Development of cattle of superior genotypes: Novel approaches to increasing tolerance of dairy cows to extended milking intervals

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ABSTRACT

The primary constraint to extended milking intervals of dairy cattle without loss of solids production is the storage capacity of the udder needed to accommodate the milk produced over the extended period. This constraint may be overcome by the production of more concentrated milk as the volume of production is a major determinant of udder capacity.

The production of more concentrated milk is being explored by two approaches. The first exploits the natural variation in milk solids' concentration present in commercial dairy herds. The herd test records from LIC, NZDB were used to identify 2 groups of 19 high breeding index Jersey cows with either high or low milk solids content, which were subsequently established at Ruakura in June 1988 along with 19 high BI Friesians. Mean fat concentration in November 1988 was 6.61, 5.32 and 4.74 for high and low solids and Friesian groups respectively. Corresponding values for protein content were 4.34, 3.72 and 3.42%. Effective hours worth of udder capacity in October-November 1988 was 27.4, 24.8 and 22.9 hours for high solids, low solids and Friesian groups respectively.

The second approach involves the use of recombinant DNA techniques to modify the expression of the a-lactalbumin gene, a key component in water secretion in milk. Alpha-lactalbumin, one of the major whey proteins and a component of the enzyme lactose synthetase, plays a major role in regulation and synthesis of lactose, which in turn is the major osmole of milk. Antisense RNA and RNA enzymes can be used for highly specific inhibition of gene expression. These gene blocking agents bind to the 'natural' mRNA and block translation of the message into a protein. An antisense RNA construct with RNA enzyme cleavage activity against the a-lactalbumin gene has been constructed and is currently under evaluation. This construct will be placed downstream to a mammary specific promoter region and inserted into the bovine genome by gene transfer techniques to produce a transgenic animal. These techniques have considerable potential as tools in physiological studies and for producing animals of superior genotypes.

Keywords Milk; milking interval; cows; lactose; ribozyme; a-lactalbumin; antisense DNA

INTRODUCTION

Twice daily milking of the lactating herd is routinely practised on dairy farms throughout New Zealand, but the maintenance of high production under extended milking intervals would have major economic and lifestyle benefits for New Zealand dairy farmers. Most milking interval studies have demonstrated that 16-20 hours is the maximum period between milkings that can be tolerated without a loss of production (Elliot, 1959). In practice, milking cows once daily generally decreases milk yield by up to 50% depending on the stage of lactation. As an explanation, Davis *et al.* (1987) recently found that the capacity of the udder to store milk is likely to be a primary constraint to extended milking intervals. On average lactating cows have only 20-30 hours worth of capacity in the udder. The yield of milk solids under extended milking intervals is limited by the associated volume of production of water (as the major volume component). The production of more concentrated milk may overcome this constraint.

In this paper we wish to outline two approaches being taken to develop dairy cows that produce more concentrated milk and thereby may be able to tolerate extended milking intervals. The first exploits cows in the national herd that naturally produce milk with a higher content of solids. The second approach involves the use of recombinant DNA technology to manipulate the level of key enzymes involved in the secretion of water into milk.

UDDER CAPACITY AS A CONSTRAINT TO EXTENDED MILKING INTERVALS

The loss in milk yield associated with once daily or extended milking intervals is widely recognised.

However, whether the production loss is due to a lack of udder storage capacity and/or chemical or hormonal inhibition of secretion is not well defined. Recently, Davis *et al.* (1987) measured udder storage capacity in Jersey and Friesian cattle.

Hours worth of udder capacity was greater in Jerseys than Friesians and in late than peak lactation (Table 1). Similarly, udder capacity was greater in older than younger cows (data not shown).

TABLE 1 Milk yield and udder capacity of Jerseys and Friesians in peak and late lactation. (From Davis *et al.*, 1987).

	Peak		Late	
	Jersey	Friesian	Jersey	Friesian
Milk yield (litre/d)	15.1	20.2	9.1	11.9
Udder capacity (litre)[1]	16.7	19.9	11.8	12.7
Udder capacity (hours worth)	26.7	23.2	31.7	26.1

[1] measured after a 40 hour milking interval

Regression analysis of effective udder capacity corrected for residual milk volume, showed that yield loss (litres) in cows with 16-20 h, 24-28 h and 28 plus hours worth of capacity were 1.14 (±.25 SEM), 0.95 (± 0.41), 0.81 (± 0.15) and 0.04 (± 0.39) in late lactation. These data indicate that 30+ hours worth of total udder capacity is required to prevent loss of production when milking at 24 hour intervals.

Chemical inhibition of milk secretion has been recently proposed by Wilde *et al.* (1988), who have suggested that milk contains a locally active chemical inhibitor which decreases milk secretion by negative feed-back. A milk fraction containing whey proteins of 10-30 kDa inhibits lactose and casein synthesis in cultured rabbit mammary explants (Wilde and Gamble, 1984) and has also reduced milk secretion and net protein production when introduced into the mammary gland of lactating goats (Wilde *et al.*, 1988). This autocrine feed back mechanism has recently been shown to act by a post-translational degradation of synthesised caseins (Stewart *et al.*, 1988). The location and mode of action of an autocrine factor within the mammary gland however, has not been determined.

PHYSIOLOGY OF LACTOSE AND WATER SYNTHESIS AND SECRETION

Lactose, the major osmole of milk, is the primary determinant of milk water content. Lactose is synthesised in the Golgi lumina of secretory cells from UDP galactose and glucose. This reaction is catalysed by the enzyme lactose synthetase (Takase and Ebner, 1984), which in turn is composed of β1, 4 galactosyltransferase and the whey proteinα -lactalbumin. Alpha-lactalbumin binds to galactosyltransferase increasing its affinity for glucose and this favours the formation of lactose over N acetyl-glucosamine. Lactose formed in the Golgi lumen is unable to permeate the Golgi membrane and, as the major osmole of milk, draws water into the lactose containing vesicles. This osmotic passage is believed to be the main mechanism for bulk water movement into milk (Linzell and Peaker, 1971) and by this means a potential difference is established across the Golgi vesicle and apical cell membrane which induces movement of ions. Golgi derived vesicles are the major pathway of secretion of lactose, water,α-lactalbumin, β-lactoglobulin, caseins and almost all other major organic components of skim milk. The contents of Golgi derived vesicles are secreted at the apical surface by exocytosis.

The regulation of lactose synthesis is not clearly defined, although α-lactalbumin is generally regarded as the major regulating factor. The onset of lactose synthesis is accompanied by the appearance of α-lactalbumin from very low prevailing levels during pregnancy (Turkington *et al.*, 1968). During established lactation however the concentration of α-lactalbumin in milk of some species (up to 5 mg/ml) is such that galactosyltransferase is likely to be always fully saturated in the Golgi (Kuhn *et al.*, 1980). In bovine milk, the α-lactalbumin concentration is around 1-1.2 mg/ml milk. Further, daily injection of 0-100 IU of pituitary-derived bovine growth hormone in lactating cows resulted in a parallel increases (up to 32%) in milk yield, lactose yield

and milk α-lactalbumin concentration over control animals. The increase in α-lactalbumin was proportionately greater than for other mammary synthesised proteins (Eppard *et al.*, 1985). Brew (1970) has suggested the flux of α-lactalbumin through the secretory cells is responsible for the regulation of lactose synthesis at various stages of lactation.

Several other possibilities have been postulated for the control of lactose synthesis. At least in rats, the total galactosyltransferase activity of the tissue increases roughly in proportion to the increasing yield of lactose (Kuhn *et al.*, 1980). However there is no clear means of stimulus for such increases. Lactose synthesis may also be influenced by variations in the concentrations of the substrate UDP-galactose or intracellular glucose. Both are present at concentrations below saturation (based on Michaelis-Menten kinetics). Difficulties associated with assaying UDP-galactose preclude precise measurement of its concentration (Kuhn *et al.*, 1980). The intracellular glucose concentration is likely to reflect factors which affect the rate of glucose transport across the plasma membrane, including variation in extracellular glucose concentration.

RELATIONSHIPS BETWEEN LACTOSE AND α-LACTALBUMIN, FAT AND PROTEIN CONCENTRATION IN MILK AND SUCKLING INTERVAL

The essential role of α-lactalbumin in the biosynthesis of lactose suggests that all milks containing lactose also contain α-lactalbumin. Indeed α-lactalbumin has been found in all milks that have been tested except that of the California sea lion which contains no lactose (Jenness, 1982). A comparison across six, mainly domestic species by Ley and Jenness (1970) found a positive linear relationship (correlation coefficient +0.95) between lactose and α-lactalbumin concentrations in the milk.

The concentration of protein and fat in milk depends primarily on their rates of secretion in relation to that of lactose. As lactose is formed inside the Golgi vesicles of the secretory cell, water is drawn in to maintain a constant osmotic pressure. Protein and subsequently fat are diluted to their final concentration in milk by this mechanism.

There are wide variations in frequency at which young suckle; Northern fur seals suckle about once a week, whilst pigs and humans suckle every few hours (Mepham, 1987). In general the frequency of suckling is inversely related to the concentration of nutrients or solids in the milk (Davies *et al.*, 1983). It is likely that the high concentration of solids in species with long suckling intervals enhances the nutrient storage ability of the mammary gland.

SELECTION AND BREEDING OF A HIGH-PRODUCING COW TOLERANT OF EXTENDED MILKING INTERVALS

An experiment was initiated in 1987 at Ruakura Agricultural Centre to examine the potential of cows with high milk solids content to tolerate extended milking intervals. A survey was carried out of cow herd test data recorded by the Livestock Improvement Corporation, New Zealand Dairy Board. Positive or negative selection pressure for fat and protein %, but balanced for breeding index BI in fat yield was applied across the national herd. Preliminary results identified 430 cows (out of 456 000) that were tested during the 1986/87 season with an average milk protein content greater than 4.8%. This group of 430 cows was further classified based on the following criteria:

1. Breeding index > 125
2. Protein % average > 4.8
3. Peak milk yield > 15.5 litre/d
4. Protein % at peak > 4.5%
5. No test showing < 4.2% protein.

From the larger group of 430 cows a sub group of 43 fulfilled all the above criteria. In addition to this group, the herd test data was screened for a high producing (>125 BI), low milk protein % (<3.7%)group, to be used as a control.

Two groups, each of 19 mixed age Jersey cows were subsequently purchased from 19 commercial herds throughout the North Island and transferred

to No. 5 Dairy, Ruakura in June-July 1988. In addition, a herd of 19 high BI Friesian cows was established with the two Jersey herds. The mean milkfat % of these groups in the previous season (1987/88) was 6.89, 5.23 and 4.47% and protein % was 4.94, 3.62 and 3.45% for the 'high' and 'low' and Friesian groups respectively. For the purchased Jerseys average fat BIs were 132 and 134 respectively.

Feeding, reproduction and milking management practices during 1988/89 were those used routinely at Ruakura (refer Bryant *et al.*, 1985). Measurements of udder capacity were carried out in early lactation using the procedure described by Davis *et al.* (1987). Udder storage capacity was determined as the total volume of milk contained in the udder 40 h after the last milking. Residual milk was removed after intravenous injection of 5 IU oxytocin. Udder capacity in terms of hours-worth of secretion was calculated by dividing total udder capacity by the hourly milk secretion rate determined during twice daily milking. The ability of the cows to tolerate once daily milking (defined by yield loss) was assessed by comparing yields of milk and milk solids when on once daily milking for 2 weeks with those on twice daily milking.

RESULTS AND DISCUSSION

TABLE 2 Milk composition in mid lactation during 1988/89.

	High solids	Low solids	Friesian
Milk yield (litre/d)	12.7	15.0	19.5
Fat %	6.61	5.32	4.74
Protein %	4.34	3.72	3.42
Lactose %	4.92	4.99	4.87
Fat yield (kg/d)	0.84	0.80	0.92
Protein yield (kg/d)	0.55	0.56	0.66
Lactose yield (kg/d)	0.63	0.75	0.95

Milk Composition and Yield

Milk composition differences observed in the 1987/88 season for cows in commercial herds were maintained in mid lactation of the subsequent season (Table 2). Fat and protein yields were not significantly different between the high and low solids herds, but were greater in the Friesian herd. Lactose yield of the high solids herd was 16 and 34% less than the low solids and Friesian herds respectively (Table 2). Milk yield was inversely related to milk solids' concentration.

TABLE 3 Udder capacity in October 1988.

	High solids	Low solids	Friesians	SED
Milk yield (litre/d)	13.0	15.5	19.6	1.0
Total udder capacity (hrs worth)	31.4	29.0	25.9	1.5
Residual (hrs worth)	4.1	4.1	3.0	0.9
Effective udder capacity[1] (hrs worth)	27.4	24.8	22.9	1.2

[1] Effective udder capacity = total udder capacity less residual hours worth of capacity.

Udder Capacity

Total and effective udder storage capacity was higher in the high solids than the low solids and Friesian herds (Table 3). Previous studies of udder capacity (Davis *et al.*, 1987) have shown that over 30 hours worth of capacity are necessary to maintain production under a 24 hr milking interval. The high solids herd averaged only 27.4 hours worth of capacity in peak lactation. In subsequent once daily milking studies conducted in November 1988 milk yield loss from once daily milking was 13.9, 17.7 and 17.7% (sed 2.4) for high and low solids and Friesian herds respectively. The combination of insufficient udder capacity and incomplete milking out may account for the loss in milk yield of the high solids herd under once daily milking, and the possible influence of the efficiency of milk removal is currently under evaluation.

USING RECOMBINANT DNA TECHNOLOGY TO MANIPULATE WATER SECRETION IN MILK

Introduction

The development of recombinant DNA

technology has allowed the identification, isolation and manipulation of specific genes. A part of these developments has included systems for the insertion of functional genes into the mammalian genome to produce transgenic livestock. The production of mice, and to a lesser extent sheep with modified genomes is now being performed in many laboratories throughout the world. These techniques for engineering and introducing foreign genes into the mammalian genome offer the potential to generate transgenic animals with modified milk production and/or composition. In this section the use of recombinant DNA techniques for the manipulation of water secretion into milk will be outlined. Techniques for insertion of modified genes into the mammalian genome to produce transgenic livestock are reviewed by Thompson and Tervit (1989).

Strategy for Manipulation of Water Secretion

As outlined earlier, lactose is the major osmole of milk, and as such regulates the secretion of water into the milk. Alpha-lactalbumin is a component of the key enzyme lactose synthetase and plays a major role in regulation and synthesis of lactose. The interaction of α-lactalbumin with UDP-galactosyltransferase lowers the km of this enzyme for glucose so that lactose synthesis can take place at physiological concentrations of glucose.

In principle, a decrease in the amount of α-lactalbumin available in the Golgi apparatus might decrease lactose synthesis, and thus in turn, the amount of water drawn into the Golgi vesicles and ultimately in milk. The amount of α-lactalbumin in the Golgi is likely to be lowered by reducing the expression of the α-lactalbumin gene. Two techniques are now available that could be used to achieve this:

1. Manipulation of α-Lactalbumin Gene Expression by Antisense RNA

Antisense RNA is produced by constructing a piece of DNA that is complementary to the negative strand of the α-lactalbumin gene. RNA produced from this construct binds to the complementary 'natural' mRNA to form a stable duplex. This hybrid formation between mRNA and the antisense RNA blocks translation of mRNA to the protein.

Antisense RNA is one of the natural mechanisms whereby prokaryotes regulate gene expression (Stout and Caskey, 1987). In *E. coli* antisense RNA is involved with the control of plasmid replication. Inhibition of gene expression by artificial antisense RNA was first successfully demonstrated in eukaryotes by Izant and Weintraub (1984). In general, the use of antisense RNA in regulation of expression results in a reduction, but not total inhibition of expression of the target gene. For example, even with a 300 fold excess of antisense RNA over sense mRNA (for thymidine kinase gene), Kim and Wold (1985) found expression was not reduced below 11%. The most effective antisense RNA constructs for reducing gene expression in eukaryotes target the 5' region, or start of the gene and/or exon-intron boundaries. Although recently antisense constructs targeting the 3' region or end of the human creatine kinase-b gene have also been shown to be effectively reduce gene expression (Ch'ng *et al.*, 1988).

2. Manipulation of α-Lactalbumin Gene Expression with Self Cleaving RNA Enzymes

The recent discovery and characterisation of RNA enzymes (ribozymes) with self catalysed cleavage or endoribonuclease activity potentially provides another approach for the manipulation of expression of specific genes (Haseloff and Gerlach, 1988). RNA enzymes which occur naturally in plant virus satellite and viroid RNA, are composed of three regions. Two regions of complementary sequence flanking the cleavage site and a region of high conserved sequence that is responsible for cleavage of the mRNA. The ribozyme flanking regions base pair to the complementary sequence of the substrate mRNA (at the target site) and cleavage of the substrate mRNA occurs by breakage of the 3' 5' phosphodiester linkages. The reaction requires divalent metal ions and is pH dependent.

DNA constructs that produce either an antisense or ribozyme RNA molecule effective

against the α-lactalbumin mRNA will ultimately be attached to a mammary specific promoter region. This construct, containing the antisense or ribozyme message located downstream from a promoter, will be inserted into the bovine genome by gene injection techniques to produce transgenic animals.

Preliminary experiments are carried out to determine the most effective antisense or RNA enzyme constructs, their stability, efficiency and the ratio of antisense or RNA enzyme transcripts to natural mRNA. Eventually, the combination of antisense and RNA cleavage enzymes together in the one construct may provide highly efficient blockage of gene expression, particularly with highly expressed proteins such as α-lactalbumin.

CONCLUSIONS

The primary constraint to high production under extended milking intervals is the storage capacity of the udder. Cows have only 20-30 hours worth of capacity, but require 30+ hours worth of storage under once daily milking, to avoid production loss. The production of more concentrated milk or a reduction in milk volume (or water) content may overcome this constraint.

The two approaches to producing a concentrated milk described above will, above all else, focus research on the physiological constraints to milk production under extended milking intervals and hopefully will lead to the breeding of cattle tolerant of once-daily milking.

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