OPTIMIZATION OF LIGHTING CONDITIONS IN DAIRY BARNS

By

Pushpam Muthuramalingam

A Thesis Submitted to

The Faculty of Graduate Studies

In Partial Fulfillment of the Requirements for the Degree of

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Department of Animal Science

The University of Manitoba

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

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Optimization of Lighting Conditions in Dairy Barns. Major Professor; Dr. A. D. Kennedy.

The effect of dim night light (0, 5, 10 and 50 lx) on plasma melatonin and insulin like growth factor-1 (IGF-1) levels was determined in experiment 1, using 12 pre-pubertal Holstein heifers (245 ± 16 d age) held in individual pens in four windowless rooms. The heifers were exposed for 14 consecutive eight hour nights to each light treatment using a 4x4 Latin Square Design and were exposed to ≥ 200 lx light intensity during the16 h day. Heifers were exposed to a 16 h d^{-1} (200 lx) photoperiod for 14 d between treatments. Jugular vein catheters were inserted on day 0, and 14 and blood was sampled on days 0, 4 and 14 of each treatment period at 23:00 h (prior to night-light treatment) and at 01:00, 02:00, 04:00 and 08:00 h the next day (during night-light treatment). Plasma was analyzed by radioimmunoassay for melatonin (all samples) and for IGF-1 (day14, 04:00 h only). Treatment (P = 0.03) and Treatment x Hour (P = 0.02) were significant for plasma melatonin. Exposure to 50 lx suppressed plasma melatonin level by 50 % during the initial two hours of the night, but not thereafter. Light of 5 and 10 lx had no effect on plasma melatonin level during the night. The plasma melatonin response to 50 lx was found on all treatment days studied (Treatment x Hour x Day; P = 0.99). There was no significant night light treatment effect on plasma IGF-1 level (P = 0.89), but plasma IGF-1 level was higher (P = 0.0008) during period 4. To our knowledge, this is the first study to suggest that plasma IGF-1 level is negatively correlated (P = 0.10) to plasma melatonin level at night. In conclusion, the light intensity of 50, but not 5 or 10 lx, suppressed plasma melatonin level

for the initial two hours of the night. Light intensities of 50 lx or less had no effect on plasma IGF-1 level. The results indicated that light intensities of 10 lx or less will not affect plasma melatonin and IGF-1 levels at night in dairy heifers.

In experiment 2, the effects of different photoperiods (14, 16 18 and 20 h d^{-1}) on milk production, composition and plasma IGF-1 levels were determined using 12 lactating dairy cows (four primiparous and eight multiparous). Cows, 109 ± 14 days (Mean ± SD) in milk at the start of the trial, were balanced for residual effects and were assigned to a 4x4 Latin Square Design with six week periods. The cows were assigned to four treatment groups of three cows balanced for parity. The cows were housed in a tie stall barn and curtains were used to control the light environment of the various light treatment groups. During a two weeks pre-treatment period, each group was exposed to an 18 h d^{-1} photoperiod with total darkness (< 1 lx) at night. Cows had *ad libitum* access to a total mixed ration and water and were milked at 04:30 h and 16:30 h daily. Milk production was recorded daily and milk samples from weeks 5 and 6 of each period were analyzed for composition. Body weight was measured on two consecutive days during week 6 of each period. Blood was collected on day 7 of week 3 and week 6 of each period for plasma insulin-like growth factor-1 (IGF-1) analysis. Body condition score was measured during week 6 of each period. Milk production was similar among treatments (P = 0.87). Plasma IGF-1 level did not differ among treatments (P = 0.90). Milk fat tended (P = 0.09) to be elevated by the 18 h d^{-1} photoperiod treatment. Although dry matter intake (DMI) was numerically increased (13 %) for the18 h d^{-1} photoperiod treatment, the effect was non-significant (P = 0.27) and milk produced per kg feed intake (FI) did not differ (P = 0.71) among treatments. Body weight (P = 0.42) and body

condition scoring (P = 0.80) did not differ among treatments. In conclusion, milk production and plasma IGF-1 level did not differ among cows exposed to 14, 16, 18 and 20 h d^{-1} photoperiods with light intensity of ≥ 200 lx and preconditioning to a18 h d^{-1} photoperiod. DMI and milk fat tended to be higher for cows exposed to an 18 h d^{-1} rather than 20, 16 and 14 h d^{-1} photoperiods. All photoperiods (14, 16, 18 and 20 h d^{-1}) may have been perceived as long day by the dairy cows resulting in similar results for milk production, milk composition, DMI, and IGF-1 among treatments. Alternatively, high milk production variance (SE), carry over within the Latin Square, an inadequate treatment period and a long (18 h d^{-1}) pre-conditioning photoperiod may be possible reasons why no differences among treatments were observed.

DEDICATION

I am honoured to dedicate this thesis to my mother, SWARNALAKSMI MUTHURAMALINGAM and to my brother, JEEVA MUTHURAMALINGAM, for their unflinching support and encouragement throughout my domestic and academic life.

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LIST OF ABBREVIATIONS

ADG	average daily gain
cAMP	cyclic adenylate monophosphate
DIM	days in milk
DMI	dry matter intake
FCM	fat corrected milk
FSH	follicular stimulating hormone
GnRH	gonadotropin releasing hormone
HIOMT	hydroxyindole-O-methyltransferase
IGF-1	insulin like growth factor 1
LH	luteinizing hormone
LPP	long photoperiod
lx	lux
NAT	N-acetyltransferase
NE	Noradrenalin
PRL	prolactin
RIA	radioimmunoassay
SCN	suprachiasmatic nucleus
SD	standard deviation
SE	standard error
SPP	short photoperiod
TMR	total mixed ration

1 *GENERAL INTRODUCTION*

Manipulation of photoperiod is an effective way to improve productivity in cattle. Many studies have shown that extending photoperiod increased milk production in dairy cattle and proper lighting provides a safer and more pleasant working environment (Peters et al. 1978; Tanida et al. 1984; Stanisiewski et al. 1985; Piva et al. 1992; Dahl et al. 1997). To maximize the milk production response to extended photoperiod it is necessary to ensure that both the duration of photoperiod and lighting conditions at night are optimum. Day length of 16-18 h increased milk production compared to13 h d^{-1} or less and a 24 h day is not advisable (Dahl et al. 2000). There has been no study that has compared 14 h d^{-1} photoperiod with 13 h d^{-1} or less.

It has been reported that the circadian pattern of melatonin secretion conveys the information about the day and night cycle to all the body tissues and thereby influences other physiological functions of the body (Arendt 1995). Light is the major factor that alters the melatonin secretory pattern in animals and humans. Surprisingly, there has been very little research on the minimum light intensity required to suppress melatonin in cattle. Recently, Lawson and Kennedy (2001) found that a light intensity of 50 lx suppressed melatonin secretion for the first three hours of the dark period in cattle. Stanisiewski et al. (1987) reported that a light intensity of 11 to 16 lx during the night caused an increase in serum prolactin (PRL) level in cattle when the night was preceded by bright light (> 449 lx for 8 or 16 h d^{-1} photoperiod) during the day but, unfortunately, plasma melatonin level was not measured. Because it is feared that exposure to white light for even a few minutes during the night might affect melatonin

production, it is now recommended that only red light (4 – 7.5 W) be used during the night when it is necessary to inspect cows (Dahl et al. 2000). The knowledge of the threshold light intensity of cattle is important for the following reasons: to minimize the number of fixtures required during the day, to reduce power consumption during the day and to facilitate cattle inspection during the night by using a low white light intensity which does not affect circulating melatonin level. One objective of this thesis was to determine the lowest light intensity that can be used in dairy barns without altering the melatonin level at night in cattle.

The current recommendation is that six to eight hours of darkness be provided to dairy cattle to maintain normal physiological and production functions (Dahl et al. 1997; 2000; Chastain and Hiatt. 1998). In Manitoba, photoperiod in dairy barns likely will not be less than 13 to14 h as twice daily milking at 12 h intervals is very common. It would be interesting to determine if milk production is greater with 16 and 18 h d^{-1} photoperiods compared to a photoperiod of 14 h d^{-1}, which is the minimum practical photoperiod. Although some dairy producers may prefer to use a minimum duration of light in their barn to reduce electricity costs, some dairy producers may prefer greater than 18 h d^{-1} of light in their barn to facilitate cow management purposes. Rather than the recommended duration of 6 to 8 h of darkness per 24 h, it is feasible that 4 h of darkness per day would be sufficient to maintain normal physiological functions and milk production in dairy cattle. Therefore, the second objective of this thesis was to compare milk production in lactating dairy cows exposed to 14, 16, 18 and 20 h d^{-1} photoperiods.

2 *LITERATURE REVIEW*

2.1 Neuroendocrine Responses to Light

It has been well established that light has a major impact on the body physiology because melatonin, the pineal hormone, is only produced in the absence of light (Arendt et al. 1981). Melatonin release is regulated by the suprachiasmatic nucleus of the hypothalamus (SCN), the circadian pacemaker, by regulating the activity of N-acetyltransferase (NAT), the rate-limiting enzyme in pineal melatonin production (Klein and Moore 1979). In mammals, light is perceived by the retinal receptors of the eye and a neural signal is conveyed to SCN via the retinohypothalamic tract (Pickard 1982) (Figure 1). In the presence of light, the neural output from the SCN is inhibited and consequently melatonin secretion from the pineal gland is inhibited. During darkness, the SCN is relieved of the inhibitory effect of light and the neural output is transferred to the preganglionic neurons in the upper thoracic spinal cord and postganglionic sympathetic fibres from the superior cervical ganglia to the pineal gland. The sympathetic nerve endings release the neurotransmitter norepinephrine (NE) in the pineal gland (Klein 1985). NE interacts mainly with β-adrenergic receptors and to a lesser extent with α-adrenergic receptors of pinealocytes (Pangerl et al. 1990). The positive action of β-adrenergic receptor stimulation on pineal melatonin synthesis is enhanced by α-adrenergic receptors (Reiter 1990), but the significance of action of α-adrenergic receptor stimulation in melatonin synthesis is unknown. The NE has two actions in the pinealocytes: a) it increases cyclic adenylate monophosphate via β-adrenergic receptors, which in turn

increases activity of NAT; b) it increases uptake of the precursor amino acid tryptophan in the pinealocytes. Tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase, which in turn is converted to 5-hydroxytryptamin (serotonin) by decarboxylation and acetylated to N-acetylserotonin by NAT action. Melatonin is formed by the methylation of N-acetylserotonin by hydroxyindole-O-methyltransferase (HIOMT) action (Illnerova 1988; Reiter 1991).

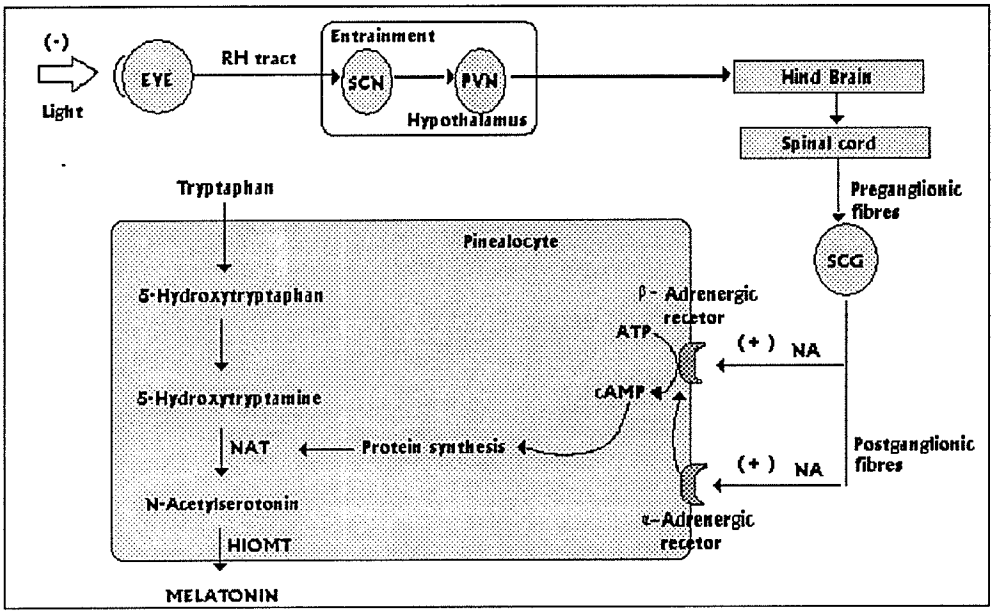

Figure 1. Schematic illustration of the major controlling mechanisms in melatonin synthesis (adapted from Arendt 1995).

During the night, the activity of NAT in the rat pineal is increased more than a hundred fold compared to the day, which results in a high rate of melatonin synthesis at night (Roseboom et al. 1996). Since there is no storage for melatonin (Kokkola and Laitinen 1998), the circulating concentration reflects the pineal melatonin synthesis.

2.1.1 Entrainment of Endogenous Rhythms

2.1.1.1 Circadian Rhythm

A circadian rhythm is a self sustained biological rhythm with a period of 24 h (Saunders 1977). The SCN of the hypothalamus acts as a biological clock to co-ordinate circadian rhythms. Light is considered as a major factor that entrains circadian rhythms via melatonin. In the presence of natural light:dark (L:D) cycles the biological clock is adjusted to an exact 24 h circadian rhythm due to entrainment (Saunders 1977).

It has been reported that an endogenous melatonin rhythm can be reset by a single bright light pulse applied during continuous darkness (Ebling et al. 1988). These authors found that the application of a 1 h light (1 h d^{-1} photoperiod) for two weeks after eight weeks of total darkness reset the melatonin rhythm similar to that found with 8 h d^{-1} photoperiod in rams. Phase shifts in melatonin rhythm have also been reported in humans, but the shift in the rhythm depends on the time of application of light (Decon and Arendt 1996). Phase delay in the melatonin rhythm is induced by exposure to a light in the first half of the night, whereas phase advance is induced by exposure to light in the second half of the night in rats (Illnerova 1988; Illnerova and Sumova 1997). This light induced phase shift in melatonin rhythm is used as an aid for adaptation to shift work in humans (Eastman et al. 1995). The sleep/wake cycle in human is an example of a circadian rhythm associated with melatonin secretion. In some species the rhythm in melatonin production persists in constant darkness, but it does not persist in constant light (Ralph et al. 1971; Tamarkin et al. 1979). Continuous exposure to light abolished increase in pineal melatonin

production at night in calves (Stanisiewski et al. 1988), rats (Ralph et al. 1971), sheep (Rollag and Niswender 1976) and in pre-pubertal heifers (Buchanan et al. 1992).

2.1.1.2 Circannual Rhythm

The SCN also uses photoperiod information to control annual cycles (circannual) of reproduction, especially in seasonal breeders. The melatonin secretory pattern synchronizes reproductive function with the appropriate season to maximize the survival of offspring (Reiter 1993). Studies have reported that the duration of elevated melatonin is the critical factor for both stimulatory and inhibitory effects on reproductive functions in seasonal breeders such as sheep and hamsters (Carter and Goldman 1983; Bittman et al. 1984). The action of melatonin on the gonads varies depending on the seasonal reproduction of animals (Hastings et al. 1989). In short day breeders such as sheep, short photoperiod (SPP) or melatonin administration stimulates gonadal development, whereas, in long day breeders (hamsters) it did not (Hastings et al. 1989).

The daily pattern of melatonin synthesis, and particularly the amplitude of its night peak, appears to be highly variable among individuals of the same species (Arendt et al. 1979). Recently, Coon et al. (1999) reported that the individual variation in melatonin production in sheep was due to the genetic variation in the pineal weight and not due to enzymatic activity and catabolism of circulating melatonin. It was also found that night melatonin level influenced fertility in sheep (Notter and Chemineau 2001). Sheep with low (143 ± 17 pg/ml; mean ± SE) melatonin level at night had

higher fertility than sheep with higher (184 ± 13 pg/ml; mean ± SE) melatonin level at night. It is not known if the level of melatonin at night is important in cattle.

2.2 Factors Controlling Melatonin Synthesis

2.2.1 Light Intensity, Duration, Quality and Time of Administration at Night

A species-specific dose response relationship between light intensity and its suppressive effect on pineal melatonin production has been demonstrated in humans and in animals (Rollag and Niswender 1976; Lewy et al.1980). However, the degree of melatonin suppression depends on many factors, such as, intensity of light, duration and wave length (Lewy et al. 1980; Brainard et al. 1985), exposure to light during the preconditioning period (Reiter 1985; Brainard et al. 1983), time of application of the light pulse during the dark phase (Reiter et al. 1986), vitamin deficiency (Fournier et al. 2002) and age (Reiter et al. 1980; 1981).

In a study of humans, exposure to light intensity of 2500 lx (administered between 0200 h and 0400 h) suppressed plasma melatonin fully, whereas intensity of 500 lx did not (Lewy et al. 1980). In a later study, Bojkowski et al. (1987) found dim light of 300 lx (administered between 0030 h and 0100 h with a 15 h d^{-1} photoperiod) suppressed melatonin secretion partially during darkness in humans. In Syrian hamsters, a light intensity of 1.08 lx or more (administered at 0300 h to 0600 h with a 10 h d^{-1} photoperiod) for 8 minutes suppressed the plasma melatonin secretion fully (Brainard et al. 1982). In rats, the intensity of white light required to suppress night melatonin synthesis is 0.5 $\mu W/Cm^2$ (0.15 lx), which is about 10 times more than full moonlight (Minneman et al. 1974). In goats 2.3 lx suppressed plasma melatonin,

whereas intensities of 0.8 lx or less were ineffective (Deveson et al. 1990). A light intensity of 0.15 lx partially (17 %) reduced plasma melatonin in Ill-De-France rams exposed to 350 lx during the day (Arendt and Ravault 1988). Sheep and goats are therefore more sensitive to light than cattle. This may be because sheep and goat are seasonal breeders and are more dependent on pineal melatonin secretion for their seasonal reproduction than non-seasonal breeders, such as cattle.

In pre-pubertal heifers, exposure to 400, 800 and 1200 lx light intensity during the second half (6 h) of the dark phase (12 h d^{-1} photoperiod) suppressed melatonin level fully and continuous exposure to light of 1200 lx fully suppressed the night melatonin level for the entire 4 months experimental period (Buchanan et al. 1992). Recently, Lawson and Kennedy (2001) reported that a light intensity of 50 lx partially (70 %) suppressed the rise in melatonin level at night for the first three hours of the dark phase. To our knowledge, no other studies on the threshold light intensity for the suppression of melatonin level at night in cattle have been conducted.

The return of normal melatonin level at night after the removal of a light pulse applied during the dark phase depends on the time of application of light during the dark phase (Reiter et al. 1986; Deveson et al. 1990; Earl et al. 1985). In ewes, a one hour light pulse after three hour of darkness cause decreased melatonin level for one hour and then rapid return to normal high night level (Deveson et al. 1990), whereas when a one hour light pulse was applied after 10 h of darkness, the melatonin level fell and then did not return to the normal high night level (Earl et al. 1985). This suggests that the response to light differs between early and late dark phase. These findings were in agreement with Reiter et al. (1986), who found that melatonin returned to the

normal level at night in hamsters when the light pulse (3200 μW/Cm^2; 10900 lx for 5 sec) was applied at eight hour but not at 12 h of darkness.

Studies have shown that the wavelength of light also plays an important role in the suppression of melatonin (Brainard et al. 1986; Reiter 1985). A wavelength of red light (700 nm) is found least suppressive to pineal melatonin secretion (Reiter 1985). Blue light with the wavelength of 475-500 nm has the most suppressive effect on pineal melatonin secretion compared to green (515-550 nm), red and yellow (560-700 nm) light. It has been reported that hamsters are very sensitive to low (340-405 nm) wavelengths of light (Brainard et al. 1986).

Lamps (fluorescent, metal halide and high pressure sodium vapour lamps) with different spectral properties have similar effects on serum PRL in cattle (Leining et al. 1979; Stanisiewski et al. 1984). Chastain and Hiatt (1998) stated that all bulbs (fluorescent, metal halide and high pressure sodium vapour lamps) are acceptable for use in dairy barns and the fixture of choice depends upon the space and height of the barn.

2.2.2 Previous Lighting History

Previous lighting history also determines sensitivity to light (Reiter et al. 1983; Brainard et al. 1983). Reiter et al. (1983) found that previous lighting history influenced sensitivity to light during the dark phase. Laboratory-raised squirrels exposed to artificial light intensity of 700 to 1100 lx during the day time had 100 % suppression in melatonin level with 1360 lx at night, but captured wild squirrels

adapted to natural light (122,400 lx) required > 1360 lx of light intensity for 100 % suppression of melatonin level at night (Reiter et al. 1983).

2.2.3 Stress, Age and Nutrition

Stress is also a factor that influences pineal melatonin release in animals. Recently, Barriga et al. (2002) reported that stress due to immobilization during the night reduced plasma melatonin level, whereas stress during the day increased plasma melatonin level in birds.

An age associated change in melatonin secretion was reported in both animals and humans (Reiter et al.1980; Reiter 1995). In humans, the melatonin level at night was low during the first six months of age, increased to a peak at one to three years of age and declines progressively with age thereafter (Waldhauser et al. 1988).

The vitamin folate is necessary for the methylation of N-acetyl serotonin to melatonin. Deficiency of folate reduces the methylation process and results in decreased melatonin production from the pineal gland of rats (Fournier et al. 2002).

2.3 Biological response to Light

Melatonin acts as a neuroendocrine transducer through the SCN and pituitary and alters release of other hormones, physiology and behaviour in animals (Hedlund et al. 1977; Kennaway et al. 1983). Melatonin acts via the hypothalamo-pitutary-gonadal axis (Reiter 1991; Hedlund et al. 1977) and alters the release of GnRH from the hypothalamus and thereby LH and FSH from the pituitary, resulting in changes in ovarian function in seasonal breeders.

Melatonin also acts on the pituitary-adreno-cortical-axis to influence adrenal secretions (Maestroni et al. 1986). Maestroni et al. (1986; 1989) reported that melatonin enhances immune functions and possessed anti-stress properties. They found that exogenous melatonin (1μg/mouse/day for 4 days) in the evening restored immune functions in mice where immune function was suppressed due to stress.

3 PHYSIOLOGICAL AND PRODUCTION RESPONSES TO LIGHT

3.1 Physiological Responses

3.1.1 Insulin-like Growth Factor-1

IGF-1 is a potent mitogen. Locally produced IGF-1 is responsible for mammary parenchyma growth (Gabler et al. 2001), whereas endocrine or circulating IGF-1 released from the liver is responsible for increased milk production in lactating animals (Dahl et al. 1997). Dahl et al. (1997) found that cows required two to four weeks to show an increase in IGF-1 level in response to extended photoperiod (18 h d^{-1}). IGF-1 levels return to normal after two weeks of shortened ($\leq$ 13 h d^{-1}) photoperiod.

It has been suggested that changes in plasma melatonin level acted as a timing signal to mediate the effect of long photoperiod (LPP) on IGF-1 in many species (Sanchez – Barcelo et al. 1991; Spicer et al. 1994). Spicer et al. (1994) found an increased concentration of circulating IGF-1 in cows exposed to photoperiod of 16 h d^{-1} (159 ng/ml) and 18 h d^{-1} (133 ng/ml) compared to 8 h d^{-1} (111 ng/ml) and there was no difference in IGF-1 level in cows exposed to photoperiod of 8 h d^{-1} (111 ng/ml) and 24 h d^{-1} (113 ng/ml).

3.1.2 Reproduction

3.1.2.1 Gonadotropin Releasing Hormone

The physiological basis for the effects of light on gonadotropin releasing hormone (GnRH) is well understood in seasonal breeders (Karsch et al. 1986; Hastings et al. 1989). As the days get shorter in the autumn, the prolonged peak in melatonin level at night acts on the hypothalamus and inhibits the release of GnRH and gonadal activity in long day breeders such as rodents, whereas in short day breeders like sheep, the prolonged peak in melatonin level at night stimulates GnRH release and gonadal activity (Karsch et al. 1986; Hastings et al. 1989). Although cattle are considered to be non-seasonal breeders, Reksen et al. (1999) found increased fertility in terms of reduced days open in cattle exposed to greater than 12 h d^{-1} (ranges from 12 h d^{-1} to 21 h d^{-1} photoperiod) of day length with dim illumination at night compared to cows exposed to total darkness at night.

Pinealectomy or denervation of sympathetic nerve supply caused inhibition of melatonin release from the pineal gland and there by abolishes the response to change in day length and causes changes in reproductive activity in mammals (Arendt 1986). A reduction in development of the testicles was observed in supraganlionectomized Soay rams (Lincoln et al. 1982), failure in reproductive behaviour was observed in pinealectomized ewes (Bittman et al. 1983) and early onset of puberty occurred in melatonin implanted hamsters (Reiter 1980).

Critical day length is a species specific photoperiod length, which is necessary to trigger seasonal events in animals. For example for reproduction, short day breeders will reproduce when the photoperiod falls below the critical day length, whereas long

day breeders will reproduce when the photoperiod is longer than the critical day length. Elliott (1976) found gonadal regression in male Syrian hamsters when exposed to a photoperiod of 12 h d^{-1} but not on 12.5 h d^{-1}. He concluded that the critical day length for reproduction in male Syrian hamsters was between 12 and 12.5 h d^{-1}. Evans et al. (1991) found that there was a critical period, 13 to 15 h after subjective dawn (6 h d^{-1} photoperiod), with respect to plasma PRL and somatotropin increases in response to a skeletal long day in cattle. A two hour light stimuli at either 10 to 12 h or 16 to 18 h was ineffective. Lawson and Kennedy (2001) found that the critical period for melatonin, with respect to 50 lx light, occurs immediately following the end of the photoperiod.

3.1.3 Prolactin

Early research by Bourne and Tucker (1975) reported a four-fold increase in serum PRL level in bull calves exposed to a 16 h d^{-1} photoperiod compared to 8 h d^{-1} photoperiod. Leining et al. (1979) found no difference in plasma PRL level for16 h d^{-1} and 20 h d^{-1} photoperiod, but exposure to continuous light (24 h d^{-1}) reduced serum PRL level to that of a 8 h d^{-1} photoperiod. Stanisiewski et al. (1988) also found increased serum PRL level in cattle exposed to a16 h d^{-1} photoperiod for four weeks compared to a 8 h d^{-1} photoperiod. Many studies confirmed the positive effect of photoperiod on serum PRL level in heifers (Petitclerc et al. 1983; Phillips et al. 1997) steers (Phillips et al. 1997), and in post-pubertal heifers (Zinn et al. 1986b). However, Peters et al. (1980) found no PRL response to an increase in day length and attributed this to cold environmental temperatures. It has also been reported that stress, due to

jugular vein puncture during blood sampling, increased serum PRL level in animals (Stanisiewski et al. 1984). Therefore, it is important to consider factors other than light that may influence serum PRL level.

3.2 Production Responses

Extended photoperiod usually stimulates growth in young cattle (Peters et al. 1978; 1980) and milk production in lactating cattle (Dahl et al. 1997; Peters et al. 1978; Piva et al. 1992). Photoperiod manipulation has been used to increase productivity of livestock for many years and the responses to photoperiod are dependent on duration and intensity of the light administered.

3.2.1 Growth

3.2.1.1 Body Growth

Heifers exposed to a 16 h d^{-1} photoperiod grew 11 % and 17 % faster than heifers exposed to a 24 h d^{-1} and natural photoperiod, respectively (Peters et al. 1980). Petitclerc et al. (1983) found similar response in peri-pubertal heifers. Although positive results have been shown by many studies, that is not always the case. A long photoperiod (16 h d^{-1}) reduced growth rate (Zinn et al. 1986b) or had no effect (Petitclerc et al. 1984; Phillips et al. 1997) compared to short photoperiod (8 h d^{-1}) or natural photoperiod (< 14 h d^{-1}) in heifers. The unexpected response was attributed to differences in age, sex, climate and season.

3.2.1.2 Mammary Growth

Photoperiod plays a significant role in mammary gland development in young animals. Exposure to LPP stimulates mammary parenchyma growth and reduces mammary fat tissue growth in heifers (Petitclerc et al.1984). Sanchez – Barcelo et al. (1991) reported an increased mammary fat tissue content and decreased mammary parenchyma level in heifers fed melatonin to mimic short photoperiod compared to heifers that were not fed melatonin. The effect of LPP on mammary growth was thought to be due to the mitogenic effect of locally produced IGF-1. Baumrucker and Stamberger (1989) found that IGF-1 increased mammary cell numbers *in vitro*.

3.2.2 Milk Production

An extended photoperiod has been shown to increase milk production by 6 –16 % with little effect on milk composition (Table 1).

Table 1. Effects of long photoperiod on milk production in lactating dairy cattle (NP = Natural photoperiod of $\leq$ 13 h d^{-1}. N = number of cows used in the experiment. NC = No change. ↓ = Decrease. Sig = significantly different. NS = Not significantly different).

Researcher	Treatments	N	Milk production increase Kg (%)	SE (kg)	Sig/NS	Fat %
Peters et al. (1978)	16 h vs NP	46	2.0 (10 %)	0.4	Sig	NC
Peters et al. (1981)	16 h vs NP	42	1.4 (6.7 %)	0.9	Sig	NC
Stanisiewski et al. (1985)	≥16 h vs NP	216	2.2 (8 %)	0.7	Sig	↓
Phillips and Schofield. (1989) (Expt.1)	18 h vs NP	12	3.3 (15.8 %)	1.2	Sig	NC
(Expt.2)	19 h vs NP	16	NC	0.44	NS	↓
Piva et al. (1992; abstr)	16 h vs NP	6	2.4 (13.7 %)	0.8	Sig	↓
Dahl et al. (1997)	18 h vs NP	20	2.2 (6.4 %)	1	Sig	_

It has been suggested that a 24 h d^{-1} photoperiod for cattle is detrimental to milk production (Dahl et al. 1997), but evidence for this is not convincing. Tanida et al. (1984) found that cows exposed to 18 h d^{-1} or 24 h d^{-1} photoperiod had a similar milk production over a three months experimental period. By the third month of the experiment the cows exposed to 24 h d^{-1} photoperiod produced 12 % less milk (non-significant) than cows exposed to 18 h d^{-1} photoperiod. Tanida et al. (1984) postulated that the difference might have been significant if the treatments were continued. Similar milk production was observed for cows exposed to 16 h d^{-1} and 24 h d^{-1} photoperiod (Dahl 1998). Spicer et al. (1994) found an increased plasma IGF-1 level in cows exposed to photoperiods of 16 h d^{-1} and 18 h d^{-1} compared to 8 h d^{-1} and similar plasma IGF-1 level in cows exposed to 8 h d^{-1} and 24 h d^{-1} photoperiods. Leining et al. (1979) reported that bull calves exposed to a 24 h d^{-1} photoperiod had reduced serum prolactin concentration compared to bull calves exposed to a 16 h d^{-1} photoperiod, but similar to that of bulls with a 8 h d^{-1} photoperiod. Peters et al. (1978) found that exposure to continuous light (24 h d^{-1}) produced similar average daily gain to that with natural photoperiod (9 h to 12 h per day) in dairy heifers. Since there is no additional beneficial effect observed using 24 h d^{-1} photoperiod on milk production response, a 16 to18 h d^{-1} photoperiod with $\geq$ 200 lx intensity is generally recommended and it is also recommended that dim red light be used during night when lighting is necessary (Dahl et al. 1997) for dairy cattle. However, many dairy farmers prefer to keep the lights on for 24 h, because it facilitates checking of cows during the night for heat and for general inspection and three times per day milking (Tucker et al. 1984).

Dahl et al. (1997) examined the mechanism involved in LPP induced milk production response in lactating cows. Dahl et al. (1997) found that exposure to long photoperiod (18 h d^{-1}) increased plasma IGF-1 and milk production (6.3 %) in cattle compared to controls (natural photoperiod of < 13 h d^{-1}). Prosser et al. (1990) found that infusion of IGF-1 into the mammary blood supply increased (11 %) milk secretion in goats.

Miller et al. (2000) found that the milk production response of cows exposed to 16 h d^{-1} was greater if the cows had been exposed to 8 h d^{-1} photoperiod rather than 16 h d^{-1} photoperiod during the dry period. These results suggest that an increase in photoperiod is necessary to achieve an increase in milk production.

3.2.2.1 Feed Intake

Changes in nutritional status and animal behavior cannot be overlooked as factors that mediate a milk production response to LPP. Exposure of dairy cows to 16 h d^{-1} or 18 h d^{-1} photoperiod compared to 13.5 h or less increased dry matter intake (Tanida et al. 1984; Peters et al. 1981; Phillips et al. 1998). In contrast, 24 h d^{-1} photoperiod did not increase feed intake in dairy cattle compared to16 h d^{-1} or 18 h d^{-1} photoperiod. Tanida et al. (1984) found similar feed intake in cows exposed to photoperiod of 18 h d^{-1} and 24 h d^{-1}. Peters et al. (1981) found a decrease in feed intake in cows exposed to 24 h d^{-1} photoperiod compared to 16 h d^{-1} photoperiod.

3.2.2.2 Milk Composition

Changes in milk composition in response to LPP have not been consistent among studies. A decrease in fat content with 16 h d^{-1} vs 8 h d^{-1} photoperiod was found by Stanisiewski et al. (1985), Phillips and Schofield (1989) and by Piva et al. (1992). No changes in milk composition were found by Peters et al. (1978; 1981) and Dahl et al. (1997). To our knowledge, there are no reports on photoperiod-induced changes in milk protein and lactose.

3.2.2.3 Light Intensity

There is very little information about the intensity of light required to cause physiological or production responses in cattle. The minimum effective intensity of light required for lactation responses in cows has not been determined, but responses to light of low intensity have been found when the light of low intensity is preceded by 16 h or 8 h of bright light per day (Stanisiewski et al. 1987). Exposure of pre-pubertal bulls to either 8 or 16 h of dim illumination (11-16 lx) preceded by bright light (> 449 lx) during the day increased PRL level (Stanisiewski et al. 1987). Recently, in an epidemiological survey, Reksen et al. (1999) found increased fertility and milk production in cows exposed to LPP of 12 h or more per day with dim illumination at night (mean =36 lx; ranges from 4-160 lx). Unfortunately, these authors did not separate out the effect of the extended photoperiod and the effect of the dim light.

It is not known if provisions of an extended day using dim light with increased milk production and it is not known if a 16 h day of bright light followed by 8 h of dim light will be perceived as a 24 h day by cattle. Consequently, dim white light at

night is not recommended for use with dairy cows (Dahl et al. 2000). Dahl et al. (2000) recommended that a red light (4-7.5 Watt) can be used during the 6-8 h dark period when cows are checked. The use of dim red light is very inconvenient and dim white light would be more preferable if no negative effects were present. If cows are not responsive to dim white light then it could be used at night instead of red light. Alternatively, if cows are responsive to dim white light, it could be used during the day, or part of the day, for the purpose of minimizing the cost of fixtures and electricity in the dairy barn.

4 *MANUSCRIPT I - PLASMA MELATONIN AND IGF- 1 LEVELS IN DAIRY HEIFERS EXPOSED TO DIM LIGHT AT NIGHT*

4.1 Abstract

The effect of dim night light (0, 5, 10 and 50 lx) on plasma melatonin and insulin like growth factor-1 (IGF-1) levels was determined in12 pre-pubertal Holstein heifers (245 ± 16 d age) held in individual pens in four windowless rooms. The heifers were exposed for 14 consecutive eight hour nights to each light treatment using a 4x4 Latin Square Design and were exposed to ≥ 200 lx light intensity during the16 h day. Heifers were exposed to a 16 h d^{-1} (200 lx) photoperiod for 14 d between treatments. Jugular vein catheters were inserted on day 0, and 14 and blood was sampled on days 0, 4 and 14 of each treatment period at 23:00 h (prior to night-light treatment) and at 01:00, 02:00, 04:00 and 08:00 h the next day (during night-light treatment). Plasma was analyzed by radioimmunoassay for melatonin (all samples) and for IGF-1 (day14, 04:00 h only). Treatment (P = 0.03) and Treatment x Hour (P = 0.02) were significant for plasma melatonin. Exposure to 50 lx suppressed plasma melatonin level by 50 % during the initial two hours of the night, but not thereafter. Light of 5 and 10 lx had no effect on plasma melatonin level during the night. The plasma melatonin response to 50 lx was found on all treatment days studied (Treatment x Hour x Day; P = 0.99). There was no significant night light treatment effect on plasma IGF-1 level (P = 0.89), but plasma IGF-1 level was higher (P = 0.0008) during period 4. To our knowledge, this is the first study to suggest that plasma IGF-1 level is negatively correlated (P = 0.10) to plasma melatonin level at night. In conclusion, the light intensity of 50, but not 5 or 10 lx, suppressed plasma melatonin level for the initial two hours of the night. Light intensities of 50 lx or less had no effect on plasma IGF-1 level. The results indicated that light intensities of 10 lx or less will not affect plasma melatonin and IGF-1 levels at night in dairy heifers.

4.2 Introduction

Light is an important controller of the mammalian pineal gland (Wurtman 1975) and regulates a variety of endocrine functions in mammals (Reiter 1980). Photoperiod information is conveyed by the pineal hormone melatonin to all tissues (Arendt et al. 1981). In mammals, melatonin is secreted from the pineal gland at a high level during the night or dark phase and a low level during the day or light phase of a 24 h light:dark cycle (Kennaway et al. 1977; Hedlund et al. 1977). The duration of the peak in melatonin level at night is read as the duration of night in a light:dark (L:D) cycle in mammals (Arendt 1995). Inhibition of melatonin secretion by light is intensity dependent and sensitivity varies among species. As little as a 1-min light pulse of 150 lx fully suppressed melatonin production at night in rats (Illnerova and Vanecek 1979). In humans, a light intensity of 2500 lx fully (Lewy et al. 1980), and 300 lx partially (Bojkowski et al. 1987) suppressed plasma melatonin level. Deveson et al. (1990) reported that a light intensity of 150 lx for one hour suppressed plasma melatonin level by 80 % in Saanen goats and 0.15 lx partially (17 %) suppressed plasma melatonin level in Ill-de-France-rams (Arendt and Ravault 1988).

The threshold light intensity for suppression of melatonin secretion has not been determined in dairy cattle, but there is evidence to suggest that cattle are more sensitive to light than humans. In pre-pubertal heifers, light intensities of 400, 800 and 1200 lx suppressed plasma melatonin level fully and continuous administration of 1200 lx light intensity (24 h d^{-1} photoperiod) abolished the melatonin peak at night throughout a four month treatment period (Buchanan et al. 1992). Stanisiewski et al. (1987) reported that continuous low intensity (11-16 lx) of light proceeded by 16 or 8 h of high intensity

(> 449 lx) light increased serum prolactin (PRL) level in pre-pubertal bulls compared to bulls that had total darkness during the night. Recently, Lawson and Kennedy (2001) reported that a light intensity of 50 lx suppressed (70 %) plasma melatonin level but only during the first three hours of night in pre-pubertal heifers.

Some dairy farmers like to provide 24 h of light in their barns, because they believe that the cows exposed to 24 h of light will consume more feed or because it aides in animal health inspections and estrus detection at night. Exposure to 24 h d^{-1} photoperiod did not affect feed intake compared to 16 h d^{-1} (Peters et al. 1981) or 18 h d^{-1} photoperiod (Tanida et al. 1984) and milk production with 24 h d^{-1} was similar to that of 18 h d^{-1} photoperiod (Dahl et al. 1997; Tanida et al. 1984). However, Dahl et al. (2000) recommended that 6 h to 8 h of darkness per day be provided to dairy cattle and recommended the use of dim red light at night when dairy farmers need to inspect their animals. It seems there is a fear that exposure to white light of any brightness during the night will affect milk production although there is no direct evidence to create this fear. Indirect evidence, that 24 h d^{-1} photoperiod reduced serum PRL level (Leining et al. 1979) and plasma IGF-1 level (Spicer et al. 1994) in cattle, suggests that the fear may be justified. Chastain and Hiatt (1998) stated that the light intensity of one to two foot candle was adequate to read newspaper print by an average person. It may be possible that dim white light can be used to facilitate management requirements for light at night without affecting hormone levels in cattle. Therefore, the knowledge of threshold light intensity for inhibition of melatonin secretion during night in cattle has important economic consequences to dairy farmers. The objective of this study was to determine the plasma melatonin and IGF-1 responses to various intensities of dim light at night in dairy heifers.

4.3 Materials and Methods

4.3.1 Animals and Housing

Twelve pre-pubertal heifers, which were 245 ± 16 d (mean ± SD) of age and weighed 261.9 ± 17.2 kg (mean ± SD) at the beginning of the experiment, were used in this study. Heifers were brought into the University of Manitoba Animal Science Research Unit (ASRU) on February 15, 2001. Heifers were divided into four groups (balanced by age and body weight) of three animals in each group and were housed unrestrained in individual pens (3.4 m W x 3.2 m L) in four rooms. Each pen had a rubber mat bedded with sawdust and had an individual feeder and waterer. Heifers were fed 2 kg d^{-1} each of 19.7 % CP dairy grower ration and alfalfa hay and water were provided *ad libitum* (Table. 2). The amount of feed offered was adjusted once per week according to the body weight of the heifers. Heifers were acclimated to the room environment (16 h d^{-1}) and diet for 40 d prior to commencement of the experiment. Heifers were cared for in accordance with the Canadian Council on Animal Care (CCAC) guidelines (1993).

4.3.2 Experimental Procedure

The heifers were exposed to a 16 h d^{-1} photoperiod. During the day (08:00 h to 24:00 h) the heifers were exposed to a light intensity of 200 lx (Dahl et al. 2000). During the eight hour night, heifers were exposed to 0 lx in the pre-treatment period and at four different light intensities (0, 5, 10 and 50 lx), according to a Latin Square Design, during the treatment periods. Each period (row) of the Latin Square consisted of a 14 d pre-treatment period followed by a 14 d experimental period and each column of the Latin

Square contained three heifers. Fluorescent light bulbs (Phillips, Hi-Vision, Alto collections, Somerset, NJ, USA,) were used to provide lighting during the day. Bulbs were blocked with duck tape to achieve the required intensity of light. Tape adjustments were made during each period to ensure that the animals received adequate intensity of light during the day. Each treatment room had four light fixtures, each with two fluorescent bulbs, which were hung 2.3 m above the animal pens. During the night, incandescent bulbs were used to provide the night light treatments. Incandescent fixtures were situated evenly throughout the rooms at a ceiling height of 3 m. Bulbs of 7.5 and 15 Watt (Sylvania, Osram Sylvania Ltd, Quebec, Canada) were used to provide the 5 lx and 10 lx light treatments, respectively. Forty-Watt bulbs (Philips Electronics Ltd, ON, Canada) were used to provide the 50 lx light treatment. Timers (TORK E103, Vernon, NY) were used to turn lights on and off automatically. The fluorescent lights were automatically turned on at 08:00 h and turned off at 24:00 h daily. The incandescent lights were turned on automatically once the fluorescent lights turned off. The room temperature was maintained at 20 ± 2 °C throughout the pre-treatment and experimental periods.

After each 28 d period (14 d pre-treatment, 14 d treatment) the heifers were moved to a new room and allowed to acclimate over the next 14 d pre-treatment phase with a 16 h d^{-1} photoperiod. Treatments were randomized across rooms to avoid the confounding effect of room and light treatment. During each treatment period, day and night light intensity was measured weekly at nine different locations (horizontally) within each pen at a height of 1.2 m (the distance halfway between lying and standing) using a light meter (Minolta Professional, Ramsey, NJ, USA). Catheters (0.62" I.D x 0.82" O.D –

Bekton-Dickenson Company, Canlab) were inserted into the jugular vein prior to 16:00 h on day 0 (the day prior to the first night of dim light treatment which commenced at 0:00 h on day 1) of each light treatment phase. The catheters were flushed with sterile heparinized (50 units) physiological saline. Heparinized (200 units) blood (10 ml) was collected at 23:00 h (during day time) and throughout the night at 01:00, 02:00, 03:00, 04:00, 06:00 and 08:00 h. The sampling procedure was repeated commencing at 23:00 h on day 3 and catheters were removed following the 08:00 h sample collection. Heifers were recatheterized on day 13 and were again sampled as described above with collection ending and catheters removed at 08:00 on day 14. Dim red light (0 lx) (head light and torch covered with red plastic filters) was used to allow blood sampling in the 0 lx light treatment. Blood samples were stored at 4 °C overnight and were then centrifuged at 750 x g for 30 minutes. Plasma was collected and stored at – 20 °C until assayed for melatonin and IGF-1 by radioimmunoassay (RIA). Plasma from day 14, hour 4 (night light treatment phase) for each of the 4 periods was used for IGF-1 analysis.

4.3.3 Hormone Assays

The IGF-1 RIA (Kerr et al. 1990) was conducted at the Western College of Veterinary Medicine, Saskatoon, SK. All samples were analyzed in one assay. The intraassay coefficient variation (CV) was 2.5 % and sensitivity was 0.1 ng/ml. Melatonin was extracted from plasma using columns and was assayed by direct RIA using the Buhlmann kit (Alpco, Windham, NH). Only blood samples collected at 23:00 h, 01:00 h, 02:00 h, 04:00 h and 08:00 h were analyzed due to the financial limitations. The non-specific binding of all assays was 5.7 % ± 1.3 % (mean ± SD) and the maximum binding

of all assays was 34.1 % ± 2 % (mean ± SD). The sensitivity of the assay (88 % binding) was 0.75 pg/ml. A high bovine standard was diluted 1:1 and 1:20 in the incubation buffer to generate medium and low standards, respectively. The intraassay coefficient of variation for the high standard (41.6 ± 6.9 pg/ml) from 4 different runs (3 values/run) was 9.9 %, for the medium standard (18.7 ± 1.5 pg/ ml) from 3 different runs (2 values/run) was 14.7 % and for the low standard (3.2 ± 0.5 pg/ml) from 3 different runs (2 values/run) was 21.2 %. The interassay coefficient of variation for the high standard (4 pairs and 4 triple samples in 8 different assays) was 16.6 %, for the medium standard (3 pairs from 3 different assays) was 8.2 % and for the low standard (3 pairs from 3 different assays) was 29 % (Appendix II, Table 1). The extraction efficiency variability for column reuse was tested using labelled plasma (Appendix III, Table 1).

4.3.4 Statistical Analysis

ANOVA of melatonin data was carried out using Proc GLM procedure for a Latin Square Design (SAS Institute, Cary, NC. 1999). Data were tested for normality using Bartlett tests. Plasma melatonin values were $\log_{10}$ transformed (Steel et al. 1997) and ANOVA was done to determine significance of main and interaction effects. Main and interaction effects were tested against mean squares as shown below. Blood samples collected during the night when exposed to 0 lx treatments were used for the determination of individual variation in plasma melatonin level. Tukey's mean comparison (Steels et al. 1997) test was used to detect significant difference ($P < 0.05$) among treatment means.

Source	Error term
Animal	Animal x Period x Treatment
Period	"
Treatment	"
Day	Animal x Period x Treatment x Day
Treat x Day	"
Hour	Animal x Period x Treatment x Day x Hour
Treat x Hour	"
Day x Hour	"
Treat x Hour x Day	"

Since neither the 5 or 10 lx treatments affected plasma melatonin and the plasma melatonin level reached its maximum concentration at hour 4 of the darkness period, values for 4:00 h of day 14 from the 0, 5 and 10 lx light treatments were used for the estimation of the correlation between night IGF-1 and melatonin levels by using linear and quadratic regression analysis (SAS Analyst 1999).

ANOVA of IGF-1 data was carried out using Proc GLM procedure for a Latin Square Design (SAS Institute, Cary, NC. 1999). The main effects were tested against the residual error to determine the significance at $P = 0.05$ as shown below.

Source	Error term
Animal	Animal x Period x Treatment
Period	"
Treatment	"

4.4 Results

Light intensities (mean ± SD) for the 5, 10 and 50 lx treatment were 5.5 ± 0.8, 10.5 ± 1.2 and 54 ± 6.6 respectively (Appendix I, Table.1).

During the day (23:00 h), before the beginning of the nightlight treatment, plasma melatonin level was < 3 pg/ml and was not different among treatments. There was significant ($P < 0.0001$) variation in plasma melatonin level among heifers exposed to 0 lx during the night with plasma melatonin level ranging from 10 pg/ml to 81 pg/ml. Plasma melatonin level for the treatments of 5 and 10 lx was similar to 0 lx during the 8 h night phase, whereas 50 lx significantly ($P < 0.02$) suppressed plasma melatonin level for the first two hours of light treatment (Figure 2). The degree of plasma melatonin suppression by 50 lx at 1:00 h was 48 %, 42 %, and 54 % and at 2:00 h was 45 %, 39 %, and 33 % compared to the 0, 5 and 10 lx treatments, respectively. The treatment x day and day x hour interactions were not significant ($P > 0.22$) and there was no effect of day ($P = 0.17$). The treatment x hour x day interaction (Appendix I, Figure.1) was not significant ($P = 0.99$). Lack of significance of this interaction indicates that the suppressive effect of 50 lx was not altered with time. There was no ($P = 0.89$) treatment effect on plasma IGF-1 level. Plasma IGF-1 level was higher ($P = 0.0008$) during period 4 (310 ± 12.6 ng/ml) than during period 1 (249 ± 12.6 ng/ml), period 2 (232 ± 12.6 ng/ml), and period 3 (251 ± 12.6 ng/ml), which were similar. There was significant ($P < 0.0001$) variation in plasma IGF-1 level among heifers and plasma IGF-1 level ranged from174 ng/ml to 284 ng/ml among heifers. Analysis by linear regression showed that there tended ($P = 0.10$) to be a negative correlation between plasma IGF-1 and

plasma melatonin levels (Figure. 3), but the quadratic equation was not significant ($P = 0.37$).

4.5 Discussions

Stanisiewski et al. (1987) and Buchanan et al. (1992) demonstrated that exposure to light intensity of > 400 lx abolished the rise of plasma melatonin level at night in cattle. Lawson and Kennedy (2001) have shown that light as dim as 50 lx could prevent the plasma melatonin increase at night to some degree (70 %) for part of night. Similarly we have also shown that a light intensity of 50 lx partially (50 %) suppressed the plasma melatonin increase at night in heifers for the first two hours of night treatment but was ineffective in maintaining its suppressive effect on plasma melatonin level thereafter. Lawson and Kennedy (2001) found that the intensities of 100, 200 and 400 lx maintained at least some suppressive effects on plasma melatonin level for the entire 16 h night. Their light treatments were only applied for one night and they speculated that the cattle might have developed a sustained and more pronounced overnight response if the light treatments were continued for additional nights. Our results show that is not the case as we found that 50 lx suppressed the nightly peak in plasma melatonin for two hours on day 1, 4 and 14. Together with our study, these studies suggest that sustained suppression of melatonin is not possible with 50 lx in cattle.

The amount of suppression of the plasma melatonin peak at night by 50 lx was 70 % in the study of Lawson and Kennedy (2001), but approximately 50 % in the present study. The reason for the lesser suppressive effect in the present study is unclear. Reiter et al. (1983) found that previous lighting history influenced sensitivity

to light during the dark phase. Laboratory-raised squirrels maintained under artificial light intensity of 700 to 1100 lx during the day time had 100 % suppression in melatonin level with 1360 lx at night, but captured wild squirrels adapted to natural light (122,400 lx) required > 1360 lx of light intensity for 100 % suppression of melatonin level at night (Reiter et al. 1983). There was a difference in the pre-conditioning day light intensity, light:dark cycle and type of night light lamp used in the current study (200 lx, 16 h d^{-1}, incandescent) and that of Lawson and Kennedy (2001) (400 lx, 8 h d^{-1}, fluorescent). Based on the preconditioning theory of Reiter et al. (1983), it would be expected, because of 200 lx pre-conditioning in our study, that there would be a greater suppressive effect of 50 lx in our study compared to Lawson and Kennedy (2001; pre-conditioned to 400 lx), but the opposite was the case. It has been reported that the spectral properties of the light sources influenced the pineal melatonin secretion (Brainard et al. 1984; 1985; Cardinali et al. 1972). The blue light with a wavelength of 435 to 500 nm was most inhibitory to pineal melatonin level in rats (Cardinali et al. 1972) and in hamsters (Brainard et al. 1984) compared to green, yellow and red lights. The green wavelength (515 to 550 nm) was 25 % less effective than blue light, whereas red and yellow (560 to 700 nm) lights did not affect melatonin level (Brainard et al. 1984). Because incandescent bulbs have less blue and green colour spectrum than fluorescent (Thorington 1985), the use of incandescent bulbs may have been responsible for the relatively lower suppressive effect of 50 lx on plasma melatonin level in the present study. If so, it is possible that the use of incandescent lights in dairy barns during night hours would affect night plasma

melatonin level less than the use of fluorescent lights. This requires further investigation.

In the present study and that of Lawson and Kennedy (2001), the effect of 50 lx on plasma melatonin level was seen during the early part of the night but not during the latter part of the night. This suggests refractoriness to dim light after a few hours during the night or the existence of a critical period for photosensitivity. The development of refractoriness to light occurred at 12 or 21 weeks of exposure in cattle (Stanisiewski et al.1987) and in rams (Almeida and Lincoln. 1984), respectively. Development of refractoriness to light within each night, as appeared to be the case in this study and that of Lawson and Kennedy (2001), has not been reported previously in cattle or any other species. However, others have shown that the response to light during the night depends on when the light is applied during the night. In rats (Illnerova 1988), a phase delay in melatonin rhythm could be induced by exposure to pulses of light early in the night, whereas a phase advance occurred with exposure to light during the latter part of the night. In ewes, a one hour light pulse after three hours of darkness cause decreased melatonin for one hour and then rapid return to normal high night level (Deveson et al. 1990), whereas when a one hour light pulse was applied after 10 hour of darkness, the melatonin level fell and then did not return to the normal high night level (Earl et al. 1985). These findings were in agreement with Reiter et al. (1986), who found that melatonin returned to the normal night level in hamsters when the light pulse (3200 $\mu W/Cm^2$; 10900 lx for 5 sec) was applied at eight hour but not at 12 hour of darkness.

Evans et al. (1991) found that there was a critical period, 13 to 15 h after subjective dawn (6 h d^{-1} photoperiod), with respect to plasma PRL and somatotropin increases in response to a skeletal long day in cattle. A two hour light stimuli at either 10 to 12 h or 16 to 18 h after subjective dawn was ineffective. It is possible that, in the present study and that of Lawson and Kennedy (2001), the lack of melatonin response to light during the latter part of the night indicated periods of photo-insensitivity 20 to 24 h (16 h d^{-1} photoperiod) and 11 to 16 h (8 h d^{-1} photoperiod) post-dawn, respectively. Melatonin results suggest that the critical period, with respect to 50 lx, was immediately follows the end of the photoperiod. With brighter light (400 lx), Lawson and Kennedy found no evidence of a critical period as melatonin was significantly suppressed throughout the entire 8 h night. To confirm that there is a critical period for photosensitivity to dim light in cattle, a study is required where the plasma melatonin response to 50 lx is examined when the light treatment commences at 0, 2, 4, and 6 h of darkness with a photoperiod of 16 h d^{-1}. Blood samples would be collected once during the day and at 30 min intervals during the night. This study would confirm the existence of times of night when it is safe for the dairy producer to use 50 lx dim light in the barn.

In the present study, the effect of daily exposure to 50 lx light on plasma melatonin was maintained for 14 days. This expands upon the findings of Stanisiewski et al. (1988), where there was a similar melatonin response to light on day 1 and 2 of treatment in cattle and does not support the suggestion (Lawson and Kennedy 2001) that the plasma melatonin response to light would increase with repeated exposure.

This is the first study to show that the light intensities of 5 and 10 lx have no effect on plasma melatonin level in cattle and that a response to these intensities does not develop over time. In sheep, a light intensity of 1.08 lx partially suppressed plasma melatonin level (Arendt and Ravault 1988). In goats 2.3 lx suppressed plasma melatonin, whereas intensities of 0.8 lx or less were ineffective (Deveson et al. 1990). Sheep and goats are therefore more sensitive to light than cattle. This may be because sheep and goat are seasonal breeders and are more dependent on pineal melatonin secretion for their seasonal reproduction compared to non-seasonal breeders such as cattle. An increase in PRL was found in cattle exposed to 11-16 lx at night when the night was preceded by 16 h or 8 h of light of high intensity (> 449 lx) in calves (Stanisiewski et al. 1987). Based on the study by Stanisiewski et al. (1987), we expect a light threshold of < 11 lx for cattle. However, light of 10 lx or less did not affect plasma melatonin in this study. In the present study, the threshold for the suppression of plasma melatonin by light in cattle lies between 10 and 50 lx. It is concluded that dairy farmers can safely use 5 and 10 lx light during the night for heifers with no endocrinological effect. Chastian and Hiatt (1998) stated that, the light intensity of one to two foot candles was adequate to read newspaper print by an average person. Thus, with the use of 5 or 10 lx light one could easily examine animals visually for estrus or general health during the night without affecting plasma melatonin level. Further study is required to determine the precise threshold for the suppression of plasma melatonin using fluorescent and incandescent lamps during night. Such a study could be conducted using cow sunglasses to regulate the brightness of light (range = 0 lx to 40 lx) during night for cattle.

In the present study, there was considerable variation in plasma melatonin level among heifers. This was consistent with previous studies by Lawson and Kennedy (2001) in pre-pubertal heifers and by Coon et al. (1999) in sheep, in which genetic variability in pineal weight was responsible for the individual variation in plasma melatonin. Recently it has been reported that night melatonin level influences fertility in sheep (Notter and Chemineau 2001). The authors found that sheep with low (143 ± 17 pg/ml) melatonin level at night had higher fertility than sheep with high (184 ± 13 pg/ml) melatonin level at night. Sanchez–Barcelo et al. (1991) reported an increased mammary fat tissue content and decreased mammary parenchyma level in heifers fed melatonin to mimic short photoperiod, but there have been no studies of cattle fertility or mammary development. Further research is required to find out the relationship between the plasma melatonin level at night and productivity in cattle.

Reduced duration of the night melatonin peak is thought to be the mechanism whereby light increases plasma IGF-1 (Dahl et al. 1997). To our knowledge, this is the first study to suggest that plasma IGF-1 is negatively correlated to plasma melatonin level at night. In the present study plasma IGF-1 level was higher during period 4. It was reported in male calves (Renaville et al.1993; 1996) and in heifers (Hall et al.1995) that puberty is associated with an increase in plasma IGF-1 level. Heifers were about 357 ± 16 d (mean ± SD) of age and weighed 323 ± 18 kg (mean ± SD) by the end of the present study. The increased plasma IGF-1 level at period 4 may have been because the heifers were peri-pubertal (NRC.1989).

4.6 Conclusion

Incandescent light intensities of 5 and 10 lx did not affect plasma melatonin level in dairy heifers. The light intensity of 50 lx reduced plasma melatonin level by 50 % for the first two hours of light treatment during the dark phase. The actual threshold for the suppressive effect of incandescent white light on plasma melatonin level lies between 10 and 50 lx. Further research is required to more precisely determine the threshold light intensity in cattle. This is the first study to suggest that plasma IGF-1 level is negatively correlated to plasma melatonin level at night. Further research involving more number of animals is required to confirm this. Light intensities of 50 lx or less had no effect on plasma IGF-1 level in this study. The results suggest that plasma IGF-1 and melatonin don't respond to light intensities of 10 lx or less in heifers. Further research is required to confirm this in lactating dairy cows.

Figure 2. Plasma melatonin response to dim light of 0, 5, 10 and 50 lx intensities during the 8 h night in dairy heifers. Daylight was from 08:00 h to 24:00 h. [a,b] means in the same hour are significantly different from each other ($P < 0.02$).

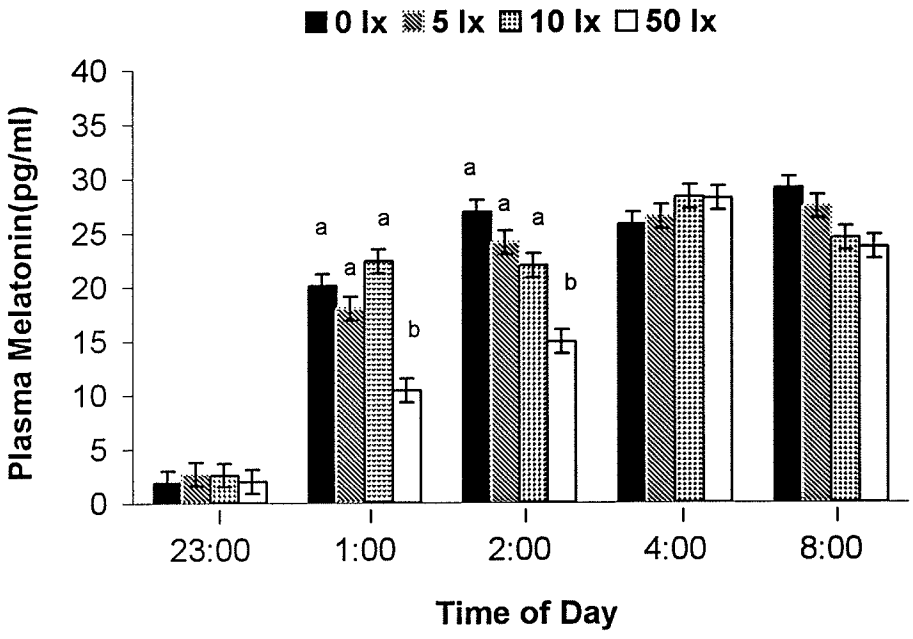
0 lx
5 lx
10 lx
50 lx
Plasma Melatonin(pg/ml)
40
35
30
25
20
15
10
5
0
a
a
a
b
a
a
a
b
23:00
1:00
2:00
4:00
8:00
Time of Day

Figure 3. Relationship between plasma melatonin and IGF-1 at 04:00 h on day 14, in dairy heifers under 0, 5 and 10 lx light intensities (P = 0.10).

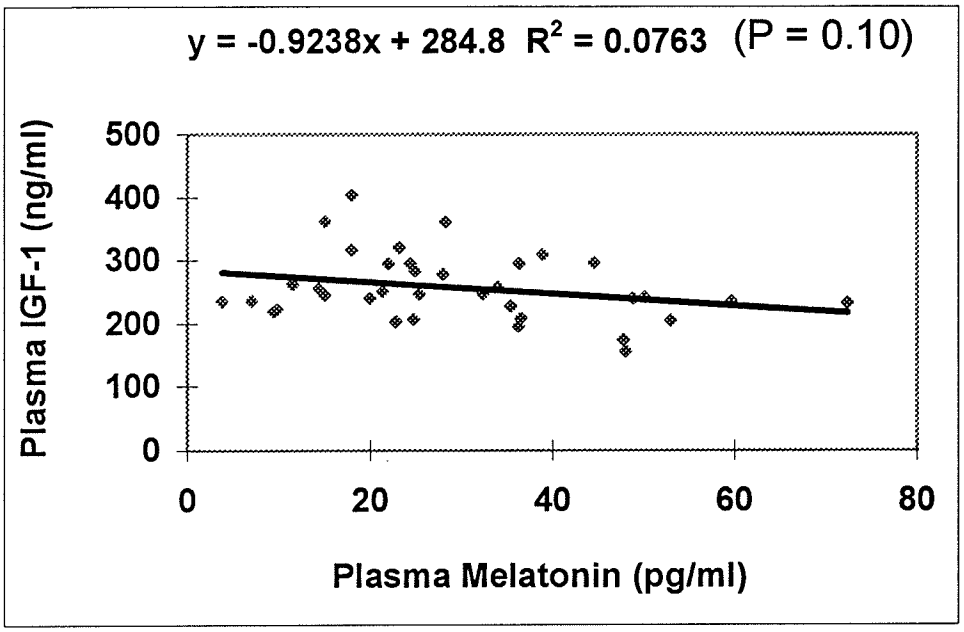
y = -0.9238x + 284.8 R2 = 0.0763 (P = 0.10)
Plasma IGF-1 (ng/ml)
500
400
300
200
100
0
0
20
40
60
80
Plasma Melatonin (pg/ml)

Table 2. Composition of the diet fed to the experimental heifers in Experiment 1.

Composition (% DM)	Alfalfa Hay	Concentrate mix[z]
Dry Matter, %	85.6	88.7
CP, % DM	12.5	19.7
ADF, % DM	49.2	86.0
NDF, % DM	65.5	25.2
NEG, Mcal kg^{-1}	0.39	1.05

[z]Containing (% DM): Ca (1.05); P (0.7); K (0.98); Mg (0.35).
(NEG was calculated by: Van Soest et al. Cornell nutrition conference 1979 and NRC 1978).

5 *MANUSCRIPT II - MILK PRODUCTION AND PLASMA IGF-1 LEVEL IN DAIRY COWS EXPOSED TO 14, 16, 18 AND 20 H PHOTOPERIODS*

5.1 Abstract

The effects of different photoperiods (14, 16 18 and 20 h d^{-1}) on milk production, composition and plasma IGF-1 level were determined using 12 lactating dairy cows (four primiparous and eight multiparous). Cows, 109 ± 14 days (Mean ± SD) in milk at the start of the trial, were balanced for residual effects and were assigned to a 4x4 Latin Square Design with six week periods. The cows were assigned to four treatment groups of three cows balanced for parity. The cows were housed in a tie stall barn and curtains were used to control the light environment of the various light treatment groups. During a two weeks pre-treatment period, each group was exposed to an 18 h d^{-1} photoperiod with total darkness (< 1 lx) at night. Cows had *ad libitum* access to a total mixed ration and water and were milked at 04:30 h and 16:30 h daily. Milk production was recorded daily and milk samples from weeks 5 and 6 of each period were analyzed for composition. Body weight was measured on two consecutive days during week 6 of each period. Blood was collected on day 7 of week 3 and week 6 of each period for plasma insulin-like growth factor-1 (IGF-1) analysis. Body condition score was measured during week 6 of each period. Milk production was similar among treatments (P = 0.87). Plasma IGF-1 level did not differ among treatments (P = 0.90). Milk fat tended (P = 0.09) to be elevated by the 18 h d^{-1} photoperiod treatment. Although dry matter intake (DMI) was numerically increased (13 %) for the18 h d^{-1} photoperiod treatment, the effect was non-significant (P = 0.27) and milk produced per kg feed intake (FI) did not differ (P = 0.71) among treatments. Body weight (P = 0.42) and body condition scoring (P = 0.80) did not differ among treatments. In conclusion, milk production and plasma IGF-1 level did not differ among cows exposed to 14, 16, 18 and 20 h d^{-1} photoperiods with light intensity of

$\geq$ 200 lx and preconditioning to a18 h d^{-1} photoperiod. DMI and milk fat tended to be higher for cows exposed to an 18 h d^{-1} rather than 20, 16 and 14 h d^{-1} photoperiods. All photoperiods (14, 16, 18 and 20 h d^{-1}) may have been perceived as long day by the dairy cows resulting in similar results for milk production, milk composition, DMI, and IGF-1 among treatments. Alternatively, high milk production variance (SE), carry over within the Latin Square, an inadequate treatment period and a long pre-conditioning photoperiod may be possible reasons why no differences among treatment were observed.

5.2 Introduction

Lactating dairy cows exposed to photoperiods of 16 or 18 h d^{-1} had increased milk production (Peters et al. 1978; Stanisiewski et al. 1985; Tanida et al. 1984; Piva et al. 1992; Dahl et al. 1997), with little (Stanisiewski et al. 1985; Piva et al. 1992) or no change in milk composition (Peters et al. 1978; Phillips and Schofield. 1989) compared to cows exposed to $\leq$ 13 h d^{-1} photoperiods. Photoperiods of 16 or 18 h d^{-1} were consequently considered to be interpreted as long days by the cows. Reksen et al. (1999) also found improved reproductive performance with long photoperiod (LPP). It has been reported that plasma IGF-1 level increased in lactating dairy cattle exposed to LPP (18 h d^{-1}) compared to SPP ($\leq$ 13 h d^{-1}) (Dahl et al. 1997). Smith et al. (1997) reported that daily feeding of melatonin in the middle of the LPP to mimic short photoperiod (SPP) increased serum melatonin and decreased circulating IGF-1 level in cattle. An infusion of IGF-1 into the mammary blood supply increased milk secretion in goats (Prosser et al. 1990). It is thought that increased photoperiod decreases the duration of the daily melatonin elevation which then triggers the liver to increase production of IGF-1, which increases milk production (Dahl et al. 1997).

It was found that exposure to light during the period13 to 15 h after subjective dawn (6 h d^{-1} photoperiod) but not at other times, resulted in increases in plasma prolactin (PRL) and somatotropin levels in cattle (Evans et al. 1991). It is possible that cattle perceive a long day only when light is present during a photosensitive phase which exists at a specific time after dawn (13 to 15 h after subjective dawn in the case of Evans et al. 1991).

A photoperiod longer than 18 h d^{-1} is not recommended (Dahl et al. 2000), but would be advantageous in some circumstances such as on farms milking 3 times a day and on farms where cows are inspected during the night. In these cases, a photoperiod >18 h d^{-1} would increase costs (Chastain and Hiatt 1998), but it has not been demonstrated in the literature that there would be other negative repercussions. It has been reported that exposure to photoperiod of 24 h d^{-1} does not result in additional milk production when compared to photoperiod of 18 h d^{-1} (Tanida et al. 1984) or 16 h d^{-1} (Dahl 1998). However, there is some concern as cows exposed to photoperiod of 24 h d^{-1} produced 12 % less milk (non-significant) by the third month of treatment (Tanida et al. 1984). The authors postulated that this difference might have been significant if the treatment had been continued. Spicer et al. (1994) found an increase in concentration of circulating IGF-1 in cows exposed to photoperiods of 16 h d^{-1} (159 ng/ml) and 18 h d^{-1} (133 ng/ml) compared to 8 h d^{-1} (111 ng/ml), but not in cows exposed to 24 h d^{-1} (113 ng/ml). Leining et al. (1979) found no difference in plasma PRL level between16 h d^{-1} and 20 h d^{-1} photoperiod treatments, but exposure to continuous light (24 h d^{-1} photoperiod) reduced serum PRL level to that of 8 h d^{-1} photoperiod. Peters et al. (1978) found that weight gains of heifers exposed to a 24 h d^{-1} photoperiod were similar to a natural photoperiod of 9 h to 12 h d^{-1}. However, a 24 h d^{-1} photoperiod reduced weight gain when compared to a 16 h d^{-1} photoperiod. Thus, although 24 h d^{-1} photoperiod has not been shown to significantly depress milk production, it is suspected that there may be undesirable endocrine effects and, to be cautious, a photoperiod of 24 h d^{-1} is not recommended during lactation. The recommended length of the dark period for lactating cows is six to eight

hour (Tucker et al. 1984; Dahl et al. 2000) and only dim red light is recommended if cow inspection is necessary during the dark period (Dahl et al. 2000).

In Manitoba, photoperiod in dairy barns will not likely be less than 13 to 14 h d^{-1} as twice daily milking at 12 h intervals is very common. It is not known if milk production is greater with 16 and 18 h d^{-1} photoperiods than with a 14 h d^{-1} photoperiod. Boris (2001) stated that reducing the usage of electricity for lighting for 2 h daily would save the Manitoba dairy industry approximately $ 277,500/Year. Although some dairy producers may prefer to minimize the duration of photoperiod in the barn to reduce electricity costs, others may prefer a photoperiod greater than 18 h in duration to facilitate cow management. Rather than the recommended duration of six to eight hour of darkness per day, it is feasible that four hour of darkness per day would be sufficient to maintain normal physiological functions and milk production in dairy cattle. Therefore, the objective of this study was to compare milk production and plasma IGF-1 level in lactating dairy cows exposed to photoperiods of 14, 16, 18 and 20 h d^{-1} at $\geq$ 200 lx light intensity.

5.3 Materials and Methods

5.3.1 Experimental Procedure

Twelve lactating Holstein cows (4 primiparous and 8 multiparous) at 109 ± 14 d (mean ± SD) in milk were used. Uniformity in calving date at the beginning of the experiment was achieved by synchronization of cows prior to breeding. The cows, weighing 695.6 ± 57.8 kg (mean ± SD) at the beginning of the experiment were assigned to one of four groups (three cows /group) balanced for parity. Cows were housed at the Glenlea Research Station, University of Manitoba (Glenlea, Manitoba,Canada) in a tie stall barn. A specialized 4x4 Latin Square Design (Cochran and Cox 1992), which balanced for carry over effect of previous treatments, was used. Four photoperiod treatments (14, 16, 18 and 20 h d^{-1} photoperiod), each with a six weeks duration, were randomly assigned to the four groups of cows during four consecutive periods. Cows within a group were maintained in adjacent stalls and curtains (Winkler Canvas Ltd, Winkler, MB) were used to separate the groups to apply photoperiod treatments (Figure 4 and 5). Each group area had six light fixtures with two fluorescent bulbs per fixture (Philips, Hi-Vision, Alto Collections, Somerset, NJ, USA). Fixtures were hung 2.3 m above the feeding area. Each group of animals was exposed to an 18 h d^{-1} photoperiod with complete darkness at night prior to the commencement of the experiment on October 26, 2001. A digital timer (TORK E103, Vernon, NY) was used to turn the lights on and off automatically for each group of cows. Output from the lights was restricted using duct tape to achieve the required daytime intensity of approximately 200 lx. Light impenetrable, rubberized nylon curtains were used to cover three sides of each group area and an adjacent wall was parallel to the feed area on the fourth side.

The curtains were raised and lowered as required to permit the application of the different photoperiod treatments to the four groups of cows simultaneously. Curtains at the sides and back end of each pen were raised at 04:00 h and lowered at 17:45 h daily by the barn staff to allow for daily management. Once the curtains were lowered, each group of cows was under separate light control. Light intensity during each period was measured once using a light meter (Minolta Professional, Ramsey, NJ, USA) (Appendix IV, Table 1) during the day and night at four locations around the left and right side of each cow 1m from the feeding area at animal eye level. Every week during the experimental period curtains were visually inspected for leaks with lights off and curtains down all sides to ensure all cows received their assigned light treatments (Appendix IV, Table 1). Stalls were bedded with chopped straw over rubber mats. Cows received a total mixed ration (TMR) (Table 3), which was balanced for the production of 35 kg of milk cow $^{-1}$ d $^{-1}$. The TMR was fed *ad libitum* between 8:45 – 11:15 h allowing for 10 % weighback (orts) and approximately 1 kg alfalfa hay cow $^{-1}$ d $^{-1}$ was provided at the same time. Cows had unlimited access to fresh water. Cows were fed the TMR diet two weeks before the beginning of the treatment periods. Cows were milked at 04:30 h and 16:30 h daily. Using a data logger (Campbell Scientific, Edmonton, AB), ambient temperature was measured at 20 sec intervals and average temperature was recorded every 20 min. From these recordings, the daily minimum, maximum and mean temperature was calculated. Cows were cared for in accordance with the Canadian Council on Animal Care (CCAC) guidelines (1993).

5.3.2 Data Collection

Milk production of each cow was recorded daily using a Milk Master (AlfaLaval Agri, Tumba, Sweden) from weeks 1 to 4 and a Tru Test milk meter (Tru Test Distributors, New Zealand) during week 5 and 6 of each experimental period. Fifty ml of milk was collected from the afternoon milking on day 5, at both milkings on day 6, and at the morning milking on day 7 of week 5 and 6 of each period. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol and stored at 4 °C until analyzed for composition at the laboratory of the Manitoba Milk Producers (Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerod, Denmark).

The TMR given to each group of cows was recorded daily during period 2, 3 and 4. Weighbacks for each group were recorded weekly once during weeks 1 to 4 and daily during week 5 and 6. Representative samples of weighbacks and of TMR were collected. A core sample of hay was taken from each new bale fed during experimental period 2, 3 and 4. Daily samples of the TMR and weighback were pooled for week 5 and 6 separately and hay samples from all periods were pooled for proximate analysis. Dry matter content of feed and weighback were determined by drying at 60 °C for 48 h (AOAC, 1990. Method No. 934.01). All feed samples were analyzed for crude protein (Mixed Catalyst Kjeldahl procedure, AOAC, 1990. Method No. 984.13), NDF (National Forage Testing Association, 1993) and ADF (AOAC, 1990. Method No. 973.18). Ca, P, K, Mg and Na were measured using inductively coupled plasma emission spectroscopy using an Atomscan 16 (Thermo elemental, Franklin, MA, USA). Feed analysis was carried out by Norwest Laboratory (Winnipeg, MB). Feed efficiency (kg milk/kg DMI) was calculated for individual cows.

Cows were weighed on two consecutive days during week 6 of each experimental period and an average body weight was calculated. Cows were body condition scored during week 6 of each period and the condition score scale ranged from 1 to 5, with 1 being an emaciated animal and 5 being an extremely obese animal (Edmondson et al. 1989).

Blood samples were collected by tail vein puncture on day 7 of week 3 and week 6 of each period using 10 ml heparinized vacutainer tubes and 20 G needles. Plasma was collected after centrifugation (750 x g for 30 minutes) and stored at –20 °C until assayed for IGF-1 (Kerr et al. 1990) at the Western College of Veterinary Medicine, Saskatoon, SK. Two cows were removed from the trial, one due to an udder injury and subsequent mastitis and another due to dramatic drop in milk production during period 2 of the trial. Data for these cows were not included in the statistical analysis.

5.3.3 Statistical Analysis

Analysis of variance was carried out to determine the significance of treatment on the average of milk production and milk composition in weeks 5 and 6. ANOVA was also carried out for body weight, body condition scoring, feed intake and plasma IGF-1 level. The experimental design was a repeated Latin Square Design. Statistical analysis of the data was performed using the Proc Mixed Model procedure of SAS (SAS Institute, Cary, NY, 1999). Results for week 5 and 6 were averaged and week was removed from the model when initial analysis showed the effect of week was not significant ($P > 0.05$). Cows were at 192 ± 15 (mean ± SD) days in milk by the beginning of period 4 by which time a few cows exhibited a large drop in milk production. It was considered that light

treatments might not be as effective during period 4 for these cows. For this reason, statistical analysis was performed with and without period 4 data for all variables except for DMI. For DMI, period 1 data was not available. Therefore, when removing period 4 data from the model, only arithmetic means could be calculated for periods 2 and 3. All effects were tested against residual error. Initial milk production (week 1) was used as a covariate in the analysis, but was non significant ($P > 0.05$) and consequently dropped from subsequent analysis. The covariates, parity, days of pregnancy and days in milk, were significant ($P \leq 0.05$) in the model, but were dropped from subsequent analysis because their inclusion had no discernable effect on the results of the ANOVA.

Source	**Error term**
Random effect	
Animal	Animal x Period x Treatment
Period	"
Fixed effect	
Treatment	"

Regression analysis on mean (mean of weeks 5 and 6) milk production vs photoperiod was performed using Proc GLM procedure of SAS Analyst (SAS Institute, Cary, NY, 1999).

5.4 Results

The mean, minimum and maximum barn temperatures (mean ± SD) were 13.7 ± 2.4, 9.7 ± 3.1 and 16.5 ± 2.7, respectively in the pre-treatment period and 8.6 ± 2.3, 5.3 ±

2.3 and 10.5 ± 2.6 in the treatment period. The curtains were found to be completely impermeable to light as light intensity during the dark phase for any particular treatment was always found to be < 0.03 lx.

Milk production (P = 0.87) and plasma IGF-1 (P = 0.90) levels were not affected by treatment (Table 4) and removing Period 4 did not alter these results (Appendix IV, Table 2). Regression analysis (R^2 = 0.38) showed that the milk production was not significantly influenced by the photoperiod treatments (P = 0.27). Cows exposed to the 18 h d^{-1} photoperiod tended (P = 0.09) to produce more milk fat (1.2 kg d^{-1}) than cows exposed to14 h d^{-1} (1.0 kg d^{-1}), 16 h d^{-1} (1.0 kg d^{-1}) and 20 h d^{-1} (1.1 kg d^{-1}) photoperiods when results for Period 4 were omitted (Appendix IV, Table 2), but the effect was much less apparent (P = 0.44) when the results for Period 4 were included (Table 4). With and without Period 4 included, photoperiod had no effect on milk protein, dry matter intake, feed efficiency, body weight and body condition score ($P \geq 0.27$) (Table 4 and Appendix IV, Table 2).

5.5 Discussions

A system of movable curtains was developed to allow the comparison of four photoperiods simultaneously in a tie stall dairy barn. Curtains were simple to manipulate, were impermeable to light and needed very little maintenance. In the present study, we observed no significant differences in milk production among treatments. To our knowledge, this is the first experiment where four potentially long day photoperiods have been studied simultaneously in one tie stall barn. Whereas photoperiods of 16 and 18 h d^{-1} have been previously shown to increase dairy cow milk production compared to

$\leq$ 13 h d $^{-1}$, photoperiods 14 and 20 h d $^{-1}$ have not been examined to date. In the present experiment, photoperiods of 14 and 20 h d $^{-1}$ resulted in similar milk production to that found with photoperiods of 16 and 18 h d $^{-1}$. Similarly, plasma IGF-1 level was not affect by photoperiod in this study.

There are a number of possible reasons for similarity among treatments in this study. In previous studies (Peters et al. 1978; 1981; Stanisiewski et al. 1985; Phillips and Schofield. 1989; Dahl et al. 1997), milk production was significantly elevated by photoperiods of 16 or 18 h d $^{-1}$ compared to natural photoperiods of $\leq$13 h d $^{-1}$. However, the minimum photoperiod required for this effect has not been determined. The day length above which an animal perceives it to be a long day and below which an animal perceives it to be a short day is called the critical day length. This day length is species specific (Elliott 1976). For example, Elliott (1976) stated that the critical day length of male Syrian hamsters for reproduction lies between 12 and 12.5 h d $^{-1}$. He found gonadal regression in male Syrian hamsters when exposed to a 12 h d $^{-1}$ photoperiod, but no such regression when the hamsters were exposed to a 12.5 h d $^{-1}$ photoperiod. Evans et al. (1991) have suggested that the critical day length for a plasma PRL response to photoperiod in dairy cows is between 13 to 15 h. Exposure of dairy cows to light for 2 h applied 13 to 15 h after dawn raised plasma PRL but 2 h light exposure applied at 10 to 12 h or 16 to 18 h after dawn did not. It is possible that dairy cows perceived all the photoperiod treatments as long days in the present study and so there were no treatment effects on milk production. Results of Leining et al. (1979) (elevated plasma PRL level) suggests that a photoperiod of 20 h d $^{-1}$ is considered a LPP by dairy cows, but Stanisiewski et al. (1987) found that this was not the case with a 24 h d $^{-1}$ photoperiod.

However, the milk production results of our study with a 20 h d^{-1} photoperiod and results of others who compared a 24 h d^{-1} photoperiod to an 18 h d^{-1} photoperiod in early lactating cows (Tanida et al. 1984) or 16 h d^{-1} (Dahl 1998) in mid to late lactating cows, respectively, suggest that photoperiods greater than 18 h d^{-1} are not detrimental to milk production.

Another possible reason why treatment effects were not found in this study is that the cows may have been refractory to LPP during our experimental period. Refractoriness to photoperiod has been reported in cattle (Stanisiewski et al. 1987), where plasma PRL level began to decline after 12 weeks exposure to LPP. In most LPP lactation studies, cows are exposed to natural SPP (< 13 h d^{-1}) prior to application of the LPP treatment (Dahl et al. 1997; Peters et al. 1978; 1981). In contrast, Tanida et al. (1984) exposed cows to a 24 h d^{-1} photoperiod prior to the beginning of the LPP treatment and found no difference in milk production between 18 h d^{-1} and 24 h d^{-1}photoperiods. The practical implication of this is that indoor housed dairy cows with an artificial photoperiod of >13 h d^{-1} (12 h milking schedule) may always be refractory. If so, to avoid this refractoriness, day length would have to be artificially reduced to below the critical day length (below 13 h d^{-1} based on Evans et al. 1991) prior to application of LPP. Also, the milk production response to LPP might only last three months. In the present study, prior to the experimental period, cows were exposed to a 18 h d^{-1} photoperiod for 109 ± 14 d (mean ± SD). This pre-experimental long photoperiod may have rendered the cows refractory to all of our photoperiod treatments.

The milk production variance (SE) in our experiment was relatively high as it was 8% of the mean milk production. To minimize variance, we had synchronized the cows

prior to the experiment to create as much uniformity in calving date (and DIM) as possible. As well, cows in each treatment group were balanced for parity. In other studies a 6.4% (Dahl et al. 1997) and 6.7% (Peters et al. 1981) increase in milk production with 16 h d^{-1} or 18 h d^{-1} photoperiod (compared to natural short photoperiod of $\leq$ 13 h d^{-1}) was significant using n = 42 (Peters et al. 1981) or n = 20 (Dahl et al. 1997) cows, respectively, and in both experiments the design was Randomized Complete Block Design. With the high level of variance in the present study, a difference in milk production among treatments of approximately 25% would be necessary to be significant at the $P < 0.05$ level. The reason for the high variance in the present study was unclear. In future studies, a larger number of cows will be required. Use of a Latin Square Design over 24 weeks (6 weeks per treatment period) of lactation may be another reason for high variance in this study. A special Latin Square Design (Cochran and Cox 1992) was used to balance for carry over effect, but it is possible that a high level of carry over contributed to the high level of variance found. From the results of Dahl et al. (1997), it appears that cows require two and four weeks for the IGF-1 and milk production responses to photoperiod, respectively, to develop. As well, Phillips and Schofield (1989) found that there was a milk production response to eight weeks (Randomized Complete Block Design) but not to three weeks of LPP (Latin Square Design) in dairy cows. Dahl et al. (1997) found that the IGF-1 response to LPP disappeared within two weeks but the time for the milk production response to disappear was not reported. Based on the above literature cited, six weeks was considered to be a sufficient treatment duration for the stimulation of milk production by LPP in dairy cows. A six weeks period was chosen to allow two weeks for carry over to disappear and four weeks for a response to develop.

However, it is possible, under our experimental conditions, that either the disappearance of carry over or development of response took longer than the time expected. If this were the case, then the use of a Latin Square Design with a period of six weeks may have introduced considerable unexplained variance. Further study is required to determine the number days needed to eliminate carry over effects of previous light treatment on milk production in dairy cows. It is also possible that six weeks was not long enough for a milk production response to develop in this study.

Exposure of dairy cows to photoperiods of 16 h d^{-1} or 18 h d^{-1} compared to 13 h d^{-1} or less increased dry matter intake (Tanida et al. 1984; Peters et al. 1981; Phillips et al. 1998). Peters et al. (1981) found that exposure to a photoperiod of 24 h d^{-1} reduced dry matter intake (DMI) compared to16 h d^{-1} photoperiod. Whereas, Tanida et al. (1984) found DMI was similar in dairy cows with 24 h d^{-1} and 18 h d^{-1} photoperiods. Although there was no significant effect of treatment on DMI in the present study, DMI with an 18 h d^{-1} photoperiod was numerically elevated by 7% (Period 4 included) or 13% (Period 4 excluded) compared to a 20 h d^{-1} photoperiod. It is possible that high variance in intake response in Period 4 made it difficult to show DMI intake difference in this study.

It was surprising to find a tendency ($P = 0.09$) for elevated milk fat with the 18 h d^{-1} (with Period 4 excluded) photoperiod compared to other photoperiod treatments. Others have found no effect (Peters et al. 1978; 1981; Dahl et al. 1997) or a decrease (Stanisiewski et al. 1985) in milk fat with LPP. In the present study adding period 4 results eliminated the milk fat trend ($P > 0.44$). The tendency for photoperiod to influence milk fat might be related to animal behavior. Dairy cows exposed to LPP (18 h d^{-1}) vs

natural SPP (8 h 6 min d^{-1}) had a 20 % increase in lying time (Phillips and Schofield 1989). Phillips et al. (1997) reported that steers exposed to a 16 h d^{-1} photoperiod spent more time lying and ruminating with no change in feed intake compared to steers exposed to natural (mean 9 h 43 min d^{-1}) photoperiod. Synthesis of milk fat in the present study may have been increased by the 18 h d^{-1} photoperiod if this treatment altered time spent ruminating and consequently the ruminal acetate to propionate ratio. Further research is required to determine the cause of a tendency for an increase in milk fat with a photoperiod of 18 h d^{-1}.

In the present study we did not find any difference in IGF-1 level in cows under different photoperiod treatments. It is possible that all photoperiods (14, 16, 18 and 20 h d^{-1}) tested were perceived as long days by the cows which resulted in similar IGF-1 level among treatments as was described for milk production. It has been reported that exposure to either a 16 h d^{-1} photoperiod (Spicer et al. 1994) or a 18 h d^{-1} photoperiod (Dahl et al. 1997) in heifers and cows, respectively, compared to a natural photoperiod of $\leq$ 13 h d^{-1} increased IGF-1 level. A detectable increase in IGF-1 level was found by four weeks of light treatment (Dahl et al. 1997). During the present study the relative change in photoperiod between the pre-experimental and experimental period was less than in the above studies. In addition, the pre-experimental photoperiod was 18 h d^{-1}. This was considerably longer than other experimental pre-conditioning photoperiods of $\leq$ 13 h d^{-1}. It is possible that cattle have to be pre-conditioned to a SPP of $\leq$ 13 h d^{-1} before LPP exposure stimulates an increase in IGF-1. It is also possible that carry over effect may account for the similarity in IGF-1 among treatments.

5.6 Conclusion

The curtains were found to be completely impermeable to light, simple to manipulate and needed very little maintenance in this study. Milk production and IGF-1 level were similar among 14 h to 20 h d^{-1} photoperiod treatments. Although it is possible that all photoperiods (14, 16, 18 and 20 h d^{-1}) tested were perceived as long days by cows and this resulted in the similarity in milk production and IGF-1 levels among all photoperiod treatments, there are a number of other possible reasons why treatment effects were not found. The variance (SE) for milk production was much higher than in other photoperiod studies, making it difficult to detect a milk production response to LPP. The reason for the high variance in the present study was unclear, but possibly there was treatment carry over as a Latin Square Design was used. Another possible reason is that exposure to an 18 h d^{-1} photoperiod for three months prior to the beginning of the experiment may have rendered the cows refractory to all of the photoperiod treatments tested in this study. DMI and milk fat tended to be higher for cows exposed to a 18 h d^{-1} photoperiod in this experiment compared to 14, 16 and 20 h d^{-1} photoperiods.

Table 3. Composition of the diet fed to experimental cows in Experiment 2.

Composition	Alfalfa Hay	Total Mixed Ration[z]
Dry Matter, %	87.5	48.8
CP, % DM	7.6	18.3
ADF, % DM	39.1	23.7
NDF, % DM	60.5	38.2
NEL[x]	1.2	1.61

[z] Containing (g kg^{-1}): Alfalfa silage (315); Corn silage (320); [1]Dairy 16 (260); [2]DPE (95); Sun Flower Seeds (10).

[x] Energy content expressed as NEL (Mcal kg^{-1}) (Van Soest et al. Cornell nutrition conference 1979 and NRC 1978).

[1] Dairy 16 contains rolled barley (54 %), luprosil salt (0.2 %), protein pellet (1.9 %), dairy supplement (40 %), and tallow (4 %).

[2] DPE contains dried distiller grain (42 %), fish meal (7 %), canola meal (22.8 %), soybean meal (20 %), beet molasses (3 %), niacin (0.3 %) and sodium bicarbonate (5 %).

Table 4. Effects of photoperiod on milk production, milk composition and plasma IGF-1 levels in dairy cows.

	Photoperiod (h d^{-1})					
Items	14	16	18	20	SE	P-Value
Milk Production, kg d^{-1}	30.6	30.6	31.5	31.4	2.5	0.87
Milk Fat, kg d^{-1}	1.0	1.0	1.1	1.1	0.09	0.44
Milk Protein, kg d^{-1}	1.1	1.0	1.1	1.1	0.09	0.38
Dry Matter Intake, kg d^{-1} *	20.7	21.0	21.8	20.3	0.83	0.27
FE, (kg milk/kg feed)*	1.4	1.4	1.4	1.5	0.08	0.71
Plasma IGF -1 conc., ng/ml^{-1}	147.0	155.0	153.0	152.0	11	0.90
Body Weight, kg	715.0	705.0	710.0	716.0	19	0.42
Body Condition Score	3.7	3.7	3.7	3.7	0.10	0.80

* DMI and FE were calculated only for 3 periods (period 2 to 4).

Figure 4. Cows within the tie-stall with rear and side curtains down.

Figure 5. Posterior end of the tie-stalls with curtains down.

6 *GENERAL DISCUSSION AND CONCLUSION*

This study was unique in that, to our knowledge, it is one of the first to examine the lowest light intensity that does not affect the normal rise in plasma melatonin level at night in cattle. It has been found that exposure to LPP increased IGF-1 level in dairy cows (Dahl et al. 1997) and melatonin feeding reduced IGF-1 level in heifers (Smith et al. 1997). Therefore, the results of this study with dairy heifers can add to understanding of adult dairy cows. In manuscript one, incandescent light intensities of 5 and 10 lx did not affect plasma melatonin level at night in heifers. Chastain and Hiatt (1998) stated that, the light intensity of 1 to 2 foot candles was adequate to read newspaper print by an average person. Thus, with the use of 5 or 10 lx (0.46 to 0.93 foot candles) light one could easily examine animals visually for estrus or general health during the night without affecting plasma melatonin level. The light intensity of 50 lx reduced plasma melatonin level by 50 % during hour 1 and 2 but not during hour 4 and 8 during the dark phase. Therefore, the actual threshold for the action of incandescent white light on the suppression of plasma melatonin level lies between 50 and 10 lx. Further study is required to determine the actual threshold light intensity for plasma melatonin suppression, possibly examining 10, 20, 30 and 40 lx. It is concluded that farmers can safely use 5 and 10 lx light during the night for dairy heifers with no endocrinological effect.

In the present study, the suppressive effect of 50 lx light on plasma melatonin level was maintained over two weeks. The degree of suppression of plasma melatonin in dairy heifers by 50 lx was 70% in the study of Lawson and Kennedy (2001), but only

approximately 50% in the present study. The difference may be due to the difference in the pre-conditioning day light intensity, light:dark cycle, and type of night light lamp used in the current study (200 lx, 16L:8D, incandescent bulbs) and that of Lawson and Kennedy (2001) (400 lx, 8L:16D, fluorescent bulbs). The variation in the spectral properties (Brainard et al. 1984; 1985) and wavelength (Cardinali et al. 1972; Brainard et al. 1984; Thorington 1985) of the light source has influence on pineal melatonin secretion. Blue light with a wavelength of 435 to 500 nm was most inhibitory to pineal melatonin level in rats (Cardinali et al. 1972) and in hamsters (Brainard et al. 1984) compared to green, yellow and red lights. The green wavelength (515 to 550 nm) was 25 % less effective than blue light, whereas red and yellow (560 to 700 nm) lights did not affect melatonin level (Brainard et al. 1984). Because incandescent bulbs have less blue and green colour spectrum than fluorescent bulbs (Thorington 1985), the use of incandescent bulbs may have been responsible for the relatively lower suppressive effect of 50 lx on plasma melatonin level in the present study.

In the present study and that of Lawson and Kennedy (2001), the effect of 50 lx on plasma melatonin level was seen during the early part of the night but not during the latter part of the night. This suggests refractoriness to dim light after a few hours during the night or the existence of photo-insensitivity periods during the night in cattle. An additional study is required to compare the plasma melatonin response to 50 lx when the light treatment commences at 2, 4, and 6 h of darkness or at night with a photoperiod of 16 h d^{-1}. This study would indicate if there are hours at night when it is safe for the dairy producer to use 50 lx dim light in the barn. Light intensity of $\leq$ 50 lx had no effect on plasma IGF-1 level in this study. To our knowledge, this is the first

study to suggest that plasma IGF-1 is negatively correlated to plasma melatonin level at night. Finally, in the present study plasma IGF-1 was higher during period 4. It was reported in male calves (Renaville et al.1993; 1996) and in heifers (Hall et al.1995) that puberty is associated with an increase in plasma IGF-1. The increased plasma IGF-1 level at period 4 may have been because the heifers were peri-pubertal (NRC 1989).

It is widely accepted that extending photoperiod to 16 to 18 h d^{-1} increases milk production compared to $\leq$ 13 h d^{-1} photoperiod in cattle. However, cows exposed to 14, 16, 18 and 20 h d^{-1} photoperiod had similar milk production, milk composition, DMI and IGF-1 level in experiment 2. There are a number of possible reasons for similarity among treatments in this study. In previous studies (Peters et al. 1978; 1981; Stanisiewski et al. 1985; Dahl et al. 1997) milk production was significantly elevated by photoperiods of 16 or 18 h d^{-1} compared to natural photoperiods of $\leq$13 h d^{-1}. However, the minimum photoperiod required for this effect has not been determined. The day length above which an animal perceives it to be a long day and below which an animal perceives it to be a short day is called the critical day length. This day length is species specific (Elliott 1976). For example, the critical day length of male Syrian hamsters for reproduction (gonadal function) lies between 12 and 12.5 h d^{-1} photoperiod (Elliott 1976), where as the critical day length of dairy cows for a plasma PRL response to photoperiod is between 13 to 15 h d^{-1}(Evans et al. 1991) photoperiod. In the present study, it is possible that dairy cows perceived all the photoperiod treatments (14, 16, 18 and 20 h d^{-1}) as long days resulting in similarity among treatments. Results of Leining et al. (1979) (elevated plasma PRL level) suggest that a photoperiod of 20 h d^{-1} is considered a LPP by dairy

cows, but Stanisiewski et al. (1987) found that this was not the case with a 24 h d^{-1} photoperiod. However, the milk production results of our study with a 20 h d^{-1} photoperiod and results of others who compared a 24 h d^{-1} to an 18 h d^{-1} photoperiod (Tanida et al. 1984) or 16 h d^{-1} photoperiod (Dahl 1998) in dairy cows, respectively, suggest that photoperiods greater than 18 h d^{-1} are not detrimental to milk production.

Another possible reason why a treatment effect was not found in this study is that the cows may have been refractory to LPP during our experimental period. Prior to the experimental period, cows were exposed to a 18 h d^{-1} photoperiod for 109 ± 14 d (mean ± SD). During this study, the relative change in photoperiod between the pre-experimental and experimental period was less than for other photoperiod studies. Therefore, it is possible that cows exposed to a 18 h d^{-1} prior to treatment will not respond to photoperiods ranging from 14 to 20 h d^{-1}.

The milk production variance (SE) in our experiment was relatively high as it was 8 % of the mean milk production. To minimize variance, we had synchronized the cows prior to the experiment to create as much uniformity in calving date (and DIM) as possible. As well, cows in each treatment group were balanced for parity. With the high level of variance in the present study, a difference in milk production among treatments of approximately 25 % would be necessary to be significant at the $P < 0.05$ level. The reason for the high variance in the present study was unclear. Either unexpected variation among cows or the experimental design (Latin Square Design) with inadequate photoperiod treatment period (6 weeks/treatment), or both, may be other reasons for high variability that was observed in this experiment. A special Latin Square Design (Cochran and Cox 1992) was used to balance for carry over effect, but it is possible that a

significant amount of carry over masked the presence of treatment effects. Based on the results of Dahl et al. (1997), 6 weeks was considered to be a sufficient treatment duration for the stimulation of milk production by LPP in dairy cows. However, it is possible, under our experimental conditions, that either the disappearance of carry over or development of response took longer than the time expected, resulting in a similarity among treatments.

Although there was no significant effect of treatment on DMI in the present study, DMI with an18 h d^{-1} photoperiod was numerically elevated by 7 % (Period 4 included) or 13 % (Period 4 excluded) compared to a 20 h d^{-1} photoperiod. It is possible that high variance in intake response in Period 4 made it difficult to show DMI intake difference in this study. It was surprising to find a tendency ($P = 0.09$) for a elevated milk fat with the 18 h d^{-1} (with Period 4 excluded) photoperiod compared to other photoperiod treatments. Synthesis of milk fat in the present study may have been increased by the 18 h d^{-1} photoperiod if this treatment altered time spent ruminating (Phillips and Schofield 1989; Phillips et al.1997) and consequently the ruminal acetate to propionate ratio. Further research is required to determine the cause of a tendency for an increase in milk fat in response to altered photoperiod. In conclusion, milk production and plasma IGF-1 level did not differ among cows exposed to 14, 16, 18 and 20 h d^{-1} photoperiods with light intensity of $\geq$ 200 lx when pre-conditioned to a 18 h d^{-1} photoperiod. DMI and milk fat tended to be higher in cows exposed to a 18 h d^{-1} photoperiod compared to 20, 16 and 14 h d^{-1} photoperiods.

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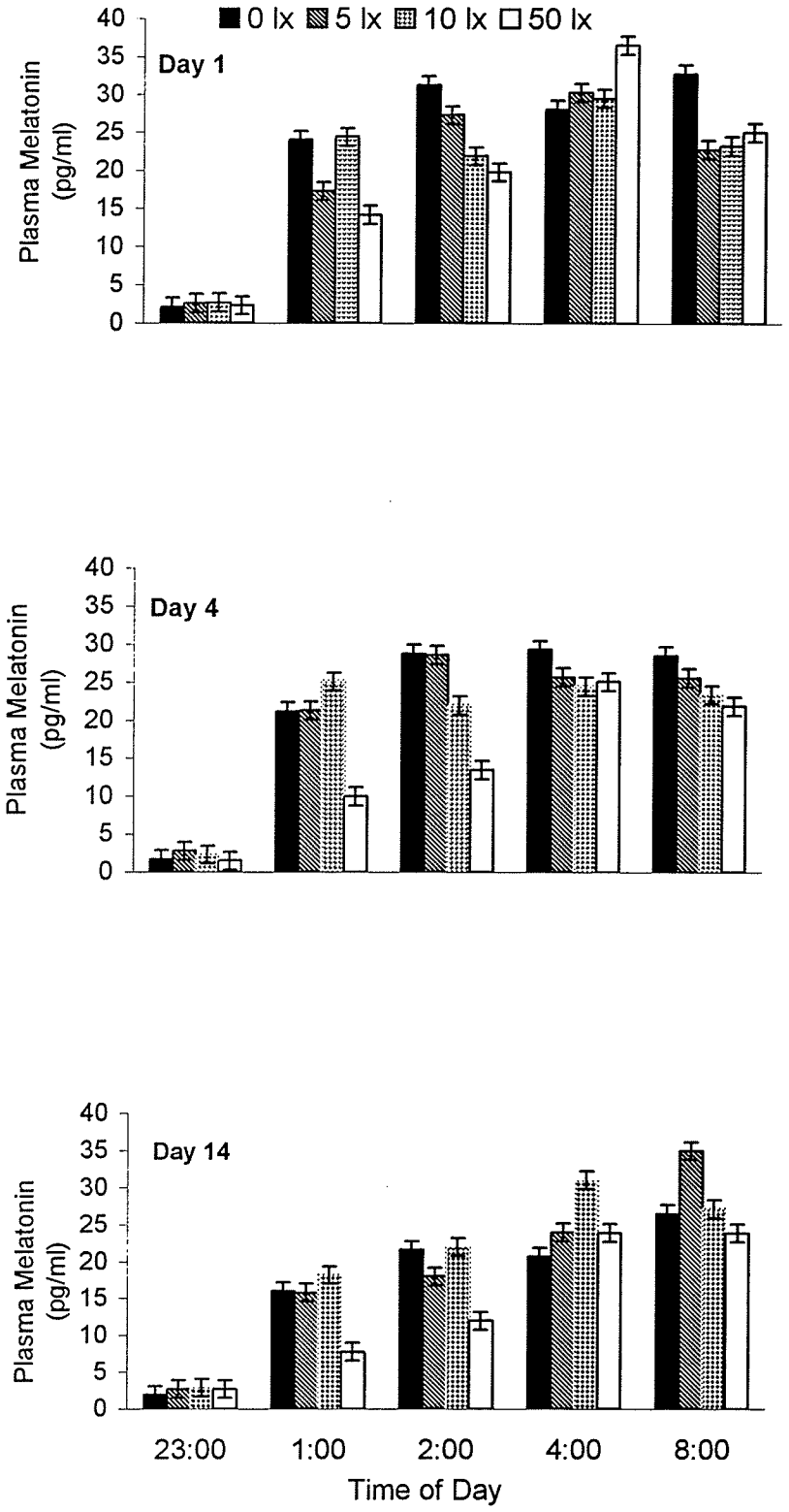
8 *APPENDICES*

Appendix I, Figure 1. Plasma melatonin response to dim light of 0, 5, 10 and 50 lx intensities during the 8 h night in dairy heifers on day 0, 4 and 14. (N=12), (Treatment x Hour x Day; P = 0.99). Darkness began at 24:00 h daily.


0 lx
5 lx
10 lx
50 lx
Day 1
Day 4
Day 14
Plasma Melatonin (pg/ml)
0
5
10
15
20
25
30
35
40
23:00
1:00
2:00
4:00
8:00
Time of Day

Appendix I, Table 1. Light intensities (mean ± SD) at night in experiment 1(from Feb 15, 2001 to July 02, 2001). Light intensities were measured horizontally using a light meter.

	Light Treatment (lx)			
Period	0	5	10	50
1	<1	5.3 ± 0.7	10.8 ± 1.2	53 ± 7.2
2	<1	5.2 ± 0.6	10.2 ± 1.0	54 ± 7.3
3	<1	5.6 ± 0.8	10.4 ± 1.4	55 ± 6.0
4	<1	5.7 ± 0.9	10.5 ± 1.0	52 ± 6.0

Appendix II Table 1. Quality control data from radioimmunoassays used to measure night plasma melatonin level.

Assay #	Pool 1	Pool 2	Pool 3	Average	SD	CV (%)	Intra-assay CV (%)	Inter assay CV (%)
				High Pool (pg/ml)				
1	45.4	44.1	54.8	48.1	5.8	12.1	9.9	16.6
2	28.4	31.3	-	29.9	2.1	6.9		
3	52.4	44.9	59.2	52.2	7.1	13.6		
4	47.6	38.7	-	43.2	6.3	14.6		
5	39.5	34.3	-	36.9	3.7	10.1		
6	39.6	35.4	37.0	37.3	2.1	5.7		
7	35.9	45.0	46.5	42.5	5.7	13.5		
8	43.4	42.0	-	42.7	1.0	2.3		
				Medium Pool (pg/ml)				
1	18.0	17.4	-	17.7	0.4	2.4	14.7	14.6
2	19.0	22.0	-	20.5	2.1	10.3		
3	22.0	14.0	-	18.0	5.7	31.4		
				Low Pool (pg/ml)				
1	1.2	1.0	-	1.1	0.1	13.0	21.0	20.8
2	1.4	2.6	-	2.0	0.8	42.0		
3	1.8	1.6	-	1.7	0.1	8.0		

Appendix III. Melatonin extraction efficiency variability

Objective

To determine the effect of person, day and reuse on extraction efficiency of melatonin extraction columns and to determine variability of extraction efficiency.

Introduction

In our experiment plasma melatonin values were highly variable among (ranged from 10 to 80 pg/ml) animals (animals within same group and also in different groups). Therefore, an experiment was conducted to determine if a considerable portion of this variation might relate to the use of extraction columns during the sample preparation for RIA.

Materials and Methods

Labeled plasma (approximately 3900 counts per minute per ml (cpm/ml)) was prepared by adding 0.2ml of I^{125} melatonin (Alpco, Windham, NH) to 5.5 ml of pooled bovine plasma. Two operators applied 0.15 ml of labeled plasma to duplicate extraction columns (Alpco, Windham, NH), which had been used previously 0, 1, 2, or 3 times for melatonin extraction from bovine plasma. The entire procedure was repeated on two consecutive days. Extractions were conducted according to the methods described in the Alpco manual (Alpco, Windham, NH) except for the final step. For the final step, extracted melatonin in methanol was collected into a tube (12x75mm) for counting directly in a Universal Gamma counter (LKB, Wallac, Campugamma).

Melatonin extraction efficiency (EE %) was calculated as follows:

$$EE\ (\%) = \frac{\text{cpm/extraction tube}}{\text{cpm applied to the column}} \times 100$$

Statistics

Data were analyzed using the General Linear Model procedure of SAS (SAS Institute Inc, 1996). Data were analyzed as a Randomized Complete Block Design with repeated measures. The column previous use was tested as a treatment and operator as a block effect. Main and interaction effects were tested against the following error term.

Source	Error term
Column previous use, operator	Column previous use x operator
Day	Day x column previous use
Day x column previous use	Residual error.

Results

There were no main or interaction effects on melatonin EE ($P > 0.05$). The results are shown in Appendix III, Table 1.

Discussion

The mean extraction efficiency of 94.1% was in agreement with kit manufacturers assessment. In this experiment, the repeated use of column did not affect the extraction efficiency for melatonin from plasma and, as indicated by the manufacturer, column reuse was not a problem. The coefficient of variation (5%) was quite low, which suggests

that EE variability is not a major contributor to variation in cattle plasma melatonin values.

Appendix III, Table 1. Effect of operator, day and column previous use on melatonin extraction efficiency (%).

	Day				
	One		Two		
	Operator				
Column previous	1	2	1	2	SE
0	92	88	93	89	1.02
1	96	98	95	97	1.02
2	93	96	95	96	1.02
3	97	96	90	97	1.02

Appendix IV, Table 1. Daytime light intensities (mean ± SD) during the four periods (from October 26, 2001 to April 11, 2002) in Experiment 2. Scotophase readings were 0.03 lx. Light intensities were measured horizontally using a light meter.

	Daytime Light Intensity (lx)			
Period	Group of Cows			
	1	2	3	4
1	249 ± 15	223 ± 10	230 ± 23	228 ± 23
2	243 ± 16	237 ± 10	233 ± 14	253 ± 19
3	237 ± 15	249 ± 15	233 ± 11	276 ± 15
4	226 ± 19	241 ± 22	220 ± 18	278 ± 43

Appendix IV, Table 2. Effects of photoperiod on milk yield, milk composition and plasma IGF-1 level in dairy cows (first 3 periods of a 4 x 4 Latin Square Design).

	Photoperiod (h d^{-1})					
Items	14	16	18	20	SE	P-Value
Milk Production, kg d^{-1}	31.5	32.5	33.4	32.2	2.6	0.70
Milk Fat, kg d^{-1}	1.0	1.0	1.2	1.1	0.10	0.09
Milk Protein, kg d^{-1}	1.1	1.1	1.2	1.1	0.09	0.60
Dry Matter Intake, kg d^{-1} *	22.0	21.30	22.70	20.0	NA	NA
FE, (kg milk/kg feed)*	1.5	1.4	1.5	1.4	0.11	0.83
Plasma IGF -1 conc., ng ml^{-1}	141.0	157.0	152.0	158.0	11	0.45
Body Weight, kg	710.0	704.0	708.0	715.0	16	0.68
Body Condition Score	3.7	3.7	3.7	3.8	0.12	0.75

NA = feed results (per pen of 3 cows) available for only 2 periods (period 2 and period 3) and the Means are arithmetic means. * DMI and FE were calculated only for 2 periods.