating goat. In comparison to these large changes in milk glucose concentration, there were no proportionately large effects on lactose yield.

Introduction

It has been shown that systemic glucose infusion in the starved lactating goat is neither sufficient to restore lactose synthesis nor mammary blood flow to pre-starvation levels (Davis & Collier, 1985a).

It was proposed that if systemic blood glucose concentration and mammary blood flow are both restored to pre-starvation levels, then restoration of lactose synthesis may be expected if blood glucose availability to the mammary epithelium is regulating lactose

synthesis. Furthermore, if this restoration of glucose availability to the mammary epithelium results in increased intracellular glucose availability, reflected by increased milk glucose concentration, without appropriately increasing lactose output then an intracellular rate-limiting step of lactose synthesis may be implicated, rather than limitation by the level of basolateral glucose transporter activity.

This experiment investigated whether lactose yield and mammary glucose uptake in the starved lactating goat could be restored to pre-starvation levels by the restoration of blood glucose availability to the mammary gland.

Confirmations of blood flow rates and milk glucose concentrations being appropriately increased or restored to basal levels, and confirmations of normalcy by routine milk fat, protein and lactose analysis were used to help validate data sets for individual goats.

Hypothesis

Restored blood glucose availability results in restored mammary glucose uptake and lactose yield.

Methods

Animals and surgical preparation.

Ethics approval for this experiment was obtained from the Ruakura Animal Ethics Committee. Six lactating goats were surgically prepared as described below. The experiment was carried out on three occasions where there were three fasted goats, two fasted goats and three fed goats. Two of the goats were used twice, but not at the same nutritional level. This resulted in eight data sets, where each data set was treated as if it were from a different goat.

During surgery several weeks before the experiments an ultrasonic blood flow probe (Transonics Systems Inc., Ithaca, New York) was implanted on the right external pudic artery and a polyvinyl chloride catheter was inserted for infusion of adenosine at a rate of 3 mg/min.

On the day of the experiment a jugular catheter was inserted into a jugular vein to enable blood sampling. Also a further catheter was inserted into the contra-lateral jugular vein to allow infusion of glucose at a rate of 90 mg/min.

Four milkings were carried out in the 24 hour period prior to hourly milking. Feed was withheld for this twenty four hours in the case of the starved goats. Thirty minutes after each milking blood flow measurement and blood collections were carried out. A three hour control period was followed by a five or six hour period over which glucose and adenosine infusions occurred. Finally there was a further six or five hour control period.

Sampling.

Twelve milk samples (one sample per hour) from the right and left mammary glands were collected making a total of twenty-four milk samples per goat. Each sample was split and preservative (bromopol) was added to one aliquot taken for determination of fat, protein and lactose. The other aliquot was frozen and assayed for glucose using the microplate glucose assay described in Appendix 1. Twelve 10 ml plasma samples in 10 ml fluoride/oxalate tubes were collected per goat. Plasma samples were assayed for glucose on an automated medical/veterinary analyser by MAFqual using a glucose oxidase based colourimetric enzyme assay.

Results

The data have been tabulated in Appendix 4.

Blood flow and systemic plasma glucose (Fig. 11) were appropriately elevated during the infusions, and they returned to basal levels after the cessation of infusions.

The results from the two samples where the sodium to potassium ratio (Fig 12.) exceeded 0.8 were not included in the generation of summary statistics.

Typical macro-composition of the milk samples (Figs. 12&13) was observed.

Lactose yield for the three fed goats appeared to be low for the third hour of infusion (B, Fig.13). This was because of inconsistent milking technique on that occasion. The balance of the milk was recovered at the next milking.

Figure 11. Graphs of blood flow in right gland and systemic plasma glucose concentration vs time (h) from the commencement of infusion of adenosine and glucose for each animal

Infusions occurred from t=0h to t=6h.

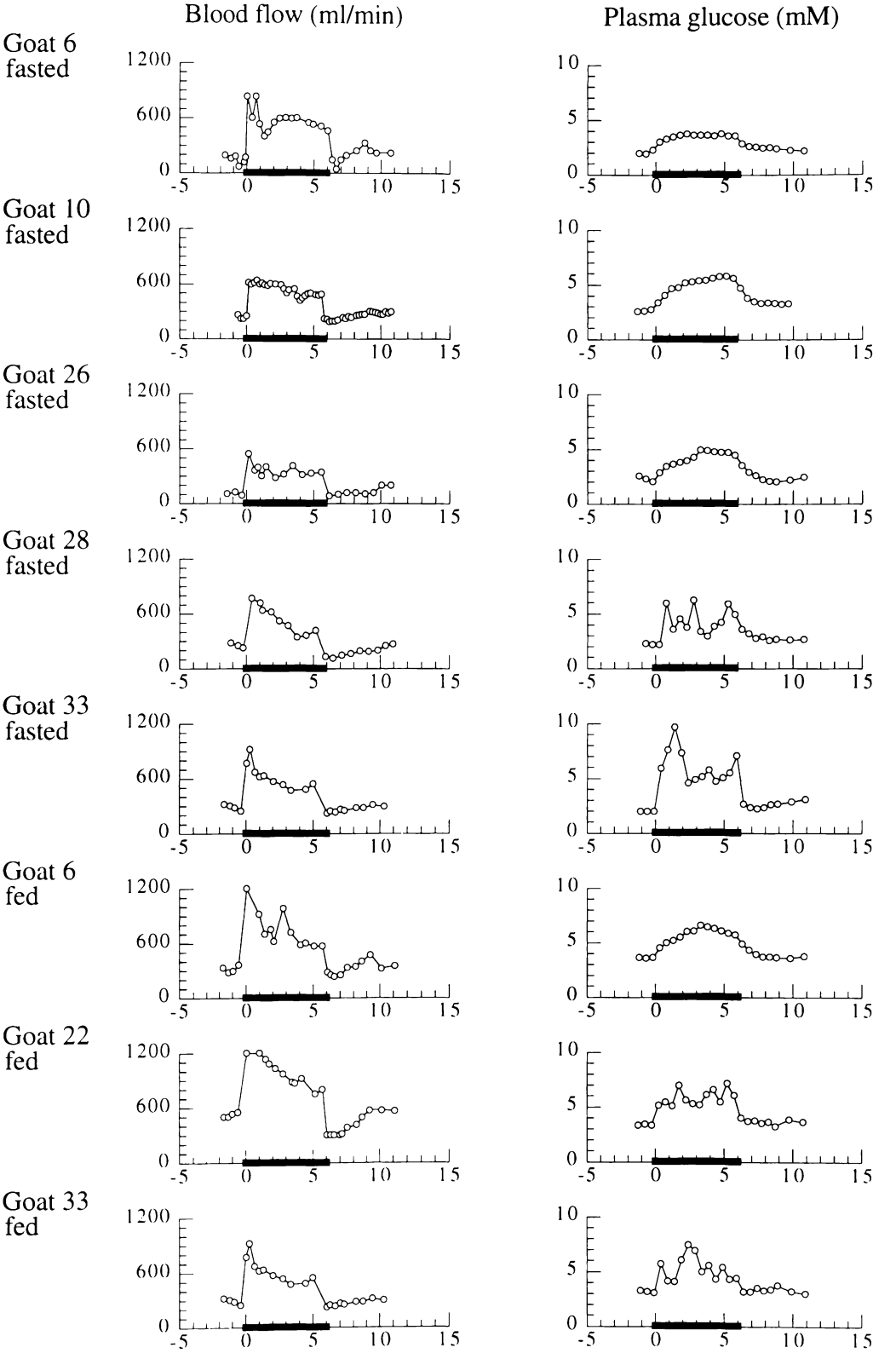
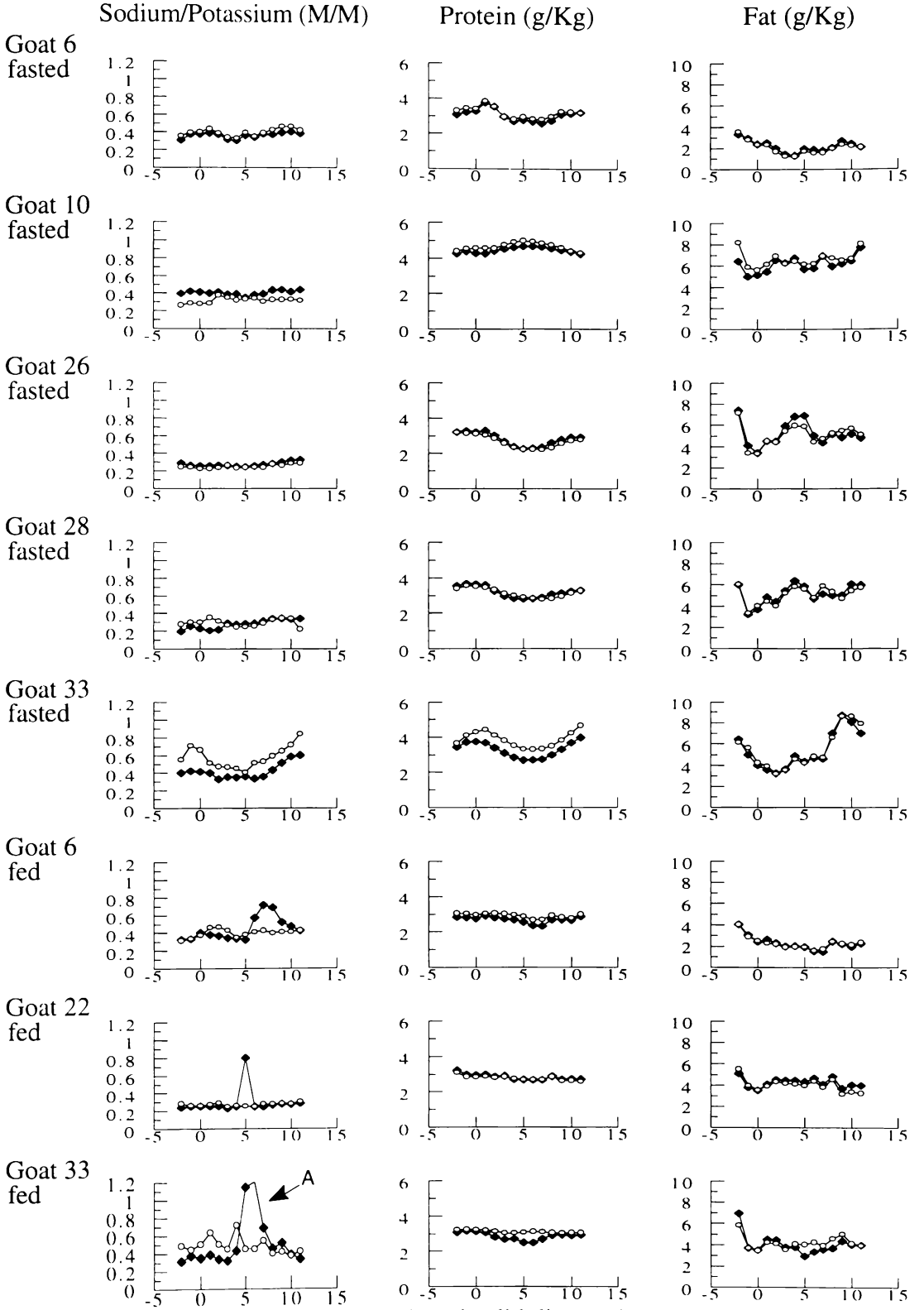


Figure 12. Graphs of sodium/potassium ratio, protein and fat content vs time (h) from the commencement of infusion of adenosine and glucose for each animal

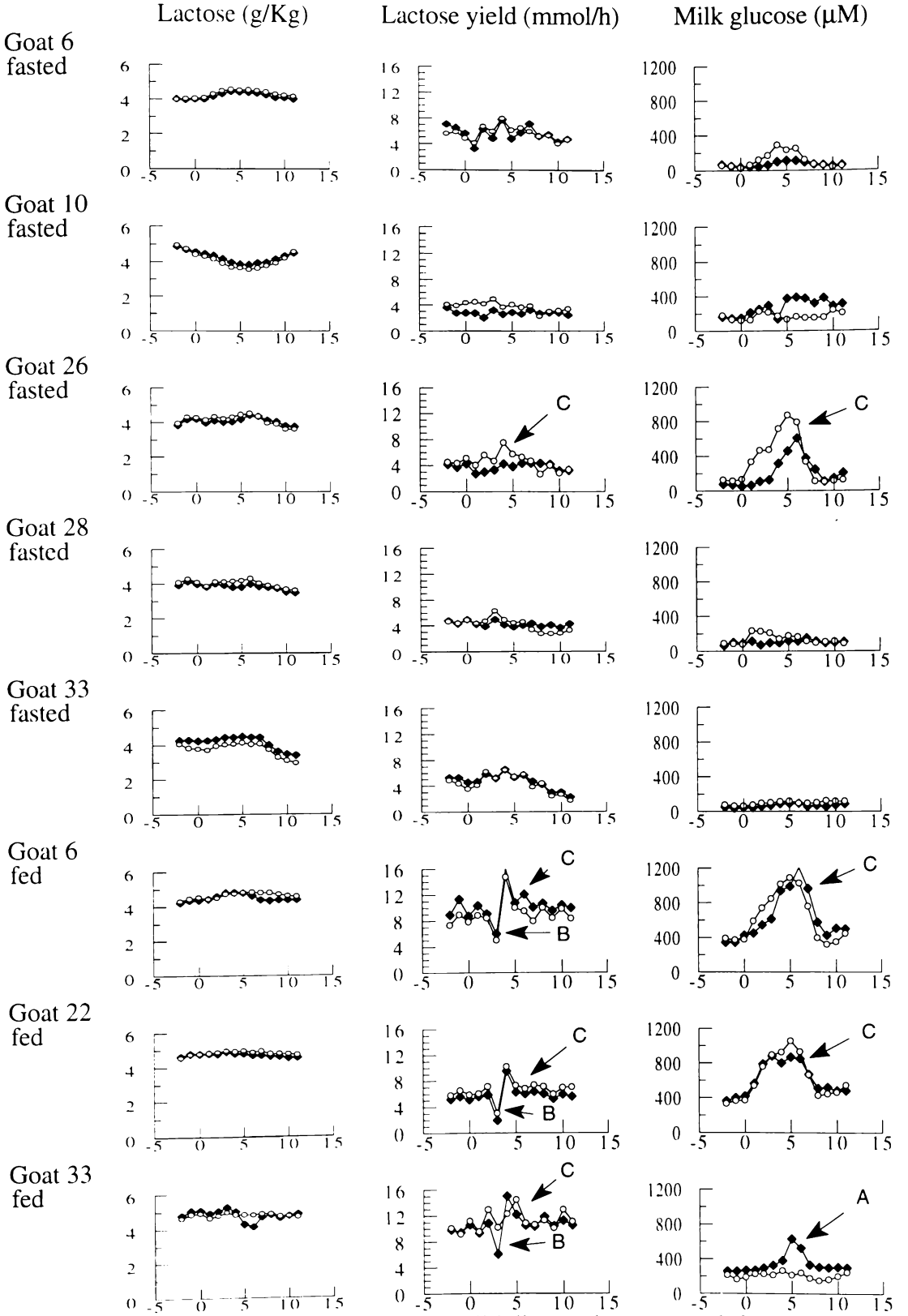
Infusions occurred from t=0h to t=6h.



Circles (°) represent right gland, and solid diamonds represent left gland.
'A' is referred to in the discussion.

Figure 13. Graphs of lactose content and lactose yield vs time (h) from the commencement of infusion of adenosine and glucose for each animal

Infusions occurred from t=0h to t=6h.



Circles (°) represent right gland, and solid diamonds represent left gland (•).
'A' and 'C' are referred to in the discussion section.
'B' is referred to at the end of the results section.

Sodium and potassium validated results indicated that milk glucose increased to above 500μM bilaterally in two of the three fed goats and in one of the five starved animals. In the remaining animals neither gland went above 500μM milk glucose concentration.

An approximate ANOVA was carried out due to the non-orthogonal design of this experiment. There was no strong evidence that elevating plasma glucose concentration and mammary blood flow were associated with observed variation in lactose output.

Discussion

Milk glucose is considered to be a useful predictor of intracellular glucose concentration (Faulkner, 1981; Grigor, 1989). This measurement tests the integrity of the epithelial tight junctions.

The observation of stable sodium:potassium ratios well below unity, on a molar basis, suggested that the tight junctions of the mammary epithelium usually maintained their integrity for the durations of these experiments. A single case at 5 hours in the left (normal flow) gland of goat 22 (See Fig.12) exceeded 0.8. Goat 33 exhibited an increase of sodium:potassium ratio (A, Fig. 12 and Fig. 13) in the left gland involving several points which coincided with an increase of milk glucose (A, Fig. 12 and Fig. 13) also on the left at the same time. This coincidence suggested a temporary loss of tight junction integrity could not be ruled out and the two points were not included in statistical summaries.

The contents of protein and lactose remained stable throughout the experiment. Fat content exhibited greater variability as is typical for milk fat measurements. There were no consistent trends amongst these measurements, other than the initial decline in fat content, and there were no data sets which indicated the production of abnormal milk from any of the individual goats.

Measurement of plasma glucose in individual goats has shown that, in each goat, plasma glucose concentration was appropriately increased by the infusion protocol. Plasma glucose returned to an approximately basal level within about an hour of stopping the infusions.

Similarly, measurements of blood flow have confirmed that, in each goat, blood flow abruptly and greatly increased during the infusion of adenosine. The sharp decrease

observed, in each goat, at the cessation of infusion resulted in a temporary overshooting to below the initial level, as is commonly seen, in about half of the animals.

Milk glucose concentration was successfully increased convincingly on three occasions (C, Fig. 13) and moderately on another three of the eight experiments. One of these first three goats (C, Fig. 13) showed a pronounced unilateral difference in favour of the gland with artificially increased blood flow. On the whole most increases in milk glucose concentration coincided with the infusion of glucose, but this was not a consistent increase among all of the goats. The associated lactose yields (C, Fig. 13) showed no increases related to these changes in milk glucose concentration, nor did any of the others exhibit increases and certainly not of the size expected from consideration of the comparison between fed and starved lactose yields.

It is puzzling as to why the milk glucose results vary so much from animal to animal. Consider the results from Chapter 2 were adenosine increased blood flow appeared to result in increased glucose uptake in some animals but not in others. On the surface it would seem that adenosine resulted in an increase in nutritive blood flow in some of the goats and not others. Goat 6 and 26 showed no or little increase in glucose uptake in response to adenosine when normally fed a month or two prior to this experiment. These two goats showed the greatest responsiveness in terms of milk glucose concentration when fasted in this experiment.

Regardless of the milk glucose measurements the desired changes in blood glucose concentration and arterial blood flow were achieved. A fuller experiment of this nature might include measurements of glucose uptake by the mammary gland in order to help confirm that increases in blood flow in that goat at that time are nutritive. It should be noted that increased blood flow may feasibly be nutritive without the observation of an increase in glucose uptake but the converse situation where an increase in glucose uptake results from increased blood flow is strong evidence for the increase being of a nutritive nature.

Conclusions

Stable synthesis of milk and in particular lactose occurred under a variety of concentrations of milk glucose, blood flow rates and plasma glucose concentrations. It

follows that lactose synthesis has been observed to be insensitive to these induced fluctuations in interstitial and intracellular glucose availability.

Chapter 5. INVESTIGATION OF BIOCHEMICAL DIFFERENCES BETWEEN JERSEY AND FRIESIAN COWS WHICH RESULT IN DIFFERENT RATES OF WATER SECRETION

Summary

The measurement of milk glucose concentration in ten animals from each of three bovine genetic groups, Friesian, high solids content Jersey and low solids content Jersey has resulted in the observation of statistically significant group differences. Of the four milkings three show significant differences and the other one milking showed no significant differences. Because one milking appeared quite different to the others it was decided to repeat the milk collections and glucose analyses. The most consistent difference over the genetic groups was that for morning milkings. Jerseys with high solids or a lower water secretion level exhibited a significantly lower concentration of milk glucose than the other Jersey group.

For samples from two separate years (120 samples from each collection resulted in 240 in total) morning milkings gave significantly and consistently higher milk glucose concentrations.

Glucose-6-phosphate on one day was significantly higher at the afternoon milking. Glucose-6-phosphate and glucose-1-phosphate were both significantly affected by day-to-day changes.

UDP-hexoses were not detected in bovine milk when the limit of detection was reduced from 10 μM (Faulkner 1980) to 4 μM.

Introduction

Two groups of 19 high fat yield breeding index (BI) Jersey cows, with either high or low milk solids content, had been established at Ruakura in June 1988 along with 19 high BI Friesians. Mean fat concentrations in November 1988 were 6.61, 5.32 and 4.74 for high (HS%J) and low (LS%J) solids Jersey groups and a Friesian group respectively. Corresponding values for protein content were 4.34, 3.72 and 3.42 percent. (L'Huillier *et al.*, 1989). These data reflected different levels of water secretion between these groups: the

water secretion of the Friesian group is higher than that of LS%J and both of these are higher than that of HS%J.

This experiment was undertaken to investigate whether significant differences in bovine milk concentrations of relevant metabolites for lactose synthesis and secretion occur between these small samples of animals from the three genetic groups mentioned above. It was hoped that such differences would indicate the point at which synthesis of lactose was being regulated.

Confirmations of normalcy by measurement of milk sodium:potassium ratio and contents of milk fat, protein and lactose were used to help validate data sets for individual cows.

Hypothesis

The different rates of lactose synthesis exhibited by the samples of animals from the three genetic groups is related to differences in average milk concentrations of some of the metabolites of lactose synthesis.

Methods

Animals

Ethics approval for this experiment was obtained from the Ruakura Animal Ethics Committee. Ten animals from each of these three groups were used, giving thirty for this trial. Some of the same animals were available in the second year and further ones were selected to give a second lot of thirty animals.

Sampling and analyses

Milk samples were from the high and low water-secreting groups of Jerseys and from Holstein-Friesians at No. 5 Dairy. The cows were under similar feeding and handling regimes. The same cows were sampled on two separate days resulting in 120 samples from four milkings. A second lot of 120 samples were collected a year later from similar groups of cows.

Milk samples were collected using herd-testing equipment. Milk samples were split into two aliquots using a metal funnel with two outlets. One aliquot had preservative added and

the other was frozen immediately in liquid nitrogen. The samples were later centrifuged and the resulting defatted milk split into aliquots and stored frozen at -20°C. The second group of samples were collected in the following year. These were collected on ice, centrifuged at 4°C in order to remove fat, deproteinised by addition of zinc sulphate and sodium hydroxide, and stored frozen at -20°C.

Milk samples from 1990 were assayed for glucose, glucose-1-phosphate, glucose-6-phosphate, UDP-glucose, UDP-galactose, sodium and potassium. Composite herd-testing milk samples from night and morning were assayed for fat, protein and lactose. Milk samples from 1991 were assayed for glucose, sodium and potassium. Composite herd-testing milk samples from night and morning were assayed for fat, protein and lactose by infra-red reflectance.

Results

The data have been tabulated in Appendix 5.

1990 Samples

Looking vertically through the graphs shown in Fig. 14, all of the results for several different metabolites from one animal may be viewed in relation to one another. The data from the samples which exhibited sodium:potassium ratios greater than 0.8 (first cow in HS% Jersey group) were removed before carrying out statistical summaries.

Summary graphs of glucose concentration in milk are shown in Fig. 15. The LS%J group exhibited a significantly higher mean glucose concentration in milk than HS%J at the two 8 a.m. milkings. The three groups were significantly different from each other at one 4 p.m. milking, increasing in the following sequence: HS%J, LS%J and Friesian. The results from the afternoon milking of Day 1 all are very low in comparison with the other milkings, suggesting that some type of analytical or sample storage problem had occurred. For this reason another sample set was collected late in 1991.

Means of neither glucose-6-phosphate nor glucose-1-phosphate concentrations (Fig. 14) exhibited significant differences between the samples of animals. These results for glucose phosphates exhibited distributional skew.

The coefficient of determination (r^2) for regression of glucose on glucose-6-phosphate was less than 1% (regression not significant), and for glucose-1-phosphate on glucose-6-phosphate it was 45% (significant regression, $p<0.001$).

1991 Samples

As before the data from the samples which exhibited high sodium:potassium ratios (Fig. 17) were removed before carrying out statistical summaries. Inspection of the deleted milk glucose concentrations *etc.* (still present in the raw data in Appendix 5) from either sample set did not lead to discovery of extreme values.

Summary graphs of glucose concentration in milk are shown in Fig. 18. As before the LS%J group exhibited a significantly higher mean glucose concentration in milk than HS%J at the two 8 a.m. milkings. Mean milk glucose concentration was significantly higher ($p<0.001$) at the 8 a.m. milkings.

Plasma glucose concentration data are shown in Fig. 17 and summarised in Fig. 19. No significant differences were observed between the means of plasma glucose concentration for the genetic groups, however the mean for 8 a.m. was significantly higher than for 4 p.m. ($p<0.001$).

The coefficient of determination for regression of plasma glucose concentration on milk glucose concentration was 30% (significant regression, $p<0.001$).

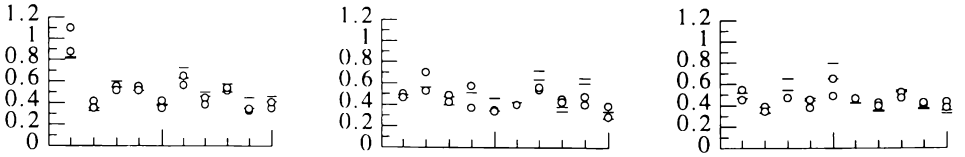
General Results

The ratio of protein to lactose content showed the typical pattern of genetic group differences (Figs. 16 & 20) which are the subject of this investigation. The ratio of protein to lactose content in the samples showed no strong relationship to plasma glucose, milk glucose, glucose-6-phosphate and glucose-1-phosphate, although this parameter itself was strongly stratified by genetic group.

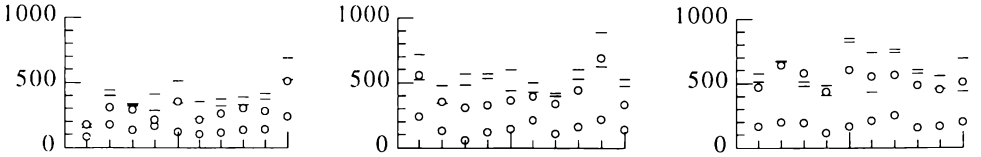
The mean rate of lactose production for the pre-milking period did not vary significantly between the morning and evening milkings.

Figure 14. Graphs of measurements of milk components in samples collected from thirty cows each sampled late in 1990 at four milkings.
HS% Jersey LS% Jersey Friesian.

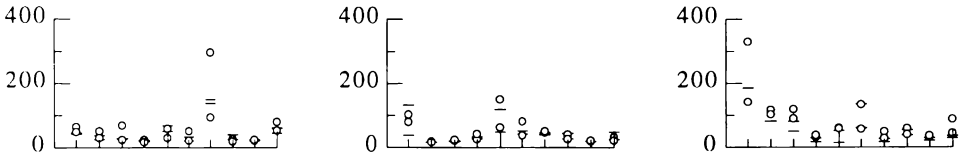
Sodium potassium ratio (on a molar basis).



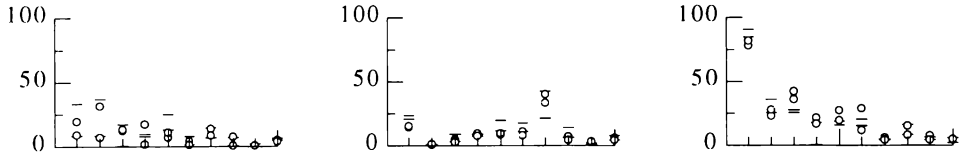
Milk glucose (μM).



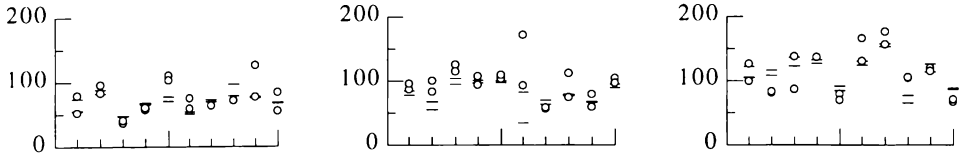
Milk glucose 6 phosphate (μM).



Milk glucose 1 phosphate (μM).



Lactose yield (mmol/h).



Pm milkings are represented by 'o' and am milkings by '-'.

Figure 15. Milk glucose concentration (μM) from 1990 summarised.
Mean ± L.S.D. (For comparison within each graph)

Day1, 8am Day1, 4pm Day2, 8am Day2, 4pm

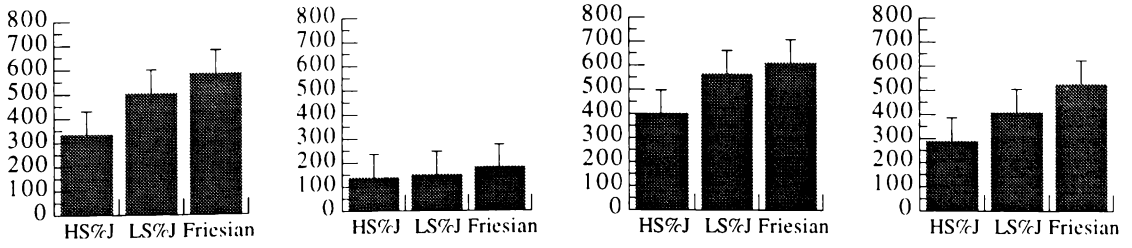


Figure 16. Protein to lactose ratio in 30 cows in 1990.
Mean ± L.S.D. (For comparison between genetic groups)

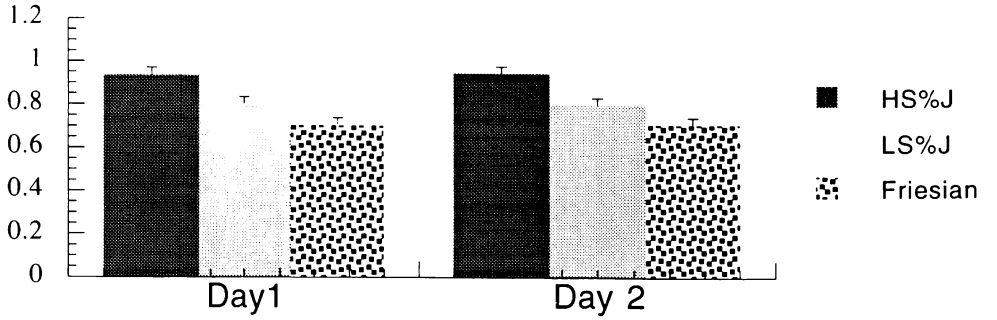
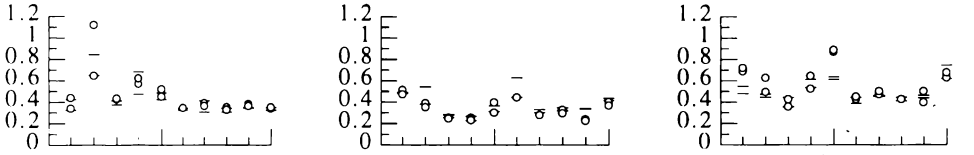
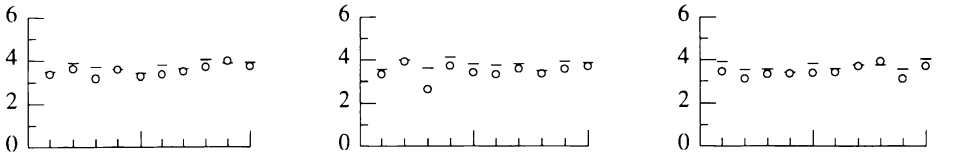


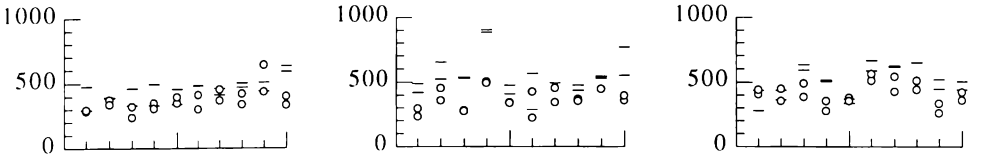
Figure 17. Graphs of measurements of milk components in samples collected from thirty cows late in 1991 each sampled at four milkings.
HS% Jersey LS% Jersey Friesian.
Sodium potassium ratio (on a molar basis).



Plasma glucose (mM).



Milk glucose (μM).



Lactose yield (mmol/h).

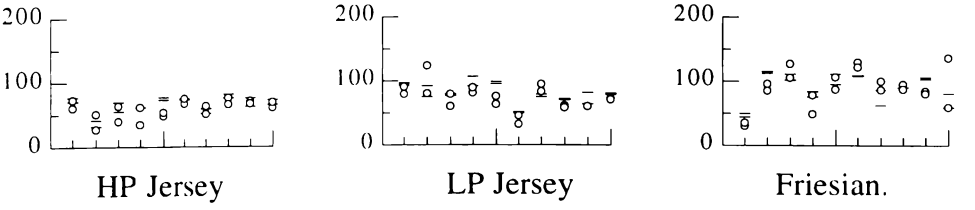


Figure 18. Milk glucose concentration(μM) from 1991 summarised.
Mean ± L.S.D. (For comparison within each graph)

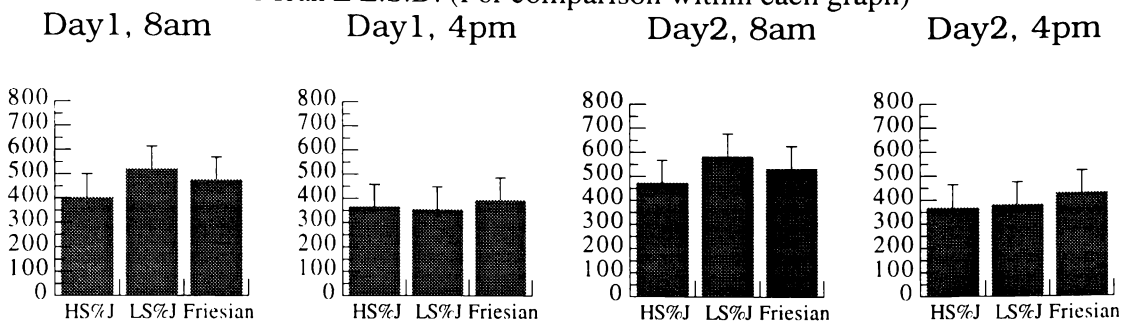


Figure 19. Plasma glucose concentration (μM) on Day 2.
Mean±L.S.D. (for comparison between genetic groups)

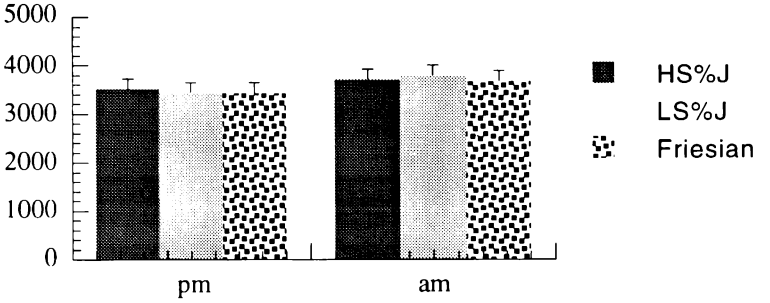
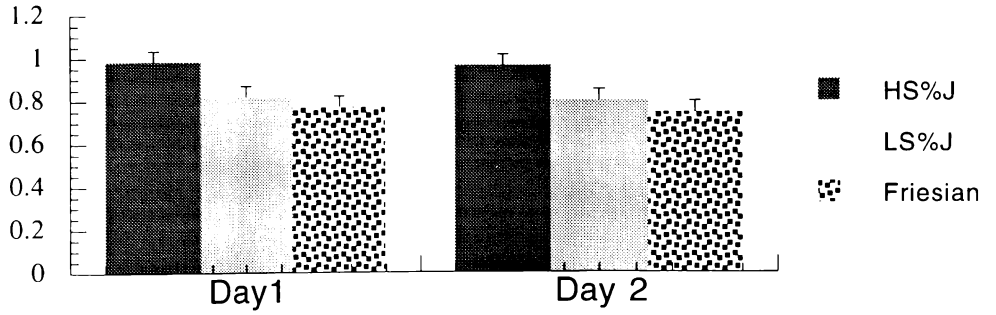


Figure 20. Protein to lactose ratio in 30 cows in 1991.
Mean ± L.S.D. (For comparison between genetic groups)



Discussion

The most consistently observed genetic group contrast on the basis of milk glucose concentration was between low and high water secreting jerseys where high water secretors (LS%Jersey) show a higher concentration of milk glucose at the morning milkings, on average, than low water secretors (HS%Jersey).

The most consistent observation involving the measurement of milk glucose was that 8 a.m. milking means were higher than the 4 p.m. milking means from the same day. This increase coincided with an increase in plasma glucose in the mornings also on the one day

that plasma glucose samples were collected, but not with any change in the mean rate of lactose synthesis. It is interesting to note that the genetic group differences in milk glucose, when observed, coincided with water secretion differences between the groups, however the morning and afternoon differences did not coincide with differences in the mean rate of lactose output. Conceivably, on the other hand, there may be a source of variation associated with milk glucose concentration which makes it a biased estimator of cytosolic glucose concentration, which in turn, really does regulate lactose output. Some other explanatory models are: that a slight reduction in lactogenesis is occurring after the 16 hour interval due to some mammary inhibition resulting in less drain on systemic glucose; that the ability of the system to supply glucose for lactogenesis is greatest at night.

The fluctuations seen in intracellular glucose availability are not reflected in glucose-6-phosphate or glucose-1-phosphate concentrations. Further analyses and investigations of skewness with more animals may be worthwhile since the means of values from Friesians were always the highest though not significant.

Though UDP-hexoses do not freely equilibrate across the apical membranes of the mammary secretory cells of bovines (Faulkner, 1980) it may still be of use to again try to measure them when assay methodology improves. They may show fluctuations in concentration related to some intra-cellular concentrations.

No attempt was made to formally say in what way these small numbers of animals represent the Friesian populations and Jersey populations at large. It is unlikely to be appropriate to say that all Friesians are distinctly representative of the Friesian breed in New Zealand particularly when one is considering quantitative traits with unknown numbers of associated loci and alleles and also an unknown component of environmental variance. However the degree of population difference does set a minimum expectation for the degree of separation for the causal parameters in concert.

Differences of metabolite concentrations measured in milk between the samples of animals from the genetic groups may reflect differences in milk composition between those populations, or alternatively may only reflect differences between the small samples of animals. Population differences do not necessarily imply important mechanistic differences associated with the control of lactose synthesis.

Conclusions

Significant differences in milk glucose concentration associated with the genetic groups seem to become manifest at milkings where higher levels of milk glucose concentration occur.

The inconsistent nature of the differences in milk glucose concentration between the groups of cows makes it hard to associate them with differences in lactose output. This may reflect the ability of steps later in the lactose synthetic pathway to smooth the effects of substrate supply. (N.B. Glucose feeds in at two places.) Indeed the fact that consistent daily fluctuations have been observed, in the absence of similar fluctuations in the rate of lactose synthesis demonstrates the unresponsiveness of these systems to variations in glucose availability, estimated by milk glucose concentration, under physiological conditions.

The significant interaction between genetic group and time of milking suggests that milk glucose concentration may be lower from less full udders and perhaps higher in hind milk from full udders, if one assumes that milking interval is the more important parameter. This experiment does not however, distinguish between milking interval and time of day since they were not the focus of this experiment. It is therefore difficult to conclude much about the causes of observed morning and afternoon variation in milk and plasma glucose other than to say that it would be worthy of further investigation.

Collection of milk fractions over time within a milking particularly hind milk and residual-milk, thrice and twice daily milking and the collection of more structured and representative sample of animals would be some logical extensions of this experiment.

Chapter 6. GENERAL DISCUSSION

The ratio of sodium and potassium has been used as a confirmation of epithelial integrity. In this thesis there have been some values of this ratio greater than unity and they have not coincided with elevated levels of milk glucose due to influx of interstitial fluid. Sodium and potassium are controlled by a complex cellular ion pumping system, therefore it is conceivable that this confirmation of normalcy is not based on a reliable assumption. Simpler to justify is the use of serum albumin concentrations for this purpose, since it is unlikely to be recovered by the apical membrane from milk.

The strong likelihood remains that there is a steep glucose concentration gradient across the baso-lateral membranes of the mammary epithelial cells and also that there must usually be a considerable amount of nutritive mammary blood flow during normal lactation, hence intracellular glucose concentration is regulated by the baso-lateral membrane. Nevertheless fluctuations in milk glucose have been achieved by the experimental systems used for these experiments.

Grigor (1989) suggested that rat mammary epithelium is normally leaky because milk glucose concentration was not observed to correlate with lactose output under differing nutritional regimens. Faulkner *et al.* (1981, 1985b) claimed that the metabolic significance of milk glucose concentration from goats was that it represents intracellular glucose concentration, and that the rate of lactose synthesis was decreased by this glucose concentration under starvation conditions and increased under inhibiting conditions of one gland resulting in supra-normal elevation of the other gland. The fluctuations in milk glucose concentration observed in these experiments in goats and cows have not correlated with fluctuations in the rate of lactose synthesis (Faulkner *et al.*, 1984; Henderson *et al.*, 1983).

Davis (1988) suggested that increased blood flow might be a metabolic consequence of increased milk synthesis when probing the galactopoietic effects of exogenous bovine

somatotropin administration in cattle. That concept would not seem to be in conflict with these results although it would be helpful to be better able to distinguish between nutritive and non-nutritive blood flow.

The apparent unresponsiveness of the lactose synthetic pathway to the modifications of glucose availability in these experiments suggests that mid-lactational steady-state control may be being exerted downstream of glucose-1-phosphate and not by the influence of the cytoplasmic concentration of glucose on the lactose synthetase complex. Regulation of the movement of glucose and UDP-galactose into the Golgi apparatus and through changes in the concentrations of divalent ions in this compartment are possibilities. The calcium binding site of α-lactalbumin is interesting in this regard due to the function of calcium as a second messenger in many cell types and organelles. Unfortunately it was beyond the scope of this project to probe sub-cellular compartmentation in any more detail. Part of the difficulty here is to fix a system for analysis of these highly diffusible molecules. The process of vesicle swelling due to osmosis attracts ones attention at this point and further questions regarding the permeability of the Golgi derived membranes of these vesicles arise. The processes of formation of vesicles, their migration and the release of their contents via the apical membranes of secretory cells is also a possibility for regulation late in the lactose synthetic pathway.

The regulatory mechanism of lactose synthesis is likely to yield a close relationship with the regulation of protein synthesis since these experiments have not succeeded in perturbing the close relationship between lactose synthesis and protein synthesis.

Short-term starvation may influence lactose synthesis through the short supply of other milk precursors besides glucose combined with a stringent intra-epithelial commitment to a fairly constant ratio of secretion of lactose and milk proteins.

Hormonal activation of a regulatory mechanism within the mammary secretory cell has not been implicated by these experiments but it is a possibility. Adrenaline remains a candidate

for helping to co-ordinate mammary regulation under starvation conditions after observing its profound effect on glucose uptake in goats, which operates not only to reduce blood flow but also to divert some nutritive blood flow to non-nutritive and/or to modify the glucose-extracting characteristics of the mammary epithelium. Fed goats in mid-lactation infused with adrenaline could in this regard be in interesting experimental system to investigate further.

GENERAL CONCLUSIONS

All of the experiments in the present study suggest that published correlations between milk glucose, at physiologically acceptable levels, and lactose output are largely coincidental or that normal fluctuations in milk glucose concentration causes only very small fluctuations in lactose output. There consequently appears to be a negation of notions that the intracellular glucose availability is usually a major control point of lactose synthesis in stable mid-lactation. It would appear that intracellular glucose availability can fluctuate over quite a wide physiological range and not impact greatly on the rate of lactose synthesis.

APPENDIX 1. ANALYTICAL METHODS.

1. Extraction procedures.

1.1 Defatting milk samples.

Milk samples were thawed at room temperature, cooled on ice, mixed, and centrifuged at greater than 2000*g* for 5 min at 4° C in 1.5ml microcentrifuge tubes or in the 5ml screw cap 'serum vials'. The defatted phase was removed by piercing the firm fat layer on each side of the tube and gently pouring out the liquid underneath through one hole using the other hole to let air in.

1.2 Deproteinisation with $ZnSO_4$/NaOH.

Reagents. 0.5M ZnSO4 and NaOH equivalent to this were prepared (Arthur *et al.*, 1989).

Method. Defatted milk (1000 µl) was mixed with $ZnSO_4$ solution (400 µl), then mixed with NaOH solution (400 µl), and centrifuged at greater than 4000*g* for 5 min. The supernatant (500 µl) was carefully removed, stored at -20° C and used for glucose analyses.

1.3 Deproteinisation with perchloric acid.

Reagents. A 1M perchloric acid solution and neutralising buffer producing equivalence when used at an addition volume ratio of 1:3 respectively were prepared (Arthur *et al.*, 1989).

Method. Defatted milk, or plasma, was deproteinised by mixing with an equal volume (normally 500 µl) of perchloric acid solution. After standing for 5 min, then centrifuging at greater than 4000 g for 5 min, the supernatant was removed (600 µl) and mixed with the one-third volume (200 µl) of neutralizing buffer. After standing for 5 min and centrifuging at greater than 4000*g* for 5 min, the supernatant was removed, stored at -20° C, and used for metabolite analyses.

2. Non-enzymatic milk analyses.

2.1 Fat, protein and lactose by infra-red reflectance.

Bovine and goat milk samples were submitted to routine milk analysis used in the dairy industry.

2.2 Sodium and potassium.

These analyses were carried out by the Dairying Research Corporations analytical biochemistry personnel. The method of Gunther *et al.* (1965), as modified by Hartmann and Prosser (1982), and adapted for a flame photometer was used. The extraction was scaled up ten-fold.

Method. Defatted milk samples (100µl) were mixed with 1000µl of 25% nitric acid (v/v) and 1% H_2O_2 (w/v) in acid-washed plastic test tubes (Arthur *et al.,* 1989; Hartmann *et al.,* 1982). The tubes were placed in a boiling water bath or heating block (80-100°C) for 30 min. After being cooled, 9ml of 15mM lithium chloride was added. After calibrating the flame photometer with sodium and potassium standards, the concentrations of sodium and potassium in each sample were measured.

3. Non-luminescent enzymatic milk analysis methods.

3.1 Milk glucose

Assay principle.

ß-D-Glucose ------(Glucose oxidase)------> D-gluconic acid + H_2O_2

H_2O_2 + (ABTS)H_2 ---(Horseradish Peroxidase)---> H_2O + ABTS

Materials. 2.2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (A-1888), horseradish peroxidase (P-6140) and glucose oxidase (G-6125) were purchased from the Sigma chemical company.

Glucose reagent. 100mM potassium phosphate (pH 7.2), glucose oxidase (4.8 U/ml), peroxidase (1.25 U/ml) and 500 mg/mL ABTS, (Bergmeyer *et al.,* 1965; Arthur *et al.,* 1989).

Method. A milk sample treated with zinc sulphate (10µl) was mixed with 200µl of glucose reagent in 96 well microplates. After incubating at room temperature for 45 min, the

dual wavelength absorbance was measured at λ_{sample} = 405 nm and $\lambda_{reference}$ = 490 nm with a Biorad microplate reader, and the results were analysed numerically with the accompanying Microplate Manager regression software.

3.2 Blood glucose.

Glucose concentration in plasma (floride-oxalate treated) was analysed using a Beckman glucose analyser, measuring the disappearance of oxygen, caused by glucose oxidase (above), using an oxygen electrode. Plasma samples were also submitted to MAFQual for standard veterinary automated enzymatic glucose analysis.

4. Luminescent enzymatic analysis methods.

4.1 Buffers and other solutions used.

B1	3.2 M ammonium sulphate (pH 6).
B2	75 mM triethanolamine HCl and 5 mM $MgCl_2$ (pH 7.8).
B3	90 mM NAD.
B4	200 mM EDTA pH 7.5.
B5	100 mM potassium phosphate buffer (pH 7.5).
B6	100 mM potassium phosphate buffer (pH 7.0).
B7	100 mM glycine buffer and 5 mM EDTA (pH 8.7).
B8	75 mM tris(hydroxymethyl) methylamine and 10 mM $MgCl_2$ (pH 8.8).
B9	90 mM uridine triphosphate in tris buffer (B8).
B10	100 mM potassium phosphate buffer and 0.1 mM $MgSO_4$ (pH 7.5).
B11	50% glycerol in tris buffer (B8) (v/v).

4.2 Enzyme preparations.

E1	Hexokinase suspended in ammonium sulphate (B1) - 350 U/ml.
E2	Glucose-6-P dehydrogenase suspended in ammonium sulphate (B1) - 350 U/ml.
E3	UDP-glucose epimerase dissolved in phosphate buffer (B6) - 4 U/ml).
E4	UDP-glucose dehyrogenase suspended in ammonium sulphate (B1) - 3 U/ml.
E5	UDP-glucose pyrophosphorylase dissolved in tris/glycerol (B11) - 200 U/ml.
E6	Galactosidase dissolved in phosphate buffer (B5) - 125 U/ml.
E7	Galactose dehydrogenase in ammonium sulphate (B1) - 50 U/ml.

4.3 Overview of enzyme assays.

The following luminescent methods are a minimal description of methods published by *Arthur et al.* (1989). However only a manual luminometer (LKB 1250) was available for my milk analyses. Satisfactory performance was attained in this simpler configuration, though this has not been a focus of experimentation.

Samples were deproteinised with perchloric acid, the standards were also treated with perchloric acid. Metabolite assays were carried out in microtitre plates and samples of well contents transferred to luminometer tubes.

4.4 Glucose-6-phosphate.

Assay principle.

Glucose-6-P + NAD -------(G6P dehydrogenase)-------> NADH + D-glucono-8-lactone-6-phosphate

Enzyme reagent. 1ml triethanolamine buffer (B2), 5µl NAD, 4µl of G6P dehydrogenase (E2), 5µL of EDTA (B4).

Method. A 10µl sample of deproteinised milk was loaded into a microplate well, then 50µl of enzyme reagent was added. After incubating for 20 min, a 20µl aliquot was taken for the luciferase assay.

4.5 Glucose-1-phosphate.

Assay principle.

Glucose-1-P + UTP -------(UDP-glucose pyrophosphorylase)-------> UDP glucose + PPi

UDP-glucose + 2 NAD -------(UDP-glucose dehydrogenase)-------> UDP-glucuronate + 2NADH

Enzyme reagent. 1ml tris buffer (B8), 10µl UTP (B9), 4µl UDP-glucose dehydrogenase (E4), 0.4µl UDP-glucose pyrophosphorylase (E5), 10µl NAD (B3), 5µl EDTA (B4).

Procedure: A 10µl sample of deproteinised milk was loaded into a microplate well, then 50µl of enzyme reagent was added. After incubating for 1h, a 20µl aliquot was taken for the luciferase assay. The results were corrected for the concentration of UDP-glucose in the sample.

4.6 UDP-glucose and UDP-galactose.

Assay principle.

UDP-galactose -------(UDP glucose-4-epimerase)-------> UDP-glucose

UDP-glucose + 2NAD -------(UDP-glucose dehydrogenase)-------> UDP-glucuronate + 2NADH

Enzyme reagent. 988µl of glycine buffer (B7), 10µl of NAD (B3), 2µl of UDP-glucose dehydrogenase (E4), 50µl of UDP-glucose-4-epimerase (E3).

Procedure. For the UDP-hexose assay, 5µl of deproteinised milk was incubated with 50µl of enzyme reagent for 45 min. A 20µl aliquot was taken for the luciferase assay. For the UDP-glucose assay the same procedure was followed except that UDP-glucose-4-epimerase (E3) was not added to the enzyme reagent. The UDP-galactose concentration was the difference between the concentration of these two UDP-hexoses and UDP-glucose in the sample.

4.7 Bioluminescent assay for NAD(P)H.

Assay principle.

$$NADH + H^+ + FMN \text{ -------(FMN oxidoreductase)-------> } FMNH_2 + NAD$$

$$FMNH_2 + CH_3(CH_2)_{12}CHO + O_2 \text{ ------(Luciferase)------> } FMN + H_2O + light + CH_3(CH_2)_{12}CO_2H$$

Luciferase reagent. 16ml phosphate buffer (B2), 50µl FMN (B1), 1ml tetradecanal (B4), 100µl luciferase (E1), and 33µl FMN oxidoreductase (E2) or equivalently initially a kitset from Boehringer Mannheim was used. This reagent was prepared at least 30 minutes before use. The reagent was kept at 4°C protected from light and was usable for up to 3 days after preparation.

20µl of samples of reaction product from a series wells in the microtitre plate were loaded into 3ml plastic tubes. A sample tube was placed into the luminometer and the chamber closed. Ice-cold luciferase reagent (200µl) was squirted in through a syringe pump resulting in immediate mixing. In the absence of a heat exchanger *etc.* the reaction performed adequately at approximately 7°C.

5. Chromatographic analysis of concentrated sugars mixed with milk.

5.1. Glucose, sucrose and lactose.

Materials. Analytical high pressure liquid chromatography (HPLC) was carried out using a Waters M45 pump coupled to a Zorbax®Sil 4.6mm x 25cm stainless steel column. HPLC grade acetonitrile was obtained from BDH and diethylenetriamine (DETA) was obtained from the Aldrich Chemical Company. A Waters differential refractometer was used as a detector connected to a chart recorder.

Method. Defatted milk samples (500µl) were diluted with filtered (0.22µm) Milli-Q water (500µM). Absolute ethanol (1.5µl) was then added to each sample, vortexed thoroughly and centrifuged at approximately 10,000g for more than 5 minutes (Kowalski *et al.*, 1986). Supernatant was carefully removed and loaded into autosampler tubes. It was found that filtration of these sample extracts could be avoided using careful technique to avoid the transfer of particulate matter.

Isochratic conditions of 70% aqueous acetonitrile and 0.01% DETA were used, and a flow rate of 1ml/min (Wheals et al., 1979). The difference between the injection solvent and the mobile phase did not cause a problem under the conditions of these analyses.

A Perkin Elmer ISS-100 autosampler was used to load 200µl of sample extracts. A wire from the fraction collector to the chart recorder was used to mark the sample injection point on the chart allowing the system to produce meaningful information whilst operating unattended.

APPENDIX 2.

Data tables from chapter 2: 'Investigation of the effect of mammary blood flow...'.

Data from the pilot experiments.

Goat	Right Heart mM	Ex Pudic Artery mM	blood sample pair
6a	3.7000	3.7400	1
6a	*	*	1
6a	3.5600	3.6700	2
6a	*	*	2
10	4.2778	4.0000	3
10	4.2222	4.0000	3
10	4.0556	4.3889	4
10	4.1667	4.2778	4
10	4.1111	3.9444	5
10	4.1667	4.0000	5
6b	4.2222	4.2222	6
6b	4.2778	4.2222	6
6b	4.2778	4.1111	7
6b	4.2222	4.1667	7
6b	4.1111	4.3333	8
6b	4.3333	4.3333	8
6b	4.0556	4.5000	9
6b	4.0556	4.3889	9
6b	4.0556	4.1667	10
6b	4.0556	4.1667	10
6b	4.1667	4.2778	11
6b	4.1667	4.3889	11
30	3.9444	4.0000	12
30	3.9444	4.0000	12
30	4.2222	4.1667	13
30	4.1667	4.2222	13
30	4.1111	4.2222	14
30	4.1111	4.2222	14
30	4.1667	4.3333	15
30	4.1667	4.2778	15
30	4.0556	4.0000	16
30	4.0556	4.2222	16

Goat 6, Haematocrit = 20 %

Milk weights for night and morning before before the experiment were 780g and 1727g respectively. There was a very slow drip from the infusion catheter of no consequence. A slow flow rate from the milk vein catheter of this goat resulted in slightly more variable glucose results because clotting and haemolysis were problems. The second case of the adrenaline treatment was not used for statistical analysis since the blood flow was informally noted as too high.

Time (h)	Treatment	Plasma flow ml/min	Right Atrium mM	Mammary vein mM	AV diff mM	Uptake mmol/min
-0.58	Control	208	3.99	2.95	1.040	0.216
-0.48	Control	192	3.88	2.95	0.930	0.179
-0.40	Control	192	3.93	2.92	1.010	0.194
-0.31	Control	224	3.76	3.07	0.690	0.155
-0.11	Control	224	3.92	2.75	1.170	0.262
0.37	Adenosine	416	3.84	3.44	0.400	0.166

0.50	Adenosine	436	3.96	3.30	0.660	0.288
0.62	Adenosine	408	4.05	3.33	0.720	0.294
0.77	Adenosine	400	4.03	3.62	0.410	0.164
0.89	Adenosine	456	4.02	3.66	0.360	0.164
1.19	Restore	176	4.00	3.44	0.560	0.099
1.30	Restore	160	4.22	3.44	0.780	0.125
1.40	Restore	216	4.37	3.23	1.140	0.246
1.47	Restore	168	4.33	3.78	0.550	0.092
1.57	Restore	200	4.15	3.56	0.590	0.118
2.00	Adrenaline	96	4.47	3.77	0.700	0.067
2.09	Adrenaline	232	4.57	3.76	0.810	0.188
2.42	Adrenaline	48	4.66	*	*	*
2.54	Adrenaline	32	4.60	4.16	0.439	0.014
2.74	Adrenaline	36	4.90	4.32	0.580	0.021

Goat 10, Haematocrit = 22%. A composite milk sample from the night and morning before the experiment gave the following results for fat, protein and lactose concentrations 3.79, 3.59, 4.77 g/100g respectively. Milk weights were 870g and 1860g for night and morning before respectively. Old fluoride-oxalate vacutainer tubes proved unreliable in their ability to prevent clotting and thus introduced more variability into the plasma glucose measurements.

Time (h)	Treatment	Plasma flow ml/min	Right Atrium mM	Mammary vein mM	AV diff mM	Uptake mmol/min
-0.34	Control	172	3.83	2.83	1.000	0.172
-0.25	Control	199	3.72	2.78	0.944	0.188
-0.17	Control	218	3.92	2.83	1.083	0.237
-0.09	Control	242	3.75	3.11	0.639	0.154
0.00	Control	250	3.94	2.81	1.139	0.284
0.25	Adenosine	601	3.97	2.83	1.139	0.684
0.33	Adenosine	577	4.00	3.42	0.583	0.337
0.41	Adenosine	577	*	*	*	*
0.50	Adenosine	577	3.94	3.42	0.528	0.305
0.58	Adenosine	587	4.11	3.22	0.889	0.522
0.85	Restore	238	*	*	*	*
0.93	Restore	251	4.17	3.11	1.056	0.265
1.01	Restore	259	*	*	*	*
1.10	Restore	251	3.97	2.83	1.139	0.286
1.18	Restore	264	3.64	2.44	1.194	0.315
1.50	Adrenaline	94	*	*	*	*
1.58	Adrenaline	86	*	*	*	*
1.66	Adrenaline	125	3.92	3.47	0.444	0.055
1.75	Adrenaline	83	3.89	3.58	0.306	0.025
1.83	Adrenaline	115	4.19	3.42	0.778	0.090

Goat 26, Haematocrit = 28%. Milk samples from the night and morning before the experiment gave the following results for fat, protein and lactose concentrations (percentages) respectively: 4.86, 3.00, 4.52 (afternoon 930g) and 4.03, 2.94, 4.53 (morning 1580g). No technical problems.

Time (h)	Treatment	Plasma flow ml/min	Right Atrium mM	Mammary vein mM	AV diff mM	Uptake mmol/min
-0.43	Control	108	3.39	2.67	0.722	0.078
-0.33	Control	112	3.17	2.61	0.556	0.062
-0.25	Control	110	3.31	2.47	0.833	0.092
-0.18	Control	110	3.25	2.44	0.806	0.089
-0.08	Control	108	3.33	2.44	0.889	0.096
0.42	Adenosine	266	3.14	2.83	0.306	0.081
0.52	Adenosine	281	3.08	2.81	0.278	0.078
0.62	Adenosine	259	2.94	2.58	0.361	0.094
0.72	Adenosine	261	3.33	2.92	0.417	0.109
0.82	Adenosine	260	3.06	2.69	0.361	0.094

1.09	Restore	127	3.03	2.56	0.472	0.060
1.37	Restore	109	3.14	2.42	0.722	0.079
1.52	Restore	108	3.50	2.67	0.833	0.090
1.60	Restore	109	3.31	2.53	0.778	0.085
1.69	Restore	113	3.28	2.50	0.778	0.088
2.14	Adrenaline	39	3.17	2.47	0.694	0.027
2.24	Adrenaline	40	2.94	2.33	0.611	0.025
2.32	Adrenaline	38	3.36	2.42	0.944	0.036
2.40	Adrenaline	43	3.25	2.39	0.861	0.037
2.50	Adrenaline	48	3.06	2.31	0.750	0.036

Goat 28, Haematocrit = 21%. Milk samples from the night and morning before the experiment gave the following results for fat, protein and lactose concentrations (percentages) respectively: 6.30, 2.86, 4.23 (afternoon 1290g) and 5.09, 3.08, 4.29 (morning 2060g). Spontaneous choking and associated distress commenced at the end of the control blood collections.

Time (h)	Treatment	Plasma flow ml/min	Right Atrium mM	Mammary vein mM	AV diff mM	Uptake mmol/min
-0.68	Control	254	3.53	2.61	0.917	0.233
-0.58	Control	251	3.61	2.64	0.972	0.244
-0.51	Control	303	3.67	2.61	1.056	0.319
-0.38	Control	294	3.56	2.61	0.944	0.278
-0.28	Control	*	3.58	2.33	1.250	*
0.30	Adenosine	659	3.72	3.06	0.667	0.439
0.42	Adenosine	650	3.78	3.22	0.556	0.361
0.51	Adenosine	607	3.72	2.94	0.778	0.472
0.60	Adenosine	602	3.61	2.86	0.750	0.451
0.69	Adenosine	559	3.78	3.03	0.750	0.419
1.02	Restore	144	3.83	2.72	1.111	0.160
1.12	Restore	125	3.89	2.78	1.111	0.139
1.22	Restore	133	4.00	2.86	1.139	0.151
1.32	Restore	160	4.00	2.94	1.056	0.168
1.42	Restore	192	3.97	2.92	1.056	0.203
1.72	Adrenaline	121	4.00	2.81	1.194	0.144
1.80	Adrenaline	117	4.11	3.06	1.056	0.123
1.89	Adrenaline	120	4.22	3.06	1.167	0.140
2.00	Adrenaline	137	4.25	3.08	1.167	0.159
2.08	Adrenaline	142	4.03	3.08	0.944	0.134

Goat 33, Haematocrit = 20 %. Milk weights for night and morning before before the experiment were 2100g and 3346g respectively. No technical problems.

Time (h)	Treatment	Plasma flow ml/min	Right Atrium mM	Mammary vein mM	AV diff mM	Uptake mmol/min
-0.70	Control	280	3.87	2.53	1.340	0.375
-0.58	Control	280	3.80	2.48	1.320	0.370
-0.40	Control	264	3.83	2.31	1.520	0.401
-0.28	Control	288	3.77	2.38	1.390	0.400
-0.13	Control	312	3.80	2.35	1.450	0.452
0.34	Adenosine	392	4.05	2.75	1.300	0.510
0.40	Adenosine	392	4.07	2.83	1.240	0.486
0.50	Adenosine	432	4.08	2.94	1.140	0.492
0.59	Adenosine	432	4.10	2.83	1.270	0.549
0.65	Adenosine	456	4.10	2.96	1.140	0.520
1.61	Restore	360	4.20	2.76	1.440	0.518
1.79	Restore	352	4.03	2.76	1.270	0.447
1.90	Restore	368	3.97	2.68	1.290	0.475
1.99	Restore	344	3.90	2.62	1.280	0.440
2.09	Restore	336	3.70	2.48	1.220	0.410
3.17	Adrenaline	72	4.23	2.53	1.700	0.122
3.30	Adrenaline	32	4.49	3.21	1.280	0.041
3.40	Adrenaline	32	4.64	3.15	1.490	0.048
3.49	Adrenaline	24	4.73	2.95	1.780	0.043
3.59	Adrenaline	40	4.57	3.06	1.510	0.060

APPENDIX 3.

Data tables from chapter 3: 'Does intraductal glucose injection...'.

The following data from analyses of milk samples from the individuals right and left mammary glands were grouped on opposite sides of the table. The time in hours represented the time of day, which in the first instance has not been converted to time with reference to the beginning of the experiment in order that the questions of diurnal variation or extraneous longitudinal effects might be addressed if necessary.

Milk yield from a gland was expressed in mL. Glucose and lactose have been expressed as mmol amounts present in the sample collected. The lactose synthesis rate has been expressed in g/h.

Time h	Right mL	Glucose mmol	Lactose mmol	Lactose g/h	Left mL	Sucrose mmol	Lactose mmol	Lactose g/h
Goat 20 fed								
7								
8	40	0.00	5.89	2.12	40	0.00	5.89	2.12
9	50	0.00	7.28	2.62	44	0.00	6.33	2.28
10	50	0.00	7.44	2.68	41	0.00	6.03	2.17
11	47	0.00	6.76	2.43	40	0.00	5.75	2.07
12	154	26.81	8.08	2.91	150	26.16	6.60	2.38
13	160	27.57	5.96	2.15	160	28.03	5.14	1.85
14	160	28.71	6.77	2.44	162	29.70	4.93	1.77
15	160	24.73	5.96	2.15	162	26.41	4.11	1.48
16	47	0.50	5.33	1.92	41	1.56	4.51	1.62
17	49	0.00	6.96	2.51	37	0.27	5.01	1.80
18	58	0.00	7.46	2.69	45	0.00	6.40	2.30
19	50	0.00	6.51	2.34	38	0.00	5.08	1.83
Goat 26 fed								
7								
8	70	0.00	9.71	3.50	73	0.00	10.25	3.69
9	54	0.00	7.86	2.83	54	0.00	7.58	2.73
10	62	0.00	9.65	3.47	60	0.00	9.03	3.25
11	56	0.00	8.62	3.10	51	0.00	7.51	2.70
12	166	25.66	9.55	3.44	172	25.95	8.73	3.14
13	176	28.14	8.93	3.21	184	30.00	8.09	2.91
14	174	27.82	8.83	3.18	184	27.31	8.09	2.91
15	177	24.84	8.09	2.91	200	30.18	7.11	2.56
16	76	0.68	10.03	3.61	80	2.01	10.56	3.80
17	71	0.00	9.73	3.50	71	0.17	9.01	3.24
18	77	0.00	11.73	4.22	80	0.00	10.83	3.90
19	79	0.00	11.23	4.04	79	0.00	10.83	3.90
Goat 28 fed								
7								
8	68	0.00	8.86	3.19	82	0.00	10.82	3.90
9	58	0.00	8.05	2.90	69	0.00	9.69	3.49
10	68	0.00	10.01	3.60	73	0.00	10.37	3.73
11	50	0.00	7.19	2.59	65	0.00	9.24	3.33
12	164	28.84	8.60	3.10	173	27.93	9.07	3.27
13	160	27.00	7.04	2.53	173	27.93	9.07	3.27
14	172	25.67	7.86	2.83	184	27.02	8.41	3.03
15	169	24.62	6.29	2.26	193	28.65	7.51	2.70
16	68	0.12	10.01	3.60	81	0.46	10.00	3.60
17	69	0.00	9.22	3.32	85	0.00	11.07	3.99
18	70	0.00	9.12	3.28	82	0.00	10.96	3.95
19	67	0.00	9.64	3.47	77	0.00	10.55	3.80

Time	Right mL	Glucose mmol	Lactose mmol	Lactose g/h	Left mL	Sucrose mmol	Lactose mmol	Lactose g/h
Goat 20 fasted								
8	30	0.00	4.37	1.57	28	0.00	3.74	1.35
9	29	0.00	4.02	1.45	25	0.00	3.47	1.25
10	32	0.00	3.84	1.38	27	0.00	3.24	1.17
11	33	0.00	3.63	1.31	28	0.00	3.17	1.14
12	146	26.45	4.20	1.51	148	25.69	3.00	1.08
13	148	26.82	4.01	1.44	152	28.98	3.60	1.30
14	148	24.98	4.26	1.53	151	29.40	3.32	1.20
15	144	28.91	3.65	1.31	143			
16	30	0.80	2.84	1.02	28			
17	31	0.00	4.04	1.45	23			
18	33	0.00	4.75	1.71	27			
19	22	0.00	2.98	1.07	22			
Goat 26 fasted								
8	44	0.00	6.03	2.17	45	0.00	5.86	2.11
9	36	0.00	4.63	1.67	31	0.00	3.46	1.25
10	35	0.00	3.97	1.43	34	0.00	3.11	1.12
11	33	0.00	3.63	1.31	33	0.00	3.35	1.21
12	144	24.30	4.14	1.49	148	25.09	3.00	1.08
13	161	31.75	4.90	1.76	170	31.03	3.74	1.35
14	148	24.98	3.51	1.26	150	27.38	3.05	1.10
15	144	29.16	3.90	1.40	156	28.47	2.64	0.95
16	42	2.01	3.98	1.43	39	2.69	2.44	0.88
17	30	0.00	3.15	1.13	31	0.55	2.73	0.98
18	32	0.00	3.79	1.36	29	0.09	3.29	1.18
19	30	0.00	3.40	1.22	25	0.00	2.62	0.94
Goat 28 fasted.								
8	33	0.00	3.68	1.32	40	0.00	3.72	1.34
9	36	0.00	3.47	1.25	37	0.00	3.32	1.20
10	37	0.00	3.07	1.11	46	0.00		
11	35	0.00	3.20	1.15	38	0.00	3.15	1.13
12	142	25.73	3.84	1.38	160	28.55	4.06	1.46
13	142	34.05	4.32	1.56	156	29.74	3.70	1.33
14	145	21.38	3.19	1.15	162	31.54	5.21	1.88
15	140	25.12	3.55	1.28	158	21.28	2.41	0.87
16	38	0.74	3.21	1.16	43	1.15	3.93	1.41
17	40	0.00	3.38	1.22	50	0.00	4.65	1.67
18	40	0.00	3.79	1.36	45	0.00	4.95	1.78
19	35	0.00	3.38	1.22	39	0.00	3.04	1.09

The following data from analyses of milk samples from the individuals right and left mammary glands were grouped on opposite sides of the table. Milk yield from a gland was expressed in mL.

The following where measured and used as confirmations of normalcy where possible: fat and protein concentrations were expressed in g/100g, sodium and potassium were expressed in mM concentrations and the ratio of these was calculated.

Time hrs	Right mL	Fat gm%	Prot gm%	Na mM	K mM	Na:K ratio	Left mL	Fat gm%	Prot gm%	Na mM	K mM	Na:K ratio
fed 20												
7		4.24	3.52	9.8	0.36	48.1		4.27	3.47	9.8	47.6	0.35
8	40	6.09	3.59	10.8	0.33	47.1	40	5.94	3.49	11.1	47.6	0.31
9	50	4.50	3.63	14.8	0.25	47.1	44	4.53	3.62	10.6	49.1	0.34
10	50	3.94	3.55	10.4	0.34	41.6	41	3.99	3.52	11.6	50.1	0.30
11	47	4.08	3.68	12.8	0.29	46.6	40	4.37	3.74	13.4	51.1	0.28
12	154	1.30	1.19	7.8	0.15	21.0	150	1.23	1.20	7.3	19.5	0.16
13	160	1.20	1.01	7.3	0.14	20.0	160	1.18	1.02	11.1	20.0	0.09
14	160	1.34	1.05	7.8	0.13	21.0	162	1.10	0.96	12.6	19.0	0.08
15	160	1.41	1.16	12.4	0.09	22.5	162	1.17	1.07	12.4	20.5	0.09
16	47	3.28	3.41	14.3	0.24	42.6	41	3.29	3.05	14.8	42.6	0.21
17	49	4.04	3.95	16.1	0.25	51.1	37	3.73	3.82	16.6	46.1	0.23
18	58	4.74	3.82	15.3	0.25	45.6	45	4.37	3.80	17.6	46.6	0.22
19	50	4.11	3.96	17.8	0.22	47.1	38	4.09	3.91	18.1	45.1	0.22
fed 26												
7		4.19	2.92	8.8	0.33	54.6		4.04	2.89	7.8	54.2	0.37
8	70	7.90	2.64	10.1	0.26	48.1	73	8.34	2.44	9.4	48.6	0.26
9	54	4.59	2.70	10.8	0.25	49.1	54	4.68	2.66	10.8	49.1	0.25
10	62	4.36	2.81	11.4	0.25	52.6	60	4.08	2.77	10.1	49.1	0.27
11	56	4.06	3.08	11.8	0.26	51.6	51	3.78	3.08	14.6	54.2	0.21
12	166	1.88	1.12	6.6	0.17	25.1	172	1.69	1.11	6.8	24.6	0.16
13	176	1.49	1.07	6.1	0.18	24.6	184	1.51	1.06	6.1	23.6	0.17
14	174	1.69	1.14	7.3	0.16	26.5	184	1.58	1.15	7.6	26.0	0.15
15	177	2.06	1.21	8.6	0.14	23.0	200	1.90	1.18	8.1	23.5	0.15
16	76	4.09	2.94	12.3	0.24	46.1	80	3.91	2.83	13.3	47.6	0.21
17	71	4.68	3.12	13.1	0.24	50.6	71	4.65	3.07	13.3	49.1	0.23
18	77	4.17	3.14	14.1	0.22	50.6	80	3.87	3.07	16.3	53.6	0.19
19	79	3.90	3.02	16.1	0.19	51.6	79	3.53	2.95	18.6	52.6	0.16
fed 28												
7		4.57	3.13	10.6	0.30	55.6		4.45	3.09	9.6	50.1	0.32
8	68	8.52	2.70	13.8	0.20	50.6	82	9.21	2.55	14.6	53.1	0.17
9	58	6.12	2.82	15.4	0.18	51.1	69	5.37	2.81	17.1	51.6	0.16
10	68	5.47	2.95	14.1	0.21	51.1	73	5.12	2.95	16.8	50.1	0.18
11	50	4.91	3.14	16.1	0.20	52.1	65	4.75	3.10	14.8	49.1	0.21
12	164	1.53	1.11	7.8	0.14	23.0	173	1.72	1.24	9.1	24.0	0.14
13	160	1.53	1.06	8.4	0.13	21.6	173	1.55	1.25	9.4	21.6	0.13
14	172	1.69	1.22	9.6	0.13	26.0	184	1.84	1.37	11.6	26.0	0.12
15	169	1.92	1.16	10.3	0.11	19.0	193	2.13	1.42	12.1	23.0	0.12
16	68	3.76	3.11	17.1	0.18	45.1	81	3.84	3.10	24.3	52.6	0.13
17	69	5.23	3.26	17.1	0.19	48.1	85	4.91	3.24	22.0	47.1	0.15
18	70	5.72	3.07	17.1	0.18	49.6	82	5.15	3.00	24.6	51.6	0.12
19	67	4.86	3.12	19.3	0.16	51.1	77	4.65	3.04	22.0	47.6	0.14

Time hrs	Right mL	Fat gm%	Prot gm%	Na mM	K mM	ratio	Left mL	Fat gm%	Prot gm%	Na mM	K mM	Na/K ratio
fasted 20												
8	30	7.90	3.98	15.5	0.26	38.1	28	9.43	4.09	15.8	39.1	0.26
9	29	5.86	4.32	15.3	0.28	38.6	25	6.07	4.43	27.0	36.6	0.16
10	32	5.27	4.41	13.6	0.32	37.1	27	4.89	4.46	14.3	34.1	0.31
11	33	7.16	4.21	13.3	0.32	36.6	28	7.14	4.26	14.0	35.6	0.30
12	146	1.85	0.95	4.0	0.24	7.5	148	1.71	0.95	4.6	7.0	0.21
13	148	1.42	0.76	6.8	0.11	9.5	152	1.20	0.68	5.0	6.5	0.14
14	148	4.66	0.78	7.6	0.10	8.5	151	1.52	0.74	6.0	7.5	0.12
15	144	2.10	0.80	6.3	0.13	7.0	143	1.84	0.75	8.3	10.6	0.09
16	30	8.02	3.02	13.0	0.23	29.6	28	7.21	2.51	12.3	28.1	0.20
17	31	3.82	3.49	16.0	0.22	42.7	23	8.23	3.26	17.0	4.1	0.19
18	33	7.47	3.42	14.8	0.23	42.7	27	7.23	3.37	14.5	38.2	0.23
19	22	8.76	3.29	14.8	0.22	39.2	22	8.13	3.30	16.3	39.6	0.20
fasted 26												
8	44	8.35	3.15	15.0	0.21	40.6	45	8.64	3.24	16.0	41.1	0.20
9	36	5.54	3.53	15.8	0.22	43.1	31	5.90	3.74	17.8	43.6	0.21
10	35	5.66	3.46	15.0	0.23	42.6	34	5.71	3.64	16.8	41.6	0.22
11	33	6.45	3.27	14.8	0.22	42.1	33	6.32	3.45	15.5	40.6	0.22
12	144	2.55	0.97	7.0	0.14	12.5	148	2.46	1.02	7.3	11.0	0.14
13	161	2.26	0.76	8.6	0.09	14.0	170	2.15	0.76	11.3	12.0	0.07
14	148	1.91	0.64	9.6	0.07	12.0	150	1.60	0.64	12.0	10.5	0.05
15	144	2.04	0.61	11.0	0.06	12.1	156	1.73	0.62	12.0	11.1	0.05
16	42	5.67	2.11	18.5	0.11	36.6	39	5.01	1.89	13.6	25.6	0.14
17	30	7.99	2.71	21.0	0.13	42.7	31	7.29	2.59	19.8	42.7	0.13
18	32	8.16	2.76	17.0	0.16	42.2	29	7.52	2.75	17.5	41.7	0.16
19	30	8.16	2.70	20.8	0.13	43.2	25	7.89	2.78	15.3	36.2	0.18
fasted 28												
8	33	9.50	4.30	31.8	0.14	35.6	40	10.20	4.31	31.8	35.6	0.14
9	36	6.10	4.88	33.5	0.15	35.1	37	6.64	5.04	33.5	35.1	0.15
10	37	5.39	4.79	30.8	0.16	35.6	46	5.31	4.94	30.8	35.6	0.16
11	35	4.23	4.71	24.3	0.19	31.6	38	4.02	4.80	24.3	31.6	0.20
12	142	1.98	1.30	12.0	0.11	13.0	160	2.05	1.56	12.0	13.0	0.13
13	142	1.86	0.99	11.3	0.09	10.0	156	1.91	1.09	11.3	10.0	0.10
14	145	1.84	0.94	12.3	0.08	12.5	162	2.17	1.12	12.3	12.5	0.09
15	140	1.87	0.89	11.8	0.08	10.6	158	2.05	1.01	11.8	10.6	0.09
16	38	5.59	3.19	22.0	0.14	33.6	43	6.13	3.09	22.0	33.6	0.14
17	40	7.12	3.59	28.5	0.13	42.7	50	6.62	3.40	28.5	42.7	0.12
18	40	7.44	3.69	26.3	0.14	41.2	45	7.64	3.47	26.3	41.2	0.13
19	35	8.02	3.60	27.0	0.13	40.2	39	8.73	3.46	27.0	40.2	0.13

APPENDIX 4.

Data tables from chapter 4: 'Investigation of the effect of simultaneous modification...'.

The boundaries of the treatment periods were the activation and deactivation of the infusion pumps respectively, glucose and adenosine infusion were started and stopped together. The blocks of missing values indicate that these parameters were only measured once for each animal at each time point.

Time	Goat	Nutrition	Udder Side	Period	Milk Yield ml/h	Lactose g/h	Milk Glucose mM	Blood Flow ml/min	Blood Glucose mM	Fat g/100g	Protein g/100g	Lactose g/100g	Na mM	K mM
-2.09	6	fast	right	BeforeInf	49.1	2.01	0.057	190	1.97	3.54	3.30	3.97	12.5	35
-1.07	6	fast	right	BeforeInf	50.9	2.10	0.051	174	1.95	2.83	3.44	4.01	19.8	50
-0.07	6	fast	right	BeforeInf	42.4	1.76	0.029	140	2.10	2.34	3.41	4.02	16.0	40
0.93	6	fast	right	Infusion	35.8	1.50	0.059	715	3.38	2.34	3.84	4.07	15.2	35
1.93	6	fast	right	Infusion	54.5	2.40	0.116	420	3.56	1.69	3.53	4.28	15.5	40
2.91	6	fast	right	Infusion	46.4	2.14	0.168	570	3.64	1.24	2.96	4.47	17.0	50
4.00	6	fast	right	Infusion	60.9	2.85	0.289	595	3.64	1.22	2.83	4.55	10.0	30
4.93	6	fast	right	Infusion	47.5	2.21	0.231	573	3.65	1.71	2.96	4.51	11.8	30
5.95	6	fast	right	AfterInf	50.1	2.33	0.250	520	3.58	1.58	2.84	4.52	10.5	30
6.98	6	fast	right	AfterInf	46.5	2.14	0.120	90	2.57	1.59	2.81	4.47	9.8	25
7.98	6	fast	right	AfterInf	41.4	1.88	0.065	165	2.48	1.97	2.98	4.40	12.8	30
8.98	6	fast	right	AfterInf	43.8	1.93	0.057	285	2.33	2.35	3.26	4.28	18.5	40
9.95	6	fast	right	AfterInf	34.0	1.48	0.036	230	2.29	2.25	3.24	4.23	23.2	50
10.93	6	fast	right	AfterInf	39.6	1.70	0.051	220	2.18	2.11	3.19	4.17	19.0	45
-2.09	6	fast	left	BeforeInf	61.6	2.54	0.068	*	*	3.32	3.07	4.00	14.0	45
-1.07	6	fast	left	BeforeInf	57.4	2.34	0.048	*	*	2.94	3.22	3.96	16.8	45
-0.07	6	fast	left	BeforeInf	49.4	2.03	0.035	*	*	2.39	3.27	3.99	15.0	40
0.93	6	fast	left	Infusion	28.7	1.18	0.039	*	*	2.50	3.76	4.00	15.5	40
1.93	6	fast	left	Infusion	53.2	2.27	0.044	*	*	2.00	3.53	4.15	16.8	45
2.91	6	fast	left	Infusion	39.8	1.76	0.063	*	*	1.44	2.96	4.30	11.0	35
4.00	6	fast	left	Infusion	61.3	2.80	0.099	*	*	1.30	2.72	4.43	12.2	40
4.93	6	fast	left	Infusion	38.6	1.75	0.113	*	*	1.93	2.79	4.39	14.5	40
5.95	6	fast	left	AfterInf	46.0	2.08	0.113	*	*	1.87	2.69	4.38	12.0	35
6.98	6	fast	left	AfterInf	57.5	2.57	0.091	*	*	1.76	2.60	4.34	13.2	35
7.98	6	fast	left	AfterInf	43.0	1.87	0.066	*	*	2.06	2.74	4.23	13.0	35
8.98	6	fast	left	AfterInf	46.8	1.97	0.060	*	*	2.68	3.10	4.08	13.8	35
9.95	6	fast	left	AfterInf	37.4	1.57	0.045	*	*	2.41	3.14	4.08	14.2	35
10.93	6	fast	left	AfterInf	41.7	1.72	0.061	*	*	2.12	3.20	4.01	13.5	35
-2.06	6	fed	right	BeforeInf	59.8	2.64	0.383	220	2.20	4.05	3.05	4.29	14.2	45
-1.03	6	fed	right	BeforeInf	70.5	3.22	0.366	285	3.61	2.90	3.03	4.43	15.2	45
-0.05	6	fed	right	BeforeInf	61.0	2.83	0.372	325	3.63	2.52	2.97	4.50	15.0	40
0.97	6	fed	right	Infusion	69.9	3.18	0.586	809	5.09	2.29	3.04	4.41	18.5	40
1.95	6	fed	right	Infusion	65.6	3.03	0.736	725	5.36	2.21	3.05	4.48	18.8	40
2.99	6	fed	right	Infusion	37.2	1.80	0.843	850	6.34	1.90	3.02	4.71	17.2	40
3.97	6	fed	right	Infusion	109.1	5.30	1.011	784	6.53	1.95	2.97	4.72	14.0	40
4.97	6	fed	right	Infusion	72.9	3.60	1.083	584	5.97	1.94	2.88	4.79	15.2	40
5.97	6	fed	right	Infusion	69.2	3.42	1.025	567	5.79	1.61	2.68	4.80	14.5	35
6.97	6	fed	right	AfterInf	57.6	2.83	0.752	242	4.14	1.74	2.70	4.77	15.0	35
7.99	6	fed	right	AfterInf	73.0	3.59	0.392	290	3.82	2.44	2.93	4.77	14.0	35
8.99	6	fed	right	AfterInf	62.3	3.01	0.318	435	3.61	2.27	2.83	4.69	16.5	40
10.00	6	fed	right	AfterInf	74.4	3.53	0.351	453	3.60	2.17	2.78	4.61	14.5	35
11.02	6	fed	right	AfterInf	64.1	3.00	0.444	335	3.73	2.38	2.99	4.55	15.0	35
-2.06	6	fed	left	BeforeInf	73.4	3.19	0.342	*	*	4.05	2.84	4.22	14.8	45
-1.03	6	fed	left	BeforeInf	91.2	4.08	0.344	*	*	3.03	2.82	4.34	15.0	45
-0.05	6	fed	left	BeforeInf	69.8	3.13	0.423	*	*	2.42	2.76	4.35	16.2	40
0.97	6	fed	left	Infusion	81.7	3.71	0.449	*	*	2.61	2.91	4.41	17.2	45
1.95	6	fed	left	Infusion	69.0	3.26	0.541	*	*	2.30	2.82	4.58	14.8	40
2.99	6	fed	left	Infusion	43.7	2.15	0.609	*	*	1.98	2.74	4.78	15.5	45

Time	Goat	Nutrition	Udder Side	Period	Milk Yield ml/h	Lactose g/h	Milk Glucose mM	Blood Flow ml/min	Blood Glucose mM	Fat g/100g	Protein g/100g	Lactose g/100g	Na mM	K mM
3.97	6	fed	left	Infusion	117.4	5.79	0.935	*	*	2.02	2.69	4.79	13.5	40
4.97	6	fed	left	Infusion	78.4	3.85	0.988	*	*	1.93	2.56	4.77	13.0	40
5.97	6	fed	left	Infusion	91.9	4.35	1.202	*	*	1.56	2.36	4.60	22.8	40
6.97	6	fed	left	AfterInf	80.8	3.63	0.962	*	*	1.48	2.33	4.36	25.0	35
7.99	6	fed	left	AfterInf	86.4	3.84	0.570	*	*	2.45	2.69	4.32	27.5	40
8.99	6	fed	left	AfterInf	76.5	3.44	0.421	*	*	2.22	2.69	4.37	20.8	40
10.00	6	fed	left	AfterInf	83.3	3.75	0.503	*	*	1.95	2.66	4.37	19.0	40
11.02	6	fed	left	AfterInf	79.6	3.57	0.496	*	*	2.28	2.87	4.36	17.0	40
-2.19	10	fast	right	BeforeInf	29.0	1.48	0.174	343	3.70	8.20	4.40	4.94	10.8	40.5
-1.19	10	fast	right	BeforeInf	29.0	1.41	0.131	301	2.56	5.88	4.53	4.72	12.0	41.5
-0.15	10	fast	right	BeforeInf	34.3	1.55	0.117	218	2.67	5.61	4.56	4.40	11.6	40.9
0.76	10	fast	right	Infusion	36.4	1.61	0.125	623	4.36	6.16	4.57	4.29	12.8	44.5
1.74	10	fast	right	Infusion	35.3	1.51	0.222	583	4.71	6.90	4.55	4.14	16.5	43.5
2.74	10	fast	right	Infusion	44.0	1.77	0.211	563	5.36	6.20	4.75	3.90	14.3	40.9
3.74	10	fast	right	Infusion	35.0	1.32	0.168	535	5.43	6.44	4.91	3.67	12.6	39
4.74	10	fast	right	Infusion	39.0	1.46	0.129	495	5.78	6.17	4.99	3.63	13.6	40.5
5.74	10	fast	right	AfterInf	36.0	1.31	0.162	480	5.71	6.21	4.94	3.54	12.0	35
6.74	10	fast	right	AfterInf	37.0	1.38	0.150	198	3.64	6.91	4.85	3.61	10.8	35
7.74	10	fast	right	AfterInf	21.0	0.81	0.149	230	3.40	6.73	4.75	3.75	10.8	32.9
8.74	10	fast	right	AfterInf	27.0	1.09	0.156	268	3.28	6.51	4.60	3.91	11.3	34.5
9.74	10	fast	right	AfterInf	26.0	1.12	0.234	283	3.24	6.69	4.41	4.19	11.6	35
10.74	10	fast	right	AfterInf	26.0	1.22	0.204	288	3.24	8.06	4.32	4.56	12.0	37.4
-2.19	10	fast	left	BeforeInf	26.0	1.30	0.163	*	*	6.43	4.26	4.86	15.5	39
-1.19	10	fast	left	BeforeInf	21.0	1.01	0.139	*	*	5.00	4.38	4.67	15.8	37.4
-0.15	10	fast	left	BeforeInf	21.8	1.02	0.150	*	*	5.15	4.28	4.54	15.3	37
0.76	10	fast	left	Infusion	21.8	1.00	0.212	*	*	5.47	4.26	4.44	14.8	37
1.74	10	fast	left	Infusion	16.7	0.75	0.248	*	*	6.53	4.41	4.34	16.1	39
2.74	10	fast	left	Infusion	27.0	1.15	0.291	*	*	6.28	4.53	4.15	14.5	37
3.74	10	fast	left	Infusion	23.0	0.94	0.136	*	*	6.71	4.61	3.95	14.3	36.4
4.74	10	fast	left	Infusion	26.0	1.03	0.376	*	*	5.69	4.68	3.84	14.5	40.9
5.74	10	fast	left	AfterInf	24.0	0.94	0.383	*	*	5.78	4.68	3.81	14.5	38
6.74	10	fast	left	AfterInf	29.0	1.17	0.373	*	*	6.92	4.65	3.91	14.3	36.4
7.74	10	fast	left	AfterInf	24.0	0.98	0.318	*	*	5.95	4.56	3.95	16.8	38.4
8.74	10	fast	left	AfterInf	24.0	1.02	0.380	*	*	6.17	4.45	4.13	17.8	40.5
9.74	10	fast	left	AfterInf	23.0	1.03	0.288	*	*	6.44	4.38	4.33	16.3	39
10.74	10	fast	left	AfterInf	19.0	0.88	0.310	*	*	7.72	4.26	4.50	16.7	38
-2.05	22	fed	right	BeforeInf	44.0	2.05	0.331	395	3.24	5.50	3.09	4.53	12.8	45
-1.04	22	fed	right	BeforeInf	47.6	2.32	0.360	500	3.38	3.97	2.86	4.73	11.8	45
-0.05	22	fed	right	BeforeInf	43.0	2.11	0.365	875	3.36	3.56	2.84	4.76	11.8	45
0.95	22	fed	right	Infusion	43.5	2.16	0.533	1038	5.28	3.91	2.88	4.81	11.0	40
1.96	22	fed	right	Infusion	51.0	2.55	0.752	1058	6.03	4.34	2.82	4.85	11.8	40
3.01	22	fed	right	Infusion	20.9	1.06	0.893	1000	5.26	4.17	2.87	4.94	11.2	45
3.96	22	fed	right	Infusion	72.5	3.66	0.926	892	5.67	4.13	2.75	4.90	11.8	45
4.96	22	fed	right	Infusion	51.3	2.62	1.053	905	6.32	3.98	2.70	4.95	10.2	40
5.96	22	fed	right	Infusion	48.1	2.41	0.927	550	6.64	4.39	2.69	4.86	10.0	40
7.00	22	fed	right	AfterInf	52.0	2.64	0.663	300	3.71	3.82	2.69	4.93	9.8	35
8.03	22	fed	right	AfterInf	51.7	2.54	0.421	395	3.63	4.54	2.87	4.77	11.2	40
8.98	22	fed	right	AfterInf	42.9	2.11	0.441	450	3.55	3.18	2.63	4.78	11.5	40
10.03	22	fed	right	AfterInf	50.4	2.47	0.464	570	3.71	3.39	2.64	4.76	11.2	40
11.03	22	fed	right	AfterInf	52.1	2.53	0.540	570	3.62	3.25	2.60	4.71	10.8	35
-2.05	22	fed	left	BeforeInf	39.0	1.85	0.359	*	*	5.10	3.22	4.60	10.8	45
-1.04	22	fed	left	BeforeInf	40.5	1.99	0.400	*	*	3.80	2.98	4.76	11.5	45
-0.05	22	fed	left	BeforeInf	37.0	1.81	0.418	*	*	3.51	2.96	4.76	10.2	40
0.95	22	fed	left	Infusion	40.6	2.00	0.559	*	*	4.07	2.98	4.78	10.2	40
1.96	22	fed	left	Infusion	42.9	2.10	0.786	*	*	4.50	2.86	4.76	10.2	40
3.01	22	fed	left	Infusion	13.4	0.67	0.877	*	*	4.45	2.90	4.88	9.2	40
3.96	22	fed	left	Infusion	69.4	3.42	0.795	*	*	4.46	2.70	4.78	13.8	55
4.96	22	fed	left	Infusion	45.6	2.24	0.865	*	*	4.32	2.69	4.77	4.0	5
5.96	22	fed	left	Infusion	44.3	2.14	0.846	*	*	4.64	2.68	4.69	12.5	50
7.00	22	fed	left	AfterInf	46.9	2.27	0.669	*	*	4.07	2.67	4.69	11.2	45
8.03	22	fed	left	AfterInf	44.9	2.14	0.506	*	*	4.81	2.88	4.63	10.8	40
8.98	22	fed	left	AfterInf	39.5	1.88	0.518	*	*	3.70	2.71	4.61	11.2	40
10.03	22	fed	left	AfterInf	45.6	2.13	0.482	*	*	4.04	2.74	4.54	11.0	40
11.03	22	fed	left	AfterInf	42.7	2.01	0.479	*	*	3.99	2.72	4.56	12.8	45
-2.15	26	fast	right	BeforeInf	40.0	1.62	0.123	333	3.62	7.18	3.19	3.93	12.3	49.9
-1.17	26	fast	right	BeforeInf	35.3	1.56	0.109	219	2.40	3.38	3.14	4.28	13.0	53.5
-0.15	26	fast	right	BeforeInf	41.8	1.83	0.131	312	2.15	3.34	3.11	4.26	10.6	47
0.85	26	fast	right	Infusion	34.0	1.44	0.327	376	3.53	4.51	3.04	4.12	11.3	49.5
1.87	26	fast	right	Infusion	44.9	2.00	0.462	340	3.72	4.41	2.87	4.32	11.6	48.4
2.83	26	fast	right	Infusion	38.4	1.66	0.468	298	4.60	5.45	2.58	4.20	12.0	45

Time	Goat	Nutrition	Udder Side	Period	Milk Yield ml/h	Lactose g/h	Milk Glucose mM	Blood Flow ml/min	Blood Glucose mM	Fat g/100g	Protein g/100g	Lactose g/100g	Na mM	K mM
3.88	26	fast	right	Infusion	60.9	2.69	0.713	361	4.92	5.97	2.34	4.29	10.8	46
4.87	26	fast	right	Infusion	45.5	2.08	0.869	320	4.72	5.88	2.24	4.43	10.6	44
5.87	26	fast	right	Infusion	41.0	1.90	0.789	209	4.60	4.45	2.24	4.49	10.0	40.9
6.83	26	fast	right	AfterInf	37.4	1.67	0.331	87	2.75	4.74	2.23	4.33	9.6	39.9
7.85	26	fast	right	AfterInf	23.5	0.96	0.107	118	2.40	5.24	2.33	3.95	11.6	41.5
8.87	26	fast	right	AfterInf	35.7	1.43	0.098	110	2.08	5.48	2.58	3.89	12.0	45
9.87	26	fast	right	AfterInf	27.0	1.00	0.114	158	2.13	5.69	2.74	3.59	13.0	45.4
10.87	26	fast	right	AfterInf	32.0	1.19	0.122	199	2.42	5.12	2.78	3.60	12.8	44
-2.15	26	fast	left	BeforeInf	38.0	1.50	0.074	*	*	7.40	3.20	3.82	13.0	45
-1.17	26	fast	left	BeforeInf	31.4	1.34	0.067	*	*	4.10	3.26	4.15	14.5	55.4
-0.15	26	fast	left	BeforeInf	35.7	1.53	0.051	*	*	3.39	3.20	4.17	13.3	51.5
0.85	26	fast	left	Infusion	24.0	0.98	0.063	*	*	4.52	3.28	3.97	12.3	48
1.87	26	fast	left	Infusion	25.5	1.07	0.102	*	*	4.47	3.02	4.08	12.6	48
2.83	26	fast	left	Infusion	28.8	1.19	0.120	*	*	5.93	2.67	4.01	12.3	47
3.88	26	fast	left	Infusion	36.8	1.52	0.310	*	*	6.82	2.39	4.02	12.0	47.4
4.87	26	fast	left	Infusion	31.7	1.36	0.457	*	*	6.90	2.26	4.16	12.0	48.4
5.87	26	fast	left	Infusion	34.0	1.54	0.603	*	*	5.03	2.29	4.40	11.8	46
6.83	26	fast	left	AfterInf	34.6	1.54	0.370	*	*	4.35	2.34	4.33	12.0	44.5
7.85	26	fast	left	AfterInf	36.7	1.55	0.240	*	*	5.14	2.61	4.11	13.3	47
8.87	26	fast	left	AfterInf	35.7	1.48	0.107	*	*	4.86	2.78	4.03	16.1	52.9
9.87	26	fast	left	AfterInf	29.0	1.13	0.144	*	*	5.16	2.90	3.79	15.1	47
10.87	26	fast	left	AfterInf	30.0	1.16	0.204	*	*	4.80	2.91	3.75	14.5	44.5
-2.07	28	fast	right	BeforeInf	40.0	1.67	0.084	241	2.42	6.06	3.40	4.05	13.6	49
-1.07	28	fast	right	BeforeInf	36.0	1.58	0.079	261	2.42	3.34	3.56	4.26	14.3	47.4
-0.05	28	fast	right	BeforeInf	41.8	1.75	0.071	496	2.18	4.01	3.52	4.07	14.8	49.5
0.93	28	fast	right	Infusion	39.2	1.55	0.227	631	4.08	4.45	3.46	3.85	16.8	47.4
1.93	28	fast	right	Infusion	40.0	1.69	0.223	568	4.11	4.01	3.33	4.10	14.8	47
2.92	28	fast	right	Infusion	53.5	2.25	0.204	544	4.97	5.23	3.11	4.09	12.3	45.4
3.95	28	fast	right	Infusion	41.2	1.75	0.134	351	3.42	5.84	3.00	4.13	10.8	43.5
4.93	28	fast	right	Infusion	37.2	1.59	0.175	356	4.04	5.61	2.92	4.16	10.8	42.9
5.95	28	fast	right	Infusion	37.7	1.66	0.162	120	4.28	4.80	2.84	4.27	11.6	44.5
6.97	28	fast	right	AfterInf	29.6	1.22	0.109	115	3.39	5.90	2.79	4.01	11.8	40.5
7.93	28	fast	right	AfterInf	25.0	1.00	0.091	175	2.72	5.35	2.83	3.88	12.0	36
8.92	28	fast	right	AfterInf	25.7	1.00	0.106	183	2.60	4.73	2.96	3.77	13.0	37.4
9.82	28	fast	right	AfterInf	27.0	1.02	0.108	226	2.61	5.45	3.14	3.65	13.8	39.9
10.92	28	fast	right	AfterInf	31.9	1.18	0.083	239	2.63	5.77	3.27	3.60	9.0	40.5
-2.07	28	fast	left	BeforeInf	43.0	1.73	0.058	*	*	6.01	3.54	3.90	9.6	48.4
-1.07	28	fast	left	BeforeInf	37.0	1.57	0.098	*	*	3.22	3.68	4.11	11.3	44
-0.05	28	fast	left	BeforeInf	43.9	1.79	0.085	*	*	3.70	3.64	3.95	10.0	43.5
0.93	28	fast	left	Infusion	39.2	1.53	0.111	*	*	4.84	3.59	3.80	9.3	44.5
1.93	28	fast	left	Infusion	34.0	1.39	0.067	*	*	4.41	3.26	3.98	9.6	44
2.92	28	fast	left	Infusion	44.5	1.78	0.089	*	*	5.44	2.97	3.89	12.6	43.5
3.95	28	fast	left	Infusion	38.1	1.48	0.094	*	*	6.37	2.84	3.78	12.8	46.4
4.93	28	fast	left	Infusion	35.3	1.38	0.114	*	*	5.87	2.81	3.79	12.6	44.5
5.95	28	fast	left	Infusion	35.7	1.45	0.111	*	*	4.70	2.82	3.95	13.0	45.4
6.97	28	fast	left	AfterInf	39.8	1.57	0.147	*	*	5.14	2.88	3.82	14.3	46
7.93	28	fast	left	AfterInf	35.5	1.38	0.111	*	*	4.98	3.07	3.78	15.5	45.4
8.92	28	fast	left	AfterInf	38.6	1.48	0.090	*	*	5.01	3.13	3.71	15.1	44
9.82	28	fast	left	AfterInf	36.9	1.33	0.100	*	*	6.08	3.23	3.51	14.5	43.5
10.92	28	fast	left	AfterInf	42.9	1.53	0.105	*	*	5.99	3.29	3.47	15.3	45
-2.01	33	fast	right	BeforeInf	42.3	1.76	0.072	292	2.31	6.14	3.64	4.04	16.5	30
-1.01	33	fast	right	BeforeInf	40.3	1.58	0.059	308	2.15	5.62	4.09	3.80	21.2	30
-0.03	33	fast	right	BeforeInf	32.9	1.27	0.059	505	3.96	4.26	4.28	3.76	19.8	30
0.97	33	fast	right	Infusion	38.6	1.47	0.072	795	6.78	3.91	4.41	3.70	20.5	40
1.97	33	fast	right	Infusion	55.0	2.23	0.094	600	5.97	3.18	4.10	3.93	14.2	30
2.97	33	fast	right	Infusion	45.1	1.86	0.096	550	4.73	3.54	3.80	4.00	14.0	30
4.00	33	fast	right	Infusion	55.6	2.31	0.110	475	5.24	4.59	3.52	4.03	13.5	30
4.99	33	fast	right	Infusion	45.8	1.94	0.115	478	4.89	4.22	3.32	4.12	12.2	30
5.99	33	fast	right	Infusion	50.0	2.07	0.095	380	4.86	4.83	3.30	4.02	12.8	25
7.00	33	fast	right	AfterInf	33.3	1.39	0.094	235	2.50	4.74	3.33	4.05	13.2	25
8.02	33	fast	right	AfterInf	41.1	1.57	0.100	265	2.45	6.63	3.49	3.72	14.8	25
9.02	33	fast	right	AfterInf	25.9	0.87	0.126	280	2.61	8.61	3.82	3.27	16.2	25
10.02	33	fast	right	AfterInf	30.4	0.96	0.116	300	2.96	8.62	4.23	3.08	21.5	30
11.00	33	fast	right	AfterInf	20.6	0.63	0.116	295	3.02	7.94	4.65	2.95	25.2	30
-2.01	33	fast	left	BeforeInf	43.4	1.90	0.049	*	*	6.41	3.43	4.25	14.0	35
-1.01	33	fast	left	BeforeInf	43.3	1.90	0.037	*	*	4.98	3.72	4.26	14.8	35
-0.03	33	fast	left	BeforeInf	37.9	1.65	0.044	*	*	4.00	3.74	4.22	12.5	30
0.97	33	fast	left	Infusion	38.4	1.67	0.044	*	*	3.58	3.67	4.23	12.0	30
1.97	33	fast	left	Infusion	47.3	2.09	0.055	*	*	3.23	3.38	4.30	13.2	40
2.97	33	fast	left	Infusion	41.1	1.87	0.064	*	*	3.60	3.09	4.41	14.2	40

Time	Goat	Nutrition	Udder Side	Period	Milk Yield ml/h	Lactose g/h	Milk Glucose mM	Blood Flow ml/min	Blood Glucose mM	Fat g/100g	Protein g/100g	Lactose g/100g	Na mM	K mM
4.00	33	fast	left	Infusion	51.6	2.34	0.083	*	*	4.85	2.85	4.41	10.5	30
4.99	33	fast	left	Infusion	41.6	1.92	0.092	*	*	4.33	2.69	4.47	10.8	30
5.99	33	fast	left	Infusion	44.7	2.04	0.091	*	*	4.70	2.70	4.43	11.8	35
7.00	33	fast	left	AfterInf	37.1	1.69	0.057	*	*	4.62	2.73	4.41	9.0	25
8.02	33	fast	left	AfterInf	37.2	1.52	0.064	*	*	7.00	2.98	3.97	10.8	25
9.02	33	fast	left	AfterInf	28.9	1.07	0.055	*	*	8.67	3.30	3.61	18.0	35
10.02	33	fast	left	AfterInf	30.7	1.09	0.073	*	*	8.08	3.67	3.45	17.5	30
11.00	33	fast	left	AfterInf	23.6	0.82	0.083	*	*	7.00	3.97	3.38	18.0	30
-2.01	33	fed	right	BeforeInf	76.2	3.62	0.212	295	3.15	5.84	3.22	4.61	19.5	40
-1.03	33	fed	right	BeforeInf	66.5	3.31	0.163	295	3.22	3.72	3.24	4.83	15.5	35
-0.01	33	fed	right	BeforeInf	79.9	4.02	0.183	565	4.38	3.47	3.23	4.88	15.2	30
0.97	33	fed	right	Infusion	72.0	3.43	0.223	815	4.92	4.22	3.20	4.62	19.0	30
1.97	33	fed	right	Infusion	94.5	4.63	0.221	775	6.76	4.11	3.13	4.76	15.2	30
2.97	33	fed	right	Infusion	72.1	3.67	0.211	742	7.19	3.56	3.03	4.94	13.5	30
3.97	33	fed	right	Infusion	89.1	4.41	0.261	600	4.93	4.10	3.03	4.81	25.2	35
5.00	33	fed	right	Infusion	104.6	5.17	0.212	567	4.85	4.04	3.07	4.80	15.8	35
5.99	33	fed	right	Infusion	79.2	3.91	0.233	509	3.81	4.25	3.11	4.79	15.8	35
6.99	33	fed	right	AfterInf	76.2	3.81	0.172	233	3.18	3.95	3.09	4.85	19.0	35
8.00	33	fed	right	AfterInf	81.2	4.06	0.146	310	3.34	4.58	3.05	4.85	12.0	30
8.99	33	fed	right	AfterInf	74.5	3.61	0.158	330	3.57	5.02	3.03	4.71	12.5	30
10.00	33	fed	right	AfterInf	95.3	4.64	0.198	320	3.13	4.14	3.05	4.73	11.2	30
11.00	33	fed	right	AfterInf	82.8	3.99	0.240	330	3.08	3.98	3.05	4.68	14.8	35
-2.01	33	fed	left	BeforeInf	72.2	3.54	0.255	*	*	6.94	3.07	4.76	12.5	40
-1.03	33	fed	left	BeforeInf	65.5	3.39	0.253	*	*	3.71	3.15	5.03	13.0	35
-0.01	33	fed	left	BeforeInf	72.8	3.79	0.265	*	*	3.52	3.11	5.05	13.8	40
0.97	33	fed	left	Infusion	66.5	3.36	0.265	*	*	4.49	3.06	4.90	13.5	35
1.97	33	fed	left	Infusion	75.0	3.88	0.289	*	*	4.45	2.81	5.02	13.5	40
2.97	33	fed	left	Infusion	40.4	2.18	0.324	*	*	3.76	2.67	5.23	14.2	45
3.97	33	fed	left	Infusion	104.9	5.38	0.380	*	*	3.80	2.70	4.98	15.0	35
5.00	33	fed	left	Infusion	99.6	4.37	0.630	*	*	2.94	2.50	4.26	34.2	30
5.99	33	fed	left	Infusion	89.2	3.78	0.524	*	*	3.36	2.49	4.11	36.2	30
6.99	33	fed	left	AfterInf	77.2	3.72	0.329	*	*	3.59	2.69	4.68	20.5	30
8.00	33	fed	left	AfterInf	85.7	4.24	0.305	*	*	3.70	2.91	4.80	22.8	50
8.99	33	fed	left	AfterInf	78.7	3.74	0.299	*	*	4.37	2.94	4.61	18.0	35
10.00	33	fed	left	AfterInf	82.9	4.05	0.307	*	*	4.05	2.90	4.74	13.5	35
11.00	33	fed	left	AfterInf	76.2	3.78	0.299	*	*	3.99	2.93	4.82	11.8	35

APPENDIX 5.

Data tables from chapter 5: 'Investigation of biochemical differences between Jersey ...'.

The following data have resulted from multiple analyses of two sets of 120 milk samples.

120 Milk samples from four milkings late in 1990 .

Each line of this table represents one milk sample. Four milk samples where collected from each animal over four milkings.

The concentrations of glucose, glucose-6-phosphate and glucose-1-phosphate were expressed in µM units. Sodium and potassium were expressed in mM units and their ratio was calculated. Fat protein and lactase concentrations were expressed in g/100g. Lactose synthesis rate for that interval was expressed in g/h units.

The column named 'Tag' contains the ear tag numbers of the cows, 'Genetic Group' contains the codes for the three groups of cows mentioned earlier, 'pm/am' refers to whether a milking is afternoon (4pm) or morning (8am) and 'day' refers to whether the milk sample is from the first or second collection day.

Glucose µM	G6P µM	G1P µM	Sodium mM	Potassium mM	Na:K ratio	Milk kg	Fat g/100g	Protein g/100g	Lactose g/100g	Lactose g/h	Tag	pm/am	day
Jerseys with high milk solids.													
84.4	63.87	19.35	19.43	22.20	0.88	4.67	7.03	4.69	4.84	28.25	900	pm	1
152.3	44.98	33.25	18.04	22.20	0.81	6.48	7.03	4.69	4.84	19.59	900	am	1
175.7	46.48	8.89	18.32	16.65	1.10	3.00	5.33	4.91	5.03	18.83	900	pm	2
178.1	40.33	8.21	18.32	22.20	0.82	8.41	5.33	4.91	5.03	26.45	900	am	2
173.4	49.92	31.32	9.16	22.20	0.41	4.54	5.34	4.59	5.30	30.06	1902	pm	1
440.5	37.63	37.00	10.82	33.30	0.33	8.94	5.34	4.59	5.30	29.62	1902	am	1
304.6	28.63	6.42	9.71	27.75	0.35	5.42	6.83	4.33	5.08	34.41	1902	pm	2
400.7	24.74	8.41	7.77	22.20	0.35	10.00	6.83	4.33	5.08	31.75	1902	am	2
132.4	67.46	11.83	18.32	33.30	0.55	2.16	6.26	4.80	4.79	12.92	2903	pm	1
319.8	25.49	16.77	19.98	33.30	0.60	5.55	6.26	4.80	4.79	16.62	2903	am	1
291.7	22.19	12.72	14.15	27.75	0.51	2.42	5.71	4.83	4.83	14.63	2903	pm	2
335.1	28.93	0.26	14.99	27.75	0.54	5.68	5.71	4.83	4.83	17.16	2903	am	2
166.4	22.79	17.08	12.21	22.20	0.55	3.30	6.26	4.68	5.01	20.69	3901	pm	1
284.7	21.59	9.27	16.93	33.30	0.51	7.71	6.26	4.68	5.01	24.14	3901	am	1
210.9	15.89	1.25	11.38	22.20	0.51	3.52	6.77	4.78	4.91	21.63	3901	pm	2
411.2	17.84	7.72	*	*		7.71	6.77	4.78	4.91	23.66	3901	am	2
117.2	57.27	6.47	11.66	27.75	0.42	6.43	7.11	4.52	4.94	39.72	3904	pm	1
343.3	67.46	12.76	12.77	33.30	0.38	9.03	7.11	4.52	4.94	27.88	3904	am	1
353.8	28.63	10.56	7.77	22.20	0.35	5.90	6.51	4.79	5.02	37.04	3904	pm	2
513.1	48.42	25.07	12.49	33.30	0.38	8.11	6.51	4.79	5.02	25.43	3904	am	2
103.1	49.62	4.24	14.43	22.20	0.65	3.13	4.86	5.14	5.44	21.27	3912	pm	1
223.8	31.93	7.50	17.48	27.75	0.63	5.33	4.86	5.14	5.44	18.12	3912	am	1
209.7	20.54	1.10	15.54	27.75	0.56	4.19	6.07	4.95	5.14	26.89	3912	pm	2
355.0	20.99	1.17	16.10	22.20	0.72	6.08	6.07	4.95	5.14	19.53	3912	am	2
111.3	294.44	13.51	12.21	27.75	0.44	3.79	6.47	4.55	4.85	22.97	4904	pm	1

Glucose µM	G6P µM	G1P µM	Sodium mM	Potassium mM	Na:K ratio	Milk kg	Fat g/100g	Protein g/100g	Lactose g/100g	Lactose g/h	Tag	pm/am	day
321.0	149.62	14.56	16.65	33.30	0.50	8.63	6.47	4.55	4.85	26.17	4904	am	1
258.9	92.35	8.38	10.55	27.75	0.38	3.70	6.40	4.81	4.91	22.71	4904	pm	2
372.6	137.33	6.72	12.77	27.75	0.46	8.11	6.40	4.81	4.91	24.87	4904	am	2
135.9	24.44	7.01	11.38	22.20	0.51	4.23	7.09	4.42	4.86	25.69	5901	pm	1
329.2	39.43	0.23	22.20	38.85	0.57	11.50	7.09	4.42	4.86	34.92	5901	am	1
302.3	17.84	0.24	8.88	16.65	0.53	12.82	6.89	4.59	5.06	81.08	5901	pm	2
386.6	23.84	6.45	11.10	22.20	0.50	8.99	6.89	4.59	5.06	28.42	5901	am	2
140.6	21.44	0.46	10.82	33.30	0.33	4.41	6.54	4.82	5.03	27.70	5902	pm	1
412.4	23.54	1.85	9.99	22.20	0.45	8.85	6.54	4.82	5.03	27.84	5902	am	1
278.8	22.64	0.47	13.32	38.85	0.34	7.27	6.59	4.77	4.98	45.25	5902	pm	2
372.6	19.34	2.10	9.71	27.75	0.35	8.72	6.59	4.77	4.98	27.15	5902	am	2
236.7	51.87	4.79	9.71	27.75	0.35	4.67	6.66	4.79	5.16	30.12	6900	pm	1
526.0	42.13	6.32	15.26	33.30	0.46	7.49	6.66	4.79	5.16	24.15	6900	am	1
508.5	79.16	3.33	15.82	38.85	0.41	3.00	5.46	4.92	5.30	19.85	6900	pm	2
690.0	59.37	4.32	18.32	44.40	0.41	7.40	5.46	4.92	5.30	24.52	6900	am	2
Jerseys with low milk solids.													
240.2	78.56	14.12	8.33	16.65	0.50	5.02	5.06	3.99	4.93	30.95	1	pm	1
526.0	132.83	23.20	10.27	22.20	0.46	9.82	5.06	3.99	4.93	30.27	1	am	1
558.8	101.20	14.91	10.27	22.20	0.46	5.55	5.46	4.02	4.95	34.34	1	pm	2
714.7	37.63	20.71	10.82	22.20	0.49	9.03	5.46	4.02	4.95	27.94	1	am	2
128.9	17.39	0.38	11.66	16.65	0.70	5.64	6.50	4.02	5.08	35.81	2002	pm	1
479.2	16.79	0.86	12.49	22.20	0.56	6.26	6.50	4.02	5.08	19.86	2002	am	1
355.0	15.29	0.78	11.66	22.20	0.53	4.54	5.12	4.15	5.28	29.95	2002	pm	2
343.3	15.44	0.19	*	*		7.36	5.12	4.15	5.28	24.28	2002	am	2
57.4	19.79	4.45	8.05	16.65	0.48	7.40	5.71	3.56	4.87	45.05	2005	pm	1
482.7	19.64	8.67	10.55	22.20	0.47	12.25	5.71	3.56	4.87	37.28	2005	am	1
306.9	21.89	2.46	9.44	22.20	0.42	6.70	5.01	3.54	4.90	41.01	2005	pm	2
572.9	17.69	2.80	8.88	22.20	0.40	11.15	5.01	3.54	4.90	34.13	2005	am	2
117.2	24.44	9.41	6.38	11.10	0.57	5.20	5.73	4.04	5.21	33.85	3003	pm	1
569.4	33.43	7.26	11.38	22.20	0.51	10.66	5.73	4.04	5.21	34.71	3003	am	1
324.5	40.48	7.03	10.27	27.75	0.37	5.86	5.64	4.07	5.24	38.38	3003	pm	2
535.4	26.09	8.53	*	*		11.10	5.64	4.07	5.24	36.36	3003	am	2
144.1	148.72	8.14	9.71	27.75	0.35	6.04	5.95	4.04	4.91	37.04	3004	pm	1
441.7	117.54	11.23	12.77	27.75	0.46	11.28	5.95	4.04	4.91	34.61	3004	am	1
364.4	62.52	9.41	7.49	22.20	0.34	6.26	5.60	4.16	5.03	39.33	3004	pm	2
599.8	46.18	19.38	9.44	27.75	0.34	11.37	5.60	4.16	5.03	35.73	3004	am	2
209.7	80.51	11.01	*	*		5.24	5.47	4.07	5.06	33.16	5637	pm	1
428.8	51.57	17.67	11.66	27.75	0.42	9.30	5.47	4.07	5.06	29.40	5637	am	1
392.5	37.33	7.88	8.88	22.20	0.40	9.82	6.31	4.16	5.04	61.89	5637	pm	2
504.9	41.68	10.59	9.44	22.20	0.42	3.92	6.31	4.16	5.04	12.35	5637	am	2
106.6	45.58	33.40	11.93	22.20	0.54	3.08	4.68	4.44	5.24	20.20	6576	pm	1
420.6	38.98	42.46	11.93	16.65	0.72	7.00	4.68	4.44	5.24	22.94	6576	am	1
336.2	49.92	39.93	12.49	22.20	0.56	3.26	6.20	4.36	5.16	21.03	6576	pm	2
399.5	44.98	21.25	10.55	16.65	0.63	7.71	6.20	4.36	5.16	24.86	6576	am	2
158.2	38.68	6.87	5.00	11.10	0.45	4.05	4.20	4.14	5.22	26.44	6628	pm	1
528.4	43.93	14.07	10.55	27.75	0.38	8.46	4.20	4.14	5.22	27.59	6628	am	1
444.0	24.29	3.99	9.44	22.20	0.42	6.26	5.11	4.11	5.10	39.88	6628	pm	2
604.5	26.54	6.10	7.49	22.20	0.34	8.85	5.11	4.11	5.10	28.22	6628	am	2
215.6	18.74	3.26	10.55	22.20	0.47	3.30	4.87	4.35	5.12	21.15	6630	pm	1
626.8	19.04	0.54	14.43	22.20	0.65	7.40	4.87	4.35	5.12	23.68	6630	am	1
693.6	20.84	2.86	13.32	33.30	0.40	4.54	4.86	4.20	5.04	28.59	6630	pm	2
896.2	18.59	2.11	9.99	16.65	0.60	7.75	4.86	4.20	5.04	24.42	6630	am	2
141.8	31.03	5.65	6.38	16.65	0.38	5.90	5.98	3.92	5.05	37.26	7625	pm	1
531.9	47.97	7.88	7.49	22.20	0.34	10.75	5.98	3.92	5.05	33.93	7625	am	1
331.6	19.94	3.96	6.38	22.20	0.29	5.64	6.48	3.93	4.93	34.75	7625	pm	2
478.0	24.59	4.24	6.11	22.20	0.28	10.40	6.48	3.93	4.93	32.03	7625	am	2
Friesians.													
164.0	329.67	78.24	14.99	27.75	0.54	5.68	4.46	3.63	5.00	35.52	4161	pm	1
515.5	400.74	84.46	17.21	33.30	0.52	14.05	4.46	3.63	5.00	43.92	4161	am	1
465.1	140.33	81.38	14.99	33.30	0.45	7.22	4.40	3.71	5.00	45.15	4161	pm	2
578.8	185.75	90.60	15.54	33.30	0.47	12.11	4.40	3.71	5.00	37.86	4161	am	2
196.8	116.94	22.88	12.77	33.30	0.38	5.02	4.29	3.34	4.67	29.32	5170	pm	1
672.5	80.06	25.43	14.99	38.85	0.39	14.36	4.29	3.34	4.67	41.92	5170	am	1
642.0	102.25	27.35	13.32	38.85	0.34	5.20	4.88	3.26	4.62	30.02	5170	pm	2
674.8	82.31	35.94	12.77	38.85	0.33	13.48	4.88	3.26	4.62	38.92	5170	am	2
192.1	89.05	35.82	13.04	27.75	0.47	7.53	4.85	3.48	5.24	49.34	5195	pm	1
514.3	49.77	25.52	18.04	27.75	0.65	13.48	4.85	3.48	5.24	44.15	5195	am	1
577.6	119.04	41.87	13.04	27.75	0.47	4.67	4.31	3.56	5.31	30.99	5195	pm	2
478.0	79.76	27.49	14.99	27.75	0.54	14.71	4.31	3.56	5.31	48.83	5195	am	2
113.6	30.43	21.38	12.49	27.75	0.45	7.89	4.09	3.35	4.96	48.89	6103	pm	1
403.0	28.93	17.42	15.54	33.30	0.47	14.76	4.09	3.35	4.96	45.75	6103	am	1

Glucose µM	G6P µM	G1P µM	Sodium mM	Potassium mM	Na:K ratio	Milk kg	Fat g/100g	Protein g/100g	Lactose g/100g	Lactose g/h	Tag	pm/am	day
433.5	36.73	16.44	12.49	33.30	0.38	8.15	4.40	3.35	4.82	49.10	6103	pm	2
480.3	15.59	0.60	14.71	33.30	0.44	15.81	4.40	3.35	4.82	47.64	6103	am	2
167.5	59.52	27.11	18.04	27.75	0.65	4.58	4.28	3.56	4.95	28.35	6119	pm	1
848.2	14.84	18.48	21.92	27.75	0.79	9.82	4.28	3.56	4.95	30.39	6119	am	1
604.5	58.62	19.22	16.10	33.30	0.48	4.01	4.01	3.61	4.99	25.00	6119	pm	2
820.1	51.72	15.28	21.65	33.30	0.65	10.57	4.01	3.61	4.99	32.97	6119	am	2
205.0	132.38	28.59	15.26	33.30	0.46	7.22	4.05	3.46	5.18	46.78	6141	pm	1
430.0	58.77	19.95	14.15	33.30	0.42	14.41	4.05	3.46	5.18	46.64	6141	am	1
551.8	55.77	11.24	15.26	33.30	0.46	9.47	4.79	3.43	5.03	59.55	6141	pm	2
736.9	134.03	14.82	13.88	33.30	0.42	14.14	4.79	3.43	5.03	44.46	6141	am	2
249.5	47.07	5.78	16.37	38.85	0.42	9.56	3.80	3.03	4.69	56.04	6151	pm	1
738.1	16.79	4.27	13.88	38.85	0.36	19.30	3.80	3.03	4.69	56.56	6151	am	1
562.3	25.79	4.07	15.26	38.85	0.39	10.88	3.70	3.11	4.64	63.11	6151	pm	2
763.9	26.99	3.81	13.32	38.85	0.34	18.99	3.70	3.11	4.64	55.06	6151	am	2
157.0	57.12	14.91	17.21	33.30	0.52	5.99	4.45	4.02	4.99	37.37	7106	pm	1
577.6	52.62	15.84	14.99	27.75	0.54	7.53	4.45	4.02	4.99	23.49	7106	am	1
485.0	38.83	7.50	15.54	33.30	0.47	5.99	4.05	3.98	5.05	37.82	7106	pm	2
603.4	37.18	8.19	14.43	27.75	0.52	8.77	4.05	3.98	5.05	27.67	7106	am	2
164.0	31.78	7.33	15.82	38.85	0.41	6.78	4.12	3.50	5.09	43.16	7141	pm	1
454.6	28.33	3.76	12.49	33.30	0.38	12.86	4.12	3.50	5.09	40.92	7141	am	1
446.4	35.98	4.27	14.15	33.30	0.42	6.52	4.04	3.52	5.07	41.32	7141	pm	2
554.1	20.54	6.31	11.93	33.30	0.36	14.23	4.04	3.52	5.07	45.09	7141	am	2
195.7	87.25	3.90	16.65	38.85	0.43	4.19	4.46	3.41	4.60	24.06	7767	pm	1
690.0	27.59	5.74	13.60	38.85	0.35	11.06	4.46	3.41	4.60	31.79	7767	am	1
502.6	43.78	4.56	14.43	38.85	0.37	4.49	4.06	3.45	4.54	25.50	7767	pm	2
434.6	33.88	2.14	12.49	38.85	0.32	10.97	4.06	3.45	4.54	31.12	7767	am	2

UDP-glucose and UDP-galactose were not detected in bovine milk with more sensitive measurements than previously published. The concentrations were thus measured to be less than 4µM.

120 Milk samples from four milkings late in 1991 .

The concentration of glucose was expressed in μM units. Lactose synthesis rate was expressed in g/h units. Milk yields per milking were given as weights in kg. Sodium and potassium concentrations were expressed in units of molarity and their ratio was calculated.

The column named 'Tag' contains the ear tag numbers of the cows, 'Genetic Group' contains the codes for the three groups of cows mentioned earlier 'Individual' is equivalent to tag but conveniently numbers the animals, 'pm/am' refers to whether a milking is 4 p.m. or 8 a.m and 'day' refers to whether the milk sample is from the first or second collection day.

Plasma gluc μM	glucose μM	Lactose g/h	Milk kg	Na/K ratio	Tag	Genetic Group	Individual	pm/am	day
	286.7	26.35	4.41	0.433	3904	HS%J	1	pm	1
	296.1	27.51	9.21	0.425	3904	HS%J	1	am	1
3340	297	21.84	3.61	0.336	3904	HS%J	1	pm	2
3460	476.5	25.32	8.37	0.321	3904	HS%J	1	am	2
	371.8	18.38	3.26	1.12	4902	HS%J	2	pm	1
	388	11.56	4.1	0.84	4902	HS%J	2	am	1
3600	337.8	9.48	1.85	0.642	4902	HS%J	2	pm	2
3880	394.8	14.79	5.77	0.65	4902	HS%J	2	am	2
	236.5	22.89	3.88	0.421	4904	HS%J	3	pm	1
	332.7	25.08	8.5	0.41	4904	HS%J	3	am	1
3160	319.9	14.31	2.42	0.429	4904	HS%J	3	pm	2
3680	462.9	19.81	6.7	0.371	4904	HS%J	3	am	2
	350.6	12.26	2.07	0.567	4905	HS%J	4	pm	1
	329.3	21.92	7.4	0.68	4905	HS%J	4	am	1
3540	304.6	22.07	3.74	0.617	4905	HS%J	4	pm	2
3570	491	22.1	7.49	0.475	4905	HS%J	4	am	2
	348.9	17.02	2.91	0.45	5901	HS%J	5	pm	1
	340.4	26.3	8.99	0.483	5901	HS%J	5	am	1
3250	395.7	19.08	3.22	0.517	5901	HS%J	5	pm	2
3390	455.2	27.55	9.3	0.425	5901	HS%J	5	am	2
	300.4	23.9	4.05	0.343	5902	HS%J	6	pm	1
	*	26.11	8.85	0.321	5902	HS%J	6	am	1
3340	412.7	26.81	4.36	*	5902	HS%J	6	pm	2
3770	480.7	26.97	8.77	0.343	5902	HS%J	6	am	2
	451	22.94	3.88	0.383	5903	HS%J	7	pm	1
	416.9	21.23	7.18	0.408	5903	HS%J	7	am	1
3450	367.6	18.17	3.08	0.358	5903	HS%J	7	pm	2
3600	459.5	18.47	6.26	0.307	5903	HS%J	7	am	2
	339.5	27.13	4.54	0.35	5907	HS%J	8	pm	1
	503.7	27.37	9.16	0.307	5907	HS%J	8	am	1
3690	420.3	23.61	3.96	0.321	5907	HS%J	8	pm	2
4010	473.9	29.42	9.87	0.343	5907	HS%J	8	am	2
	639.9	25.32	4.1	0.379	6900	HS%J	9	pm	1
	506.3	27.2	8.81	0.35	6900	HS%J	9	am	1
3960	435.6	24.01	3.88	0.363	6900	HS%J	9	pm	2
3880	439.1	22.49	7.27	0.343	6900	HS%J	9	am	2
	332.7	21.55	3.66	0.333	6901	HS%J	10	pm	1
	633.1	26.2	8.9	0.35	6901	HS%J	10	am	1
3710	397.4	24.72	4.19	0.35	6901	HS%J	10	pm	2
3910	590.5	23.39	7.93	0.321	6901	HS%J	10	am	2
	235.7	28.36	4.63	0.51	3003	LS%J	11	pm	1
	419.5	34.67	11.32	0.49	3003	LS%J	11	am	1
3320	297.8	32.38	5.11	0.47	3003	LS%J	11	pm	2
3540	489.3	33.65	10.62	0.48	3003	LS%J	11	am	2
	357.4	44.62	7.53	0.386	3004	LS%J	12	pm	1
	526.7	33.27	11.23	0.54	3004	LS%J	12	am	1

Plasma gluc µM	glucose µM	Lactose g/h	Milk kg	Na/K ratio	Tag	Genetic Group	Individual	pm/am	day
3900	451	28.8	4.8	0.35	3004	LS%J	12	pm	2
4000	655.2	26.97	8.99	0.379	3004	LS%J	12	am	2
	279.1	28.36	4.63	0.25	4001	LS%J	13	pm	1
	539.5	27.78	9.07	0.257	4001	LS%J	13	am	1
2660	275.7	21.61	3.61	0.24	4001	LS%J	13	pm	2
3600	529.2	28.62	9.56	0.28	4001	LS%J	13	am	2
	502	29.16	4.85	0.25	4002	LS%J	14	pm	1
	881.5	38.42	12.78	0.264	4002	LS%J	14	am	1
3710	492.7	32.23	5.24	0.23	4002	LS%J	14	pm	2
4130	903.6	33.18	10.79	0.22	4002	LS%J	14	am	2
	343.8	22.79	3.79	0.3	5626	LS%J	15	pm	1
	411	35.62	11.85	0.31	5626	LS%J	15	am	1
3390	336.1	27.11	4.49	0.39	5626	LS%J	15	pm	2
3810	478.2	34.44	11.41	0.358	5626	LS%J	15	am	2
	220.4	11.53	1.89	0.442	6628	LS%J	16	pm	1
	287.6	18.67	6.12	0.442	6628	LS%J	16	am	1
3310	424.6	16.48	2.69	0.442	6628	LS%J	16	pm	2
3750	566.7	17.79	5.81	0.63	6628	LS%J	16	am	2
	451	30.07	4.93	0.272	7625	LS%J	17	pm	1
	*	28.36	9.3	0.3	7625	LS%J	17	am	1
3540	343.8	34.01	5.68	*	7625	LS%J	17	pm	2
3800	494.4	26.91	8.99	0.33	7625	LS%J	17	am	2
	366.7	21.96	3.66	0.321	8002	LS%J	18	pm	1
	475.6	25.77	8.59	0.314	8002	LS%J	18	am	1
3340	354.8	20.81	3.44	0.293	8002	LS%J	18	pm	2
3480	438.2	25.32	8.37	0.35	8002	LS%J	18	am	2
	445.9	21.72	3.44	0.237	8003	LS%J	19	pm	1
	544.6	29.35	9.3	0.342	8003	LS%J	19	am	1
3540	444.2	21.59	3.44	0.225	8003	LS%J	19	pm	2
3930	528.4	23.22	7.4	0.333	8003	LS%J	19	am	2
	355.7	26.24	4.19	0.4	8005	LS%J	20	pm	1
	552.2	29.25	9.34	0.433	8005	LS%J	20	am	1
3680	395.7	25.36	4.01	0.367	8005	LS%J	20	pm	2
3880	767.5	28.56	9.03	0.417	8005	LS%J	20	am	2
	406.7	10.69	1.98	0.683	5170	Friesian	21	pm	1
	280.8	17.95	6.65	0.55	5170	Friesian	21	am	1
3430	441.6	12.85	2.38	0.717	5170	Friesian	21	pm	2
3900	433.9	16.17	5.99	0.479	5170	Friesian	21	am	2
	449.3	30.65	5.24	0.492	6103	Friesian	22	pm	1
	360.8	41.36	14.14	0.475	6103	Friesian	22	am	1
3080	353.1	34.42	5.81	0.625	6103	Friesian	22	pm	2
3530	434.8	40.32	13.61	0.442	6103	Friesian	22	am	2
	382	45.62	7.62	0.425	6141	Friesian	23	pm	1
	634.8	36.67	12.25	0.442	6141	Friesian	23	am	1
3320	486.7	37.96	6.21	0.357	6141	Friesian	23	pm	2
3540	593.9	39.85	13.04	0.357	6141	Friesian	23	am	2
	272.3	17.65	3.13	0.525	6142	Friesian	24	pm	1
	503.7	30.3	10.75	0.533	6142	Friesian	24	am	1
3330	352.3	27.91	4.76	0.642	6142	Friesian	24	pm	2
3400	513.9	27.11	9.25	0.608	6142	Friesian	24	am	2
	381.2	38.16	6.83	0.867	6148	Friesian	25	pm	1
	334.4	34.45	12.33	0.608	6148	Friesian	25	am	1
3380	354.8	31.59	5.68	0.883	6148	Friesian	25	pm	2
3790	366.7	39.94	14.36	0.633	6148	Friesian	25	am	2
	508.8	46.56	8.37	0.421	6151	Friesian	26	pm	1
	584.6	38.97	14.01	0.386	6151	Friesian	26	am	1
3390	563.3	43.68	7.8	0.45	6151	Friesian	26	pm	2
3590	667.1	39.34	14.05	0.443	6151	Friesian	26	am	2
	427.1	31.05	5.24	0.467	7117	Friesian	27	pm	1
	611.8	31.05	10.48	0.45	7117	Friesian	27	am	1
3690	541.2	35.95	6.08	0.5	7117	Friesian	27	pm	2
3740	623.7	22.53	7.62	0.458	7117	Friesian	27	am	2
	441.6	34.19	5.77	0.425	7120	Friesian	28	pm	1
	*	32.5	10.97	0.442	7120	Friesian	28	am	1
3890	510.5	31.44	5.24	*	7120	Friesian	28	pm	2
3730	650.1	32.37	10.79	0.408	7120	Friesian	28	am	2
	261.2	30.29	5.07	0.393	7131	Friesian	29	pm	1
	445.9	38.03	12.73	0.433	7131	Friesian	29	am	1
3090	334.4	28.61	4.85	0.5	7131	Friesian	29	pm	2
3540	519.9	37.05	12.56	0.458	7131	Friesian	29	am	2

Plasma gluc µM	glucose µM	Lactose g/h	Milk kg	Na/K ratio	Tag	Genetic Group	Individual	pm/am	day
	419.5	49.04	8.68	0.625	7134	Friesian	30	pm	1
	441.6	29.13	10.31	0.742	7134	Friesian	30	am	1
3680	357.4	21.23	3.7	0.675	7134	Friesian	30	pm	2
4030	505.4	21.23	7.4	0.617	7134	Friesian	30	am	2

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