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EFFECT OF PHOTOPERIOD ON SERUM CONCENTRATIONS OF MELATONIN IN PREPUBERTAL HOLSTEIN HEIFERS.

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EFFECT OF PHOTOPERIOD ON SERUM CONCENTRATIONS OF MELATONIN IN PREPUBERTAL HOLSTEIN HEIFERS

By

Brent Alan Buchanan

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ABSTRACT

EFFECT OF PHOTOPERIOD
ON SERUM CONCENTRATIONS OF MELATONIN IN
PREPUBERTAL HOLSTEIN HEIFERS

By

Brent Alan Buchanan

My goal was to suppress the daily surge of melatonin in serum of prepubertal dairy heifers by manipulating the intensity (Experiment 1) and duration of exposure to light (Experiment 2). Heifers in Experiment 1 were exposed to either continued darkness (000 lux, control), or 400, 800, or 1200 lux of light during the last 6 h of their usual 12-h nocturnal period. During this 6-h exposure to light, melatonin concentrations of all groups were similar to daytime baseline values but were 52 to 73% lower than melatonin concentrations of control heifers receiving 6 h of continued darkness. In Experiment 2, heifers were exposed to 8L:16D, 16L:8D, 20L:4D, or 24L:0D photoperiods (1200 lux) for 4 months. Throughout treatment, concentrations and durations of the melatonin surge were suppressed in the 24L:0D group and were greatest (during the nocturnal period) in the 8L:16D group. In conclusion, continuous light for 4 months at an intensity of 1200 lux suppressed the nocturnal surge of melatonin in prepubertal heifers.

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INTRODUCTION

The world is faced with the continued challenge of producing more food with less: less labor, less land, less money, and less additives. On the other hand, more nutritious, more healthful and more natural foods are also highly desirable. The process involved in improving products in the manner described above is typically limited because very few products or practices are able to meet all criteria of the consumer. Products such as recombinant somatotropin have been extensively tested and proven to be highly efficacious in increasing both efficiency of milk production and quality of meat products (less fat, more lean). However, the mind-set and perspective of the consuming public (or a vocal minority subset of the public) has already delayed and may even prevent the adoption of this technology by commercial producers. The primary reason for the public wariness concerning recombinant somatotropin is that it appears to fail the public's litmus test of "fewer additives and more natural" methods for producing food. Even though the general public is not always well-informed, their opinions serve as the basis for acceptance or rejection of a food production method.

In this light, it is prudent to pursue alternative avenues in order to achieve the above mentioned food production goals. Although these alternatives typically yield smaller increases in efficiency than exogenous hormones, they meet a larger number of the public's requirements. Alternatives such as increasing daily durations of light from that of short days (14 h or less of daily light) to long days (approximately 16 or more hours of daily light) show promise in cattle for increasing efficiency and production of milk and meat products. Such methods should foster no public mistrust or fear of the production technology.

The biological mechanisms underlying long-day mediated increases in live weight gain (Peters et al., 1978, 1980; Petitclerc et al., 1983) and increased proportion of carcass protein with decreased proportion of fat (Zinn et al., 1986a) in cattle are not clearly understood. It is possible that these effects are due to a decrease in melatonin concentration (Zinn et al., 1988) or an interaction of melatonin with other hormonal factors. It is important, therefore, to determine which environmentally-controlled processes are important in animal production efficiency and under what conditions they can be modulated. Light-induced suppression of melatonin is one such process that has the potential to increase the efficiency of animal production. Increased melatonin decreases mammary development in prepubertal cattle (Sanchez-Barcello et al., 1991) and suppresses peak lactation in rabbits (Shani et al., 1971). Therefore, it may be beneficial to suppress melatonin in cattle, at least during certain critical life stages such as the allometric growth phases of the mammary gland. Suppression of melatonin may lead to increased growth and milk secretory capacity of the mammary gland.

My overall objective was to determine the efficacy of intensity and daily duration of light to suppress melatonin concentrations in serum of heifers.

LITERATURE REVIEW

A) Photoperiodic Effects On Animal Systems

Areas of the world other than those on the equator undergo a daily shift in the duration of daylight. These shifts are typified by earlier dawns and later dusks during the longer days of summer and later dawns and earlier dusks during the shorter days of winter. Many animals rely upon these dynamic cues

from the sun in order to time such important events as puberty, breeding activity, hibernation, and migration. Even animals thought to be non-photoperiodic use daily lighting cues to orchestrate bodily functions such as temperature and blood pressure changes, feeding and activity patterns, the timing of release of diurnal hormones and other basic internal rhythms.

Many domestic animals in a typical food production system in the United States are likely to be held under artificial indoor lighting conditions for extended periods of time. The intensity of this indoor lighting (50 to 600 lux) is seldom as bright as outdoor daylight (up to 100,000 lux) and therefore may not provide as strong a cue as natural daylight for entrainment of the internal clock of the animal. Additionally, domestic animal production systems often necessitate longer durations of daily light than would be seen naturally, and this duration is often fixed year round at a constant length. Therefore, domestic animals are exposed to a lesser intensity of light, and are not allowed to experience the natural waxing and waning cycles of annual day length, which is in contrast to wild animals living away from the equator. Therefore, the endocrine effects of the artificial environment that domestic food-producing animals are placed under are of great importance in our quest to promote gains in efficiency of food production.

1) Photoperiod Regulation of Mammary Growth and Milk Production

Mammary gland growth is augmented when cattle are exposed to long-day photoperiods. For example, mammary parenchymal DNA is increased under long days by 68 and 35% in prepubertal and pubertal heifers, respectively, and extraparenchymal mammary tissue is decreased by 12 to 35% relative to short days (Petitclerc et al., 1984).

Sixteen h of continuous daily light increases milk production in mice (Sorensen and Hacker, 1979) and cattle (Peters et al., 1978,1981; Phillips and Schofield, 1988; Bilodeau et al., 1989) relative to that observed under 8 h. Average milk yield increased about 5% in cattle exposed to 16 to 17 h of extended daylength versus cows under 12 to 15 h of natural daylength (Sorensen et al., 1986). Similarly, cattle receiving approximately 16 h of daily light produced 2.2 kg more milk and 0.16% less fat (Stanisiewski et al., 1985) than cattle receiving 9 to 12 h of light each day, and 18 h of daily light increased milk yield 7% (Marcek and Swanson, 1984). Furthermore, supplying light as a "skeleton" photoperiod that daily provides a 2 h block of light 13 to 15 h after the initiation of a 6 h block of light (6L:7D:2L:9D), increased persistency of milk production relative to 12 to 13 h of continuous light each day (Evans and Hacker, 1989).

2) Photoperiod Regulation of Growth and Body Composition

Relative to short-day photoperiods, cattle exposed to long-day photoperiods had increased protein percentages in the 9-10-11 rib section as well as increased carcass weights (Petitclerc et al., 1984). Similarly, sheep exposed to long-day photoperiods had increased carcass weights and increased muscle mass compared with animals on short days (Forbes et al., 1975, 1979b; Schanbacher and Crouse, 1980, 1981). When compared with short-day photoperiods, long-day photoperiods stimulated live-weight gains of peripubertal or pubertal Holstein heifers and bulls 9 to 17% (Peters et al., 1978,1980; Petitclerc et al., 1983; Tucker et al, 1984; Zinn et al., 1986b), but photoperiod did not affect weight gains of prepubertal animals (Roche and Boland, 1980).

Body growth is controlled by several factors including concentrations of glucocorticoids, growth hormone, and prolactin (Bates et al., 1964; Purchas et al., 1970, 1971). These hormones will respond to changes in photoperiod under certain situations (Leining et al., 1979, 1980; Brinklow and Forbes, 1984a, b). Therefore, body growth and carcass composition changes due to photoperiod exposure may be mediated through concurrent changes in prolactin, growth hormone, and(or) glucocorticoids. In other studies where melatonin was fed to heifers in the middle of a long-day photoperiod (simulating a short-day melatonin profile) decreased concentrations of prolactin (Sanchez-Barcelo et al., 1991), increased percentage of fat in rib and longissimus muscle, and decreased percentage of protein in rib (Zinn et al,. 1988), relative to control heifers fed melatonin-free vehicle in the middle of the light period. Therefore, it is not yet clear which hormones actually initiate and coordinate the photoperiod response and which hormones merely respond to the effects of the orchestrating hormones.

3) Photoperiod and Regulation of Gonadotropins

Seasonally breeding species, such as sheep, goats, and horses have reproductive cycles that are influenced by changes in daily photoperiod. These animals rely upon the changing daylengths in order to time the beginning and end of their reproductive season and are classified as long-day or short-day breeders depending on the daylength that initiates reproduction (Karsch et al., 1984). The reproductive responses of most breeds of hamsters are stimulated by long days (Hoffman and Reiter, 1965; Bittman and Zucker, 1981) whereas sheep are stimulated by short-day photoperiods (Lincoln, 1979; Bittman et al., 1983a,b). Furthermore, gonadotropins are lowest during short days in hamsters (Stegar et al., 1985; Yellon and Goldman, 1987) and during long days in sheep

(Lincoln, 1979; Lincoln et al., 1982) goats (Racey et al., 1975) and white-tailed deer (Mirarchi et al., 1978; Bubenik and Schams, 1986; Bubenik et al., 1987). Mares on long-day photoperiods had higher concentrations of LH relative to mares on short days (Oxender et al., 1977). Syrian hamsters, which are long-day breeders, have lower concentrations of LH while under shorter days. However, hamsters are also sensitive to the direction of photoperiodic change (increasing or decreasing daylength), which can make a single photoperiod either reproductively stimulatory or inhibitory, depending on the previous daylength to which they were exposed (Hastings et al., 1989). Thus, photoperiodic history is important in experimental paradigms involving photoperiod or photoperiod-sensitive systems.

The long- or short-day breeder classification generally refers to postpubertal animals. In fact, prepubertal animals typically respond differently, sometimes even oppositely, to daylength cues relative to pubertal animals. For example, 16L:8D photoperiods delay puberty in the female pony, but hasten the beginning of the breeding season in the mature pony (Wesson and Ginther, 1982). Puberty is delayed in ewe lambs under short days (Yellon and Foster, 1982), while mature ewes under similar conditions have hastened breeding seasons (Yeates, 1949; Hart, 1950; Thwaites, 1965; Vesely, 1975).

Many practical applications have been found for modulating photoperiod in animal production systems. For example, if it is advantageous for foals to be born earlier in the season than normal, the long-day ovulatory season in pony mares can be hastened by approximately 2 months. This can be accomplished simply by exposing the mares to spring-like photoperiod conditions (Sharp and Ginther, 1975) during their normal anovulatory period or to an artificial long-day photoperiod (Kooistra and Ginther, 1975). Additionally, chickens can be induced to lay eggs for a longer period of time without molting

by using continous 14 to 16 h or more of light throughout the productive life of the chicken. Actively laying hens will cease egg-laying and begin to molt if they are exposed to decreasing daylengths (Nesheim et al., 1979).

Swine and cattle have generally been considered to be non-seasonal breeders, and most reports suggest that photoperiod has no effect on secretion of gonadotropins (Ntunde et al., 1979; Rzepkowski et al., 1982; Diekman and Hoagland, 1983; Peirce et al., 1987; Stanisiewski et al., 1987a, 1988a). In fact, the estrous cycles of cattle continue throughout the year (Rzepkowski et al., 1982). However, photoperiod has been reported in some instances to influence the estrous activity of both sows and gilts (Hurtgen et al., 1980), and some other reproductive factors in cattle as well. For example, testosterone is increased in prepubertal bulls given 16 h of daily light relative to those given 8 h of daily light (Stanisiewski et al., 1987a). Prepubertal heifers exposed to either long photoperiods or spring to fall transitions in daylength and temperature, attain puberty faster than those on short-day photoperiods or fall to spring transitions (Hawk et al., 1954; Menge et al., 1960; Peters and Tucker, 1978; Roy et al., 1980; Hansen et al., 1981, 1983; Schillo et al., 1983). In ovariectomized cattle, LH increases during increasing durations of daily light relative to decreasing durations of daily light (Day et al., 1986, Critser et al., 1987b, 1988; Stumpf et al., 1988). In other studies using ovariectomized heifers treated with estradiol, concentrations of FSH and LH were higher in animals given short days (Critser et al., 1987a).

4) Photoperiod and Regulation of Prolactin

Several factors related to photoperiod affect concentrations of prolactin in serum. Season of the year generally involves both photoperiod and temperature modulations, where long days generally coincide with higher ambient temperatures and short days with lower temperatures. Serum prolactin concentrations are increased during the spring and summer months and are decreased during the fall and winter in cattle (Koprowski and Tucker, 1973; Kensinger et al., 1979; Peirce et al., 1987), sheep (Ravault, 1976; Munro et al., 1980; Kennaway et al., 1981; Bosc et al., 1982), goats (Buttle, 1974), horses (Johnson, 1986; Thompson et al., 1986), and deer (Mirarchi et al., 1978; Bubenik and Schams, 1986).

Increasing temperature while photoperiod is held constant rapidly increases serum prolactin concentrations. In contrast, decreasing temperature rapidly decreases serum prolactin concentrations in cattle (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976) and pigs (Kraeling et al., 1987). Serum concentrations of prolactin in steers kept at 30° C were increased 2.5-fold relative to steers kept at 10° C. These elevated values for prolactin were associated with both a decline in the rate of clearance and an increase in the rate of secretion of prolactin (Smith et al., 1977).

Modulating photoperiod while holding ambient temperature constant also has a major influence on serum concentrations of prolactin, although the effects of photoperiod are not as immediate as those seen with temperature modulation. Prolactin concentrations are generally higher during long days (16L:8D) and lower during short days (8L:16D to 12L:12D) at constant ambient temperature in cattle (Bourne and Tucker, 1975; Leining et al., 1979; Stanisiewski et al., 1984, 1987b; Critser et al., 1987b), sheep (Forbes et al.,1975, 1979b; Fitzgerald et al., 1982; Leshin and Jackson, 1987) and deer

(Abbott et al.,1984; Bubenik et al.; 1987). However, heifers exposed to cold temperatures (1°C) and 16L:8D photoperiods have prolactin concentrations similar to values found in heifers exposed to natural short-day photoperiods (Peters and Tucker, 1978). Thus, cold temperatures reduce the stimulatory effects of long days on prolactin.

5) Photoperiod and Regulation of Other Hormones

Not all hormones are photoperiod-dependent and few, if any, are photoperiod-dependent under all circumstances. Complex interactions between hormones and the environment play a major role in the metabolic balance of an animal.

In most studies, average growth hormone (Forbes et al., 1979 a,b; Leining et al., 1980), insulin (Forbes et al., 1979 a,b) and thyroxine (Forbes et al, 1979a) concentrations in serum are unaffected by photoperiod. However, other reports have been contradictory: for example, insulin concentrations tend to decrease (Zinn et al., 1989), and growth hormone concentrations increase in variability (Leining et al., 1980) in response to long-day photoperiods. It is not known if variability of hormonal concentrations has any influence on metabolism during body growth or milk production phases of an animal's life.

Additionally, testosterone is increased in prepubertal bulls given 16 h of daily light relative to those given 8 h of daily light (Stanisiewski et al., 1987a). The increased concentrations of testosterone may have a positive impact on metabolism and growth. Therefore, there is mixed evidence concerning the effect of photoperiod on some hormones; under certain circumstances photoperiod stimulates specific hormones of possible metabolic significance but has no influence at other times.

6) Photoperiod and Regulation of Melatonin

The indolamine hormone, melatonin, was first isolated in 1958 in bovine pineal tissue (Lerner et al., 1958). Since that time, particularly in recent years, a great deal of interest has developed in attempts to determine the role of melatonin in vertebrate systems. Even though melatonin is just one of a large class of indoleamine compounds, it has been singled out from other methoxyindoles because it has 10 times greater potency in controlling physiological functions such as photoperiodically entrained rhythmicity and reproduction in animals (Reiter et al., 1975, 1982; Goldman et al., 1984). In most animals studied, melatonin concentrations in serum are lowest during periods of light and highest during periods of dark (Rollag and Niswender, 1976; Lincoln et al., 1982). It is this diurnal change in melatonin concentrations that enables animals to interpret the relative length of the photoperiod to which they are exposed. Therefore, sheep experiencing 8 or 16 h of daily elevated melatonin are perceiving long- and short-day photoperiods, respectively (Bittman and Karsch, 1984). The mammalian pineal gland is responsible for producing and secreting most of the nocturnal melatonin found in serum, although melatonin is also produced by the gut (Quay and Ma, 1976; Bubenik et al., 1977), harderian gland (Bubenik et al., 1976), retina (Pang and Allen, 1986), red blood cells and extraorbital lacrimal glands (Reiter, 1986).

It has been commonly assumed that the melatonin rhythm in blood is a consequence of rhythmic pineal synthesis and release of melatonin because pinealectomy abolishes the 24-h cycle of melatonin in the blood (Reiter, 1988). This is purported to occur within the pineal gland because melatonin production in other tissues and organs is generally very small (as in the retina) and(or) not of a rhythmic nature. It is hypothesized that the pineal gland receives most of its neural input from the suprachiasmatic nuclei (SCN) of the anterior

hypothalamus. The retina of the eye communicates with the SCN via the retinohypothalamic pathway. The direct connection of the eye with the SCN allows the SCN to function as the primary pacemaker of the central nervous system (CNS). Thus, the SCN transduces the external light-dark cycle and generates circadian rhythmicity in mammals.

There is some debate as to the role of melatonin in photorefractory animals; for example, melatonin profiles in rams have been observed to become refractory to repetitive long-term photoperiods (Almeida and Lincoln, 1984), whereas other studies have found no alteration in melatonin profiles regardless of the photorefractory state of the animal (Malpaux et al., 1987). Although the pineal gland and SCN are connected by a circuitous neural route, there is evidence that release of melatonin from the pineal gland could be classified as functionally being part of the SCN output (Armstrong, 1989). Systemic melatonin concentrations inversely reflect the metabolic activity of the SCN which results in high concentrations of melatonin in serum at night and low concentrations during the day. The resulting periodic chemical message of melatonin may then be utilized to synchronize the daily rhythms of cells, tissues and organs within an organism (Armstrong, 1987, 1989). The pineal gland was among the first of the endocrine organs found to have distinct daily changes in hormone production (Krieger, 1979). It is hypothesized that the rhythmic production of melatonin by the pineal gland may stimulate or synchronize rhythmic changes in other endocrine glands (Binkley, 1988). Factors that can alter the size and secretory rate of the pineal gland include: duration of light and dark, time of day (relative to the onset of light and dark), season of the year, and timing of occurrence of light and dark relative to other internal ryhthms (Binkley, 1988).

7) Physiological Effects of Melatonin

Exogenous melatonin has typically been administered by one of two methods. The first is designed to yield a continuous elevation of melatonin in serum through the use of subcutaneous implants and the second utilizes a dose given once daily which causes a rapid increase, followed by a decline in melatonin concentrations in serum. Administration of a single daily dose of melatonin is typically timed in conjunction with the animal's light-dark cycle so that the daily duration of elevated melatonin is increased substantially. It may be important to note that the continuous release method of melatonin administration can completely overwhelm the animal's daily surge of endogenous melatonin which may mask the physiological response to endogenously-produced melatonin (Turek et al., 1976). The timed daily dose method of administration of melatonin can effectively mimic a normal day. However, the nocturnal period which would normally be interpreted by the animal as beginning shortly after the lights were actually turned off, would instead begin after administration of exogenous melatonin. Thus, exogenous melatonin, when given in the middle of a 16-h light period, will cause sheep to respond as if exposed to only 8 h of light per day (Kennaway et al., 1982; Symons et al., 1983).

a) Body Growth and Body Composition

The effects of feeding melatonin parallel the effects of exposure to short day photoperiods in cattle in terms of fat accretion (Zinn et al., 1988). Postpubertal beef heifers fed melatonin in the middle of a long-day photoperiod had body weight gains not different from control heifers; however, those fed melatonin had over 9% higher fat accretion and a concomitant reduction in protein accretion (Zinn et al.,1988). In another study, heifers fed melatonin had

10% more fat in the rib, 24% more fat in the longissimus muscle and 8% less protein in the rib (Zinn et al., 1988). Melatonin effects are seen in rodents as well. For example, Syrian hamsters given small doses (2.5 ug/day) of melatonin had increased body weight and fat content over that of control hamsters (Wade and Bartness, 1984). Additionally, daily injections of melatonin increased mean body weights and decreased serum thyroxine concentrations in female hamsters relative to saline-injected controls (Petterborg and Rudeen, 1989).

b) Reproduction

The most widely studied effects of exogenous melatonin have been on the reproductive characteristics of seasonally breeding species, such as hamsters and sheep. Changes in photoperiod can alter the secretion of pituitary hormones by a mechanism thought to involve an interaction between the pineal gland and hypothalamic neural activity (Lincoln et al., 1982). Long days (or short daily durations of elevated melatonin) induce gonadal recrudescence in certain breeds of hamsters. Alternatively, Mongolian gerbils are long-day breeders where exposure to 8L:16D photoperiods causes a decrease in the weight of the reproductive tract. This antigonadal effect can be mimicked in animals under normally stimulatory photoperiods by a melatonin implant placed either subcutaneously or in the anterior hypothalamus (Devries et al., 1989).

Exogenous melatonin administered to estradiol-treated ovariectomized heifers (non-seasonal breeders) had no effect on FSH concentrations and only tended to inhibit the decrease in concentrations of LH seen in vehicle-treated controls (Critser et al., 1987a). Therefore, photoperiod and melatonin have relatively little effect on reproduction in non-seasonal breeding species, such as

cattle; in contrast, the reproductive systems of the seasonal breeders rely heavily upon the changes in photoperiod and the concomitant changes in concentration of serum melatonin, in order to time their breeding seasons.

c) Mammary Gland Growth and Milk Yield

There has been only limited experimentation in the area of melatonin and milk production. Pinealectomized rats had mammary development similar to sham-operated rats (Mizuno and Sensui, 1970). However, these animals were kept under 14L:10D photoperiods which may be stimulatory to mammary development in the sham-operated control rats. Reiter (1977) claims that animals exposed to more than 12 h of light per day are "physiologically pinealectomized", therefore, results obtained from studies involving pinealectomy in animals given more than 12 h of light may be inconclusive. Lactating mice injected with bovine pineal extracts had lower litter weight gains, but only when the dose equalled 0.25 g pineal tissue equivalent (Sorensen and Hacker, 1979). In rabbits, administration of melatonin lowered the peak of the milk yield curve (Shani et al., 1971), and exogenous melatonin fed for 68 d to prepubertal dairy heifers in the middle of the 16-h light period reduced mammary gland growth (Sanchez-Barcello et al., 1991). Therefore, melatonin may have a negative impact on milk production.

8) Why Study Melatonin?

It is apparent that photoperiod has important implications on many bodily processes throughout the lifetime of an animal. Changes in daily durations of light are very accurately reflected in the pineal gland by the pattern of secretion of melatonin. This signal may be responsible for coordinating the myriad of complex biological systems that rely upon photic cues for proper timing. If

melatonin is responsible for initiating the effects of photoperiod, modulation of the melatonin signal may be an effective tool in the quest to increase efficiency of milk and meat production through photoperiodic methods.

CHAPTER 1

Suppression of nocturnal melatonin in heifers under four intensities of light

Introduction

Duration of elevated nocturnal melatonin concentrations in serum may mediate the effects of long- vs short-day photoperiods on production traits in cattle. Thus, suppression of the nocturnal surge should stimulate rates of gain, mammary development and milk yield as well as reduce carcass fat. Melatonin can be effectively suppressed by exposure to light.

In cattle, as in most other species studied, the rhythm of melatonin production is endogenously generated and entrained by the light-dark cycle (Rollag and Niswender, 1976; Lincoln and Ebling, 1985). However, different species may respond to the same photoperiodic stimuli in different ways. For example, under long days, long-day breeders are sexually active whereas short-day breeders, under long days, are sexually quiescent. Both the intensity of the light cue (Lewy et al., 1980; Lynch et al., 1981; Brainard et al., 1983; Reiter, 1985) as well as the photoperiodic history are important when considering the sensitivity of animals to a specific photoperiodic cue. For example, sheep can experience a 46% suppression of nocturnal melatonin when exposed to as little as 1 lux of light during their nocturnal period (Arendt and Ravault, 1988). In cattle, however, the light intensity that maximally suppresses nocturnal melatonin in serum is unknown. My objective was to examine the effects of light intensity on the nocturnal surge of melatonin in serum of heifers.

Materials and Methods

Sixteen prepubertal Holstein heifer calves (ranging in age from 2.2 to 7.7 months) were placed in light- and temperature-controlled environmental chambers. Heifers were blocked by body weight and were assigned randomly from within block to one of four treatments. Four heifers per chamber were acclimated to a 12L:12D photoperiod at 400 lux intensity (at 1 m above floor level) for 10 d (lights on 0700 h, lights off 1900 h). It was assumed that entrainment to the photoperiod in terms of the melatonin rhythm would be established during this period. Temperatures were held at 13.3±2°C. Heifers were fitted with indwelling jugular catheters on d 9 of the acclimation period in order to facilitate the sampling of blood. Catheter patency was maintained by flushing with 3.5% Na citrate in sterile water after collection of each blood sample. Samples of blood were taken every 30 min, beginning at 0700 h of d 10 and continued through 0800 h of d 11. Treatments were administered on d 11 (0115 h) during the second half of the normal nocturnal period and consisted of the following lighting conditions: continued darkness (000 lux), 400, 800 or 1200 lux of light. Blood samples were allowed to clot at approximately 20° C, then were held at 4° C for approximately 12 h before being centrifuged at 991 x g for 30 min. Following centrifugation, serum was decanted and stored frozen at -20° C until assayed for melatonin. A direct assay of melatonin previously validated in our laboratory (Stanisiewski et al., 1988b) was used. This assay utilizes a melatonin antibody (batch no. G/S/704-6483) from Guildhay Antisera Ltd (Guilford GU25XH, Surrey, United Kingdom) in a methodology similar to that of Fraser et al. (1983). Due to assay drift problems associated with charcoal separation of free and bound hormone, the size of individual assays was limited to 160 tubes. Therefore, all samples from an individual heifer were measured in

a single assay. The inter- and intra-assay coefficients of variation were 20.4 and 5.2%, respectively.

Statistical analyses involved both a split-plot design with the light, dark, and intensity treatment periods within a day as the split-plot for hormone analyses, and a one-way analysis of variance (ANOVA) for comparing durations of periods (Gill, 1978). The Pulsar computer program was used to determine the initiation and cessation of the nocturnal melatonin surge of each heifer (Merriam and Wachter, 1982). The Pulsar program was set to accommodate a sensitivity of 4 pg/ml which approximated the lower limit of sensitivity of the assay used. Fifty-one samples were assayed per heifer, therefore a 51 unit smoothing time was chosen for use in the program. The G(value) standard deviation settings for G(1) to G(5) of the program were 6.2, 4.2, 3.6, 2.4, and 20.4, respectively. The G(values) correspond to the number of standard deviations above a Pulsar-generated smoothed baseline required for 1 to 5 successive data points to be included as a surge value. Once the initiation and cessation points of the surge were determined by Pulsar, the duration of the surge was calculated. This information was subsequently used to divide the sampling interval (25 h total) into 3 periods: 1) daytime baseline period, 2) nocturnal surge period, and 3) treatment period. Concentrations of melatonin within the daytime, nocturnal, and treatment portions of the day were averaged within treatment groups and compared using the Bonferroni 't' test (Gill, 1978). Additionally, average duration of the surges and duration of the baselines were compared within each treatment group by the Bonferroni 't' test.

Results

Fifteen of the 16 heifers responded to the 400 lux daytime intensity of light with relatively low concentrations of melatonin, which usually increased to typical nocturnal concentrations within 30 min after the onset of darkness (Figure 1). Heifer 6 (400 lux treatment) had no discernible nocturnal surge using the Pulsar criteria (Figure 2). Thus, it was assumed that no distinct diurnal rhythm of melatonin existed in this heifer, and her data were discarded from further analysis.

Daytime baseline (0700 to 1900 h) concentrations of melatonin in serum of the 15 heifers exposed to 400 lux of light prior to treatment were not different between treatment groups ($P>0.1$) and averaged 11.2 pg/ml (Figure 3). The duration of baseline concentrations of melatonin averaged 12 h for all treatment groups.

Concentrations of melatonin in serum during the initial 6-h nocturnal period prior to initiation of treatment, averaged 58, 37, 35, and 54 pg/ml for animals to be treated with 000, 400, 800, and 1200 lux, respectively (Figure 3). Average melatonin concentrations for animals to be treated with 000 lux during the initial 6-h nocturnal period were not different from the concentrations in animals to be treated with 1200 lux ($P>0.1$) but were greater than the average concentrations in the animals to be treated with 400 ($P<.05$) and 800 lux ($P<.025$).

Following the 6-h nocturnal period, heifers in the 000 lux treatment group (controls) received 6-h of continued darkness, whereas the light-treated heifers received varying intensities of light treatment. In the 000 lux treatment group, the average concentration of melatonin during the treatment period (67 pg/ml)

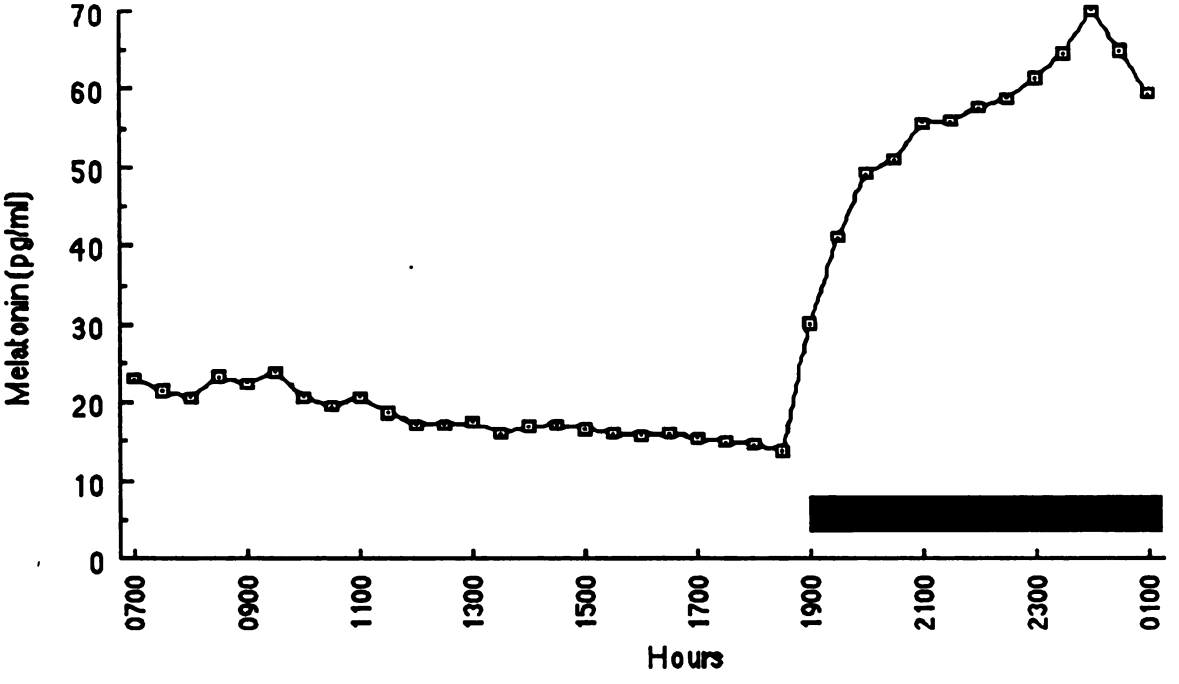

Figure 1. Average concentrations of melatonin in serum of photoperiod-responsive heifers (n=15) for 18 h prior to the treatment period. Light intensity was 400 lux. Darkness is depicted by the black rectangle. Pooled SE=4.3 pg/ml for 000, 800, and 1200 lux groups and 4.9 pg/ml for 400 lux group.

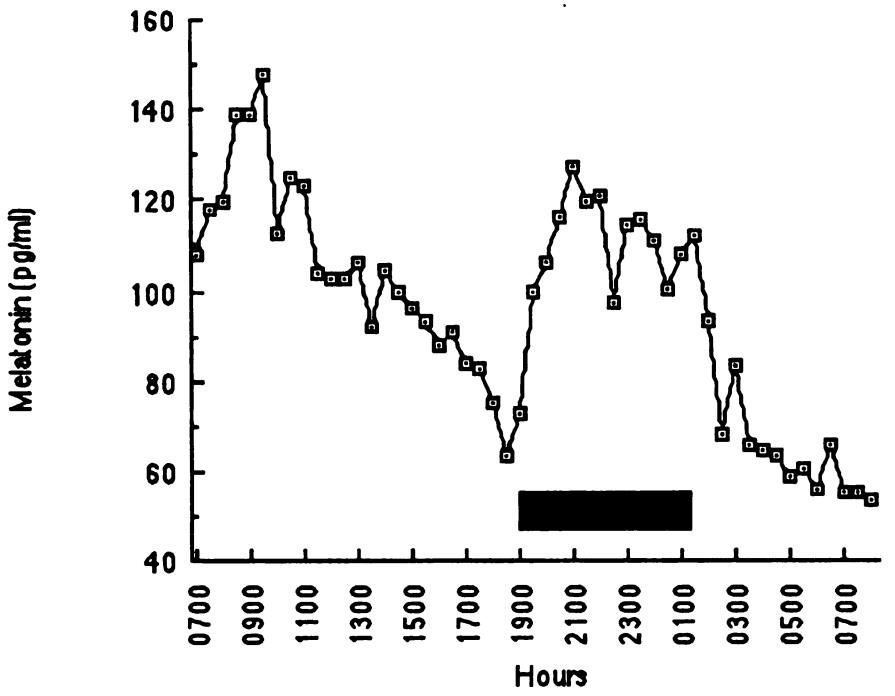

Figure 2. Melatonin concentrations over 25 h for heifer 6. This animal was non-responsive to the 400 lux intensities of light according to Pulsar criteria. Black rectangle depicts period of darkness.

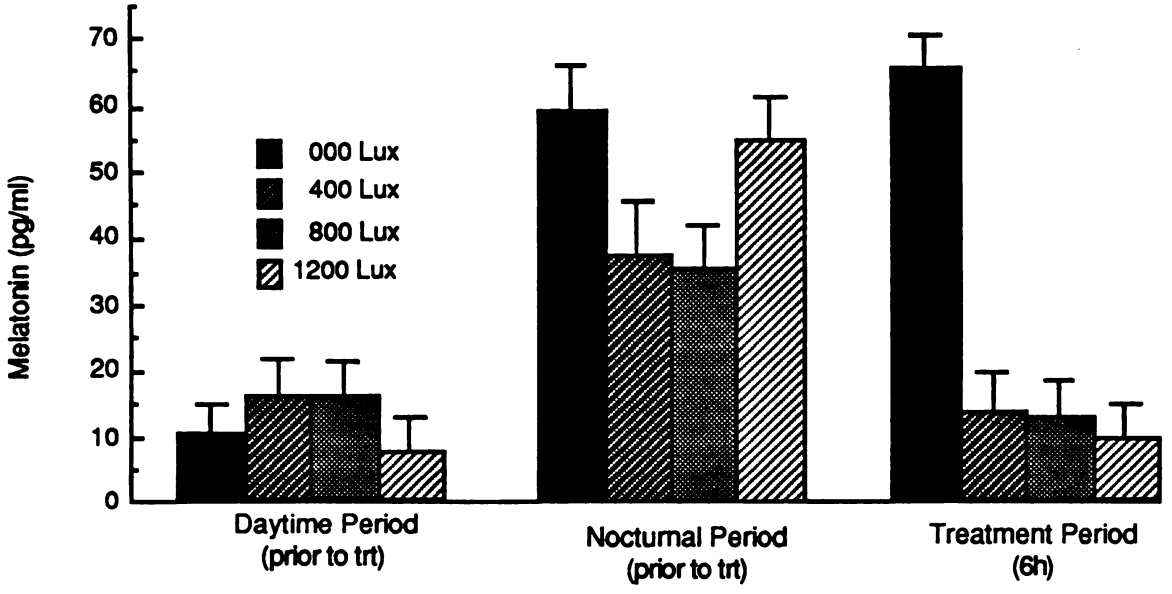

Figure 3. Average concentrations of melatonin by treatment group during the daytime period (exposed to 400 lux of light prior to treatment), nocturnal period (exposed to darkness prior to treatment), and during the treatment period (exposed to 000, 400, 800, or 1200 lux treatment intensities of light for 6 h).

was not different (P>0.1) from the average concentration during their previous 6 h period of darkness (58 pg/ml). However, for those groups receiving 400, 800, or 1200 lux of light during the treatment period, the concentrations of melatonin decreased (P<.01) to their 400 lux daytime concentrations and averaged 13.8, 13.3, and 9.8 pg/ml, respectively (Figure 3). On a percentage of their respective average nocturnal concentrations, 400, 800 and 1200 lux suppressed melatonin 63, 62, and 82%, respectively.

Following the 6-h treatment period of continued darkness, the 000 lux treatment group maintained elevated concentrations of melatonin for an average of 12.25 h (Figure 4). In contrast, the 400, 800, and 1200 lux treatment groups that received light during the second half of the nocturnal period had average durations of nocturnal surges of melatonin lasting 6.8, 6.0, and 7.6 h, respectively. These values were shorter (P<.01) than the duration of the nocturnal surge of the 000 lux group. There was a trend toward an increase in surge durations between the 800 lux (6.0 h) and 1200 lux (7.6 h) treatment groups (P<0.1). However, the 1200 lux group had to decrease from a 53.7 pg/ml nocturnal peak, whereas the 800 lux group had to decrease from 35.2 pg/ml during the nocturnal period to reach daytime baseline concentrations which averaged 11.4 pg/ml.

Discussion

Concentrations of melatonin in serum increased in response to darkness in the present study, which is in agreement with results from other cattle studies (Hedlund et al., 1977; Martin et al., 1983; Stanisiewski et al., 1988) as well as other species (Cardinali, 1981). Additionally, in agreement with Stanisiewski et al. (1988b), the present experiment showed that the duration of the nocturnal

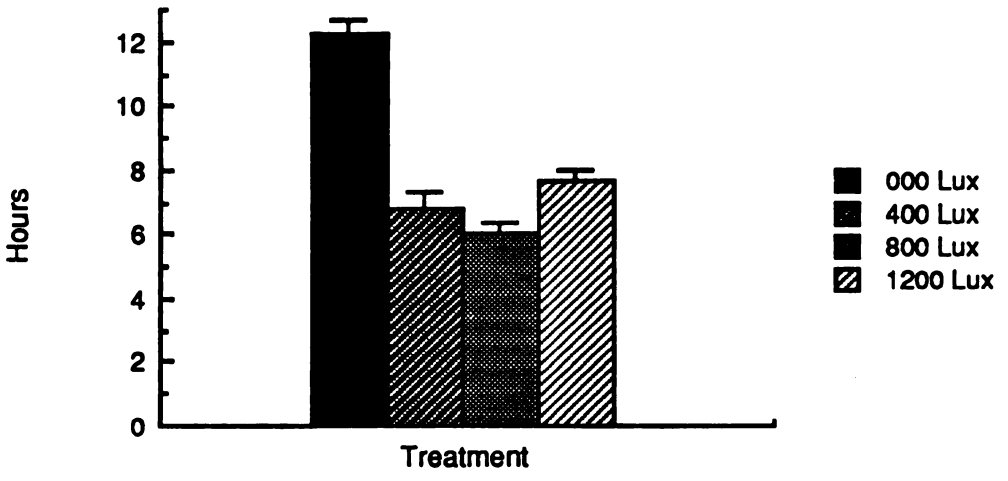

Figure 4. Average duration of the nocturnal surge for each treatment group.

surge of melatonin in cattle changes with the duration of darkness given the animal.

Fifteen of the 16 heifers in the present experiment responded to the prevailing intensity of 400 lux of light with low daytime and relatively high nocturnal concentrations of melatonin. Intensities of 400, 800 or 1200 lux of light each suppressed the nocturnal surge of melatonin to similar concentrations, but on a percentage basis 1200 lux suppressed melatonin more than either 400 or 800 lux. The greater inhibition by 1200 lux was associated with the fact that the average nocturnal concentration of melatonin before treatment was greater in this group than in the 400 or 800 lux groups. Assuming similar rates of clearance, this greater nocturnal concentration of melatonin also accounts for the trend toward a longer duration of the nocturnal surge in the 1200 vs 400 or 800 lux groups.

Duration of low daytime melatonin concentrations coincided with the duration of light at 400 lux intensity. Melatonin concentrations increased with onset of darkness and remained elevated for the entire 12 h dark period experienced by the control heifers. This is in contrast to data in rams receiving 8 or 16 h of daily dark (Lincoln et al., 1982) and bulls receiving 16 h of daily dark (Stanisiewski et al., 1988b) which experienced a decline in nocturnal melatonin concentrations prior to the onset of light, and(or) a delayed increase in melatonin after the onset of dark. The differences in response to duration of darkness between my experiment and those of others may be associated with the fact that the duration of my experiment was only 1 d and involved either a 12L:12D or 18L:6D photoperiod; whereas in other experiments, changes in nocturnal melatonin concentrations are typically noticed when animals are exposed to very long or very short-day photoperiods which are extended for many weeks. It is possible that these differences in responses may be a

function of internal time-keeping systems within the animal which can over-ride a stale photoperiod signal in the absence of changing or sufficiently strong time-keeping stimuli.

Light intensities of 400, 800, or 1200 lux did not suppress nocturnal concentrations of melatonin below their respective daytime values in the present experiment. It is possible that daytime concentrations of melatonin would not be affected by any intensity of light if daytime secretion of melatonin is from tissues that are not within the retinal-SCN-pineal axis. This may indicate that melatonin secreted during the day is not part of the diurnal variation of the melatonin profile, and therefore may be of little importance to the animal's time-keeping and entraining abilities.

The one heifer (of 16) that was photoperiodically unresponsive had melatonin concentrations 2- to 8-fold higher than any other animal in the experiment. It is possible that photic entrainment of melatonin was not yet entirely aligned with the photoperiod provided. If this heifer was capable of eventually achieving photoperiodic entrainment of melatonin, her failure to entrain in this case may be attributed to at least two possible causes. The first possibility may indicate that a 10 d acclimation period was not of sufficient length for photoperiodic entrainment to occur in this individual heifer (and perhaps a small sub-set of the bovine population). The second and perhaps more plausible possibility, is that 400 lux of light was not of sufficient intensity for this individual to effectively establish entrainment to the photoperiod to which she was exposed. Stanisiewski et al. (1988b) reported that three out of four bull calves housed under continous light at 525 lux (measured 1.2 m from the floor) for a duration of 4 weeks had no diurnal rhythm, nor any apparent surge of melatonin throughout the 24-h sampling interval. In Stanisiewski's study, the non-entraining animal apparently had a diurnal rhythm of melatonin even in the

face of the 24 h photoperiod. However, this rhythm was 180 degrees out of phase from the 8L:16D photoperiod experienced by the animal 4 weeks previously. It is possible, therefore, that some calves require more intense lighting cues than others for melatonin entrainment to occur.

Results of the present experiment indicate that 400, 800 and 1200 lux of light are sufficient to suppress nocturnal concentrations of melatonin to baseline concentrations similar to daytime values in most heifers. However, 400 lux may not be of sufficient intensity to entrain the daily melatonin rhythm of all heifers.

CHAPTER 2

Four month suppression of melatonin in Holstein heifers under four different photoperiods

Introduction

In Chapter 1, I found that 400, 800 and 1200 lux of light each suppressed the nocturnal surge of melatonin for at least 6 h. However, repetitive daily exposure to the same photoperiod frequently leads to a state of photorefractoriness. The term photorefractory is used to describe a state where an animal ceases to respond consistently to a recurring or long-term photoperiodic condition. The change in response may be behavioral, sexual, endocrine or a combination of these factors. In terms of the melatonin rhythm, photorefractoriness would occur when an animal ceases to maintain low concentrations of melatonin during the lighted portion of the daily photoperiod and(or) surge concentrations of melatonin during the hours of darkness. Such responses are frequently seen in rams (Lincoln, 1978, 1980; Howles et al., 1982; Almeida and Lincoln, 1984) and ewes (Thwaites, 1965; Ducker et al.,1973) where the reproductive activity and other corresponding hormone cycles eventually revert, even in the face of stimulatory short-day photoperiods, to levels seen only during the sexually quiescent period. However, there is conflicting evidence in terms of melatonin rhythms in photorefractory animals. Several researchers have indicated that the melatonin rhythms remain closely associated with the photoperiod, and photorefractoriness occurs because of a loss of responsiveness to melatonin (Bittman, 1978; Reiter et al., 1979; Malpaux et al., 1987). On the other hand, melatonin rhythms did vary with photorefractory responses in sheep over a 94 week period of repetitive long or short-day photoperiods (Almeida and Lincoln, 1984). Therefore, the role played by melatonin in photorefractoriness has yet to be elucidated.

Photorefractoriness is not unique to sheep; it occurs widely in other mammals, as well as in birds (Turek and Campbell, 1979; Stetson and Tate-Ostroff, 1981). There are several factors that may affect the length of time an

individual may be held on a continuously repeating photoperiod without developing photorefractoriness. Extraneous (non-photoperiodic) cues such as ambient temperature cycles, pheromones from neighboring animals, feeding patterns and intermittent noise patterns may override a stale photoperiodic cue and re-entrain an animal's endogenous rhythm (Barrell, G.K., personal communication). Therefore, a strong photoperiodic signal (such as high intensity light) may be beneficial for maintaining entrainment.

In dairy cattle, growth of the mammary gland is allometric between weaning and puberty. Potentially this is a time when growth-suppressing factors can negatively affect mammary development. In support of this concept, high rates of body weight gain during this period inhibit mammary development (Gardner et al., 1977; Little and Kay, 1979; Branning and Lundkvist, 1978; Sejrsen, 1978). In addition to high rates of gain prior to puberty, administration of exogenous melatonin also suppresses mammary development in peripubertal mice (Sanchez-Barcelo et al., 1990) and heifers (Sanchez-Barcelo et al., 1991). Conversely, long-day photoperiods (shorter nocturnal surges of melatonin) increase mammary parenchyma relative to short-days (longer nocturnal surges of melatonin) in both pre- and postpubertal heifers (Petitclerc et al., 1984). Therefore, it is possible that continuous suppression of melatonin during the prepubertal period of allometric mammary growth could augment mammary development and subsequent lactational performance in cattle.

The objective of this experiment was to determine a photoperiod which would suppress the nocturnal surge of melatonin for a period of 4 months without inducing photorefractoriness.

Materials and Methods

Sixteen prepubertal Holstein heifer calves (ranging in age from 0.5 to 3 months) were placed in light- and temperature-controlled environmental chambers. All heifers were acclimated to a 12L:12D photoperiod for 8 weeks of pretreatment. Initially, intensity of light was 400 lux at 1 m above floor level. Light intensity was increased to 1200 lux at the end of week 5 of pretreatment through the remainder of the experiment. Temperatures were held at 22.5 ± 3.5°C. Heifers were blocked by body weight and assigned randomly from within block for subsequent exposure to one of four photoperiod treatments. All heifers were fitted with indwelling jugular catheters and subsequently blood was sampled 2 d prior to the initiation of photoperiod treatments. Catheter patency was maintained between blood samples by flushing with 3.5% Na citrate in sterile water. The first sampling period included at least the last 32 h of the pretreatment period and continued through the initiation of the treatment photoperiods. Blood was sampled at 2-h intervals.

Beginning on week 8 of pretreatment (heifers ranged from 2.5 to 5 months of age at this time), treatment photoperiods were initiated at staggered intervals such that the middle of the day was kept at the same time (1200 h) for each treatment group during the pretreatment and treatment periods. I speculated that maintaining the same midpoint of each day might lessen the liklihood that extraneous (non-photoperiodic) signals would become factors to compete as entraining cues. The treatment photoperiods were: 8L:16D, 16L:8D, 20L:4D, or 24L:0D at 1200 lux intensity and continued for 4 months. Blood was sampled every 2 h from the end of pretreatment through the first two full days of the treatment photoperiods (Mo 0) and blood sampling was

repeated monthly over 2 days at the end of each of the four ensuing months of treatment (Mo 1 through Mo 4).

Blood samples were allowed to clot at approximately 22° C, then were held at 4° C for approximately 12 h before being centrifuged at 991 x g for 30 min. Following centrifugation, serum was decanted and stored frozen at -20° C until assayed for melatonin and prolactin.

Values for serum samples taken every 2 h over the first 24-h sampling interval within each animal were averaged with the corresponding values from the second 24-h sampling interval to yield a daily profile of melatonin for each sampling period at the end of months 0, 1, 2, 3, and 4 of treatment. The Pulsar computer program was used to determine the onset and cessation of the nocturnal surge of melatonin found in the average profile of melatonin from each heifer as described in Chapter 1. The G(value) standard deviation settings for G(1) to G(5) of the program were 5.5, 2.8, 2.5, 2.2, and 20.4, respectively. The information derived from Pulsar was subsequently used to divide the 24-h profiles from each sampling period (pretreatment and months 0 through 4 of treatment) into melatonin surge and baseline periods for each heifer.

The direct assay of melatonin previously validated in our laboratory (Stanisiewski et al., 1988b) was modified to accommodate a double antibody separation of free and bound tracer in a methodology similar to Webley et al. (1985). The primary differences in the present assay involved using a 1:9000 dilution of the Guildhay Antisera Ltd sheep anti-melatonin primary antiserum, and 1:70 dilution of the donkey anti-sheep secondary antiserum (Lot No. A5839). The entire assay was carried out (from initial sera dilution through addition of scintillation fluid and ß-counting) in 12x55 mm polypropylene sample tubes. To validate this assay, melatonin was quantitatively recovered in

five replicates that had melatonin (Sigma, No. m5250) added at 20, 80, 160, and 240 pg/ml concentrations; recoveries averaged 105.5, 98.0, 101.9, and 106.2%, respectively. In addition, assay parallelism was demonstrated using three serum samples at dilutions comparable to those within the useable range of the standard curve. The lower limit of sensitivity of the assay was approximately 4 pg/ml. The inter- and intra-assay coefficients of variation were 11.3 and 4.9%, respectively. The elimination of charcoal separation of free and bound tracer reduced assay variation, and increased the potential capacity of individual assays. Therefore, all samples from one heifer in each treatment group were measured in a single assay.

Samples were also assayed for prolactin (Newbold et al., 1991). Prolactin concentrations served as another indicator of the status of photoperiodic entrainment. The inter- and intra-assay coefficients of variation for the prolactin assays were 4.2 and 9.7 %, respectively.

Statistical analyses involved both a split-split-plot design with the periods of high and low concentrations of melatonin as one split-plot and time as the second plot for hormone analysis. One-way analysis of variance (ANOVA) was used to compare the durations of the periods of baseline or surge melatonin (Gill, 1978).

The 8L:16D treatment served as the short-day control and values from all other treatments were compared against this group within each sampling period. Additionally, each treatment group was compared across time (Mo 0 through 4) to their respective pretreatment values. Concentrations of melatonin from the baseline portion of the profiles from each sampling period were averaged by treatment groups and compared using the Bonferroni 't' test (Gill, 1978). The surge of melatonin was analyzed similarly. Durations of melatonin surges were averaged within sampling period for each treatment group and

were also compared using the Bonferroni 't' test. Additionally, concentrations of prolactin, averaged within the time periods described above for melatonin, were also compared using the Bonferroni 't' test.

Results

Concentrations of melatonin from the end of pretreatment through the first 2 d of photoperiod treatment.

Baseline and surge concentrations of melatonin during the last full day of pretreatment were not different across groups ($P>0.2$; Figure 5). Melatonin increased ($P<.01$) from an average of 20.2 pg/ml during the daytime baseline period to 172.3 pg/ml during the nocturnal surge period. Baseline and surge concentrations of melatonin during the period of transition into treatment photoperiods were also similar across groups ($P>0.2$). However, relative to pretreatment values, surge concentrations of melatonin decreased ($P<.01$) progressively during the first two days of treatment in the 24L:0D photoperiod group, but not in the other treatment groups. These dampened surges in the 24L:0D treated heifers were centered over the nocturnal period of their previous 12L:12D photoperiod. Values of melatonin during the baseline and surge periods of all treatment groups are summarized in Appendix Tables 1 and 2, respectively.

Concentrations of melatonin between 1 and 4 months of photoperiod treatment.

In the 8L:16D photoperiod treatment group, baseline melatonin in serum increased from an average of 26.5 pg/ml by the end of months 1 through 4 of the treatment to 172.9 pg/ml during the melatonin surge (Figure 6). These

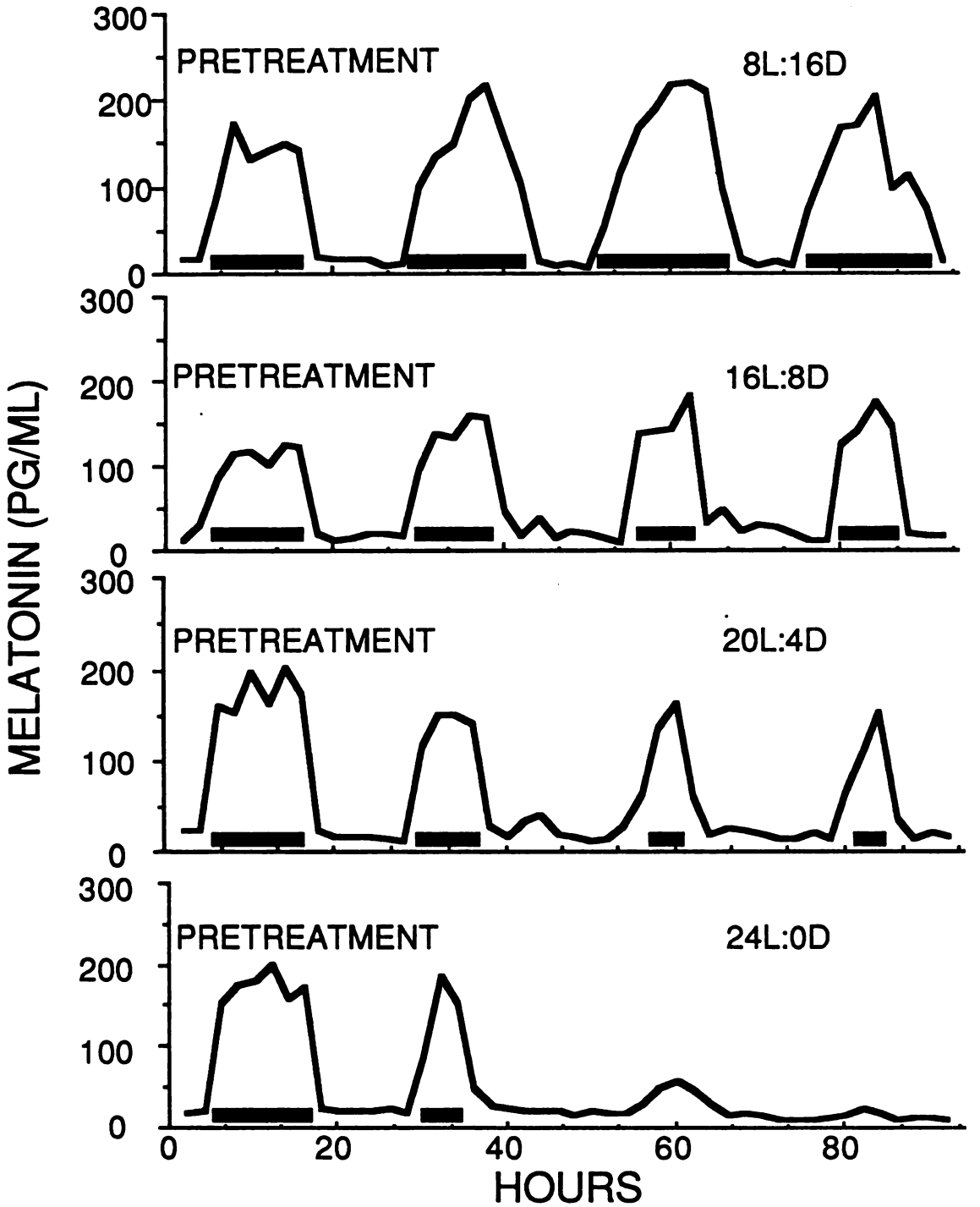

Figure 5. Average concentrations of melatonin in heifers on the last full day of pretreatment (12L:12D) through the first two full days of their respective photoperiod treatments. Black rectangles depict periods of darkness. Pooled SE=16.3 pg/ml and n=four heifers per treatment.

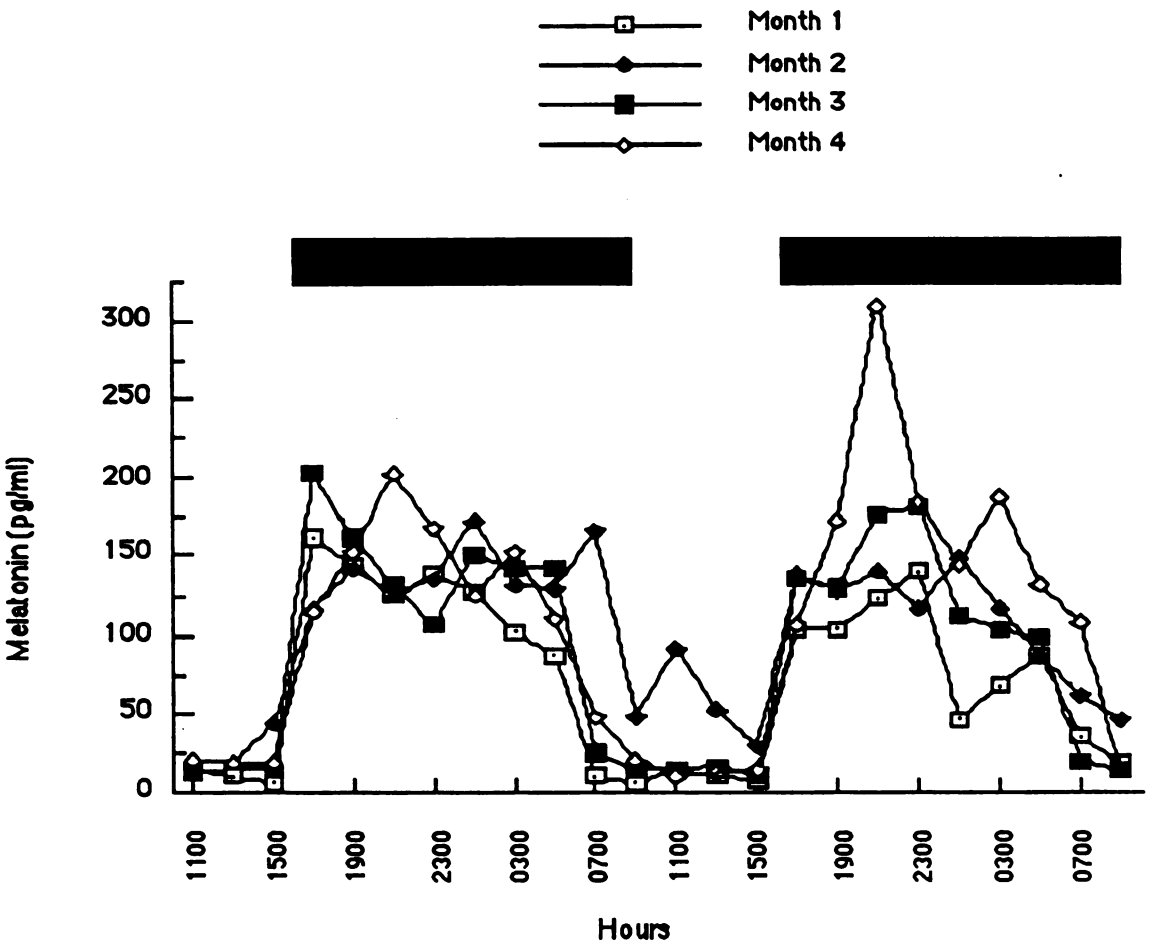

Figure 6. Average concentrations of melatonin in heifers over 4 months of 8L:16D photoperiod treatment. Black rectangles depict periods of darkness. Pooled SE=8.7 pg/ml and n=four heifers per month.

values did not change relative to respective pretreatment values (P>0.2; Figure 5).

Similarly, in the 16L:8D photoperiod treatment group, baseline melatonin in serum increased from an average of 45.6 pg/ml by the end of months 1 through 4 of treatment, to 128.8 pg/ml during the melatonin surge (Figure 7). These values did not change relative to respective pretreatment values (P>0.2; Figure 5). Additionally, all concentrations of melatonin in the16L:8D heifers during all treatment months were similar to respective values of the 8L:16D treatment group (P>0.2). It should be noted that heifer 2 had unusually high concentrations of melatonin on month 2 of treatment. Data from this animal were included in statistical comparisons because their inclusion had no effect on the significance or interpretation of the comparisons. However, her data on month 2 were deleted in Figure 7, and are shown instead in Figure 8.

In the 20L:4D photoperiod treatment group, baseline melatonin in serum increased from an average of 22.2 pg/ml by the end of months 1 through 4 of treatment, to 131.6 pg/ml during the melatonin surge (Figure 9). These values did not change relative to respective pretreatment values (P>0.2; Figure 5). Also, all concentrations of melatonin in the 20L:4D group during all treatment months were similar to respective values of the 8L:16D treatment group (P>0.2).

In the 24L:0D photoperiod treatment group, baseline melatonin in serum increased from an average of 22.9 pg/ml by the end of months 1 through 4 of treatment, to 49.7 pg/ml during the surge of melatonin (Figure 10). Surge concentrations of melatonin were decreased on months 1, 2, 3 and 4 of the 24L:0D treatment relative to pretreatment surge concentrations (P<.01). When compared with 8L:16D treatment values, concentrations of surge melatonin in the 24L:0D group were decreased on months 2 (P<0.1), 3 (P<.02), and 4 (P<.05). On month 1 of treatment two of the four heifers in the 24L:0D treatment

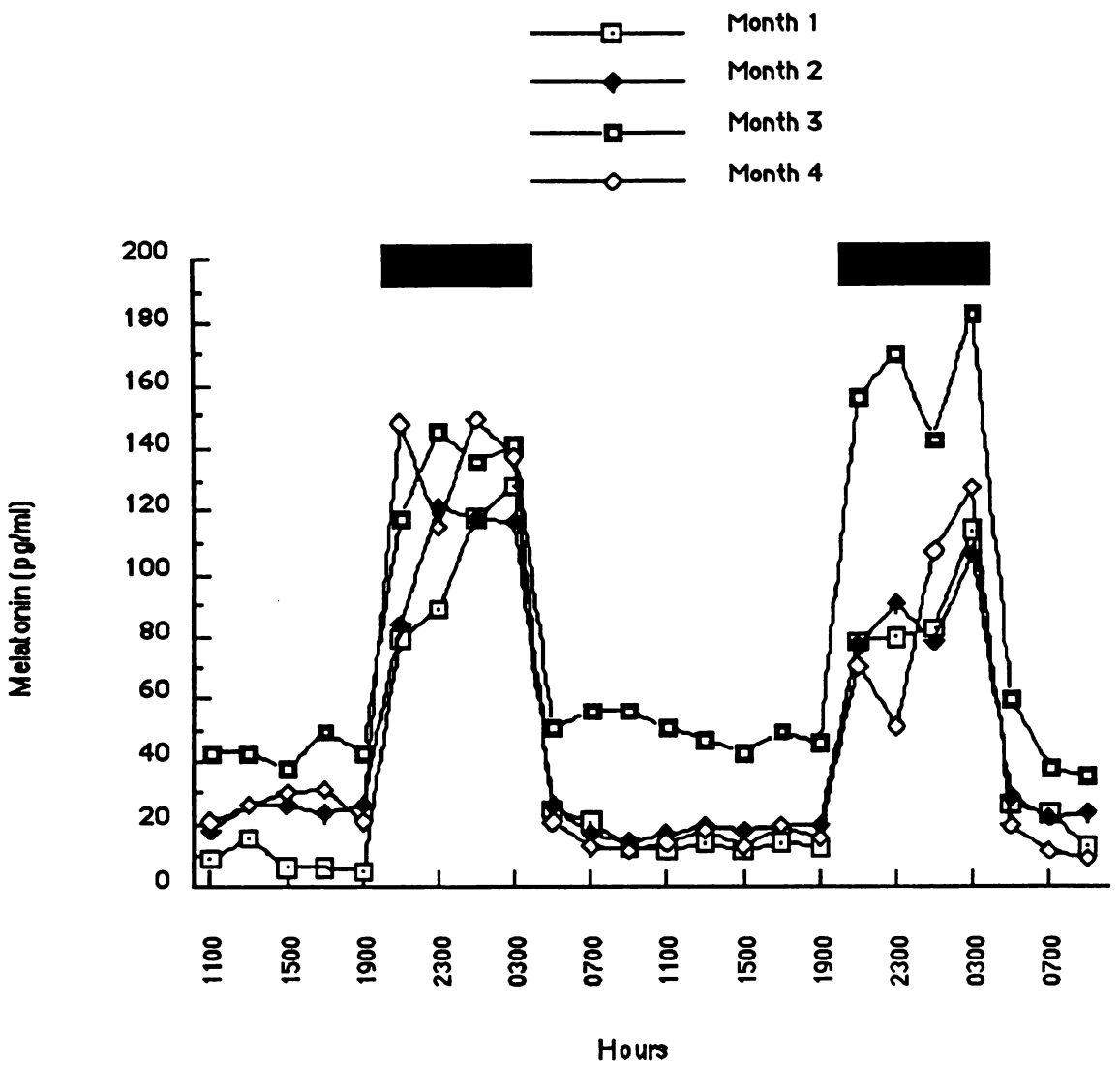

Figure 7. Average concentrations of melatonin in heifers over 4 months of 16L:8D photoperiod treatment. Black rectangles depict periods of darkness Pooled SE=9.4 pg/ml and n=four heifers per month (except for month 2 where n=three).

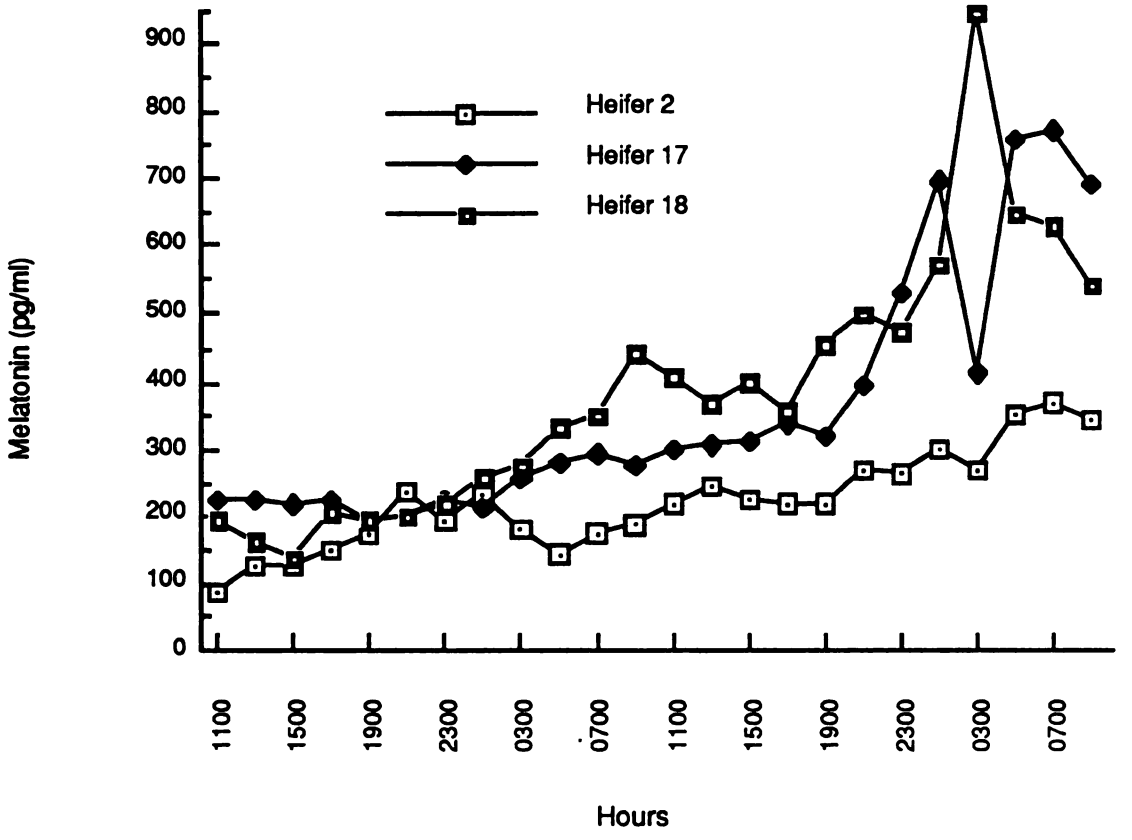

Figure 8. Average concentrations of melatonin after 2 months of 16L:8D photoperiod treatment (heifer 2; lights off from 2000 to 0400 h) or after 1 month of 24L:0D photoperiod treatment (heifers 17 and 18).

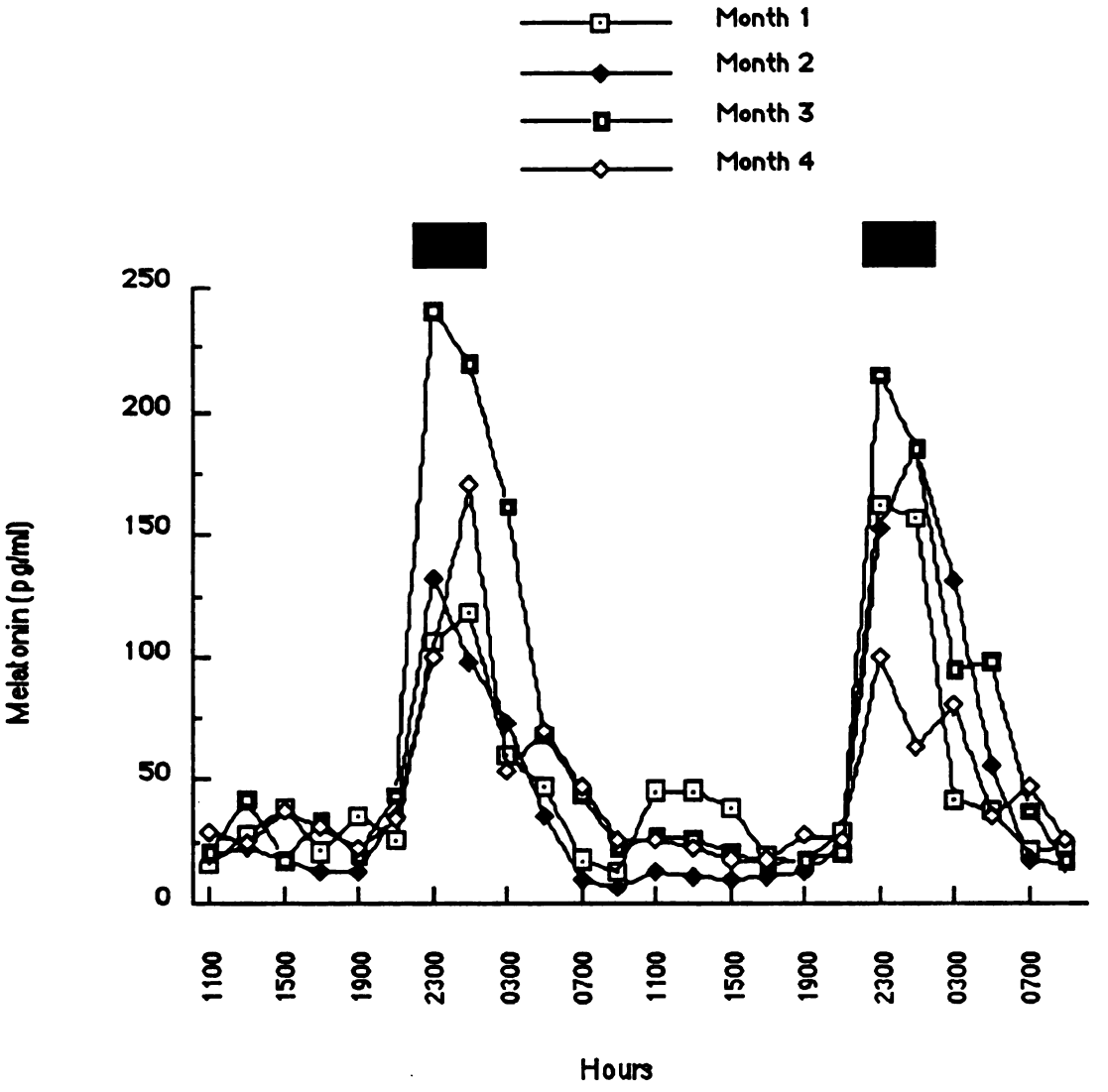

Figure 9. Average concentrations of melatonin in heifers over 4 months of 20L:4D photoperiod treatment. Black bars depict periods of darkness. Pooled SE=5.7 pg/ml and n=four heifers per month.

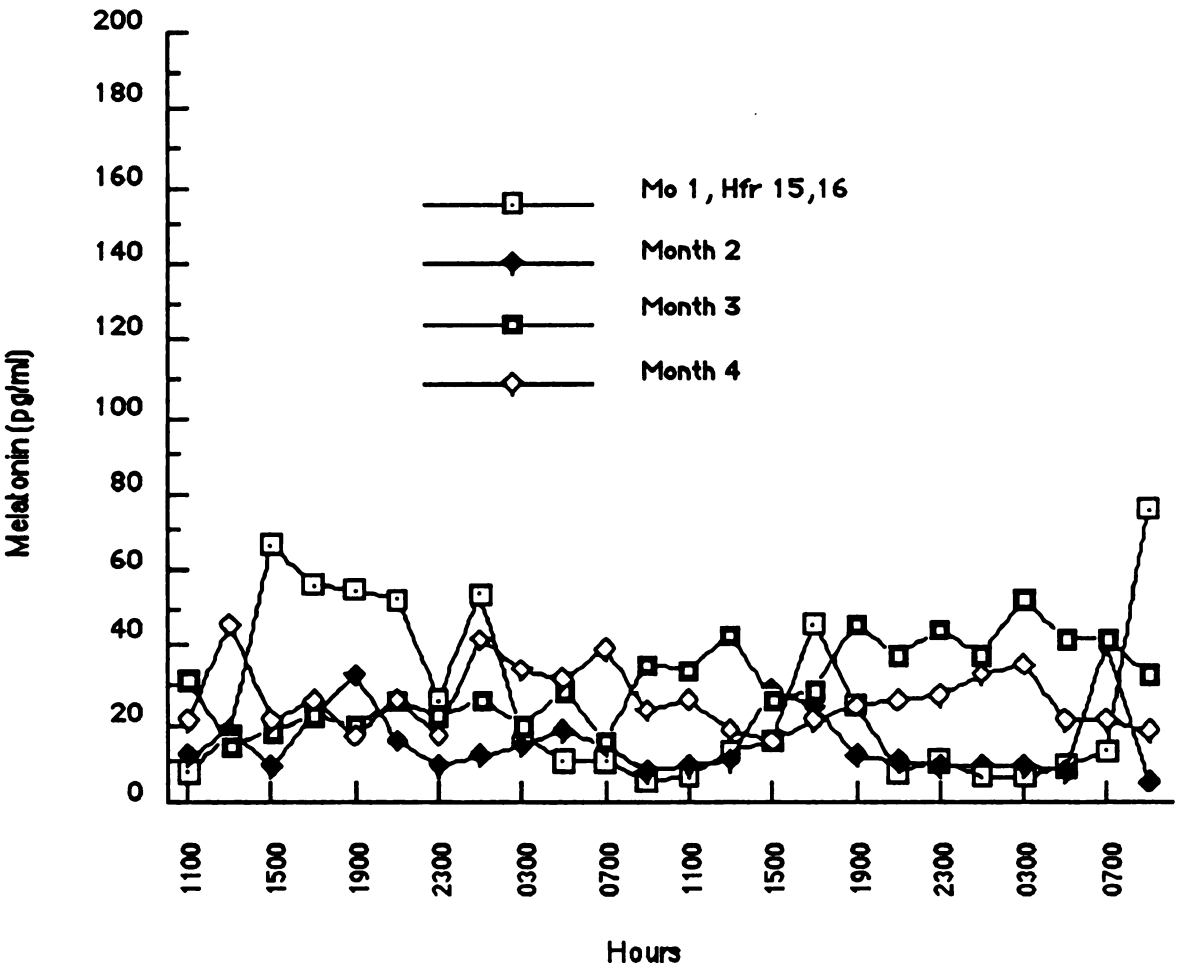

Figure 10. Average concentrations of melatonin in heifers over 4 months of 24L:0D photoperiod treatment. SE=20.8 pg/ml and n=four heifers per month (except on month 1 where n=2).

group (heifer 17 and 18) had elevated concentrations of melatonin (Figure 8). These individuals were housed in adjacent stalls and were the most nervous animals in the experiment; however, they possessed no other obvious significant characteristics. On month 1 of treatment, serum melatonin in heifers 17 and 18 averaged 359 and 384 pg/ml, respectively. In contrast, heifers 15 and 16 of the 24L:0D treatment group at 1 month of treatment had serum melatonin which averaged only 29 and 5 pg/ml, respectively. Therefore, mean comparisons of melatonin were not made on month 1 of treatment for the 24L:0D group. The melatonin profiles of heifers 17 and 18 during month 1 of treatment were similar to that described previously for heifer 2 during month 2 of the 16L:8D treatment (Figure 8). All three profiles depicted very high and steadily increasing concentrations of melatonin over the 48-h sampling period. Melatonin values in all three heifers were similar to their respective contemporaries during all other sampling periods.

Duration of the melatonin surge

The average duration of the surge of melatonin for each treatment group and sampling period is depicted in Table 1. Surge durations were not different among the groups during the 12L:12D pretreatment period ($P>0.2$). Durations of surge concentrations of melatonin were correlated ($r=0.82$; $P<.01$) with the duration of darkness throughout the experiment. Melatonin surge durations for the 16L:8D treatment group were shorter than the 8L:16D durations between month 0 and 4 of treatment. In the 20L:4D treatment group, surge durations decreased relative to durations in the 8L:16D treatment on months 0, 1, 3, and 4 of treatment. Durations of the melatonin surge were shorter for all months of treatment in the 24L:0D group. Furthermore, only one heifer per month (and it

Table 1. Average duration of surge of melatonin in heifers.

		Period					
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]	Hours						
8L:16D	12.0	15.5	14.0	11.0	12.5	15.5	13.3
16L:8D	10.0	9.0[e]	9.5[g]	6.0[f]	8.0[g]	8.0[e]	7.9[e]
20L:4D	12.0	8.0[e]	5.0[ce]	7.0[d]	8.0[g]	6.5[de]	6.6[ce]
24L:0D[b]	12.0	3.0	0.5	2.0	1.0	0.5	1.0

[a]N=four heifers per treatment and pooled SE=0.4 h.

[b]During months 1 through 4 of treatment, a different individual heifer in the 24L:0D group had a melatonin surge each month. Therefore mean comparisons were not made for this treatment group.

[c]Within a treatment, means differ from pretreatment (P<.01).

[d]Within a treatment, means differ from pretreatment (P<.10).

[e]Within a period, means differ from 8L:16D treatment (P<.01).

[f]Within a period, means differ from 8L:16D treatment (P<.05).

[g]Within a period, means differ from 8L:16D treatment (P<0.1).

was a different heifer each month) in the 24L:0D group had a defined surge. Therefore, the 24L:0D treatment group was not included in the mean comparisons.

Pretreatment durations were not different from the durations during any month of treatment in the 8L:16D or 16L:8D groups ($P>0.2$; Table 1). However, in the 20L:4D treatment group, durations were less than the pretreatment values in month 1, 2, and 4.

Average durations of the nocturnal surge of melatonin combined over months 1 through 4 were decreased in the 16L:8D and 20L:4D groups relative to 8L:16D surge durations. Additionally, overall average surge durations in the 20L:4D treatment were shorter than their respective pretreatment durations. Surge durations averaged only 1.0 h in the 24L:0D treatment group during months 1 through 4 of treatment.

Concentrations of prolactin from the end of pretreatment through the first 2 d of photoperiod treatments.

Prolactin during baseline or surge periods of melatonin were not different among the four groups during pretreatment and month 0 (Tables 2 and 3).

In the 8L:16D heifers, mean concentrations of prolactin during both the period of baseline and surge melatonin did not change relative to pretreatment values over months 1, 2, 3, or 4 (Tables 2 and 3).

In the 16L:8D group of heifers, mean concentrations of prolactin during the period of baseline melatonin were increased on months 1, 2, and 3 of treatment, when compared with pretreatment values (Table 2). Furthermore, concentrations of prolactin during the period of baseline melatonin were increased compared with the 8L:16D treatment group on months 1 and 2 as

Table 2. Average concentrations of serum prolactin in heifers during the period of baseline concentrations of melatonin.

			Period				
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]			Serum prolactin (ng/ml)				
8L:16D	12.6	14.6	9.0	13.9	13.6	11.2	11.9
16L:8D	14.5	16.4	24.6[be]	21.9[cf]	21.0[dg]	17.4	21.2[df]
20L:4D	6.9	9.1	17.2[bf]	21.6[bg]	17.4[bg]	14.6[c]	17.7[b]
24L:0D	10.6	12.0	18.2[cf]	17.2[d]	20.8[bg]	18.3[cg]	18.6[b]

[a]N= four heifers per treatment; pooled SE=1.5 ng/ml.
[b]Within a treatment, means differ from pretreatment (P<.01).
[c]Within a treatment, means differ from pretreatment (P<.02).
[d]Within a treatment, means differ from pretreatment (P<.05).
[e]Within a period, means differ from 8L:16D treatment (P<.01).
[f]Within a period, means differ from 8L:16D treatment (P<.05).
[g]Within a period, means differ from 8L:16D treatment (P<0.1).

Table 3. Average concentrations of serum prolactin in heifers during melatonin surge periods.

			Period				
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]			Serum	prolactin	(ng/ml)		
8L:16D	20.2	20.2	10.2	16.2	20.1	19.8	16.6
16L:8D	23.8	16.6	18.8	17.2	27.2	31.1	26.1
20L:4D	12.2	11.4	18.9	28.4[c]	22.5	20.8	22.7
24L:0D[b]	11.2	11.8	4.3	14.3	16.7	11.3	11.6

[a]N=four heifers per treatment and pooled SE=2.8 ng/ml.

[b]During months 1 through 4 of treatment, a different individual heifer had a melatonin surge each month.

[c]Within a treatment, means differ from pretreatment (P<.02).

well as on month 3. Interestingly, on month 4 of treatment, prolactin concentrations in the 16L:8D treatment group were not different from pretreatment concentrations, nor were they different from 8L:16D concentrations. In contrast, concentrations of prolactin in the 16L:8D treatment group during the melatonin surge were not different from pretreatment values on months 1, 2, 3 or 4 of treatment.

In the 20L:4D treatment group, mean concentrations of prolactin during the period of baseline melatonin were increased on months 1, 2, 3 and 4 of treatment when compared with pretreatment values. In contrast, prolactin during the surge period of melatonin was elevated above pretreatment values only during month 2 of treatment. Compared with 8L:16D, the 20L:4D treatment group had increased prolactin during the baseline melatonin period on months 1, 2, and 3.

In the 24L:0D treatment group, mean concentrations of prolactin during the period of baseline melatonin were increased on months 1, 2, 3 and 4 of treatment when compared with pretreatment values. Average concentrations of prolactin during the baseline period were increased compared with the 8L:16D treatment group on months 1, 3, and 4.

Discussion

Consistent with results of other studies (Cardinali, 1981; Hedlund et al., 1977; Martin et al., 1983) as well as the previous experiment as described in Chapter 1, concentrations of melatonin in serum became markedly elevated in response to darkness. Also similar to Chapter 1, melatonin concentrations quickly responded to changes in photoperiod. The melatonin rhythms in most heifers became entrained within the first day of exposure to the treatment

photoperiods. Therefore, unlike other photoperiodically-responsive hormones (such as prolactin) which require several weeks to respond to changing photoperiods, the response of melatonin is within minutes.

It has been proposed that similar amounts of melatonin are produced in an animal each day, regardless of the photoperiod exposure; for example, long nights (short days) must then have lower average concentrations during the melatonin surge and short nights (long days) must have higher surge concentrations, such that the overall exposure to melatonin over any 24-h period is not different. However, contrary to some studies where nocturnal melatonin was higher in long-day and lower in short-day photoperiods (Lincoln et al., 1982; Stanisiewski et al., 1988b), average concentrations of nocturnal (or surge) melatonin in the present study did not change under long or short photoperiods, except in the 24L:0D group which tended to have no melatonin surge. It may be postulated, therefore, that in heifers the height of the surge of melatonin in serum is not important for transmitting information about the current photoperiod.

Baseline melatonin concentrations did not differ under any photoperiod exposure. This is consistent with results given in Chapter 1, where high intensities of light were unable to suppress nocturnal melatonin concentrations below daytime baseline values. It is possible that baseline concentrations of melatonin are not of pineal origin and thus are not sensitive to photoperiodic stimuli.

Durations of surge concentrations of melatonin in the present experiment were correlated with the duration of darkness, which is consistent with other studies (Lincoln et al., 1982; Stanisiewski et al., 1988b). The timing of the initiation of the melatonin surge was consistently linked to the beginning of the dark period (in those heifers receiving dark); however, the timing of the

cessation of the surge did not adhere strictly to the end of darkness. For example, melatonin surge in the 8L:16D treatment declined an average of 2.7 h prior to lights on, which is consistent with other reports utilizing this photoperiod (Stanisiewski et al., 1988b); and the surge in the 20L:4D treatment extended an average of 2.6 h beyond the daily dark period. However, duration of the melatonin surge in the 16L:8D treatment was highly synchronous with the periods of darkness. In contrast, the 24L:0D treatment was practically devoid of surges of melatonin. It appears that the beginning and end of the scotoperiod are powerful cues that establish and maintain the photoperiodic rhythm in heifers. I postulate that duration of the surge of melatonin may be responsible for communicating entrainment information within cattle.

Photorefractoriness was not evident as far as melatonin surge durations were concerned, because duration of the surge of melatonin was constant within treatment throughout the experiment. Furthermore, in the present experiment 24L:0D was the most effective photoperiod for suppressing serum melatonin to baseline concentrations over the 4-month period, again without any obvious indication of a photorefractory response. This observation supports the hypothesis that suppression of the duration of the nocturnal surge of melatonin via exposure to light is possible for at least 4 months, which agrees with reports in sheep where refractoriness did not occur until after 21 wk of repetitive photoperiod exposure (Almeida and Lincoln, 1984).

The melatonin profiles of heifers 17 and 18 in month 1 and heifer 2 in month 2 are not readily explainable. Unlike the heifer that did not respond to the 400 lux photoperiod described in Chapter 1 (heifer 6), all three profiles of melatonin from the heifers in the present experiment continued to increase throughout most of their respective 48-h sampling periods. Therefore, this response appears to be caused by some factor other than a simple lack of

photoperiodic entrainment as suggested for heifer 6 in Chapter 1. Perhaps these heifers had somewhat lower thresholds of response to stress. Heifers 17 and 18 were nervous throughout the experiment, particularly at month 1; it is possible that this clinical sign of stress is associated with high concentrations of melatonin. Indeed, it has been proposed that melatonin may play a role in moderating stress effects from stimuli such as cold (Tannenbaum et al., 1988), immobilization (Lynch et al., 1973), and insulin-induced hypoglycemia (Champney et al., 1985). Many reports indicate that the pineal gland responds to these stressors by increasing the production of melatonin (Khan et al., 1990).

In the past, others have found that daytime concentrations of prolactin did not differ from nighttime concentrations in cattle (Petitclerc et al., 1983). However, comparing prolactin concentrations during the lighted portion of the day with prolactin concentrations during the dark period may not be the most appropriate comparison. Assuming melatonin is the driving force behind photoperiodicity, it may be appropriate to monitor photoperiodic characteristics (such as prolactin concentrations) in relation to the melatonin rhythm and not the arbitrary periods of light and dark. Therefore, prolactin concentrations in the present study were averaged from periods of baseline or surge concentrations of melatonin, instead of using overall (Appendix Table 3), diurnal or nocturnal prolactin averages.

Prolactin during baseline melatonin was much more closely associated with the prevailing photoperiod than was prolactin during the surge of melatonin. Specifically, concentrations of prolactin in serum during the periods of baseline melatonin concentrations increased in response to long-day photoperiods in the present experiment, which supports numerous studies with similar results (Bourne and Tucker, 1975; Leining et al., 1979; Petitclerc et al., 1983; Stanisiewski et al., 1984, 1987a,b; Zinn et al., 1986b; Critser et al.,

1987a,b). Alternatively, prolactin during surge melatonin generally did not differ throughout the experiment. Therefore, photoperiod effects on prolactin seem to be most easily distinguished during the periods of baseline concentrations of melatonin.

Concentrations of prolactin in the pretreatment period and in heifers treated with 8L:16D were lower than concentrations in heifers treated with 16L:8D, 20L:4D or 24L:0D throughout the 4 months of treatment. This supports the hypothesis that both 12L:12D and 8L:16D photoperiods are interpreted by heifers as short-day photoperiods. Conversely, 16L:8D, 20L:4D, and 24L:0D photoperiods were interpreted as long days after 1 month of treatment. The present results differ from some studies where 24L:0D photoperiods did not increase prolactin concentrations above 8L:16D values (Bourne and Tucker, 1975; Leining et al., 1979) or increased prolactin only after 6 or 8 weeks of photoperiod exposure (Stanisiewski et al.,1987a, 1988b). It is possible that the higher intensity of light (1200 lux) used in the present experiment may be responsible for the differences in prolactin values relative to previous studies which used 300 to 650 lux of light.

In a previous study, the mechanism involved in maintaining elevated concentrations of prolactin in cattle became refractory to 16L:8D by 3 months of photoperiod treatment (Stanisiewski et al., 1987b). In agreement, photorefractoriness in terms of prolactin occurred in the present experiment by month 4 of the 16L:8D treatment. Here again, it is possible that the higher intensity of light in the present experiment was responsible for delaying the photorefractory response of the heifers treated with 16L:8D photoperiods for an additional month, relative to Stanisiewski's study. Surprisingly, there was no evidence of refractoriness in the mechanism controlling prolactin secretion in the heifers maintained on 20 or 24 h of light. It appears that the different

photoperiods have varying abilities to prevent onset of refractoriness in prolactin secretion.

It has been hypothesized that the positive effects of long-day photoperiods on animal growth; accretion of protein, suppression of fat, and increase of mammary gland parenchymal tissue are manifested through hormonal mechanisms. High concentrations of prolactin have been associated with the desirable characteristics mentioned above. In addition, high concentrations of melatonin have been negatively associated with these desirable traits. In the present experiment, concentrations of prolactin were elevated and concentrations of melatonin were suppressed throughout the 4 months of treatment in the 24L:0D treatment group. Decreased concentrations of melatonin coupled with increased prolactin in response to continous high intensity light may be a useful tool to increase the body growth and milk production potential of dairy heifers.

SUMMARY AND CONCLUSIONS

Concentrations of melatonin in serum can be suppressed in prepubertal heifers to concentrations similar to baseline values typically seen during the day. Suppression can be achieved with either 400, 800, or 1200 lux of fluorescent light, however, it is possible that photoperiodic entrainment can be achieved more effectively and for longer periods using 1200 lux of light. When comparing the ability of 8L:16D, 16L:8D, 20L:4D, or 24L:0D photoperiods to suppress surge concentrations of melatonin, only the 24L:0D treatment supressed surge and overall melatonin concentrations below those of the 8L:16D treatment group. Additionally, the suppression was maintained in the 24L:0D treatment group for the entire 4-month treatment period. Relative to a short-day photoperiod of 8L:16D, long-day photoperiods of 16L:8D, 20L:4D or 24L:0D increased serum concentrations of prolactin for 3 to 4 months.

I speculate that decreased concentrations of melatonin coupled with increased prolactin in response to continous high intensity of light may be a useful tool to increase the body growth and milk production potential of dairy heifers.

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APPENDIX

APPENDIX

Appendix Table 1. Average baseline concentrations of serum melatonin in heifers.

			Period				
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]			Serum melatonin (pg/ml)				
8L:16D	15.8	11.4	12.8	71.1	16.6	16.6	26.5
16L:8D	22.1	19.3	12.8	46.4	46.9	18.6	45.6
20L:4D	20.8	17.0	29.8	15.4	24.8	26.5	22.2
24L:0D	22.3	17.0	194.2[b]	12.6	30.5	25.7	22.9

[a]N=4 heifers per treatment; pooled SE=12.0 pg/ml.

[b]Individual averages for this period were 25, 5, 359, and 384 pg/ml for heifers 15, 16, 17, and 18 respectively; therefore this mean was not used in comparisons.

Appendix Table 2. Average surge concentrations of serum melatonin in heifers.

	Period						
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]	Serum melatonin (pg/ml)						
8L:16D	141.8	147.9	133.1	160.6	180.6	177.4	172.9
16L:8D	135.0	174.0	89.2	98.9	168.5	119.1	128.8
20L:4D	175.1	111.6	128.9	122.7	167.2	105.0	131.6
24L:0D	237.4	55.2[b]	80.4[b]	52.6[be]	44.3[bc]	52.0[bd]	49.7[bd]

[a]N=4 heifers per treatment; pooled SE=24.7 pg/ml except for 24L:0D where n=1 heifer and SE=28.6 pg/ml.

[b]Within a treatment, means differ from pretreatment (P<.01).

[c]Within a period, means differ from 8L:16D treatment (P<.02).

[d]Within a period, means differ from 8L:16D treatment (P<.05).

[e]Within a period, means differ from 8L:16D treatment (P<0.1).

Appendix Table 3. Average overall concentrations of serum prolactin in heifers.

	Period						
	Pretrt	Mo 0	Mo 1	Mo 2	Mo 3	Mo 4	Avg Mo 1-4
Treatment[a]	Serum prolactin (ng/ml)						
8L:16D	17.0	18.0	10.1[d]	14.8	17.1	17.5	14.9
16L:8D	18.5	16.4	23.3[f]	23.3[f]	23.5	21.2	22.8[g]
20L:4D	9.6	9.8[g]	17.5[d]	23.6[bf]	19.4[b]	16.0	19.1[b]
24L:0D	12.3	12.5	18.0[g]	17.0	20.7[c]	18.3[e]	18.5[d]

[a]N=4 heifers per treatment; pooled SE= 1.8 ng/ml
[b]Within a treatment, means differ from pretreatment (P<.01).
[c]Within a treatment, means differ from pretreatment (P<.02).
[d]Within a treatment, means differ from pretreatment (P<.05).
[e]Within a treatment, means differ from pretreatment (P<0.1).
[f]Within a period, means differ from 8L:16D treatment (P<.05).
[g]Within a period, means differ from 8L:16D treatment (P<0.1).