Journal of Dairy Research (2007) **74** 52–57. *© Proprietors of Journal of Dairy Research 2006*
doi:10.1017/S0022029906002160 *First published online 15 September 2006 Printed in the United Kingdom*

Effects of melatonin on the yield and composition of milk from grazing dairy cows in New Zealand

Martin J Auldist[1]*†, Sally-Anne Turner[1], Chris D McMahon[2] and Colin G Prosser[2]‡

[1] Dexcel Ltd., Private Bag 3221, Hamilton, New Zealand
[2] AgResearch Ruakura, Private Bag 3123, Hamilton, New Zealand

Received 25 January 2006 and accepted for publication 9 June 2006

The aim was to determine whether administration of melatonin would alter the yield and composition of milk from grazing dairy cows in summer. Twelve sets of spring-calving identical twin Friesian cows were used in the experiment. In late-November (late spring), one twin from each set was given slow-release melatonin implants behind the ears (108 mg melatonin/cow). Two further implantations occurred at 4-weekly intervals to maintain increased circulating concentrations of melatonin for 12 weeks. The other twin served as a control. Milk yield and composition were measured twice prior to treatment and then four times over the following 12 weeks. Concentrations of melatonin, prolactin and insulin-like growth factor 1 (IGF-1) were measured in blood plasma twice before treatment and then either seven (melatonin and prolactin) or three (IGF-1) further times during the experiment. Management procedures for all cows were similar and cows grazed a daily pasture allowance of approximately 30 kg DM/cow as their sole feed source. In melatonin-treated cows there was a decrease in mean concentrations of prolactin in plasma, but concentrations of IGF-1 did not change. Melatonin reduced milk yield by 6 weeks after treatment and by the end of the 12-week experimental period milk yield in melatonin-treated cows had fallen by 23%. Melatonin also reduced concentrations of lactose in milk, but increased concentrations of fat, protein and casein, changes that were broadly similar to those that occur in late lactation in seasonally calving dairy cows. Thus, the results suggest that some of the variation in the volume and quality of milk throughout the season in New Zealand dairy systems may be due to changes in photoperiod mediated by increased concentrations of plasma melatonin in association with decreased concentrations of plasma prolactin.

Keywords: Melatonin, photoperiod, dairy cows, prolactin, insulin-like growth factor 1.

The New Zealand dairy industry is based around the use of pasture as a low-cost feed, which has led to the adoption of seasonal calving to maximize pasture utilization. Most cows calve just prior to spring, and are dried off (lactation ceased) for periods of 8–10 weeks during winter. This production system is characterized by irregularities in the supply of milk to processors in terms of both quantity and composition, and is accompanied by seasonal variations in the manufacturing potential of the milk (Auldist et al. 1998). In particular, milk from late in the production season (autumn and winter) can have manufacturing properties that differ from those of early and mid-season milk, and some products cannot be made at all during this time (Lucey, 1996). This reduces the ability of manufacturing companies to react to market forces, and necessitates storage of product to meet demand out of season.

Seasonal variation in milk composition in pasture-based, seasonally calving dairying systems has been associated with several factors. Nutritional factors associated with changing availability and quality of pasture through the year (Kefford et al. 1995; Auldist et al. 2000) and physiological changes associated with the stage of lactation of cows (Auldist et al. 1998) are commonly regarded as playing a role (Lucey, 1996). Other environmental and climatic conditions can also affect the seasonality of milk production, but information on the impact on these factors in grazing cows is scarce.

*For correspondence; e-mail: Martin.Auldist@dpi.vic.gov.au
†Present address and address for correspondence: Department of Primary Industries, 1301 Hazeldean Road, Ellinbank VIC 3821, Australia
‡Present address: Dairy Goat Co-operative (N.Z.) Private Bag 1398, Hamilton, New Zealand

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press

Table 1. Ages, days in milk and liveweights for control cows and cows treated with Regulin® melatonin implants. Values are group means (±SD) for $n=12$ at the first implantation

	Control	Treatment
Age (years)	3·5 (±2·4)	3·5 (±2·4)
Days in milk	125 (±13·6)	132 (±14·5)
Liveweight (kg)	426 (±31·0)	425 (±26·3)

One such environmental condition is photoperiod (duration of daylight). Several studies show that increasing the exposure of cattle to light can increase milk yield in cows (Dahl et al. 2000). This is probably due, in part, to increased dry matter intake, but generally the increases in milk yield induced by extending photoperiod cannot be fully accounted for by changes in intake. The endocrine mechanism through which this photoperiod effect is mediated, however, is uncertain. Daylight is known to inhibit the synthesis of melatonin, the hormone produced and secreted from the pineal gland that signals the duration of night. Associated with the lower amounts of melatonin secreted is an increase in the secretion of a number of other pro-lactational hormones, notably prolactin and insulin-like growth factor 1 (IGF-1). It has been proposed that these endocrine effects are responsible for the increase in milk yield reported to occur during long-day photoperiod (Dahl et al. 2000).

Whatever the mechanism, these effects of photoperiod on milk yield suggest that there are some aspects of seasonal variation in milk yield and composition that cannot be altered via the manipulation of nutrition or calving time. The current experiment determined the impact of elevated melatonin on milk yield and composition in grazing dairy cows. This was considered a first step in determining the potential contribution of reduced day-length to the seasonal variation in milk quantity and quality in New Zealand.

Materials and Methods

Animals and design

The study used 12 sets of identical twin Friesian cows sourced from the seasonally calving research herd at Dexcel (Hamilton, New Zealand). All cows had calved just prior to spring. Mean ages, liveweights and days in milk at the first implantation are presented in Table 1. All cows grazed together for the duration of the experiment and were subjected to the same management procedures. Cows were milked twice daily at approx. 7.00 and 16.00 through a common dairy. A daily pasture allowance of approximately 30 kg DM/cow formed 100% of the diet. No supplements were fed.

In late November, approximately 1 month before the summer solstice of 21 December, one twin from each set received the first of three doses of melatonin. The second and third doses of melatonin were given 4 weeks and 8 weeks after the first. The other twins from each twin-set formed the control group. They did not receive any implants but were also run through the cattle crush when the treatment cows received their implants.

Melatonin implants

Melatonin was administered as slow-release implants (Regulin®, NZ Vet, Christchurch, New Zealand), implanted subcutaneously behind the ears. Each implant contained 18 mg melatonin. Based on the dose recommended for red deer (Webster et al. 1991), and personal communication with Dr J Webster (AgResearch Invermay, New Zealand) each cow was given six capsules (108 mg) at each of the three implantation times. At each implantation, three implants were inserted per ear using an implant gun, following thorough disinfection of the implantation site.

Blood sampling

Blood samples were drawn from the coccygeal blood vessel of each cow on two occasions in the week prior to the first melatonin implantation. Thereafter, blood samples were collected on seven further occasions over the duration of the experiment (approximately 2, 4, 6, 8, 10, 11 and 12 weeks after the first melatonin implants were administered). On each occasion, samples were collected in daylight (immediately following the morning milking at approx. 7.00) into vacutainers containing either heparin (for melatonin analyses) or EDTA (for prolactin and IGF-1 analyses). Samples were chilled on ice immediately, then the plasma separated by centrifugation within 1 h of collection.

Milk Sampling

Milk yield was measured electronically on each sampling each day using the Ruakura Milk Harvester. Milk samples were collected from each individual cow in both the treatment and control groups using in-line milk meters. On each milk-sampling occasion, samples were collected at four consecutive milkings (*i.e.* am+pm+am+pm), and the samples bulked to form one sampling per cow. A blood sample was collected (as above) on one morning during each milk sampling (extra blood samples were collected on some mornings when no milk was sampled).

Two such milk samples were collected in the week immediately prior to the first melatonin implantation (so as to obtain reliable baseline data and ensure that milk composition of twins within twinsets was initially similar). Further milk samples were collected 6, 8, 11 and 12 weeks after the first melatonin implantation. Following collection, samples were immediately chilled and subsampled, then frozen, preserved or immediately analysed, depending on assay requirements.

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press

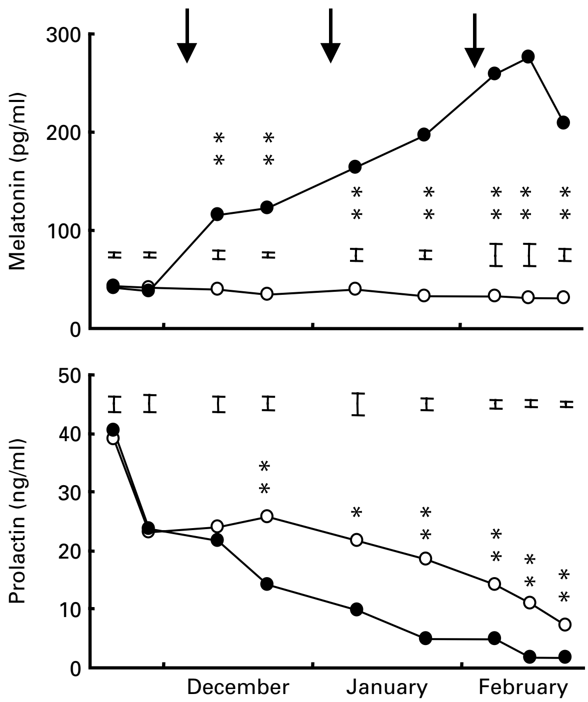

Figure 1. Plasma concentrations of melatonin and prolactin in cows treated with Regulin® melatonin implants (●) and control cows (○). Arrows show times of implantation. Vertical brackets represent the standard error of the difference between treatment means ($n=12$). Asterixes indicate significance of differences between means at particular times (*$P<0{\cdot}05$; **$P<0{\cdot}01$).

Analyses

Melatonin and prolactin were measured in all blood plasma samples, but IGF-1 was only measured in the samples collected prior to the first implantations and the three samples after the final implantations.

Melatonin was measured by radioimmunoassay as described by Fraser et al. (1983). Intra- and inter-assay CV was 7·1% and 11·0%, respectively. Prolactin was measured using the method described by Pearson et al. (1996) but using lactoperoxidase in place of iodogen for preparation of the radio-labelled prolactin. Intra- and inter-assay CV was 8·2% and 6·2%, respectively. Concentrations of IGF-1 in plasma samples were measured by RIA using antibody supplied by Drs LE Underwood and JJ van Wyk, University of North Carolina, USA through the National Hormone and Pituitary Program, National Institutes of Health. Before assay, plasma samples were extracted using the procedure of Bruce et al. (1991) to remove IGF binding proteins. Human recombinant IGF-I was used as the reference standard. Assay sensitivity was 2·3 nmol/l and intra- and inter-assay CV was 4·6% and 8·9%, respectively.

Milk samples were analysed for fat and lactose using an infra-red milk analyser (Milkoscan 133B; Foss Electric, Hillerød, Denmark). Somatic cell counts (SCC) were measured using an automated cell counter (Fossomatic 215; Foss Electric). Total N (TN) and non-casein N (NCN) were measured using macro-Kjeldahl techniques (Barbano et al. 1991), and these N fractions were then used to calculate casein protein ((TN−NCN)×6·38).

Table 2. Mean and SED ($n=12$) concentrations of insulin-like growth factor 1 in plasma of control cows and cows treated with Regulin® melatonin implants

Sampling date	Control	Treatment	SED	*P* value†
20 November	92·6	90·5	3·05	NS
27 November	94·9	95·1	2·35	NS
5 February	110·19	113·5	6·41	NS
12 February	86·8	82·7	3·65	NS
19 February	84·7	84·8	3·14	NS

† NS, difference between means is not significant ($P>0{\cdot}05$)

Statistical Analyses

Data were initially analysed as repeated measurements using the AREPMEASURES procedure in GenStat (GenStat 2002), with twinpair and cows within twin pair as blocks and treatment, age (cow *v.* heifer), date and interactions as fixed effects. Because the effect of melatonin treatment changed significantly through time for most variables (milk yield, fat, protein, casein, prolactin; $P<0{\cdot}05$), this initial analysis was followed by univariate analyses of the data from each date individually (by ANOVA). Data at each date are presented so as to illustrate better these changes of treatment effects through time. Data from a cow with SCC >400 000 cells/ml were excluded from the analysis at one sampling point (data from her twin remained in the statistical model).

Results

Blood plasma

Concentrations of melatonin in melatonin-treated cows were increased several-fold by the first sampling after implantation and remained so for the duration of the trial (Fig. 1). No changes were observed in control cows. Concentrations of plasma prolactin declined over the course of the study in both treated cows and control cows (Fig. 1). The decline was greater for melatonin-treated cows, in which concentrations of prolactin were reduced compared with control cows by 4 weeks after implantation. There was no difference between treated and control cows in plasma concentrations of IGF-1 (Table 2).

Milk yield

Compared with control cows, milk yield of melatonin-treated cows was reduced by 6 weeks after implantation and remained that way for the duration of the trial (Fig. 2). At the final measurement, 12 weeks after implantation,

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press

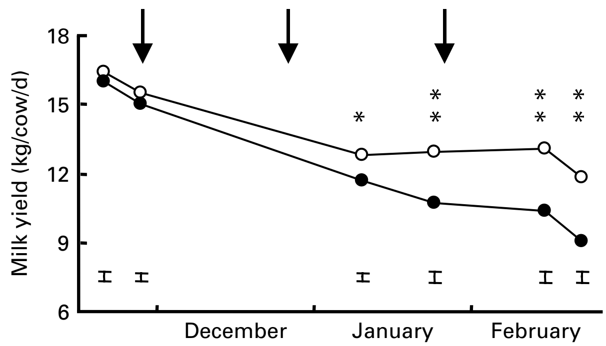

Figure 2. Mean yields of milk from cows treated with Regulin® melatonin implants (●) and control cows (○). Arrows show times of implantation. Vertical brackets represent the standard error of the difference between treatment means (n=12). Asterixes indicate significance of differences between means at particular times (*P<0·05; **P<0·01).

milk yield of the treated cows was 23% (2·8 l) less than the control cows.

Milk composition

Concentrations of fat, protein and casein were increased, while lactose concentrations decreased, in milk from cows treated with melatonin compared with control cows (Fig. 3). Generally these changes in the concentration of the major milk components were apparent by 6 or 8 weeks after implantation (with the exception of casein for which the changes did not become apparent until 11 weeks after implantation).

Discussion

An increase in milk yield of dairy cattle as a result of increasing the amount of daylight per day has been observed in at least eight studies (see Dahl et al. 2000). In many of these experiments, increased milk yield in response to long-day photoperiod was associated with a reduction in the amount of time during which melatonin was elevated in blood. Melatonin is released by the pineal gland during darkness and inhibits secretion of the lactational hormone prolactin by the pituitary gland. Thus concentrations of circulating prolactin consequently increase during daylight (Peters & Tucker, 1978; Buchanan et al. 1993; Dahl et al. 2000). Hence, other studies have attempted to mimic a reduction in photoperiod by feeding melatonin to cows in an established lactation. In these previous studies, melatonin reduced prolactin secretion, but did not change milk volume (Dahl et al. 2000).

In the Southern Hemisphere, the summer solstice occurs on 21 December. At this time, the duration of photoperiod is maximal and, presumably, melatonin secretion is at its lowest and prolactin secretion at its peak. In the current experiment, cows were treated with melatonin approximately 1 month before 21 December. This was done to maximize concentrations of melatonin and, therefore, minimize prolactin secretion in treated cows by the time of the solstice. The results showed that administration of melatonin reduced concentrations of prolactin in cows by 4 weeks after melatonin was implanted. In contrast to previous studies, however, our study showed that the administration of melatonin also caused a significant decrease in milk yield within 6 weeks of implantation, an effect that was sustained until the end of the experiment. The difference amounted to a 23% decline in milk yield in treated cows compared with control cows at the end of the experiment (12 weeks after first implantation).

This effect of melatonin on milk yield in the face of previous results showing no effect may be due to differences in the methods of administration. Implanting melatonin subcutaneously may have introduced melatonin into the circulation more directly than feeding melatonin, although the average daily dose of melatonin per cow in our study (~3·8 mg) was considerably less than that in other feeding studies (*e.g.* 22·5 mg in the study of Dahl et al. 2000). Such direct introduction to the bloodstream may be one reason that a relatively low dose of melatonin in the current experiment resulted in concentrations of melatonin that were well above normal physiological levels. Previous reports show nocturnal melatonin levels ranging between 26 and 90 pg/ml (Berthelot et al. 1990; Burchard et al. 1998; Eriksson et al. 1998). In the current study, concentrations of melatonin in treated cows at times averaged over 200 pg/ml. It is also possible that cows bred within pasture-based, seasonally calving dairying systems may be more sensitive to melatonin than cows bred within non-seasonal systems, even though *Bovidae* are not considered to be especially seasonal (Sanchez-Barcelo et al. 1991) nor sensitive to prolactin in established lactation (Knight, 2000).

It is also important to note that the constantly elevated levels of melatonin induced pharmacologically in treated cows in the current study present a different scenario to the physiological changes in the pattern of melatonin secretion that occur during actual alterations in daylength. Melatonin is elevated in response to darkness, but then reduced in response to daylight and therefore there is substantial diurnal variation of circulating melatonin concentrations even during short-day photoperiod (Gustafson 1994; Dahl et al. 2000). In our experiment, such circadian rhythm may have been overridden by the constantly high melatonin levels such that the experimental conditions may have more accurately represented constant darkness rather than short-day photoperiod. This may be another reason for the differences in our results compared with previous studies.

Implantation of exogenous melatonin not only reduced concentrations of prolactin in blood plasma and decreased milk yield, but also caused changes in milk composition that were similar to those that occur in late lactation as

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press

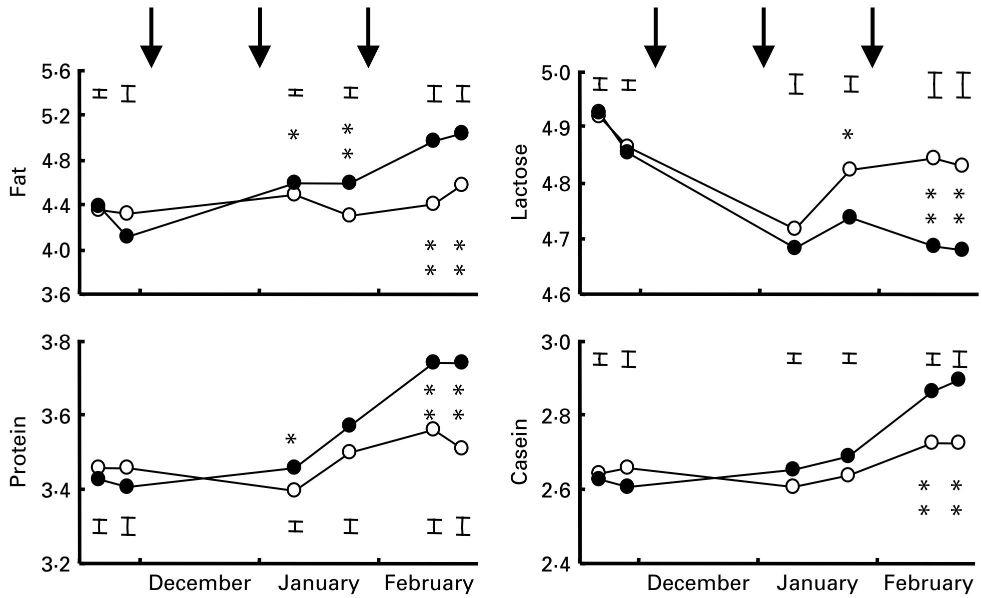

Figure 3. Mean concentrations of fat, lactose, protein and casein (g/100g) in milk from cows treated with Regulin® melatonin implants (●) and control cows (○). Arrows show times of implantation. Vertical brackets represent the standard error of the difference between treatment means (n=12). Asterixes indicate significance of differences between means at particular times (*P<0·05; **P<0·01).

mammary glands of cows approach involution (Auldist et al. 1998). Specifically, concentrations of fat, protein and casein increased, while concentrations of lactose decreased. These changes in milk composition would have offset some of the decrease in yield of the major milk solids (fat, protein and casein) induced by melatonin. Nevertheless, this observation further suggests that at least some of the seasonal variation in milk yield and composition may be due to increased amounts of circulating melatonin during the shorter days of autumn and winter.

The precise mechanism of action of melatonin in inducing a reduction in milk yield in the current experiment remains unclear. In a review of photoperiod effects on milk yield, Dahl et al. (2000) point out that the role of prolactin as a potential mediator of the galactopoietic effects of photoperiod is questioned by two key observations. The first is that the administration of exogenous prolactin to cows in established lactation has no effect on milk yield, at least not within the first 4 weeks (Plaut et al. 1987). The second is that at temperatures below freezing, long-day photoperiod does not change concentrations of prolactin but does affect milk yield (Peters et al. 1980). Dahl et al. (2000) instead put forward the theory that the increases in milk yield observed when photoperiod is increased may be mediated via IGF-1. Dahl et al. (1997) showed that concentrations of IGF-1 were lower in cows exposed to short-day compared with long-day photoperiod, while feeding melatonin has previously had a similar effect. In the current study, IGF-1 was measured in blood on three occasions after the final implantations of melatonin but there were no differences between control or treatment groups at any point. Thus, our data provide no support for the theory that photoperiod effects on milk yield are mediated through melatonin-induced changes in circulating IGF-1 concentrations.

In conclusion, our results show that the administration of melatonin via subcutaneous implants can affect milk production in grazing dairy cattle. Melatonin not only induced a reduction in milk yield, but also caused changes in milk composition that were similar to those observed in cows approaching the end of lactation. The results also showed that exogenous melatonin reduced prolactin secretion but did not affect circulating levels of IGF-1. These results imply there may be a proportion of the seasonal variation in milk yield and composition common to New Zealand dairying systems that cannot be mitigated by on-farm strategies involving manipulations to cow diet or calving pattern. Further studies inducing a more physiological variation in circulating levels of melatonin are required to confirm this observation.

The authors are grateful to B Dow for statistical analyses, M Bryant and laboratory staff for chemical analyses of milk and J Wildermoth for prolactin analyses. We thank also J Kay, B Sugar, B Clarke, A Napper and No. 1 Dairy staff for animal handling and husbandry procedures. This work was funded by the NZ Foundation for Research, Science and Technology.

References

Auldist MJ, Napper AR & Kolver ES 2000 Contribution of nutrition to seasonal variation of milk composition in NZ Friesian and US Holstein dairy cows. *Proceedings Joint Conference AAAP/ASAP*, Sydney

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press

Auldist MJ, Walsh BJ & Thomson NA 1998 Seasonal and lactational influences on bovine milk composition in New Zealand. *Journal of Dairy Research* **65** 401–411

Barbano DM, Lynch JM & Fleming JR 1991 Direct and indirect determinations of true protein content of milk by Kjeldahl analysis: collaborative study. *Journal of Associated Official Analytical Chemists* **74** 281–288

Berthelot X, Laurentie M, Ravault JP, Ferney J & Toutain PL 1990 Circadian profile and production rate of melatonin in the cow. *Domestic Animal Endocrinology* **7** 315–322

Bruce LA, Atkinson T, Hutchinson JSM, Shakespeare RA & MacRae JC 1991 The measurement of insulin-like growth factor 1 in sheep plasma. *Journal of Endocrinology* **128** R1–R4

Burchard JF, Nguyen DH & Block E 1998 Effects of electric and magnetic fields on nocturnal melatonin concentrations in dairy cows. *Journal of Dairy Science* **81** 722–727

Buchanan BA, Chapin LT & Tucker HA 1993 Effect of 12 weeks of daily melatonin on lactation and prolactin in dairy cows. *Journal of Dairy Science* **76** (Suppl. 1) 288. (Abstr.)

Dahl GE, Buchanan BA & Tucker HA 2000 Photoperiodic effects on dairy cattle: A Review. *Journal of Dairy Science* **83** 885–893

Dahl GE, Elsasser TH, Capuco AV, Erdman RA & Peters RR 1997 Effects of a long daily photoperiod on milk yield and circulating concentrations of insulin-like growth factor-1. *Journal of Dairy Science* **80** 2784–2789

Eriksson L, Valtonen M, Laitinen JT, Paananen M & Kaikkoken M 1998 Diurnal rhythm of melatonin in bovine milk: pharmacokinetics of exogenous melatonin in lactation cows and goats. *Acta Veterinaria Scandinavica* **39** 301–310

Fraser S, Cowen P, Franklin M, Franey C & Arendt J 1983 Direct radioimmunoassay for melatonin inn plasma. *Journal of Clinical Chemistry* **29** 396–397

GenStat 2002 GenStat for Windows. Release 6.1, Sixth Edition. (VSN International Ltd. Oxford, UK)

Gustafson GM 1994 Effect of changes in light on hormonal secretion and milk production of dairy cows in early lactation. *Acta Agriculturæ Scandinavica* **44** 160–168

Kefford B, Christian MP, Sutherland BJ, Mayes JJ & Grainger C 1995 Seasonal influences on Cheddar cheese manufacture: influence of diet quality and stage of lactation. *Journal of Dairy Research* **62** 529–537

Knight CH 2000 Overview of prolactin's role in farm animal lactation. *Livestock Production Science* **70** 87–93

Lucey J 1996 Cheesemaking from grass based seasonal milk and problems associated with late-lactation milk. *Journal of the Society of Dairy Technology* **49** 59–64

Pearson AJ, Parry AL, Ashby MG, Choy VJ & Wildermoth JE 1996 Inhibitory effect of increased photoperiod on wool follicle growth. *Journal of Endocrinology* **148** 157–166

Peters RR & Tucker HA 1978 Prolactin and growth hormone responses to photoperiod in heifers. *Endocrinology* **103** 229–234

Peters RR, Chapin LT, Emery RS & Tucker HA 1980 Growth and hormonal response of heifers to various photoperiods. *Journal of Animal Science* **51** 1148–1153

Plaut K, Bauman DE, Agergaard N & Akers RM 1987 Effect of exogenous prolactin administration on lactational performance of dairy cows. *Domestic Animal Endocrinology* **4** 279–290

Prosser CG, Fleet IR, Corps AN, Froesch ER & Heap RB 1990 Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. *Journal of Endocrinology* **126** 437–443

Sanchez-Barcelo EJ, Mediavilla MD, Zinn SA, Buchanan BA, Chapin LT & Tucker HA 1991 Melatonin suppression of mammary growth in heifers. *Biology of Reproduction* **44** 875–879

Webster JR, Suttie JM & Corson ID 1991 Effects of melatonin on reproductive seasonality of male red deer (*Cervus elaphus*). *Journal of Reproduction and Fertility* **92** 1–11

https://doi.org/10.1017/S0022029906002160 Published online by Cambridge University Press