Cisternal milk in the dairy cow during lactation and after preceding teat stimulation

By HANS-ULRICH PFEILSTICKER, RUPERT M. BRUCKMAIER* AND JÜRG W. BLUM

Institut für Tierzucht der Universität Bern, CH-3012 Bern, Schweiz

(*Received 13 July 1995 and accepted for publication 29 May 1996*)

Summary. Experiments were designed to determine the cisternal milk during machine milking in unfamiliar surroundings, which has previously been shown to inhibit milking-related oxytocin (OT) release and milk ejection. The first experiment was performed with 22 cows in early, mid and late lactation. After cisternal milk was removed, 10 i.u. OT were twice injected intravenously to remove the remaining milk. Total milk yield, cisternal milk yield, cisternal milk fraction (17 and 12% in early and late lactation) and the milk yield obtained in response to the first OT injection significantly decreased from early to late lactation. Cisternal milk yield was similar in front and rear udder halves. However, owing to higher total yield, the cisternal milk fraction was lower in rear than in front halves. Cisternal milk yield and cisternal milk fraction were smaller in primiparous than in older cows. In a further experiment milk distribution in the bovine udder was evaluated after preceding teat stimulation and milk ejection. Teats of 12 cows were manually stimulated for 1 min at 15, 60 or 120 min before milking. Total milk yield was not significantly different with or without teat stimulation. However, cisternal milk yield and fraction were significantly higher in teat stimulated cows than in unstimulated controls but were similar whether cows were stimulated 15, 60 or 120 min before milking.

Milk stored in the udder can be divided into two fractions with respect to its availability for milk removal. The cisternal milk fraction is located in the cisternal cavities of teat and gland and in the large milk ducts and can be removed by overriding the teat sphincter barrier. In contrast, the alveolar milk fraction, which is stored in the alveoli and smaller milk ducts, is fixed by adhesive and capillary forces. This fraction can only be removed after being shifted into the cisternal cavity by oxytocin (OT)-induced myoepithelial contraction, i.e. by milk ejection. The distribution of both milk fractions in the bovine udder has been determined by teat cannulation (Mielke, 1969; Wehowsky *et al.* 1986; Dewhurst & Knight, 1993; Knight & Dewhurst, 1994; Knight *et al.* 1994) and during machine milking in unfamiliar surroundings (Bruckmaier *et al.* 1994*a*). If the milking machine is attached without sufficient premilking teat stimulation, only the cisternal milk is available for milk removal until the liner has provoked an OT release. Our goal was to evaluate the amount of cisternal milk under machine milking conditions and how this is affected by stage of lactation and parity. In addition, the amount of cisternal milk was

* For correspondence.

measured at different time intervals from a preceding manual teat stimulation. Results should provide basic information to optimize milk removal during the first minutes of milking.

MATERIALS AND METHODS

Cows

Cows (Simmental × Red Holstein and Swiss Braunvieh × Brown Swiss breeds with an average milk yield of 7300 kg/year) were from the herd of the Swiss Federal Research Station for Animal Production, Posieux. Cows were kept in tie stalls and fed on maize silage, hay and concentrates according to their individual production levels. All experiments were performed during routine milking times, i.e. Expt 1 from 06.00 to 07.30 (14 h interval from previous milking) and Expt 2 from 16.00 to 17.30 (10 h interval from previous milking).

Materials

Milking was performed at a 45 kPa vacuum level, a 65:35 pulsation ratio and a pulsation rate of 60 cycles/min using Harmony clusters (Alfa Laval Agri, S-147 21 Tumba, Sweden). In Expt 2, in mid and late lactation, teatcups were connected with a quarter milking cluster (Surge RX, Babson Bros & Co., Naperville, IL 60563, USA). Milk flow was continuously recorded with a strain gauge system and conveyed to a strip chart recorder (Schams *et al.* 1984).

Experiments 1 and 2

All milkings were performed immediately after the cows were moved from their familiar barn to unfamiliar surroundings (an operating theatre) known to inhibit the release of OT (Bruckmaier *et al.* 1993, 1994*a*). When milk flow had fallen below 200 g/min, the cluster was removed for ~1 min and 10 i.u. OT was injected intravenously. Then milking was immediately continued. The OT injection was repeated when milk flow again fell below 200 g/min. After the milk fraction in response to the second OT injection (10 i.u., intravenous) had been removed, the udder was emptied by machine stripping. There was at least 1 d between two displacements to avoid adaptation to repeated milking in unfamiliar surroundings.

In Expt 1, the same 21 cows in their first to seventh lactations were used in experimental milkings in early (weeks 5–14), mid (weeks 21–26) and late (weeks 38–43) lactation. For technical reasons only 14 of these cows were available in late lactation. Every trial was repeated and milk yields of all experimental milkings were compared with four additional routine milkings in the familiar barn.

In Expt 2, a 1 min manual teat stimulation was applied at 15, 60 and 120 min before the evening milking. Twelve cows in their second to eighth lactation and in early to mid lactation (weeks 10–19) were milked according to a randomized block design in order to avoid influences of the experimental day.

Terminology

Different milk yields were defined as follows. Total milk yield (kg) was the total amount of milk removed from each cow in one experiment. Cisternal milk yield (kg) was milk removed in unfamiliar surroundings before OT was injected. Cisternal milk fraction (%) was defined as (cisternal milk yield × 100)/(total milk yield). In unfamiliar surroundings the OT1 milk yield (kg) was the milk removed in response to the first OT injection. OT1 milk fraction (%) was (OT1 milk yield × 100)/(total milk yield). The remaining milk yield (kg) was milk removed after the second OT

Table 1. *Milking characteristics during the course of lactation in Experiment 1*

(Values are means ± SEM)

Stage of lactation, weeks...	5–14	21–26	38–43
Milk yield, kg			
Cisternal	$3·5 \pm 0·3^{a}$	$2·3 \pm 0·3^{b}$	$1·3 \pm 0·2^{c}$
After first oxytocin injection	$15·3 \pm 0·5^{a}$	$11·7 \pm 0·4^{b}$	$8·0 \pm 0·4^{c}$
Remaining†	$1·0 \pm 0·3^{a}$	$0·6 \pm 0·1^{a}$	$0·9 \pm 0·1^{a}$
Total	$19·8 \pm 0·7^{a}$	$14·6 \pm 0·5^{b}$	$10·1 \pm 0·5^{c}$
Milk fraction, %			
Cisternal	$17·1 \pm 1·3^{a}$	$14·8 \pm 1·6^{ab}$	$12·3 \pm 1·5^{b}$
After first oxytocin injection	$78·4 \pm 1·6^{a}$	$81·0 \pm 1·5^{a}$	$79·1 \pm 1·7^{a}$
Remaining†	$4·6 \pm 1·2^{a}$	$4·3 \pm 0·4^{a}$	$8·5 \pm 0·7^{b}$

† Milk obtained after the second oxytocin injection and stripping.
[a,b,c] Values in the same line without a common superscript letter were significantly different: $P < 0·05$.

injection and by stripping. Expressed as a percentage, this was (remaining milk yield × 100)/(total milk yield).

Evaluation and statistical analyses

Results are presented as means ± SE. For statistical evaluations the SAS program package, release 6.08 (SAS, 1990), was used. Means were tested for significant differences ($P < 0·05$) using the General Linear Model procedure (GLM) and multiple t test. The repeatability ($\omega = \sigma^2(a)/(\sigma^2(a)+\sigma^2(\epsilon))$) was computed according to Essl (1987), employing the GLM randomized model procedure based on estimated values: $s^2(a) = (\text{MS}(a)-\text{MS}(e))/n$ and $s^2(e) = \text{MS}(e)$, where σ^2 = real variance, s^2 = estimated variance, MS = mean square, a = animal, e = error, n = number of animals. The model $Y = \mu + a_j + e_{ij}$ was used.

RESULTS

Experiment 1

Milk yields. Total milk yields of experimental milkings were significantly higher than in routine milking at all stages of lactation.

Effects of stage of lactation. Total, cisternal and OT1 milk yields decreased significantly from early to mid and from mid to late lactation (Table 1). The remaining milk yield did not change significantly during lactation. The cisternal milk fraction fell significantly during lactation. The OT1 milk fraction did not change, while the remaining milk fraction increased significantly from mid to late lactation. The repeatability of total milk yield, cisternal and the OT1 milk fraction was 0·92, 0·83 and 0·86 respectively.

Milk distribution between udder halves. In mid and late lactation total milk yield was significantly higher in the rear than in the front udder halves (Table 2). Cisternal milk yield was similar in front and rear halves at both stages of lactation. As a result, the cisternal milk fraction was lower in the rear than in the front halves.

Effect of parity. Total milk yield was smaller in primiparous than in multiparous cows, except in late lactation, when no parity effect was apparent (see Table 2). Cisternal milk yield and fraction appeared to be smallest in primiparous cows, however, significantly so only for cisternal milk yield. Cisternal milk yield and fraction were highest in the oldest cows (lactation 7; 3·7 ± 0·8 kg and 21 ± 4% respectively). Total milk yield of cows in their first lactation had a higher persistency (significant interaction effect) and the cisternal milk fraction tended to decrease more slowly than in older cows.

Table 2. *Distribution of milk during lactation in primiparous and multiparous cows and in the front and rear udder halves in Experiment 1*

(Values are means ± SEM)

Stage of lactation, weeks...		5–14	21–26	38–43
Cisternal milk yield, kg				
Parity	1	1·9 ± 0·2[a]	1·5 ± 0·2[ab]	1·1 ± 0·2[b]
	2–7	4·0 ± 0·4[a]	2·5 ± 0·4[b]	1·5 ± 0·3[b]
Udder half	Front	ND	1·2 ± 0·1[a]	0·7 ± 0·1[a]
	Rear	ND	1·1 ± 0·2[a]	0·6 ± 0·1[a]
Cisternal milk fraction, %				
Parity	1	13·2 ± 1·6[a]*	12·5 ± 1·3[a]	11·2 ± 1·5[a]
	2–7	18·4 ± 1·5[a]	15·5 ± 2·1[ab]	13·0 ± 2·2[b]
Udder half	Front	ND	54·4 ± 2·3[a]*	54·6 ± 4·0[a]
	Rear	ND	45·6 ± 2·3[a]	45·5 ± 4·0[a]
Total milk yield, kg				
Parity	1	14·4 ± 0·6[a]*	11·8 ± 0·7[b]*	9·6 ± 0·5[c]
	2–7	21·5 ± 0·7[a]	15·5 ± 0·5[b]	10·4 ± 0·7[c]
Udder half	Front	ND	6·7 ± 0·3[a]*	4·4 ± 0·3[a]*
	Rear	ND	7·6 ± 0·4[a]	5·8 ± 0·4[a]

ND, Not determined.
[a,b,c] Values in the same line without a common superscript letter were significantly different: $P < 0·05$.
* Values within stage of lactation were significantly different between parities or udder halves.

Table 3. *Effects of a 1 min manual teat stimulation at different intervals before milking in Experiment 2*

(Values are means ± SEM)

Interval, stimulation–milking, min...	(No stimulation)	15	60	120
Milk yield, kg				
Cisternal	1·4 ± 0·4[a]	4·1 ± 0·4[b]	3·9 ± 0·5[b]	4·4 ± 0·5[b]
After first oxytocin injection	13·1 ± 0·8[a]	10·0 ± 0·8[b]	10·4 ± 0·7[b]	11·5 ± 0·9[ab]
Remaining†	1·0 ± 0·1[a]	1·1 ± 0·1[a]	0·9 ± 0·1[a]	1·1 ± 0·2[a]
Total	15·5 ± 1·0[a]	15·2 ± 1·1[a]	15·2 ± 1·0[a]	17·0 ± 1·2[a]
Milk fraction, %				
Cisternal	8·4 ± 1·8[a]	26·9 ± 1·6[b]	25·5 ± 2·0[b]	26·1 ± 2·5[b]
After first oxytocin injection	85·3 ± 1·9[a]	65·6 ± 1·7[b]	68·3 ± 1·9[b]	67·5 ± 2·4[b]
Remaining†	6·4 ± 0·8[a]	7·4 ± 0·9[a]	6·2 ± 0·8[a]	6·5 ± 1·0[a]

† Milk obtained after the second oxytocin injection and stripping.
[a,b] Values in the same line without a common superscript letter were significantly different: $P < 0·05$.

Experiment 2

Cisternal milk yield and fraction were significantly higher after manual teat stimulation before milking (Table 2). However, the time between stimulation and milk removal (15, 60 or 120 min) did not affect the amount of cisternal milk. Therefore, OT1 milk fraction was significantly smaller when cows were stimulated before milking. The remaining milk fraction and total milk yield were not significantly different with and without stimulation.

DISCUSSION

Methods

During normal machine milking, liner movement induces oxytocin release and thus elicits milk ejection, so alveolar and cisternal milk cannot be separated. To remove cisternal milk separately, milk ejection must be avoided. Cisternal milk yield was previously determined by draining milk using teat cannulae (Mielke, 1969; Wehowsky *et al.* 1986; Knight *et al.* 1994). This method seems to be suitable in most cases although transient oxytocin release and unintended and unrecognized partial milk ejection during teat cannulation cannot be completely excluded (Mayer *et al.* 1991). In unfamiliar surroundings oxytocin release is absent (Bruckmaier *et al.* 1993; 1996*a*). Therefore, cisternal milk can be measured during machine milking, i.e. under milking vacuum conditions, after removal to unfamiliar surroundings (Bruckmaier *et al.* 1994*a*). The amount of cisternal milk, i.e. the amount of milk that is available without milk ejection, may be different if the teat is just cannulated or if milking vacuum is applied as in our experiments. In contrast to our previous findings (Bruckmaier *et al.* 1994*a*), when we investigated lower yielding cows and found smaller amounts of cisternal milk, the cisternal milk determined in this work was basically similar to that obtained by teat cannulation (Knight *et al.* 1994). Thus milking vacuum seems to have no great influence on the availability of cisternal milk. Furthermore, similar amounts of cisternal milk were obtained during milking after oxytocin receptor blockade and α-adrenergic receptor stimulation, and in unfamiliar surroundings (Bruckmaier *et al.* 1996*b*). Cisternal milk yield was shown to be highly correlated with cisternal cavity size derived from ultrasound cross sections *in vivo*, and frozen sections and corrosion casts after slaughter (Bruckmaier *et al.* 1994*a*).

Definition of milk fractions

After milk removal by normal routine milking additional milk can be obtained after OT administration (Senft *et al.* 1974; Gorewit & Sagi, 1984; Knight *et al.* 1994). In our experiment, total milk yield in response to OT injection in pharmacological amounts and very careful stripping was $\sim 120\%$ of the milk yield obtained during normal routine milkings by the usual milking staff. Therefore, the calculated cisternal milk fraction was slightly smaller compared with normal routine milking. However, even after OT injections a certain amount of milk still remains in the udder (Isaksson & Arnarp, 1988).

Milking without milk ejection

Milk accumulation in the cisternal cavity without preceding milk ejection is due to slow alveolar–cisternal milk transfer. Milk distribution in the udder depends on the cisternal cavity and alveolar tissue size and the amount of milk actually stored in the udder. During lactation cisternal milk yield was reduced to a greater extent than total milk yield, as indicated by a significant decrease in the cisternal milk fraction. Total milk yield is known to decrease during lactation, but to increase with increasing time from the previous milking. Accordingly, the cisternal milk fraction increases with increasing time from the previous milking (Mielke, 1969; Knight *et al.* 1994). Hence milk distribution between alveolar and cisternal fractions in the bovine udder depends on the total amount of stored milk.

The cisternal milk yield was similar in the front and rear udder halves. However, the cisternal milk fraction was higher in the front than in the rear halves. This is consistent with the fact that cisternal size, as derived from ultrasound cross sections, was similar in all quarters (Bruckmaier *et al.* 1994*a*) and total milk yield was higher in rear than in front quarters (Rothenanger *et al.* 1995). Thus, milk distribution in the udder is not only a function of the amount of milk stored in the udder, but also depends on cisternal cavity and alveolar tissue size.

Total milk yield, cisternal milk yield and cisternal milk fraction were smaller in primiparous than in older cows. However, total milk yield of cows in their first lactation had a greater persistency and the cisternal milk yield and fraction tended to decrease more slowly than in older cows. The smaller cisternal milk yield could partly be the cause and/or effect of the smaller total milk yield in primiparous cows (Dewhurst & Knight, 1993). Cows with higher milk yields could force more milk into the cistern. Conversely, smaller cisternal cavities might cause a higher intra-alveolar pressure and also increase the concentration of a substance that inhibits milk secretion (Wilde & Peaker, 1990). Thus it is not surprising that cows with small cisternal proportions respond to thrice-daily milking by a larger increase of milk production than cows with larger cisternal reservoirs (Dewhurst & Knight, 1994).

Milking with premilking teat stimulation

Cows used in Expt 2 were relatively low yielding. This may be the reason for a smaller cisternal fraction without stimulation in Expt 2 than in Expt 1 and agrees with our previous findings (Bruckmaier *et al.* 1994*a*) and with decreasing cisternal fractions during the course of lactation. Milk accumulation in the cisternal cavity was immediately increased by milk ejection (Bruckmaier *et al.* 1994*b*; Wellnitz *et al.* 1996). Therefore, alveolar–cisternal milk transfer during milking was due to oxytocin release and milk ejection (Bruckmaier *et al.* 1994*b*; Pfeilsticker *et al.* 1995). In our experiments a 1 min stimulation was chosen to achieve maximum milk ejection (Mayer *et al.* 1991). Teat stimulation at different times before milking did not change total milk yield, in agreement with Senkel & Mielke (1969). Milk ejection forces the transfer of alveolar milk into the cisternal cavity. Thus, cisternal milk yield and milk fraction were significantly higher with stimulation. Interestingly, there were no significant differences in amounts of cisternal milk if teats were stimulated 15, 60 or 120 min before milking, i.e. the cisternal cavity was filled up to an anatomically determined and individually different maximum. Our results indicate that, in the unmilked udder, milk once ejected into the cisternal compartment does not return to the alveolar tissue, or that an elastic recoil of cisternal milk (Knight, 1994) has already occurred before 15 min after stimulation. Immediately after teat stimulation up to 50% of the total milk yield is stored in the cistern (Bruckmaier *et al.* 1994*b*; Wellnitz *et al.* 1996). This supports the conclusion that retrograde milk flow into the alveolar tissue in unmilked udders occurs shortly after milk ejection, because only ~ 25% of the total milk was found at 15, 60 and 120 min after stimulation. In similar experiments in goats oxytocin treatment had no effect on milk distribution 1 h after injection (Peaker & Blatchford, 1988). On the other hand, further milk accumulation in the cisternal cavity was not observed. Therefore, milk secreted after stimulation was obviously stored in the alveolar tissue. Probably the storage capacity in the alveolar tissue after milk ejection was comparable to the capacity found some hours after milking. Total milk yield was not increased after milk ejection, indicating that the transfer of an autocrine inhibitor of milk secretion from the alveolar tissue to the cisternal cavity (Wilde & Peaker, 1990) did not increase milk secretion rate. It was, however, shown that the concentration of the inhibitor in aveolar milk is important for inhibition of milk secretion. This depends not necessarily only on milk movement, but also on the rate of inhibitor secretion (Wilde *et al.* 1995). It was shown in lactating goats that gland stimulation without milk removal did not stimulate milk yield (Linzell & Peaker, 1971).

This study was supported by Alfa Laval Agri, Sursee (Switzerland) and Tumba (Sweden).

REFERENCES

Bruckmaier, R. M., Pfeilsticker, H.-U. & Blum, J. W. 1996*a* Milk yield, oxytocin and β-endorphin gradually normalize during repeated milking in unfamiliar surroundings. *Journal of Dairy Research* **63** 191–200

Bruckmaier, R. M., Rothenanger, E. & Blum, J. W. 1994*a* Measurement of mammary gland cistern size and determination of the cisternal milk fraction in dairy cows. *Milchwissenschaft* **49** 543–546

Bruckmaier, R. M., Schams, D. & Blum, J. W. 1993 Milk removal in familiar and unfamiliar surroundings: concentrations of oxytocin, prolactin, cortisol and β-endorphin. *Journal of Dairy Research* **60** 449–456

Bruckmaier, R. M., Schams, D. & Blum, J. W. 1994*b* Continuously elevated concentrations of oxytoxin are necessary for complete milk removal in dairy cows. *Journal of Dairy Research* **61** 323–334

Bruckmaier, R. M., Wellnitz, O. & Blum, J. W. 1996*b* Inhibition of milk ejection in dairy cows by oxytocin receptor blockade, α-adrenergic receptor stimulation and in unfamiliar surroundings. *Journal of Dairy Science* **79** (Suppl. 1) Abstr. P157

Dewhurst, R. J. & Knight, C. H. 1993 Changes in the sites of milk storage over the lactation cycle of primiparous and multiparous dairy cows. *Animal Production* **56** 428–429

Dewhurst, R. J. & Knight, C. H. 1994 Relationship between milk storage characteristics and the short-term response of dairy cows to thrice-daily milking. *Animal Production* **58** 181–187

Essl, A. 1987 *Statistische Methoden in der Tierproduktion.* Vienna: Österreichischer Agrarverlag

Gorewit, R. C. & Sagi, R. 1984 Effects of exogenous oxytocin on production and milking variables of cows. *Journal of Dairy Science* **67** 2050–2054

Isaksson, A. & Arnarp, L. 1988 Quantitative estimation of residual milk in bovine udders – a methodological study. *Acta Veterinaria Scandinavica* **29** 259–262

Knight, C. H. 1994 Short-term oxytocin treatment increases bovine milk yield by enhancing milk removal without any direct action on mammary metabolism. *Journal of Endocrinology* **142** 471–473

Knight, C. H. & Dewhurst, R. J. 1994 Once daily milking of dairy cows: relationship between yield loss and cisternal milk storage. *Journal of Dairy Research* **61** 441–449

Knight, C. H., Hirst, D. & Dewhurst, R. J. 1994 Milk accumulation and distribution in the bovine udder during the interval between milkings. *Journal of Dairy Research* **61** 167–177

Linzell, J. L. & Peaker, M. 1971 The effects of oxytocin and milk removal on milk secretion in the goat. *Journal of Physiology* **216** 717–734

Mayer, H., Bruckmaier, R. & Schams, D. 1991 Lactational changes in oxytocin release, intramammary pressure and milking characteristics in dairy cows. *Journal of Dairy Research* **58** 159–169

Mielke, H. 1969 [Intramammary milk storage as a complex function of lactation in cattle.] *Wissenschaftliche Zeitschrift, Karl Marx Universität, Leipzig, Mathematisch-naturwissenschaftliche Reihe* **18** 421–429

Peaker, M. & Blatchford, D. R. 1988 Distribution of milk in the goat mammary gland and its relation to the rate and control of milk secretion. *Journal of Dairy Research* **55** 41–48

Pfeilsticker, H.-U., Bruckmaier, R. M. & Blum, J. 1995 Interruption of machine milking in dairy cows: effects on intramammary pressure and milking characteristics. *Journal of Dairy Research* **62** 559–566

Rothenanger, E., Bruckmaier, R. M. & Blum, J. W. 1995 Association and dissociation of single quarter and total milk flow in dairy cows: effects of milking with and without pre-stimulation. *Milchwissenschaft* **50** 63–67

SAS 1990 *SAS Users' Guide: Statistics.* Cary, NC: SAS Institute

Schams, D., Mayer, H., Prokopp, A. & Worstorff, H. 1984 Oxytocin secretion during milking in dairy cows with regard to the variation and importance of a threshold level for milk removal. *Journal of Endocrinology* **102** 337–343

Senft, B., Klobasa, F. & Pfleiderer, U. E. 1974 [Physiological and environmental factors affecting the amount of residual milk in cows.] *Züchtungskunde* **46** 265–273

Senkel, M. & Mielke, H. 1969 [Intermediate udder stimulation in cows.] *Archiv für Tierzucht* **12** 155–163

Wehowsky, G., Moritz, P., Tröger, F. & Lohr, H. 1986 [Deliberate initiation of incomplete alveolar milk ejections for determining the effect of stimuli.] *Wissenschaftliche Zeitschrift, Karl Marx Universität, Leipzig, Mathematisch-Naturwissenschaftliche Reihe* **35** 273–282

Wellnitz, O., Bruckmaier, R. M. & Blum, J. W. 1996 Inhibition of milk ejection in dairy cows before and during milking by oxytocin receptor blockade, α-adrenergic receptor stimulation and in unfamiliar surroundings. *Proceedings, Symposium on Milk Synthesis, Secretion and Removal in Ruminants*, Berne, Switzerland.

Wilde, C. J., Addey, C. V. P., Boddy, L. M. & Peaker, M. 1995 Autocrine regulation of milk secretion by a protein in milk. *Biochemical Journal* **305** 51–58

Wilde, C. J. & Peaker, M. 1990 Autocrine control in milk secretion. *Journal of Agricultural Science* **114** 235–238