To the Graduate Council:

I am submitting herewith a dissertation written by Mary Elizabeth Sowerby entitled "Effects of Managerial and Environmental Practices on Milk Yield, Hormone Levels, and Behavior of Lactating Dairy Cows". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Kelly R. Robbins, Major Professor

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and Dean of the Graduate School

EFFECTS OF MANAGERIAL AND ENVIRONMENTAL PRACTICES ON MILK YIELD, HORMONE LEVELS, AND BEHAVIOR OF LACTATING DAIRY COWS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Mary Elizabeth Sowerby

August, 1996

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Thesis
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DEDICATION

This dissertation is dedicated to John P. Hitchcock, who up to his untimely death on November 9, 1995 served as chairman of my graduate committee.

Dr. Hitchcock's unceasing encouragement through life's ups and downs is the primary reason for my completion of this doctoral program. His compassion, concern, technical assistance, and friendship were all highly valued and appreciated. His helpful spirit and good humor are much missed.

ACKNOWLEDGMENTS

Often the 4-H member who stands at the bottom of a showmanship class learns far more than the youth who stands at the top of the line-up. Over the many years I have spent working on this doctoral degree I have often felt like the 4-Her on the bottom of the line. But how I have learned life's bitter and sweet, both from personal experience and from my fellow graduate students, various professors, and other friends over this extended program. I have gained far more philosophically than I could ever have, had I completed this degree "normally".

For the opportunity to complete this degree, I am much indebted to my graduate committee for their patience and faith. Dr. John Hitchcock I have already acknowledged in the dedication. Serving in his place as my graduate committee chairman is Animal Science Department Head, Kelly Robbins. Dr. Robbins has quietly provided research funding and administrative support throughout this doctoral program and without hesitation volunteered to chair my committee in its final duties. I am most grateful.

Dr. Benny Bell has been there through thick and thin as "dairy chairman" of my committee, and just recently Acting Committee Chairman. His suggestions, help, understanding, and friendship are all very appreciated.

Dr. James K. Miller, despite not comprehending how I could have taken such a circuitous route to complete a degree much easier done in a straight line, has been a most wonderful mentor. I love his practical, down-to-earth style and manner, and intellectual curiosity. I truly appreciate his sharing of knowledge, experience, time, and work (especially during overnight blood samplings).

The ever-energetic Dr. Stephen P. Oliver, has been another great example of scientific endeavor. I am grateful for his thought-provoking questions, solid suggestions, and continuous encouragement.

Dr. Edward O'Connor left the University of Tennessee Psychology Department soon after I completed my comprehensive exam. I was very grateful for his support. I am equally grateful to Dr. Fred Hopkins, who in the past year agreed to become a committee member in Dr. O'Connor's place. Thank you, Dr. Hopkins, for taking on these graduate committee duties.

My research would never have been completed without Dr. Gary McCracken for his infrared camera and photography assistance; Dr. Richard Saudargas for behavioral analysis help; and Bob Muenchen for his statistical help. A squadron of underclass students also provided behavioral observations and many fellow graduate students helped take blood samples at all hours of the day and night. Clyde Holmes, Manager of Cherokee Dairy Research Center, and his excellent farm staff kept me and both experiments on track. Eddie Jarboe and Nancy Rohrbach provided supplies and laboratory expertise. All of this help was immensely appreciated.

I am also most thankful to the University of Tennessee faculty who have endeavored to teach me, the now countless graduate student friends I have made over the years at UT, and the wonderful helpful Animal Science staff. They have made this degree program both educational and fun.

My parents, Merton and Frances Sowerby, deserve an extra big thanks for their never-ending love and support.

And for making rainbows when life has been lowest, I want to thank my Heavenly Father for never giving up on me.

ABSTRACT

Two studies investigating managerial effects on cattle behavior and physiology were performed. In the first study, forty mid-lactation Holstein cows were divided into two groups and housed in identical, light-controlled, stanchion barns. During the 3 wk pretreatment period, incandescent lights (providing 100 lx 1 m above the floor) were on from 0300 to 2100 h in both barns. Treatments consisted of lights on from 0300 to 2100 h in one barn and lights on from 0700 to 1700 h with a skeletal light period between 0400 and 0500 h in the other for 14 wk. Milk yield; body weight; serum prolactin, cortisol, and triiodothyronine concentrations; and duration and frequency of eating and lying down and frequency of drinks were not significantly different between the two treatments. Results suggest savings in utility costs could be attained by using a skeletal light period to replace a long continuous light period without decreasing milk production or eating time.

To detect trends in behavioral feeding preference, 48 lactating cows were observed 72 continuous h during five different feed management regimes. Treatments were: hay and silage fed simultaneously at 0830, 1300, and 1630 h; hay fed at 0730, 1145, and 1530 h and silage fed at 0830, 1300, and 1630 h; silage fed at 0730, 1145, 1530 h and hay fed at 0830, 1300, and 1630 h; hay and silage fed simultaneously at 0700, 1000, 1300, and 1600 h; and hay and silage fed simultaneously at 0700 and 1630 h. Binomial *z*-scores indicated that cows had definite eating patterns which went across all treatments. Strongest feeding preferences were to eat grain, then silage, drink water, and then eat hay. However, behavioral differences between treatments were not

detected. On average, cows ate silage 9.51 times/d, 18.45 min each time; hay 5.59 times/d, 10.91 min each time; grain 7.33 times/d; and drank water 4.68 times daily.

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PART I.

REVIEW OF LITERATURE

INTRODUCTION

"If cows could talk, they would be heard all over this country calling for an improved breed of dairyman."

--W.D. Hoard (Rankin, 1925)

Living cells, which interact together as organs and tissues, intricately communicating with one another to promote a homeostatic self- and species-preserving unity, are invariably affected by their environment. In the case of domesticated animals, specifically dairy cattle, that environment is invariably affected by prevailing management practices.

Management of light and feeding will be the central focus of this dissertation. Both can be altered in an endless variety of ways. Both affect the physiology, behavior, and ultimately milk yield of lactating cows. A separate section of this literature review will be devoted to light and feeding as they relate to two reported experiments in Parts II and III.

LIGHT MANAGEMENT

What is Light?

Light, the fundamental source of energy for cellular life, is known to affect countless traits including reproductive cycles of seasonal breeders (Hansen, 1985), thickness of hair coat (Yeates, 1955), and behavior of wild and domestic species (Tucker and Ringer, 1982). As electricity made possible easy alterations of natural photoperiods,

length, intensity, and timing of lighting has been used to increase productivity in many species. These include egg-laying capacity of chickens (Weaver and Siegel, 1968), twice a year lambing of sheep (Tucker and Ringer, 1982), and fur coat thickness in mink (Martinet et al., 1992).

Light acts as a continuous electromagnetic radiation wave of discrete energy packets called photons. Photons have no mass and no electrical charge (Carlson, 1986). Light waves have a particular frequency and length. Wavelength of light determines its hue. Human eyes can detect the visual spectrum of electromagnetic radiation wavelengths between 380 nanometers **(nm)** (ultraviolet) and 760 nm (infrared) (Carlson, 1986).

Light can vary in intensity, which determines brightness (Carlson, 1986). Light intensity is measured in footcandles (a unit of illumination one-foot square from a uniform point source of light of one candle) or lux **(lx)** (a unit of illumination equal to the direct illumination on a surface one meter from a uniform point source of one candle or equal to one lumen per square meter) (Carlson, 1986).

Saturation or the relative purity of the wavelength of light also varies. If radiation has only one wavelength, the perceived color is pure or fully saturated. Radiation of all wavelengths appears white or without sensation of hue (Carlson, 1986).

Artificial Lighting

Natural light varies from a noontime illuminance level that peaks at over 100,000 lx on a summer day, to 8200 lx at the start of twilight, to a final level below 0.0006 lx at night. Transition periods of dawn and dusk each last about 3 h (Hughes et al., 1987).

Seasonal variations in day length of natural light are caused by the axial tilt of the earth toward or away from the sun. At the spring and autumn equinoxes in April and September, day and night length are equal at 12 and 12 h. Winter solstice in late December marks the shortest day in the northern hemisphere varying from 3 h 50 min in Fairbanks, AK (65° latitude), to 10 h 36 min in Key West, FL (25° latitude), with a moderate 9 h 16 min in New York City (41° latitude). Daylengths at summer solstice vary from 23 h 3 min in Fairbanks, to 15 h 8 min in New York City, to 13 h 42 min in Key West (Hughes et al., 1987).

Artificial lighting typically is either on or off with no adjustment for dawn or dusk. Standard incandescent lamps have a wavelength spectrum similar to firelight, very high in the yellow, orange, and red waves (570 to 700 nm). Warm light fluorescent bulbs are similarly high in yellow and orange (570 to 625 nm), but are far lower in red waves (625-700 nm). Natural outdoor light has relatively equal amounts of blue, green, yellow, orange, and red waves (440 to 700 nm) with about one-third less violet rays (380-440 nm). Vita-lite fluorescent lamps produce the most nearly equal spectrum to natural lights (Hughes et al., 1987).

Dannemann et al. (1985) tested effects of four levels of light intensity, 2, 20, 100, and 130 lx on the behavior of calves and found that at 2 lx calves had the longest duration and most frequent phases of resting behavior. A more distinctive daily rhythm of resting behavior was noted in the stable with better lighting along with longer lasting feeding behavior, greater duration and frequency of play-fighting and solitary play-running, and greater licking of objects. No significant differences in average daily gain

or feed efficiency were observed in gilts exposed to 16 h daily light of either cool-white light, full spectrum daylight, red light, or ultraviolet light. Gilts exposed to red light achieved puberty significantly later, and at heavier weights than gilts exposed to the other three lights (Wheelhouse and Hacker, 1982). Wheelhouse and Hacker (1982) concluded gilts were responsive to regions of the light spectrum below red.

To fully appreciate the outward manifestations of light-related effects, an internal perspective is needed to understand what happens to light as photons hit the eye and proceed to cause chemical changes which ultimately affect the entire body.

Photoreception

Photoreceptors for light in cattle are found in the retina of the eye (Prince, 1960). Predominantly rod cells are interspersed with an increasing number of cone cells towards the center of the retina, as in primates (Prince, 1960). The proportion of rod to cone cells is 15:1 for cattle, compared to 20:1 for humans (Dannemann et al., 1985). Cones detect small features in the environment providing visual acuity, in addition to discriminating light of different wavelengths for color vision. In most higher primates, each cone cell contains one of three types of cone photopigments. This variation produces cone cells which are maximally sensitive to light of long, medium, or short wavelengths and produces color vision (Carlson, 1986). Rods do not detect different colors and produce vision of poor acuity. However, rods are more sensitive to light and produce vision when light is dim (Carlson, 1986).

Behavioral data used to detect color discrimination in cattle indicated green, yellow, orange, and red are distinguished from grays of equivalent luminosity. Blue and

purple are not discriminated and orange and yellow are confused (Thines and Soffie, 1977; Soffie et al., 1980). Green, red, and blue have also been compared in pairs to note whether heifers could distinguish between them. Results demonstrated cattle can learn to discriminate between colors, indicating a wide range of color vision (Gilbert, 1986).

Photopigments consisting of opsin and retinal in rods and cones react to light by absorbing a photon, which induces a transformation from cis- to trans-retinal. During this transformation, opsin breaks into two parts activating molecules of phosphodiesterase which decreases cyclic-GMP and affects calcium concentrations, resulting in a sudden decrease in sodium permeability as sodium channels close. Net result is a hyperpolarization of the photoreceptor membrane which acts as the transmitting signal to ganglion cells (Carlson, 1986).

For vision, ganglion cells transmit light signals through the optic nerves to the dorsal lateral geniculate nucleus of the thalamus. Signals are sent from the dorsal lateral geniculate nucleus via optic radiations to the striate cortex, the primary visual cortex where vision is perceived (Carlson, 1986). In addition to the primary retino-geniculo-striate pathway, there are several other pathways taken by neurons from the retina (Carlson, 1986; and Card and Moore, 1985). Two pathways are to areas which control movements of the eye: the accessory optic nuclei and superior colliculus. A third goes to the pretectum which controls pupillary size (Card and Moore). Another pathway from the retina goes to the intergeniculate leaflet and acts as a relay for visual information to

subcortical structures: the pretectum, superior colliculus, pontine nuclei, and suprachiasmatic nucleus (Carlson, 1986; and Card and Moore, 1985).

The suprachiasmatic nucleus (SCN) also has a direct signal from the retina through the retino-hypothalamic tract. Glutamate and aspartate have been suggested as neurotransmitters for this pathway (Anderson et al., 1987). The SCN has been identified as the "mind's clock", the area of the hypothalamus which controls behaviors and physiological processes which vary across the day/night cycle (Van den Pol, 1985). The geniculohypothalamic tract, characterized by neuropeptide Y immunoreactivity, connects the ventral lateral geniculate nucleus and the intergeniculate leaflet to the SCN (Harrington et al., 1985).

Another major afferent to the SCN, which may be visual, originates in the median and dorsal raphe nuclei. Raphe terminals contain serotonin and are responsible for the high serotonin concentration in the SCN (Mosko and Jacobs, 1974).

From the SCN, efferent projections have six broad anatomical components that project to intra- and extrahypothalamic targets:

1) the pre-optic area;
2) the paraventricular nucleus;
3) through the retrochiasmatic area to the region between the arcuate and ventromedial nuclei;
4) the intrafascicular nuclei, the paraventricular nucleus of the thalamus and the paratenial nucleus;
5) the intermediate lateral septal nucleus; and

6) the intergeniculate leaflet of the lateral geniculate nucleus (Watts, 1991).

The pre-optic region of the hypothalamus may affect a number of circadian rhythms influenced by the SCN. Fluid balance, reproduction, sleep, and thermoregulation are all controlled by structures in the pre-optic region (Watts, 1991).

The major efferent pathway from the SCN is to the paraventricular nucleus, or more specifically, a region called the sub-paraventricular zone **(sPVHz)** of the hypothalamus. Efferent targets of sPVHz are very similar to those of the SCN itself. However, density of sPVHz projections is typically greater than those of the SCN (Gillette, 1991).

Watts (1991) suggested that from an anatomical standpoint, the sPVHz, beginning with the peri-SCN region appears to play an integral part of the system that transmits circadian information to the rest of the brain. In addition, the sPVHz receives an afferent projection from the intergeniculate leaflet suggesting it may receive photic input independent of the SCN. Such input may contribute to further modifications to the circadian signal after it has left the SCN (Watts, 1991).

Neuroendocrine, autonomic, and behavioral processes are all integrated through the hypothalamic paraventricular nucleus (Swanson and Sawchenko, 1983). Its efferents influence secretions of both the anterior and neural lobes of the pituitary gland in addition to the brainstem and spinal cord. For instance, plasma concentrations of corticosterone in the rat showed a well-characterized SCN-dependent diurnal rhythm (Dallman et al., 1987). Neurons containing corticotropin-releasing hormone, which control the secretion

of adrenocorticotrophic hormone and thereby corticosterone, are found in the medial paricellular part of the paraventricular nucleus (Watts, 1991). Watts (1991) speculated these cells would appear to be likely targets of information derived from SCN. Evidence from lesion experiments has also shown the paraventricular nucleus is probably the point at which the SCN influences the circadian rhythm of the pineal gland (Watts, 1991).

Activity and function of the ventromedial and dorsomedial nuclei appear to be influenced directly by the SCN (Watts, 1991). These two nuclei have been implicated in a number of physiological functions including ingestive behavior, rage, and female sexual behavior. Through the ventromedial and dorsomedial nuclei, the SCN could potentially influence a variety of rhythms (Watts, 1991).

Of the extrahypothalamic projections from the SCN, both the paraventricular nucleus of the thalamus and lateral septal nucleus have projections which ultimately go to the hippocampus (Swanson and Cowan, 1977, 1979; and Wyss et al., 1979), amygdala (Ottersin and Ben-Ari, 1979), and nucleus accumbens (Watts, 1991). The hippocampus, amygdala, and nucleus accumbens can all potentially modulate the activity of the medial forebrain bundle, which is critical in determining the level of arousal and attention, and expressing many motivated behaviors (Watts, 1991). The intergeniculate leaflet provides neuropeptide Y innervation to the ventral SCN (Card and Moore, 1982) which may regulate photic modulation of the phase of circadian rhythms (Albers et al., 1984).

Although the SCN remains a "black box" into which photo-signals enter, the bio-rhythms which exit are manifested in many more easily observable ways called circadian rhythms.

Circadian Rhythms

Moore-Ede et al. (1976) stated, "Circadian rhythms in biological variables are one outward manifestation of an important evolutionary adaptation to life on a rotating planet: the ability to measure time. This capacity enables organisms to predict the major changes in environmental conditions, and the consequent alterations in food supply and predator activity, which occur with a 24-hour periodicity because of the earth's rotation. Thus, for example, adaptative physiological and behavioral responses which may take several hours to be activated can be initiated in advance of the predicted environmental challenge, or events where timing may be critical for survival, such as the emergence of flies, can be timed to occur at the point of maximum environmental advantage."

Moore-Ede later pointed out (1986), "The concept of homeostasis should be extended to include the precisely timed mechanisms of the circadian (and circanular) timing system which enable organisms to predict when environmental challenges are most likely to occur."

Biological rhythms are characteristics of animals which vary more or less regularly with specific periods. They are classified according to their period length as ultradian (< 24 h), circadian (approximately 24 h), infradian (> 24 h) and seasonal or circannual rhythms (approximately 1 year) (Wollnik, 1989).

Some ultradian rhythms are true periodic processes with a constant period, but others are episodic with variable time lags between single events. *Mus musculus,* whose daily rhythm rate is considerably shorter than 24 h, has a more pronounced day-to-day

instability and long term lability than a hamster whose daily rhythm rate is indistinguishably close to 24 h (Wollnik).

General features of circadian rhythms include:

1) Ubiquitity: Virtually all plants and animals including unicellular organisms have been found to exhibit circadian rhythms (Pittendrigh and Minis, 1964).

2) Genetic determination: Takahashi and Zatz (1982) cited examples of single-gene mutations which altered period length of *Drosophila melanogaster, Drosophila pseudoobscura, Chlamydomonas reinhardi,* and *Neurospora crassa*. Inbred rat experiments have shown a recessive single-gene mode of inheritance for an unusual activity pattern (Wollnik et al., 1987).

3) Precision: Noctural rodents have been noted to have a variation in cycle length of < 3 min (Pittendrigh and Daan, 1976). However, species vary in "tightness" of circadian length. Serial correlation analysis indicated that the precision (day-to-day stability) of the pacemaker's period is about twice as good as the precision of the activity rhythm it drives (Pittendrigh and Daan, 1976).

Bunning (1960) observed from his plant research, "By means of diurnally periodic oscillations (the physiological clock) the cell is brought alternately into two period parts with properties differing both quantitatively and qualitatively. Each of these parts lasts approximately 11-13 h. Basic importance of this oscillation lies in the fact that the cell

is thus brought to certain extreme physiological states. Various functions are possible only when these extremes are reached. One of these extreme states is characterized by a high synthetic capacity and the other by a high catabolic capacity."

Most biological fluctuations synchronized to the environmental 24 h cycle are endogenous rhythms which persist even when no external time cues are present. These rhythms will slightly deviate from the 24 h period and eventually free-run with an intrinsic natural period without a synchronizing zeitgeber (time giver). The free-running rhythm is considered to be the basal state which reflects the period of the unrestrained endogenous oscillator (Takahashi and Zatz, 1982). The light-dark cycle is the strongest zeitgeber for most animals. Other environmental factors which synchronize or entrain (impose a period and phase control by environmental cue) rhythms are temperature cycles, food availability (Boulos and Terman, 1980), and social cues (Wever, 1982).

Entrainment of the free-running rhythm is not restricted to periods of exactly 24 h. However, there is a limited range of periods to which the internal circadian clock can be entrained by a zeitgeber (Wollnik, 1989). Aschoff and Pohl (1978) found this range to be 20 to 28 h in mammals and dependent on the strength (amplitude) of the zeitgeber as well as the strength of the endogenous circadian system of the organism. Exceed the range of entrainment and the circadian rhythm will free-run with a period close to that observed under constant conditions. Even then, in a phenomenon called relative coordination, signals of the zeitgeber can still modulate the free-running pattern by periodically entraining the rhythm (Wollnik, 1989).

Today it is widely accepted that the circadian clock of mammals is a multi-oscillatory system (Wollnik, 1989). In human and other mammalian circadian systems, multiple oscillators are usually coupled with each other. For instance, temperature and plasma catecholamine levels, which generally increase and decrease in synchrony, may change their phase relationship depending on conditions, or may even become free-running with different frequencies (Aschoff and Wever, 1976).

Photoperiodic Responses

Neural events organized rhythmically by the SCN may be categorized generally as motivated behavior (i.e. general activity, drinking, feeding, estrous behavior) or homeostatic changes (i.e. body temperature, osmoregulation, plasma hormone concentrations) (Watts, 1991). Neither motivational behaviors nor homeostatic changes are turned on and off by the SCN. However, without the SCN there is a suppression of a temporally-dependent, light-entrainable circadian pattern that can be overridden when the situation demands (Watts, 1991).

Animals housed in a constant or aperiodic environment have free running rhythms which deviate slightly from the environmental 24-hour cycle depending on individual and species differences, and variations in background light intensity (Daan and Pittendrigh, 1976b). The free-running period is usually longer with increasing light intensity for nocturnal hamsters and rats, but shorter or persisting for diurnal chipmunks and squirrels (Meijer, 1991).

Continuous exposure to light or darkness may also cause splitting, an alteration of activity patterns when animals become active twice a day instead of once (Hoffman,

1971). Splitting occurs in the nocturnal hamster when it is housed in bright light and diurnal shrews when housed in constant dim light or darkness (Hoffman, 1971; and Meijer, 1991).

Short pulses of either light (against continuous dark) or darkness (against continuous light) also modulate circadian rhythms (Boulos and Rusak, 1982). Light and dark pulses (called skeletal lighting periods) cause a phase-advancing or phase-delaying shift in circadian rhythm, causing an animal to start activity at an earlier or later phase, respectively, than its free-running rhythm. The rhythm will be advanced if a light pulse is given in late subjective night. The rhythm will be delayed if a light pulse is given during early subjective night. Little or no phase shifting occurs if a light pulse is given during subjective day. Once shifted by a light pulse, the phase remains shifted. Magnitude of the phase shift produced by a light pulse depends on:

1) Duration of the light pulse: Phase shifts have been noted to increase with longer light pulses from several minutes to 3 h or more (Daan and Pittendrigh, 1976a). Long-lasting light presentations of several minutes or more on the entire retina have been demonstrated as the most effective stimuli for responsive SCN cells.

2) Light intensity: Studies using hamsters have shown a threshold light intensity of about 1 to 10 lx exists at the lower end of a sigmoid curve-like relationship between intensity of light pulse and magnitude of phase shift change (Takahashi et al., 1984). Two SCN cell-types, light-activated and light-suppressed, are characterized by a tonic response to light (Meijer

et al., 1986). Change in discharge rate after a light pulse depends on its brightness. No visual response could be elicited below certain light intensities of 0.1 and 1 lx, respectively, for nocturnal rats and hamsters, or 1000 lx or more for diurnal thirteen-lined ground squirrels (Meijer et al., 1986, 1989).

In vitro experiments have suggested the SCN is specifically sensitive to stimulation of cAMP-analogue during subjective day and cGMP-analogue during subjective night. Phase advances or a reset of the timing of sensitivity to cAMP- and cGMP-analogue are also opposite for the two (Gillette, 1991).

Gradually increased light intensities (as occurs naturally with dawn) increase light-activated cell discharge level and decrease light-suppressed cell discharge level (Meijer et al., 1986). Saturation occurs at about 1000 lx in nocturnal hamsters and rats. Saturation has been tested in diurnal squirrels, but not achieved, probably because they were not tested up to 3500 lx (equivalent to a bright sunny day) (Meijer et al., 1986, 1989). Actual environmental light intensities vary from 4.1^{-5} to 10^{5} lx. The range of light on a bright night is approximately 0.1 to 10 lx. Light intensity of early dusk or late dawn is about 1000 lx. Diurnal animals have an intensity-response curve shifted toward higher light intensities than nocturnal animals (Meijer, 1991).

Research with humans has shown light pulses of 2500 lx immediately and profoundly decreased melatonin blood concentrations when subjects were awakened between 0200 and 0400 h. Five hundred lx had no effect on melatonin concentrations, but 1500 lx caused a 50% suppression of melatonin release (Lewy et al., 1985). Melatonin is a hormone produced by the pineal gland involved in timing of reproduction in some animals (Waller et al., 1988) and sleep and depression in humans (Reiter, 1990).

3) Wavelength of light: Wavelength of light plotted against magnitude of phase shift in hamsters indicated greatest sensitivity to light with a wavelength of 515 nm (green light) (Takashshi et al., 1984). Meijer (1991) noted the spectral sensitivity curve resembled that for rod photoreceptors, except rods were a little more sensitive to red light.

4) Circadian phase at which the light pulse is applied: A light pulse at the beginning of activity delays the phase of free-running circadian rhythm in nocturnal animals. However, a light pulse at the end of the activity period advances the rhythm. Light pulses during a nocturnal animal's inactive period have no effect on the phase of the free-running period (Daan and Pittendrigh, 1976a). Dark pulses applied 3 to 9 h before activity onset of hamsters produces a phase advance (earlier than normal activity). From hamster activity data, Elliott (1976) estimated the period of

photosensitivity begins about 0.5 h before activity onset and ends between 11 and 11.5 h after activity onset.

5) Lighting history: Reiter (1985) noted thirteen-lined ground squirrels bred in a darker than natural environment compared to those bred in natural lighting had a much lower threshold for melatonin suppression. Rats raised in a bright light intensity had a lower ratio of SCN grey, type 1 (excitatory) to grey, type 2 (inhibitory) receptors than rats raised in the dark (Meijer, 1991). Light sensitivity was affected by lighting history.

Pittendrigh and Minis (1964) reported effects of a complete photoperiod, for example, 8 h of continuous light:16 of continuous dark (8L:16D), could be almost fully simulated by two short (15 min) skeletal pulses of light 8 h apart in *Drosophila* pupal emergence rhythms. Simulation of complete long photoperiods by skeleton photoperiods was nearly perfect for all photoperiods up to 11 h. At 12 h, the simulation was fair. At 13 h, the skeleton photoperiod produced an unstable entrainment. When attempting to entrain to a skeletal 14 h photoperiod, the circadian oscillation assumed a phase characteristic of the 10 h skeleton. A clear phase jump occurred between 13 and 14 h. All skeletons greater than 14 h took on the phase of the shorter complement (i.e. 14:10 was interpreted as 10:14; 15:9 as 9:15; etc.). Pupal emergence rhythms jumped to the shorter of the two skeletal periods between 13 and 14 h; calculated at 13.8 h.

An asymmetric skeleton, the coupling of a long duration signal with a short duration signal, also produced distinctive entrainment patterns. Light cycles of 3L:21D have been complicated by short pulses through the 21 h night in the air plant, Kalanchoe

(Pittendrigh and Minis, 1964). These asymmetric skeletal photoperiods were physiologically interpreted as the night period beginning at the onset of the main photoperiod and terminating at the end of the night interruption, or as beginning with the onset of the interruption and ending at the termination of the long photoperiod (Pittendrigh and Minis, 1964).

Pittendrigh and Minis (1964) concluded: 1) asymmetric skeletons can simulate longer photoperiods better than two, short pulse, symmetric skeletons; 2) asymmetric skeletons involving a main photoperiod of 8 h or more can simulate a long photoperiod two ways, with the night interruption as terminator of the skeleton, or with the night interruption as initiator of the skeleton; and 3) simulation of long photoperiods is better when the interruption functions as initiator, rather than terminator, of the long skeleton.

Four specific bovine photoperiodic responses will next be addressed. Hormone concentrations, growth, milk yield, and behavior have all been shown to be affected by photoperiods (Stanisiewski et al., 1988; Peters et al., 1980; Peters et al., 1978; and Evans and Hacker, 1989b), but many known and unknown factors are still confounding experiments and leading to contradictory results.

Hormonal Responses

Plasma hormone concentrations are among the homeostatic changes responsive to photoperiods (Watts, 1991). Plasma hormone concentrations are regulated by feed-back and influenced by both endogenous and exogenous forces.

Glucocorticoids. Selye, who developed the General Adaptation Syndrome (Selye, 1936), characterized a standardized response to stressors by rate of glucocorticoid secretion and suggested three consecutive stages of stress response:

1) the alarm reaction (an initial surge of glucocorticosteriods followed by depletion of stored glucocorticosteroids resulting in shock);
2) the stage of resistance beginning about 48 hours after injury (period of optimal adaptation wherein the adrenal cortex regains corticoid secretory granules); and
3) the stage of exhaustion (acquired adaptation lost and glucocorticosteroids depleted) (Selye, 1936).

Mason (1971) has since proposed the pituitary-adrenocortical response may actually be a specific reaction for psychological stress rather than a nonspecific reaction for all stressors. Final effect of increased or diminished glucocorticoids occurs regardless of whether an environmental factor is producing a pituitary or adrenocortical response through psychological then physiological effects or strictly physiological effects.

Two primary glucocorticoids, cortisol and corticosterone, are secreted by the adrenal cortex of cattle. In addition to stress effects on cortisol plasma concentrations, circadian and ultradian rhythms of cortisol levels in dairy cattle have been reported by some researchers (Fulkerson et al., 1980; MacAdam and Eberhart, 1972) and refuted by others (Hudson et al., 1975; Shaw, et al., 1960).

Fulkerson et al. (1980) observed an ultradian rhythm of cortisol concentration in plasma of 18-mo-old dairy heifers in samples collected every 10 min for 24 h. An

amplitude of approximately 30 ng/ml and a frequency of about 0.6 cycles/h were reported by Fulkerson et al. (1980). By eliminating ultradian components of variation in cortisol concentration, a diurnal variation which was high between midnight and mid-morning and low in the afternoon was found. MacAdam and Eberhart (1972) also found a daily rhythm with higher concentrations of cortisol between 0230 and 0630 h, and lower at 2030 h.

Definitive circadian rhythms for cortisol have been demonstrated in humans (Aschoff and Wever, 1976), pigs (Griffith and Minton, 1992), and mares (Johnson and Malinowski, 1986). Hudson et al. (1975) theorized cattle may not show such a definitive circadian rhythm because they lack a well defined sleep-wake cycle.

Thyroid Hormones. Thyroid hormones exert effects within almost every tissue of the body throughout the life of the individual, in large part, via stimulation of cellular protein synthesis (Oppenheimer, 1979). Effects include body growth stimulation (such as mammary and brain development), regulation of basal metabolic rate, and induction of synthesis of numerous cellular enzymes and other proteins (Hadley, 1984).

Two hormones are secreted by the thyroid: thyroxine (T_4) and triiodothyronine (T_3). In the bloodstream these hormones may be protein-bound (which renders them inactive) or free. Free T_3 is considered the most physiologically active form of thyroid hormone in cattle (Kahl et al., 1991).

Seasonal variations have been found in free T_3 and T_4 plasma concentrations in cattle. Nixon et al. (1988) found free T_4 concentrations ranged from a low of 1.4 ng/dl in the summer to a high of 1.86 ng/dl in the spring. Average free T_3 plasma

concentrations ranged from a low of 2.98 pg/ml in winter to a high of 6.23 pg/ml in the fall. Free and total T_4 concentrations were high in the fall, low in the winter, and intermediate in spring and summer. Free and total T_3 concentrations had a similar pattern with greater variation than observed with T_4. Nixon et al. (1988) did not relate this work to daylength or ambient temperature. Other work (Premachandra et al., 1958) indicated that T_4 secretion rates were reduced three-fold in the summer, with considerable individual variation. However, depressed T_4 disappearance rates by high environmental and body temperatures have been suggested also (Lundgren et al., 1964).

Refsel et al. (1980) noted a diurnal pattern for T_3 plasma concentrations in cows maintained in a stanchion barn with a 16L:8D photoperiod. A low baseline of T_3 concentration occurred from 0400 to 1000 h (average 1.10 ng/ml), with a rise to an elevated plateau from noon to 2200 h (average 1.30 ng/ml) and gradual return to low baseline. Thyroxine concentration changes paralleled those observed with T_3.

Prolactin. Prolactin (**PRL**) influences a variety of physiological factors. In the bovine, PRL plays an integral role in mammogenesis and lactogenesis and is essential for maintaining lactation, but has little effect during lactation on milk yield and milk composition (Walsh et al., 1980). In addition, PRL plays a role in fluid regulation presumably via alterations in renal hemodynamics (Becker et al., 1985).

Many stimuli have been noted to increase PRL secretion from the pituitary of cattle including:

1) suckling, milking, and mechanical stimulation of teats (Karg and Schams, 1974);

2) injection of thyroid-releasing hormone (Vines et al., 1977);

3) low concentrations of estrogen, progesterone and associated parturition (Ingels et al., 1973);

4) season [serum PRL concentrations were higher during April to September (74 ng/ml) than during October to March (35 ng/ml)] (Koprowski and Tucker, 1973);

5) high plane of nutrition (Petitclerc et al., 1983a);

6) stress from jugular puncture (Raud et al., 1971);

7) parity (multiparous cows had higher concentrations of serum PRL than primiparous cows) (Koprowski and Tucker, 1973); and

8) temperature (Wettemann et al., 1982).

An increase in ambient temperature from 10 to 30° C reduced metabolic clearance rate and significantly increased secretion and disappearance rates of serum PRL concentrations (Smith et al., 1970). Serum PRL concentrations increased as ambient temperatures increased from -7 to +29° C (Peters and Tucker, 1978). When ambient temperatures were below 0° C, serum PRL concentrations were similar in heifers exposed to natural and 16L:8D photoperiods (Peters and Tucker, 1978).

Serum PRL levels during lactation have not been found to be correlated with either stage of lactation or milk yield (Walsh et al., 1980; Hart et al., 1976; and Bonczek et al., 1988). Serum PRL, measured after stimuli associated with milking, was greatest at 8 wk of lactation and gradually decreased as lactation advanced until at 32 wk no PRL was released (Koprowski and Tucker, 1973).

Photoperiod effects on PRL concentration in cattle have been studied extensively. Seventeen-wk-old bull calves exposed to 16 h of light daily had PRL concentrations significantly greater than calves receiving 8 or 24 h light daily (Stanisiewski et al., 1988). Prolactin concentrations were not different between calves receiving 24 or 8 h of light daily (Stanisiewski et al., 1988). Heifers exposed to a 16L:8D photoperiod with artificial light had a 4-fold increase in PRL concentration when compared to heifers in natural Michigan winter daylength (Peters and Tucker, 1978). Between April 30 and August 13, a 1.6-fold increase in PRL was noted in heifers receiving 16 h light compared to natural light (Peters and Tucker, 1978). Serum PRL concentrations in bull calves maintained at an ambient temperature of 22° C decreased from 57 to 8 ng/ml as light decreased from 16 to 8 h over a 12 wk period (Bourne and Tucker, 1975). Cows and first calf heifers under 17.5L:6.5D light exposure both before and after calving had significantly increased plasma concentrations of PRL, while other cows exposed to 9.5L:14.5D prior to calving and 17.5L:6.5D after calving, showed a similar increase in PRL only after calving (Gustafson, 1994).

Lights with different spectral properties have been shown to influence the concentrations of PRL. Cool-white fluorescent, incandescent, high-pressure sodium Vita-lite (which simulates natural daylight), and mercury vapor lamps increased PRL concentrations as duration of daily light increased from 8 to 16 h (Stanisiewski et al., 1984). Comparison of 8 h of cool-white light, plus 8 h of red (550 to 750 nm) or blue (300 to 425 nm) fluorescent light, on PRL concentrations in bulls showed no significant difference in their ability to increase serum PRL (Bourne and Tucker, 1975).

Prolactin secretion varies with the timing of skeletal photoperiods. When daily light exposure on prepubertal bulls was changed from 8L:16D to 16L:8D or 6L:8D:2L:8D, basal secretion of PRL increased 418% six weeks later. Changing from 8L:16D to 6L:14D:2L:2D increased basal secretion of PRL by 173% (Petitclerc et al., 1983b). Cows exposed to 6L:7D:2L:9D exhibited a circadian rhythm of PRL plasma levels unseen in photoperiods of 12L:12D or 6L:4D:2L:12D, suggesting a photosensitive phase for PRL occurs between 13 and 15 h after subjective dawn (Evans et al., 1991).

Circadian rhythms of PRL release have been implicated from blood collected from lactating cows every half hour (Koprowski et al., 1972, and Mollett and Malvern, 1982). Koprowski et al. (1972) found a circadian pattern and the highest value occured at 0400 h (58 ng/ml) and lowest values recorded between 0400 and 1000 h (28 ng/ml). Others (Mollett and Malvern, 1982) found PRL in plasma appeared to have both a 24 h rhythm and 6 h rhythm by time series analysis. Maximum PRL plasma concentrations were noted between 1030 and 1600 h. Lowest PRL occurred between 0600 and 0900 h each day.

Effects of Photoperiod on Growth

Reports of photoperiodic effects on bovine growth have been contradictory. Average daily gain of prepuberal heifers increased when 16L:8D was compared with 24 h light or natural short day length (9.3 to 11.6 h light daily) (Peters et al., 1980, and Peters et al., 1978). Heifers exposed to 6L:8D:2L:8D grew 3% faster than heifers exposed to 8L:16D (Tucker et al., 1974). Photoperiod failed to influence growth or carcass composition of steers placed 168 d in photoperiods of either 16L:8D or 8L:16D

on high or low energy planes (Zinn et al., 1989). Likewise, Petitclerc et al. (1984) found that 20 prepuberal heifers fed low and high planes of nutrition during either 16L:8D or 8L:16D photoperiods did not have a photoperiodic effect on live body weight gains. However, increased protein content in the 9-10-11th rib section of heifers on high nutrition was attributed to photoperiod. Roche and Boland (1980) concluded that extending photoperiod in winter did not increase growth rate in Friesian bull calves or steers in Ireland.

Tucker et al. (1984) suggested one potential reason for the opposite growth responses might be due to a gonad-dependent phenomenon. A 16L:8D photoperiod increased average daily gain 9.8% in intact Holstein bulls when compared to natural short-day photoperiods, but had no significant effect on Holstein steers under the same conditions (Tucker et al., 1984).

Previous lighting experience also influences growth responses to photoperiod. Zinn et al. (1986) found prepuberal heifers that received a prior photoperiod of 16L:8D and a subsequent photoperiod of 8L:16D had significantly reduced liveweight gains compared to heifers with a previous photoperiod of 16L:8D and subsequent of 16L:8D, or previous 8L:16D and subsequent 8L:16D. Interestingly, in this experiment, heifers had similar liveweight gains when exposed to previous 8L:16D and subsequent 8L:16D or previous 16L:8D and subsequent 16L:8D. Possibly heifers became refractory to photoperiod after 214 d of the same lighting period.

Gradual versus abrupt light transitions to simulate dawn and dusk was another factor investigated by Zinn et al. (1986). Prepuberal heifers exposed to an 8L:16D

gradual transition photoperiod gained significantly less than heifers exposed to abruptly changing photoperiods of 8L:16D or 16L:8D or gradually changing 16L:8D (Zinn et al., 1986). Photoperiod induced differences in liveweight gains were significantly greater between heifers exposed to long versus short days when photoperiod changes were preceded by gradual transitions in light intensity rather than by abrupt. Zinn et al. (1986) hypothesized that gradual transitions in light intensity at dawn and dusk may be a more potent cue than abrupt transitions in light intensity for growth of heifers.

Petitclerc et al. (1985) investigated mammary growth in pre- and postpubertal heifers under 8L:16D and 16L:8D photoperiods. No effect from photoperiod was found on total weight of the mammary gland (parenchymal plus extraparenchymal tissue). However, mammary parenchymal weight increased 40 and 30% in pre- and postpuberal heifers, respectively, exposed to 16L:8D conditions. Petitclerc et al. (1985) concluded 16L:8D stimulates mammary parenchymal tissue to grow into the fat pad of Holstein heifers.

Effect of Photoperiod On Milk Production

Early experiments designed to determine effects of extended light periods on milk production were not promising. Sarchet et al. (1958) reported no effect from exposing lactating dairy cows to 20 h light daily versus natural winter lighting for 6 wk. Only one of three herds in an experiment by Murrill et al. (1969) produced 0.5 kg/d more milk in the summer and tended to produce more milk in the winter with extended light.

However, after discovery of a positive photoperiod effect on prolactin (Bourne and Tucker, 1975; and Leining et al., 1979), an intensive investigation of lighting effects

on milk yield was initiated. Peters et al. (1978) first reported results of an experiment comparing Michigan natural winter day length (9 to 12 h of light/d) to 16L:8D, with 46 cows in each treatment. During the first 100 d of lactation, cows given supplemental light produced 10% more milk than cows exposed to natural light. At day 100 postpartum, treatments were reversed for 40 more days on 18 cows in each treatment. Light supplementation for 16 h was not a sufficient stimulus to cause increased milk production between days 101 and 140, but persistency of milk yield improved. The lactation curve of cows in natural light in later lactation tapered off much faster (Peters et al., 1978).

In a subsequent experiment, Peters et al. (1981) exposed 12 cows in early lactation (37 to 74 d postpartum) and nine cows in late lactation (94 to 204 d postpartum) to 16L:8D. Equal numbers of control cows were exposed to natural light photoperiods of 9 to 12 h/d plus minimal supplemental light for routine management practices. In this experiment, morning milking time was an hour earlier (0400 instead of 0500 h) than the prior experiment. Overall average daily milk yields, adjusted for parity and pretreatment production, were 6.7% higher for the cows exposed to 16L:8D compared to cows with natural photoperiods.

Meanwhile, Tanida et al. (1984) compared cows exposed to 18L:6D and 24L:0D conditions and found no significant differences between the two lighting periods. However, by the third and final month of the experiment, cows exposed to 18L:6D produced 12% more milk (a non-significant trend) than cows under continual light.

In an effort to test photoperiod effects on commercial herds, Stanisiewski et al. (1985) worked with 13 Michigan dairy herds during autumn and winter seasons. Half of each herd received natural duration photoperiods plus supplemental light (total light equaled 16 to 16.25 h each day) and the other half was exposed to natural photoperiods plus minimal supplemental light to permit routine management activities (milking and feeding). Total daily light did not exceed 13.5 h each day. Overall, cows exposed to supplemental lighting produced 2.2 kg/d more milk than herdmate controls. Considerable herd to herd variation existed.

Evans and Hacker (1989a) attempted to determine if chronobiological manipulation of the environment through use of skeletal photoperiods enhanced bovine milk yield. Cows (n = 32) 8 mo pregnant were divided equally between the following treatments:

1) Control group exposed to 12 to 13 h continuous light each day.
2) Lights on from 0500 to 1100 h and 1500 to 1700 h (6L:4D:2L:12D) - a total of 8 h light stretched over 12 h from subjective dawn at 0500.
3) Lights on from 0500 to 1100 h and 1800 to 2000 h (6L:7D:2L:9D) - a total of 8 h light stretched over 15 h from subjective dawn at 0500.
4) Lights on from 0500 to 1100 h and 2100 to 2300 h (6L:10D:2L:6D) - a total of 8 h light stretched over 18 h from subjective dawn at 0500.

After calving, cows continued under the same lighting schedule. Although not statistically significant, there was a strong trend of cows in the 1800 to 2000 h pulse regime to produce 2 - 3 kg more milk/d than controls or cows in the other two pulse

groups. Milk production of cows in the 1800-2000 h pulse regime not only peaked higher, but also were more persistent (Evans and Hacker, 1989a).

Evans and Hacker (1989a) also observed an apparent increase in efficiency of feed utilization in the higher yielding 1800-2000 h pulse group. Increased milk production was not associated with increased dry matter intake. Evans and Hacker (1989a) hypothesized the light pulse at 1800 to 2000 h coincided with and stimulated photosensitive circadian rhythm(s) involved in milk production. Light is potentially necessary to elicit a photoperiodic response in milk production somewhere between 13 and 15 h after subjective dawn.

Another experiment using a skeletal photoperiod was performed by Bilodeau et al. (1989). Photoperiods of 16L:8D to 8L:2D:2L:12D were compared. Cows exposed to 16L:8D produced 5 to 11% more milk if fed ad libitum or pair fed.

To compare effects of previous lighting experience, Marcek and Swanson (1984) initially exposed cows to continuous light. Half the group then changed to an 18L:6D photoperiod while the rest remained under continuous light. No photoperiod effect was noted in milk yield. However, when cows were given prior experience of natural daylength (9 to 12 h light) and then exposed to 18L:6D for 9 wk, first-calf heifers had no difference in 4% fat-corrected milk whereas multiparous cows produced about 7% more 4% fat-corrected milk than did controls still under natural light.

Gustafson (1994) also compared effects of previous lighting experience on milk production by exposing one group of cows and heifers to 17.5L:6.5D 4 to 8 wk before and after calving. Another group was exposed to 9.5L:14.5D before calving and

17.5L:6.5D after. First calf heifers of the long-light exposed group produced 10-12% more energy-corrected milk and milk fat than heifers with short-light exposure prior to calving.

Petitclerc et al. (1985) investigated the effect of exposing heifers in their seventh month of gestation to short days versus long days until parturition and then placing both groups on long days through the first 100 d of lactation. Milk yield of heifers with short-day light exposure prior to parturition was 9% greater than heifers kept under long-day conditions both prior and after calving.

Thus, results of previous research are contradictory and suggest that, prior lighting experience has positive, negative, and neutral effects on milk yield depending on lactation number and other unknown factors. A key question in future photoperiod research should be effects of prior photoperiod and how long it takes for a photoperiod to have a positive or negative impact on milk yield.

Effects of Photoperiod On Bovine Behavior

Under natural lighting, bovine behavior in grazing and feedlot conditions has been studied many times with similar results. Stricklin and Kautz-Scanavy (1981) summarized these results, "Cattle exhibit a diurnal rhythm, with the majority of eating occurring near the times of sunrise and sunset. This is true in both pasture and feedlot conditions and during both summer and winter. Grazing cattle have a minor eating-period in the middle of the night, which occurs to a greater extent when the darkness period is longer and forage is poor. Feedlot cattle may also exhibit an eating period in the middle of the

night particularly if the daylength is short. Lying and ruminating occur primarily during the periods of reduced eating."

Cows exposed to 24L:0D or 18L:6D photoperiods ate an average of 270 to 280 min/d (Tanida et al., 1984). Average number of times eating was 10 to 12/d, averaging 24 to 27 min in duration. Lighting regimen did not affect eating behavior or milk production. Similar eating patterns were found in both light treatments with peaks of eating activity before sunset, before and after evening milking, and after fresh feed was offered in the morning (Tanida et al., 1984).

Phillips and Schofield (1989) compared cows under natural winter daylength (mean 8 h light) to 18L:6D in North Wales. Feeding times were not affected significantly by treatment. Periodicity of feeding behavior was similar throughout the day except during the latter part of the supplemented light period when a marked increase of feeding activity occurred only for cows with supplemented light. Another peak in feeding activity was observed around midnight. Interestingly, time spent lying was significantly less for cows under natural light throughout the day, except when cows supplemented with light were eating shortly before lights were turned out. Cows significantly increased walking time under natural light.

Behavioral and calving effects due to skeletal photoperiods were studied by Evans and Hacker (1989b) on dry cows comparing 12L:12D to 6L:4D:2L:12D, 6L:7D:2L:9D, and 6L:10D:2L:6D photoperiods. All lights came on initially at 0500 h each day. In all lighting regimes, eating followed 0500 h feeding. A major bout of eating followed the 1430 h feeding in all but the 6L:10D:2L:6D photoperiod. Eating profiles of cows

in the 6L:7D:2L:9D photoperiod were unique with a peak of eating associated with the 2 h skeletal period. Evans and Hacker (1989b) suggested this light pulse had a synchronizing effect on eating behavior since such a peak was not seen in the other skeletal photoperiod treatments.

In addition, Evans and Hacker (1989b) found calving time was synchronized for all cows in the 6L:7D:2L:9D photoperiod, occurring between 1030 and 1500 h (most calved about 1400 h). In the other three light treatments, cows calved throughout the light and dark periods. Evans and Hacker (1989b) suggested the skeletal light pulse in the 6L:7D:2L:9D treatment (at 13 to 15 h after subjective dawn) coincided with and stimulated a photosensitive phase of a circadian rhythm involved in the timing of parturition.

Conclusion

Light is not only the essential energy source at the bottom of the food chain, but also the most important timing signal (Zeitgeber) for plants and animals (Boulos and Terman, 1980). Through the timing of light, both natural and artificial, circadian (daily), circanular (yearly), and other shorter and longer periods of cellular chemical change or organism behavior are organized repetitively to ultimately promote species preservation.

Man has learned to use artifical light to affect biological cycles of domestic animals and increase production of economically important traits. For dairy cattle this learning is still incomplete. Effects of dusk and dawn, previous lighting experience, and skeletal (short-time) periods remain unclear. However, it does appear that milk

production can be improved by extending light 13 to 15 h after subjective dawn each day if natural light is shorter.

To better understand how skeletal photoperiods might influence cow milk yield, body weight, hormone concentrations, and behavior, an experiment was conducted to compare a long continuous light with short dark photoperiod, to a shorter light plus a skeletal lighting period. By better understanding factors involved in improving milk production by light, it could be a simple and inexpensive method of increasing milk production.

FEEDING MANAGEMENT

Feeding Systems

Three primary feed management systems are used currently by dairy producers in the United States: 1) total mixed rations (**TMR**), 2) separately fed or layered forage and grain rations, and 3) intensive grazing plus grain rations (Coppock, 1977).

The concept behind a TMR is to make every bite nutritionally complete by mixing together all ration ingredients needed to meet specific nutritional requirements, then feeding adequate amounts (Villavicencio et al., 1968). A TMR offers the advantages of controlling the ratio of nutrients consumed and reducing feeding costs (Marshall and Voight, 1975). Drawbacks of total mixed rations include: insufficient mixing, leaving pockets of only one ingredient; over-mixing leaving forage particle size too small for optimal effectiveness; and potential to overfeed or underfeed cows whose nutritional requirements are outside the range the TMR is designed (McCoy, 1966).

Feeding ration ingredients separately or layered offers nutritional preciseness to animals individually fed. In addition, this method does not require an expensive mixer wagon for mixing. However, particularly when group fed, these separate or layered rations leave much of the ration balancing up to the individual cow who can choose one ingredient in preference to others. Also, rumen microbes may not receive necessary nutrients when various ingredients are fed throughout the day. With a TMR, vitamins, minerals, carbohydrates, and proteins are available to microbes with each bite (Coppock, 1977).

Lately there has been a resurgence of grazing (particularly rotational grazing) as a way to reduce feed expense by allowing cows to harvest their own forage. Depending on grass quality and nutritional needs of grazing cattle, supplemental grain, and possibly hay or haylage are often fed. This system generally does not maximize production, but advocates of grazing claim reduced herd health problems, lower culling rates, and less labor (N. Nickerson, personal communication).

Challenge feeders, so-called because they permit cows to eat grain in amounts individually needed for production, have also become popular in the past 12 yr. Magnets placed around cows' necks triggered the original challenge feeder models to slowly deliver grain as long as the magnet activated the feeder (Hutjens, 1976). More recent challenge feeder versions used electronic transponders which signaled grain release. Advantage of the transponder was its connection to a computer. Not only could grain be metered precisely to meet individual cow daily nutritional needs, but by using the

computerized transponder the total amount fed each day could be metered out periodically throughout the day.

When cows with varying nutritional needs are grouped together, challenge feeders offer a viable method to allow higher producing cows the adequate protein needed without overfeeding lower producing cows. Challenge feeders have been used also by many dairy producers to eliminate grain feeding in the parlor. Research by Little and Harrison (1987) indicated that body condition score and serum total protein were both significantly better for cows fed through a challenge feeder out-of-parlor compared to cows fed grain in the milking parlor.

Nutrients for Rumen Microbes

Mammals, including ruminants, do not secrete cellulase, an enzyme for breaking the β-configuration of the 1-4 glucosidic linkages of cellulose (Hungate, 1966). However, because of the symbiotic relationship between ruminants and anaerobic bacteria (who do secrete cellulase) in the ruminoreticulum, ruminants can utilize large amounts of cellulose, the most abundant plant compound (Hungate, 1966). When feeding a ruminant like a dairy cow, consideration should be given not just to the cow, but also rumen microbes which supply energy, amino acids, and vitamins.

Russell and Hespell (1981) described the rumen as an ideal anaerobic fermentation site. "In most ruminant species, the rumen is approximately one-seventh of the mass of the animal, is maintained at a relatively constant temperature (39° C), is buffered well by salivary secretions, and compared to many other microbial ecosystems is well supplied with nutrients. End products of fermentation (e.g. volatile fatty acids), which can be

toxic to microbial metabolism, are removed across the rumen wall. The microflora inhabiting the rumen is dense and contains approximately 10^{10} to 10^{11} bacterial and 10^{6} protozoal cells per ml. Diversity within this population is extensive, and approximately 200 species of bacteria and 20 species of protozoa have been isolated."

Plants are composed primarily of carbohydrate polymers, cellulose, starch, pectin, hemicellulose, and xylan (Russell and Hespell, 1981). These are hydrolyzed by enzymes produced by rumen bacteria into small saccharides (cellobiose, maltose, sucrose, xylobiose, hexoses, and pentoses). Small saccharides are fermented into terminal products (acetate, butyrate, propionate, carbon dioxide, hydrogen gas, and methane). Ratios of terminal products vary with diet and frequency of feeding which affect microbial metabolism and species composition (Russell and Hespell, 1981).

Proteins are also needed and degraded by rumen microbes into ammonia, carbon dioxide, and volatile fatty acids. Ammonia is known to be a major nitrogen source for bacterial growth. However, peptides and amino acids are also particularly important in low quality diets (high fiber, low protein) where up to 40% of the bacterial nitrogen does not come from ammonia (Nolan and Stachiw, 1979). Hespell and Bryant (1979) hypothesized insufficient ammonia, peptides, and amino acids at certain times after feeding may cause energetic uncoupling which results in continued production of fermentation products without concomitant bacterial growth.

Chemical, physical, and structural properties of feedstuffs are important factors affecting rumen degradation. Extracellular enzymes degrade feedstuff polymers before utilization by rumen bacteria. Smaller particle size leads to a greater surface to mass

ratio which generally enhances bacterial fermentation rate. However, small particles can pass through the rumen undigested. If passage rate exceeds the increase in fermentation rate, overall rumen fermentation will be reduced (Waldo et al., 1972).

Plant polymer solubility also affects rumen fermentation rates. Starch granules of most plants are resistant to bacterial hydrolases because of their inherent insolubility. Therefore, starches tend to have rather slow fermentation rates and high passage rates (Topps et al., 1968). Cellulose and hemicellulose fractions are also relatively insoluble and slowly degraded in the rumen (Van Soest, 1973). Lignin in forage fibers is in close association with cellulosic materials. Lignin protects about 1.4 times its own mass in cell wall carbohydrates from microbial cellulase digestion (Van Soest, 1981).

Ruminal protozoa also play a major role in conversion of feedstuffs to readily metabolizable compounds. Because of their larger cell volumes, protozoa are less metabolically active on a cell mass basis than bacteria. However, as fermentative anaerobes, protozoa produce acetate, butyrate, lactate, carbon dioxide, and hydrogen (Russell and Hespell, 1981).

Russsell and Hespell (1981) further attribute protozoa to sequestering carbohydrates from rapid bacterial attack by engulfment of starch grains and other particulate carbohydrates. Without this sequestering, far more carbohydrates would ferment rapidly to lactate, and a lower ruminal pH would result. Net result would be detrimental to overall rumen function. Particulate protein may also be engulfed, permitting extended proteolysis, slower release of products, and less catabolism of amino acids and peptides to volatile fatty acids (Russell and Heskell, 1981).

Fungi are also present in the rumen at numbers similar to protozoa (1×10^5) (Sniffin and Robinson, 1987). Fungi most likely compete for substrate with fiber bacteria. However, they also provide synergistic action by fracturing fibrous material (Sniffin and Robinson, 1987).

Maximum growth rates of bacteria determine microbial success in the rumen. Large variations in maximum growth rates occur between species depending on energy source and rumen pH (Russell and Hespell, 1981). Russell and Hespell (1981) noted, "Because bacteria grow exponentially it is impossible for them to maintain high rates of growth for extended time. *Streptococcus bovis* is able to achieve a doubling time of 14 min. At such a growth rate, one *S. bovis* cell with a volume of approximately 1.2×10^{-13} cm^3 would be able to fill completely a 60 l rumen in less than 14 h and would equal the mass of the earth in approximately 34 h!"

Solubility of feedstuff substrates plays a major role in controlling bacterial growth in the rumen. Rumen bacterial species preferentially utilize some substrates to the exclusion of others (e.g. ammonia instead of amino acids) (Russell and Hespell, 1981). Russell and Hespell (1981) suggested that differences in substrate affinities and preference patterns may indicate that rumen bacteria have evolved different strategies of growth and these physiological factors may affect competition among rumen bacteria. Other researchers (Bryant and Robinson, 1968) noted feeding alfalfa chopped, ground, pelleted, or ensiled made a difference in numbers of bacteria, estimated by colony counts from rumen samples collected in different positions in the rumen of four heifers fed at 12 h intervals.

Similarly, Moir and Somers (1957) investigated effects of rate and method of feeding in sheep. A daily ration given once a day resulted in greater fall-off in bacterial counts and pH, lower dry matter digestibility and nitrogen retention, and higher ruminal nitrogen levels than the same ration: 1) divided into four equal portions fed at 2 h intervals; or 2) with concentrate fed in the morning and roughage in the afternoon; or 3) roughage fed in the morning and concentrates in the afternoon, or 4) half the ration morning and afternoon. Best performance was seen when sheep were fed concentrate in the morning and roughage in the afternoon, or the whole ration in four equal portions.

Low rumen pH, which occurs when fermentation acids sometimes exceed the buffering capacity of bicarbonate, phosphate, and proteins, decreases growth of most rumen bacteria (Russell et al., 1979). Major exceptions are *S. bovis* and lactobacilli which not only thrive in a low pH environment, but produce more lactic acid which continues to lower rumen pH and ultimately may cause onset of rumen acidosis (Russell et al., 1979).

Bacterial cell death generally results from lack of nutrients during part of the feeding cycle which causes a decrease in potential growth capacity when nutrients are available at other times (Russell and Hespell, 1981). However, predation by protozoal species also is significant (Coleman and Sandford, 1979). Coleman and Sandford (1979) calculated rates of bacterial engulfment by protozoal species ranged from 130 to 21,200 bacteria/protozoan/h at bacterial densities of 10^9 cells/ml. Coleman and Sandford (1979) also determined that intracellular digestion rates of bacteria ranged from 345 to 1200 bacteria/protozoa/hour. Protozoa exhibit strong preferences for certain bacteria to engulf

and the pH of their surroundings (e.g. pH of 6.0 is optimal for *Entodinium*) (Coleman and Sanford, 1979).

Control of rumen microbes can be manipulated by chemical agents which modulate selected pathways of microbial metabolism, by controlling rumen dilution rate, and by regulating ruminal pH (Chalupa, 1977). Production of propionate from hexoses utilizing metabolic hydrogen was 31% more efficient than production of butyrate and 47% more efficient than production of acetate (Chalupa, 1977). Therefore, metabolically useful energy recovered in fermentation and useful end-products of fermentation can be increased by enhancing production of propionate versus butyrate or acetate. Chemical agents known to enhance production of propionate include monensin sodium, methane inhibitors such as halomethane analogs, and deaminase inhibitors such as diaryl iodonium (Chalupa, 1977).

Rumen dilution rate can be altered to accelerate liquid passage particularly through the rumen by inclusion of mineral salts. However, the advantage of acceleration is still questionable since propionic acid production decreased (Rodgers, et al., 1979). Types of bacteria present, growth rate, and efficiency are all known to change (Hungate, 1966) without alterations of pH, probably because of faster removal from the rumen of substances and microbes contained in the rumen fluid (Chalupa, 1977).

Feed Intake Factors

Body condition scoring has recently become the vogue method of dairy cattle appraisal. A keen eye and feel for too much or too little flesh (fat) on cows in various stages of lactation will quickly discern that cows do not innately balance dietary needs.

Many factors interplay in feed intake and feeding behavior can be influenced by a variety of external and internal factors. Sensory cues such as palatability of a feed can enhance or detract from its acceptance (Baile and Della-Fera, 1981). Dehydration inhibited ruminants from eating (Utley et al., 1970). Ruminal stasis caused by a fall in rumen fluid pH below 5.5 to 5.0 depressed feed intake (Baile and Forbes, 1974). In addition, changes in osmolarity of rumen fluid influenced feeding behavior (Blair-West and Brook, 1969). Rumen quantities of both acetate and propionate may also play major roles in control of meal size. Intraruminal injections of acetate and propionate depressed feed intake of cattle (Simkins et al., 1965).

Several mineral and vitamin deficiencies decrease feed intake of ruminants (Baile and Forbes, 1974). Diets deficient in calcium, manganese, potassium, phosphorus, sodium chloride, cobalt, copper, zinc, vitamin A, vitamin D, riboflavin, and vitamin B_{12} are all known to lower ruminant feed intake (Baile and Forbes, 1974). Excess amounts of arsenic, flouride, molybdenum, selenium, and zinc also cause decreased feed intake (Baile and Forbes, 1974).

Baile and Forbes (1974) calculated on average a 48% increase in total weight of digesta in the rumen and a 96% increase in weight of dry matter are ruminal distension cut-off points for cattle eating. However, providing fresh, palatable feed at the tail-end of a meal will cause a renewed bout of eating.

Metabolic diseases, e.g., pregnancy toxemia (Blaxter, 1957), D-lactic acidosis (Uhart and Carroll, 1967), and ketosis (Krebs, 1966) quickly reduce feed intake.

Likewise, most gastrointestinal disorders caused by infections or parasites, as well as many systemic diseases, decrease feed intake (Baile and Forbes, 1974).

Type of ration does not change diurnal feeding pattern, but definitely affects total time spent eating. Putnam and Davis (1963) found steers fed a pelleted ration spent about 30% less time feeding than when fed a coarsely ground ration.

Other factors are known to stimulate eating to balance energy needs. As energy requirements increase during exposure to low environmental temperatures, cows will increase energy intake. Conversely, when heat stressed, cows will radically decrease feed intake (Ragsdale et al., 1950). Exercise also requires greater energy for maintenance and stimulates increased eating. Grazing cattle require 20% to 70% more energy for maintenance than stall-fed animals (Huffman, 1959).

Energy expenditure of high producing cows during lactation rises to three times the maintenance requirement. In early lactation as milk yields increase rapidly, feed intake does not keep up with energy needs and weight loss normally occurs. However, over several months of lactation cows adjust energy intake to energy demands as feed intake remains high and milk production declines (Baile and Forbes, 1974). If fed ad libitum quantities of a high energy ration, dry cows will often in excess of their nutritional needs and develop dry cow syndrome. Careful monitoring of body condition scores throughout lactation and the dry period can help alleviate nutritionally caused metabolic disorders.

Feeding Frequency

In 1952, Gordon and Tribe published the first indication of a positive correlation between frequency of feeding and production traits in ruminants. Using young, growing sheep Gordon and Tribe (1952) showed a 65.8% increase in body weight attributed to feeding eight times a day instead of one. Since then, many studies on the effect of feeding frequency have been conducted using lactating cows. Gibson (1984) summarized results from 23 publications reporting 35 experiments. Milk yield increased significantly (statistically) in four experiments, was not significantly affected in 24 experiments, and decreased significantly in one experiment as a response to increased frequency of feeding. A 2.7 % proportional increase in milk yield over all reported experiments was calculated, which was significantly different than zero (Gibson, 1984).

Gibson (1984) also found seven experiments that reported significantly increased milk fat percentage when cows were fed more times per day. No significance was reported in 27 experiments. The unweighted mean proportional increase across all experiments for milk fat concentration was a significant 7.3%. Gibson (1984) noted all statistically significant responses to increased feeding frequency occurred when milk fat concentrations were depressed originally (generally due to feeding pelleted or highly concentrated diets). Milk fat concentration increases due to increased feeding frequency were generally insufficient to bring the milk fat concentration up to a commercially acceptable level. Gibson (1984) concluded cows producing commercially acceptable milk fat concentrations probably would not benefit from increased feeding frequency. Indeed, even with the added stress of daily bovine somatotropin injections, French et al. (1990)

found increasing feeding frequency of concentrates from 2 to 12 times per day (with chopped alfalfa hay and barley silage fed twice a day ad libitum) did not influence milk yield, fat, protein, or lactose.

Studies by Kaufmann (1976) and Satter and Baumgardt (1962) demonstrated a marked leveling of rumen pH throughout the day as feeding times increased to four or more times daily. Twice a day feeding resulted in comparatively high peaks and low valleys in rumen pH throughout the day. Kauffman (1976) noted higher feeding frequencies, particularly of concentrates, resulted in less decrease in ruminal pH. Rumen pH is important to the ratio of acetic acid (C_2) to propionic acid (C_3) produced by rumen bacteria. When a higher frequency (4 times or more) of feeding occurred, the acetic to propionic acid ratio was over 3:1 throughout the day. Twice a day feeding resulted in a ratio lower than 3:1 which decreased milk fat content. Milk fat content for 20 cows averaged above 4% with high feeding frequency and only 3.6% when cows were fed twice a day (Kauffman, 1976).

Water Intake

Total body water ranges from 56% (for fat, dry cows) to 81% (for lactating cows) of body weight in dairy cattle which underscores the need for this nutrient (Murphy, 1992). Water for cattle comes from three primary sources: drinking, feed, and metabolic (oxidation) water. Water is lost in milk, urine, feces, and various forms of evaporation (Murphy, 1992). Sufficient water to meet all physiological needs must be provided to maximize milk production.

Factors known to influence drinking behavior include: eating pattern, water temperature, whether water is given in a water bowl or trough, flow rates into water bowls, animal dominance if water bowls are shared, stray voltage, temperature, humidity, dry matter intake, nature of the diet, and milk production (Murphy, 1992).

Early studies in cattle concluded that water intake was a function of dry matter consumption and ambient temperature (Winchester and Morris, 1956). Castle and Thomas (1975) calculated an average daily intake of drinking water of 49.9 (range 20.1 to 87.1) kg/cow for cows yielding an average of 16.8 kg milk/d at 8.2° C and 84.8% relative humidity. Since then, Murphy et al. (1983) through stepwise multiple linear regression calculated the following equation to estimate water intake (R^2 = .59):

Free water intake, kg/d = 15.99 + 1.58 x dry matter intake, kg/d + .90 x milk production, kg/d + .05 x sodium intake, g/d + 1.20 x minimum temperature, °C.

Holter and Urban (1992) predicted free water intake using Julian days rather than ambient temperature. Dietary dry matter content again played a significant role in water intake. Holter and Urban (1992) calculated (R^2 = .69): Free water intake, l/d = -32.39 + 2.47 x dry matter intake, kg/d + .6007 x milk, kg/d + .6205 x dietary DM, % + .0911 x Julian Days - .000257 x Julian Days^2.

Eating Behavior

A long, mobile tongue conveys forage into a cow's mouth. Feeds of smaller particle size are manipulated into the mouth by relatively immobile lips (Beauchemin, 1991). Lower incisors apply pressure via lateral movements of the jaw on a tough upper dental pad to chew, since ruminants do not have upper incisors. Fibrous material is

ground rather than cut and reduced to a swallowable size. Each day, 30,000 to 50,000 chews shear, crush and fragment forage to expose internal plant structures to microbial attack (Beauchemin, 1991). Cows have been noted to eat hay steadily at a mean rate of 75-81 jaw movements/min (Balch, 1958). Large volumes of saliva are secreted (over 300 l/d) aiding in bolus formation and swallowing. Chewing during eating varies in time and pauses during a meal are erratic (Beauchemin, 1991).

Rate of eating by dairy cows is affected by several factors including: whether or not cows are lactating (dry cows eat slower) (Journet and Remond, 1976); age of cow (older cows eat faster) (Burt, 1957); type of feed consumed (legume leaves are consumed faster than stems; eating rate is negatively correlated with fiber content measured as neutral detergent fiber) (McLeod and Smith, 1989); moisture content of feed (addition of water to concentrates increases eating rate) (Suzuki et al., 1969); and feed particle size (increasing particle size of silage decreases eating rate) (Campling and Balch, 1961).

Rumination is a cyclical process of regurgitation, remastication, and reswallowing. Chewing during rumination is usually slower (50 to 55 chews/min) than during eating. Several factors inhibit rumination including low pH, high osmotic pressure, and high volatile fatty acid concentrations of rumen fluid. Diets high in grains and finely processed feeds tend to inhibit rumination (Beauchemin, 1991). Rate of regurgitation of boli in sheep was more rapid when feeding was frequent (Gordon, 1961). This is possibly because of a general increase of motility of the foregut, of secretion, and digestion (Gordon, 1961).

Hashbarger (1949) observed average eating rates for dairy cows ranged from 4.4 to 6.6 min/kg for grain, 3.85 to 6.1 min/kg for silage, and 15.4 to 35.2 min/kg for hay. Rate of eating was generally fastest for Holsteins, slowest for Jerseys, with Ayrshires, Brown Swiss, and Guernseys intermediate.

McLeod and Smith (1989) noted diets with a high content of indigestible fiber caused reduced intake if rumination was at its maximum. However, if the maximum had not been reached, indigestible fiber increased rumination chews. McLeod and Smith (1989) concluded voluntary intake is not always reduced because of restrictions in either rumen fill or rumination when a cow is given a diet of high fiber content. Instead, ease with which the forage is eaten should be considered as an intake limiting factor.

Lactating cows observed by Webb et al. (1963) spent an average of 6.4 h/d eating silage and hay in dry lot conditions with grain fed ad libitum in the milking parlor. Friend and Polan (1977) found when bunk space was decreased from 0.41 to 0.1 m/cow, time eating a total mixed ration at the bunk decreased by > 1 h/d. Vasilatos and Wangness (1980) observed cows fed ad libitum and housed in individual stalls. Cows ate an average of 12 meals/d averaging 20.9 minutes each with 4.2 h/d of defined meal time. However, only 58% of the total defined meal time was actually spent eating. Holstein cows given an ad libitum choice between excellent corn silage and alfalfa hay expressed wide variation in dry matter (DM) intake (from 23.6% to 77.7% corn silage DM) (Coppock et al., 1974). Furthermore, there was no indication cows choosing the low protein forage (corn silage) would switch over time to eating more alfalfa to overcome protein deficiency.

Jones et al. (1966) found the primary factor affecting concentrate eating time was the quantity eaten. The larger the ration (between 0.91 and 3.6 kg) the faster the rate of eating with considerable cow to cow variation. Concentrates in cube form were eaten faster than meal.

Gonyou and Strickin (1981) compared the eating behavior of beef cattle groups fed from a single stall in each pen, or a trough. Stall-fed cattle ate significantly faster (80.9 min/d compared to 119.1 min/d at the trough). Both groups ate approximately 10 kg of feed/d. Cattle fed from troughs did most of their eating during daylight hours with two peaks of eating activity at 0900 and 1900 h and a lesser peak at 0200 h. The single stalls were occupied extensively throughout the day and night except between 0400 to 0600 h. Feedlot cattle have been noted to have two major daily peaks of eating activity regardless of season: sunrise to mid-morning and afternoon to early evening hours (Ray and Roubicek, 1971).

Conclusion

Feeding management requires far more than simply putting a round bale of weathered hay out in the pasture and letting cows eat as much grain in the parlor as possible if production is to be maximized with feed costs minimized. Understanding not just the cow, but the voracious army of bacteria, protozoa, and fungi in the rumen, is but a beginning of comprehending the complexity of dairy nutrition.

Conventionally feeding hay in a hay mow, silage in a silage trough, and grain from a computer feeder gives cows considerable latitude in ration balancing. Would changing feeding frequency of ration components alter feeding patterns, consumption,

and ultimately milk yield? How can cows be fed to maximize dry matter intake? In an effort to let cows answer such questions, an experiment was conducted to observe feeding behavior under several different feeding regimes to determine if feeding frequency or order in which ration components were fed influenced intake.

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PART II.

EFFECTS OF PHOTOPERIOD ON MILK YIELD, BODY WEIGHT, HORMONE CONCENTRATIONS AND BEHAVIOR OF LACTATING DAIRY COWS

ABSTRACT

Forty mid-lactation Holstein cows were divided into two groups and housed in identical, light-controlled, stanchion barns. During the 3 wk pretreatment period, incandescent lights (providing 100 lx 1 m above the floor) were on from 0300 to 2100 h in both barns. Treatments consisted of lights on from 0300 to 2100 h in one barn and 0700 to 1700 h with a skeletal light period between 0400 and 0500 h in the other barn for 14 wk. Milk yield; body weight; serum prolactin, cortisol, and triiodothyronine concentrations; and duration and frequency of eating and lying down, and frequency of drinks were not significantly different between the two treatments. Results suggest savings in utility costs could be attained by using a skeletal light period to replace a long continuous light period without decreasing milk production or eating time.

INTRODUCTION

With the arrival of rural electricity in the 1930s and 1940s, those involved in cattle production could conveniently manipulate a new factor in livestock environment: photoperiod. For dairy producers, the convenience of turning on an electric light to milk, feed, breed, and do other management necessities has been taken for granted for many years. However, only in the past 20 yr has it been recognized photoperiod affects bovine plasma prolactin levels (Bourne and Tucker, 1975) and can positively affect milk yield (Peters et al., 1978). More recently, Evans and Hacker (1989b) demonstrated photoperiod can affect timing of parturition.

Several experiments (Peters et al., 1978; Peters et al., 1981; and Stanisiewski et al., 1985) demonstrated that cows exposed to supplemental light during short natural

winter days produced up to 10% more milk than cows exposed to natural light. Others (Tanida et al., 1984; and Marcek and Swanson, 1984) have compared exposing cows to 24 h light versus 18 h light and found no significant difference in milk yield. Using two light periods each day, Evans and Hacker (1989a) theorized that somewhere between 13 and 15 h after subjective dawn light was necessary to elicit a photoperiod response in milk production.

Objective of the following experiment was to investigate effects of two photoperiods, one with an 18 h light period and the other with a 10 h light period, plus, a later one h skeletal (short) light period, on milk yield, body weight, hormone concentrations, and behavioral traits.

MATERIALS AND METHODS

Animals

Forty Holstein cows from The University of Tennessee Cherokee Dairy Research herd were divided into two groups balanced for current milk production, lactation number, body weight and sire. Cows had calved between 2 and 4.5 mo prior to the beginning of pretreatment period and were milked twice daily at 0400 and 1600 h. Cows were fed a mixed corn silage and concentrate ration at 0700, 1100, and 1600 h, plus 2.72 kg alfalfa hay after the 0700 and 1600 h feedings. Feed quantities met or exceeded National Research Council (1988) requirements based on lactational performance. Cows received exercise for 1 to 2 h every morning during the light period.

Housing

Each group of 20 cows was housed in an identical, light-controlled, stanchion barn. All windows, gutters, and fans were covered to eliminate outdoor light. Experimental cows had previously been housed in free stalls with a large, well-lit (day and night) outdoor lot. The experiment began in February when natural light occurred 10 to 11 h each day. The barns were lit by 32 incandescent 100-watt bulbs which produced approximately 100 lux **(lx)**, as measured by photometer, at 1 m from the floor throughout the barns. One hundred lx was chosen for this experiment to give cows adequate light to see (Dannenmann et al., 1985) and for ease of management and behavioral photography.

Treatments

During the 3 wk pretreatment period, all cows were exposed to lights on from 0300 to 2100 h and lights off between 2100 and 0300 h (18 h light: 6 h dark or **18L:6D**). Following the pretreatment period, Group 1 cows (continuous) stayed on the same 18L:6D daily schedule while Group 2 cows (skeletal) had lights on from 0700 to 1700 h and again from 0400 to 0500 h (10L:11D:1L:2D) for the next 14 wk. Lighting periods were chosen to contrast a long continuous light photoperiod to a shorter light period plus a skeletal (brief) light period which occurred when cows were milked in the morning.

Data Collection

Milk weights were recorded at each milking and averaged weekly. Body weights were averaged for 3 d during the second week of pretreatment period, and at weeks 7 and 14 during treatment.

Blood samples were taken from five, randomly selected, first lactation cows in each group during the third week of pretreatment period and at weeks 6 and 14 during treatment. Sampling weeks were chosen to collect early, mid- and late study data. An indwelling jugular canula was inserted two or more hours before blood sampling began. During dark periods, a pinpoint flashlight was used if necessary. Blood (15 ml) was collected hourly for 25 h from the 10 cows into Vacutainers (Becton Dickerson, Rutherford, NJ). Serum was separated at room temperature via centrifufation at 1520 x *g* and stored in one-ml Eppendorf tubes at -40° C until analysis.

Sera were analyzed for total T_3 (both free and bound triiodothyronine) using Coat-A-Count RIA (Diagnostic Products Corp., Los Angeles, CA), and total cortisol (hydrocortisone) using GammaCoat (^{125}I) Radioimmunoassay Kit (INCSTAR Corporation, Stillwater, MN). Prolactin radioimmunoassay was run using a radioiodination procedure described by Bolt and Rollins (1983) with purified and reference grades of PRL supplied by USDA, Beltsville, MD. (See Appendices A and B for detailed protocol of PRL radioimmunoassay.)

Five randomly selected multiparous cows from each group were videotaped for 72 h on three occasions, once during the pretreatment period and again on weeks 6 and 13 during the treatment period for behavioral data collection. Due to camera limitations, cows from both groups were not videotaped on the same days. Therefore, weighing and blood sampling had to occur on different weeks. When lights were on in the barns, five videocameras, one pointed at the head of each cow, were synchronized to record each cow for 4 sec in continuous succession. During dark periods, an infrared light was

spotlighted from above the rear of all five cows while an infrared camera rotated to record behavior. Various technical difficulties (such as light bulbs burning out, and swishing cow tails unplugging cameras) resulted in only 48 h of recorded data used for data analysis. Data obtained between 0400 and 0800 h were not included in the analysis because cows were milked and exercised during that time period. From the videotapes, frequency and duration of eating, standing, and lying down were recorded for each cow, along with the number of times each cow drank water.

Statistical Analyses

Analysis of covariance was performed on the weekly averaged milk weights. Covariates used were lactation number and pre-trial 305 d mature equivalent (ME) milk weights. Body weight mean difference was calculated between groups using lactation number, pre-trial body weights, and pre-trial 305 d ME milk weights as covariates. Model used for analysis of covariance was:

$$Y_{ijk} = \mu + T_i + C_j + \beta_1(x_{1ij}) + \beta_2(x_{2ij}) + E_{ijk}$$

where μ = overall mean,

T_i = fixed treatment effects due to photoperiod differences (i = 1 to 2),

C_j = covariate effect of lactation number (j = 1 to 5),

$\beta_1(x_{1ij})$ = effect of pre-trial ME milk weights,

$\beta_2(x_{2ij})$ = effect of pre-trial body weights*, and

E_{ijk} = residual.

* Factor not used in milk weight analysis

Cortisol, T_3, and PRL were analyzed by general linear models procedure of SAS (1985) without covariates. Ambient temperature and time of sampling were included as variables in one analysis of PRL. Behavioral data were also analyzed by general linear models procedure of SAS (1985). Least squares means were compared for duration and frequency of standing and eating, and frequency of drinking water. An F-test was used to test all variables for heterogeneity of slopes and all slopes were found to be homogeneous.

RESULTS AND DISCUSSION

Least squares means for weekly milk yield (kg/wk) at weeks 7 and 14 of the treatment period are presented in Table 2-1. Weekly milk yield means at treatment weeks 7 and 14, respectively, were 204.2 and 196.4 kg for Group 1 (continuous), and 210.6 and 202.7 kg for Group 2 (skeletal). Pretreatment 305 d ME milk and lactation number were both highly significant covariates ($P < 0.001$). However, photoperiod treatment was not significant indicating cows exposed to the skeletal lighting period produced equivalent amounts of milk to cows exposed to 18 hr continuous light.

Body weight means (adjusted for covariates) at weeks 5 and 14 during the photoperiod treatments (Table 2-2) were also not significant. Pretreatment body weight ($P < 0.001$), pre-trial ME milk yield ($P < 0.002$) and lactation number ($P < 0.027$) were included in the equation. Body weight least squares means at treatment weeks 5 and 14, respectively, were 604.2 and 640.5 kg for cows in Group 1, and 610.2 and 627.9 kg for cows in Group 2.

TABLE 2-1. Milk yield (kg/wk) least squares means at treatment weeks 7 and 14 corrected for pre-trial mature equivalent milk and lactation number (n = 20 per group).

	7 weeks	14 weeks
Group 1 (continuous)	204.2	196.4
Group 2 (skeletal)	210.6	202.7

TABLE 2-2. Body weight (kg) least squares means at treatment weeks 5 and 14 corrected by lactation number, pretreatment body weights, and pretreatment mature equivalent milk yield (n = 20 per group).

	5 weeks	14 weeks
Group 1 (continuous)	604.2	640.5
Group 2 (skeletal)	610.2	627.9

Mean cow serum cortisol concentrations (μg/dl) for the three blood sampling periods are found in Table 2-3. Average serum cortisol was 1.25 and 1.22 μg/dl for the two groups under the same pretreatment lighting. At treatment week 6, cows maintained under the 18 h light still averaged 1.25 μg/dl, while cows with the skeletal lighting period averaged 1.14 μg/dl. By treatment week 14, average serum cortisol was 0.78 and 0.79 μg/dl for the continuous and skeletal groups, respectively. Serum cortisol was not significantly different between groups. Shaw et al. (1960) found similar concentrations of 17-hydroxycorticosteroid in pregnant cows and discovered concentrations of 17-hydroxycorticosteroid generally decreased as gestation advanced during the first six months of pregnancy. In this study, during pretreatment only cows 14 and 21 were pregnant. By treatment week 6, all Group 1 cows (11-15) and three cows from Group 2 (21, 24, and 25) were pregnant. By week 14, cows 22 and 23 were finally bred. Although cortisol concentrations generally decreased in later lactation, no trends were obvious relating to reproductive status.

Mean cow serum T_3 concentrations for the 25 h sampling periods are reported in Table 2-4. Average pretreatment serum T_3 concentrations were 156.1 and 164.1 ng/dl, for Groups 1 and 2, respectively. At treatment week 6, for Groups 1 and 2, respectively, serum T_3 concentrations increased slightly to 165.6 and 166.8 ng/dl. Concentrations of serum T_3 decreased to 130.9 and 141.7 ng/dl for cows in Group 1 and 2, respectively, by treatment week 14. Nixon et al. (1988) found average free and total plasma T_3 concentrations were lowest in winter, highest in the fall, and intermediate in spring and summer. This study does not support findings of Nixon et al. (1988).

TABLE 2-3. Mean cow serum cortisol concentrations (μg/dl) over a 25 h period during pretreatment period and treatment weeks 6 and 14 (n = 5 per group).

Cow	Treatment	Pretreatment		Week 6		Week 14	
		Cortisol (μg/dl)	S.D.	Cortisol	S.D.	Cortisol	S.D.
11	Continuous light	1.42	(0.82)	1.35	(0.93)	0.78	(0.39)
12		1.34	(0.58)	1.34	(0.73)	1.05	(0.40)
13		1.22	(0.65)	1.09	(0.97)	0.62	(0.23)
14		1.13	(0.56)	1.08	(0.85)	0.73	(0.24)
15		1.11	(0.56)	1.46	(1.42)	0.74	(0.24)
$\bar{x}$		1.25	(0.63)	1.25	(1.00)	0.78	(0.43)
21	Skeletal light	0.96	(0.51)	1.16	(0.95)	0.80	(0.48)
22		1.15	(0.60)	1.21	(0.82)	0.81	(0.28)
23		1.16	(0.60)	0.99	(0.70)	0.69	(0.26)
24		1.53	(0.62)	1.43	(1.22)	0.80	(0.42)
25		1.32	(0.63)	1.02	(0.78)	0.87	(0.52)
$\bar{x}$		1.22	(0.62)	1.14	(0.89)	0.79	(0.40)

TABLE 2-4. Mean cow serum triiodothyronine (T_3) concentrations (ng/dl) over a 25-hr period during pretreatment period and treatment weeks 6 and 14 (n = 5 per group).

Cow	Treatment	Pretreatment		Week 6		Week 14	
		T_3 (ng/dl)	S.D.	T_3	S.D.	T_3	S.D.
11	Continuous light	145.8	(29.8)	149.1	(17.8)	136.7	(16.4)
12		158.5	(18.8)	151.7	(9.1)	144.5	(13.8)
13		157.2	(13.6)	168.7	(17.0)	127.7	(11.2)
14		156.1	(20.2)	169.8	(17.0)	118.5	(14.7)
15		162.8	(15.4)	189.6	(13.5)	127.3	(10.8)
$\bar{x}$		156.1	(6.3)	165.8	(16.3)	130.9	(10.0)
21	Skeletal light	150.0	(13.2)	161.7	(25.7)	140.6	(13.1)
22		147.8	(15.0)	146.0	(21.8)	128.5	(15.1)
23		164.6	(14.2)	160.3	(17.3)	132.9	(13.0)
24		161.7	(42.3)	150.1	(11.8)	132.8	(27.4)
25		209.3	(50.1)	221.0	(32.5)	174.6	(26.8)
$\bar{x}$		164.1	(23.1)	166.8	(27.3)	141.7	(16.8)

However, Premachandra et al. (1958) indicated T_4 secretion rates were reduced three-fold in the summer which might explain the low serum T_3 concentrations noted in this study during the third sampling period in early June.

Stage of lactation also has a great influence on serum thyroid hormones. Generally, concentrations of serum T_4 and T_3 increase later in lactation as production decreases (Hart et al., 1978; Blum et al., 1983; and Akasha et al., 1987). Week 6 and 14 mean hourly serum T_3 data found in Tables 2-5 and 2-6 does not show this trend. However, Trenkle (1978) and Thompson et al. (1963) noted secretion of thyroid hormones increased in cold and decreased in warm environments. In early June at the conclusion of this experiment, the ambient temperature averaged 23.5° C, (range 19 to 27° C on collection day) which could have influenced serum T_3 concentrations. Previous work (Refsal et al., 1980; and Bitman et al., 1984) indicated that T_3 concentrations were lower in the morning and higher in the afternoon. Generally, lowest serum T_3 concentrations occurred in the morning in this experiment. Higher values were scattered between noon and midnight.

Mean cow serum PRL concentrations for the 25 h sampling periods are reported in Table 2-7. With wide individual differences, cows exposed to continuous light averaged 32.7, 48.1, and 98.0 ng/ml serum PRL during pretreatment, and treatment weeks 6 and 14, respectively. Cows in the skeletal light treatment averaged 34.6, 35.9, and 80.9 ng/ml, respectively, during the same three collection periods.

Mean serum PRL concentrations by group over time are shown in Figures 2-1, 2-2, and 2-3. The frequent sporadic, but inconsistent, bursts of PRL concentrations

TABLE 2-5. Mean serum triiodothyronine (T_3) (ng/dl) by hour for 5 Group 1 (continuous) cows sampled during pretreatment and on treatment period weeks 6 and 14.

Blood sampling time	Pre-treatment		Week 6		Week 14	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
1300	145.9	(9.1)	171.3	(11.5)	150.0	(14.1)
1400	140.6	(12.5)	179.3	(14.6)	149.1	(24.6)
1500	160.7	(14.7)	164.8	(15.4)	144.3	(22.6)
1600	148.5	(12.4)	180.7	(31.8)	142.4	(14.8)
1700	143.5	(14.7)	172.9	(12.7)	142.8	(14.2)
1800	163.1	(30.1)	180.1	(11.3)	135.0	(9.8)
1900	150.3	(15.1)	164.3	(11.6)	135.8	(15.1)
2000	169.6	(34.8)	164.6	(21.4)	132.3	(14.7)
2100	156.8	(11.8)	163.5	(23.7)	129.9	(5.1)
2200	165.6	(17.9)	173.1	(25.9)	129.3	(13.5)
2300	157.5	(14.8)	156.1	(22.2)	121.0	(13.3)
2400	156.8	(15.4)	159.2	(25.1)	129.0	(22.0)
0100	163.0	(13.3)	166.7	(19.6)	130.5	(4.2)
0200	161.1	(6.6)	161.1	(13.7)	137.0	(14.6)
0300	176.0	(26.2)	170.5	(26.5)	130.7	(10.2)
0400	155.5	(18.8)	165.7	(19.1)	127.5	(9.3)
0500	-----	-----	163.7	(25.6)	121.8	(4.5)
0600	182.1	(51.1)	155.3	(19.3)	121.2	(9.2)
0700	144.1	(11.6)	164.8	(20.8)	120.3	(6.2)
0800	147.1	(10.9)	155.0	(17.6)	123.7	(18.9)
0900	148.1	(10.3)	132.2	(9.2)	116.4	(13.5)
1000	131.6	(15.9)	174.8	(28.2)	123.7	(22.9)
1100	156.1	(16.5)	159.7	(7.4)	131.0	(14.3)
1200	157.6	(8.6)	140.7	(13.6)	124.8	(22.3)
1300	153.3	(9.4)	178.3	(23.2)	122.8	(11.8)
Overall $\bar{x}$	156.1	(6.3)	165.8	(16.3)	130.9	(10.0)

TABLE 2-6. Mean serum triiodothyronine (T_3) (ng/dl) by hour for 5 Group 2 (skeletal) cows sampled during pretreatment and on treatment period weeks 6 and 14.

Blood sampling time	Pre-treatment		Week 6		Week 14	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
1300	153.3	(15.8)	200.2	(74.0)	170.4	(54.7)
1400	155.7	(15.4)	171.6	(30.6)	151.7	(19.0)
1500	165.7	(14.7)	162.5	(28.1)	151.6	(13.7)
1600	158.3	(13.1)	168.5	(27.1)	151.7	(20.3)
1700	154.8	(9.6)	171.6	(35.4)	147.0	(24.6)
1800	167.7	(17.7)	157.6	(40.6)	148.5	(21.1)
1900	169.9	(21.2)	161.3	(34.8)	145.9	(12.1)
2000	171.6	(39.6)	167.4	(31.7)	153.0	(13.7)
2100	175.7	(31.1)	186.1	(33.1)	144.7	(37.8)
2200	175.5	(16.7)	170.2	(26.3)	130.6	(16.3)
2300	207.5	(75.2)	158.8	(29.7)	129.5	(18.6)
2400	176.9	(25.3)	183.4	(40.0)	150.6	(26.3)
0100	168.1	(12.9)	160.7	(28.6)	137.0	(13.1)
0200	165.6	(28.6)	158.2	(31.7)	144.9	(16.6)
0300	168.3	(19.5)	164.6	(25.0)	139.9	(16.0)
0400	188.5	(47.3)	158.5	(35.2)	134.4	(18.1)
0500	207.9	(119.4)	161.5	(29.4)	127.1	(21.1)
0600	171.7	(25.7)	145.4	(24.0)	136.8	(35.4)
0700	160.3	(21.2)	145.5	(15.1)	149.7	(46.8)
0800	145.3	(20.6)	152.5	(27.7)	133.5	(49.6)
0900	138.2	(19.6)	158.4	(49.9)	118.7	(17.9)
1000	143.0	(30.1)	171.2	(36.4)	137.9	(21.5)
1100	158.3	(37.0)	175.6	(27.3)	132.2	(7.6)
1200	155.6	(39.3)	157.7	(33.4)	130.3	(21.1)
1300	160.0	(32.0)	204.8	(45.6)	142.7	(11.8)
Overall $\bar{x}$	164.1	(23.1)	166.8	(27.3)	141.7	(16.8)

TABLE 2-7. Mean cow serum prolactin (PRL) concentrations (ng/ml) over a 25-h period during pretreatment period and treatment weeks 6 and 14 (n = 5 per group).

Cow	Treatment	Pretreatment		Week 6		Week 14	
		PRL	S.D.	PRL	S.D.	PRL	S.D.
11	Continuous light	25.1	(17.5)	27.4	(9.5)	53.5	(27.9)
12		30.4	(17.1)	76.0	(31.1)	90.2	(41.7)
13		46.2	(54.0)	41.2	(36.6)	119.6	(57.1)
14		36.8	(32.9)	57.9	(36.2)	137.0	(80.9)
15		25.5	(20.1)	34.7	(26.5)	91.7	(54.2)
$\bar{x}$		32.7	(32.2)	48.1	(34.4)	98.0	(61.2)
21	Skeletal light	15.1	(17.7)	12.4	(8.6)	40.5	(21.3)
22		44.7	(24.2)	62.8	(39.3)	133.8	(71.8)
23		36.4	(25.2)	33.2	(15.6)	81.4	(59.6)
24		39.6	(21.7)	43.6	(71.6)	81.4	(19.9)
25		36.5	(29.6)	26.6	(21.3)	65.4	(28.5)
$\bar{x}$		34.6	(25.7)	35.9	(41.8)	80.9	(54.4)

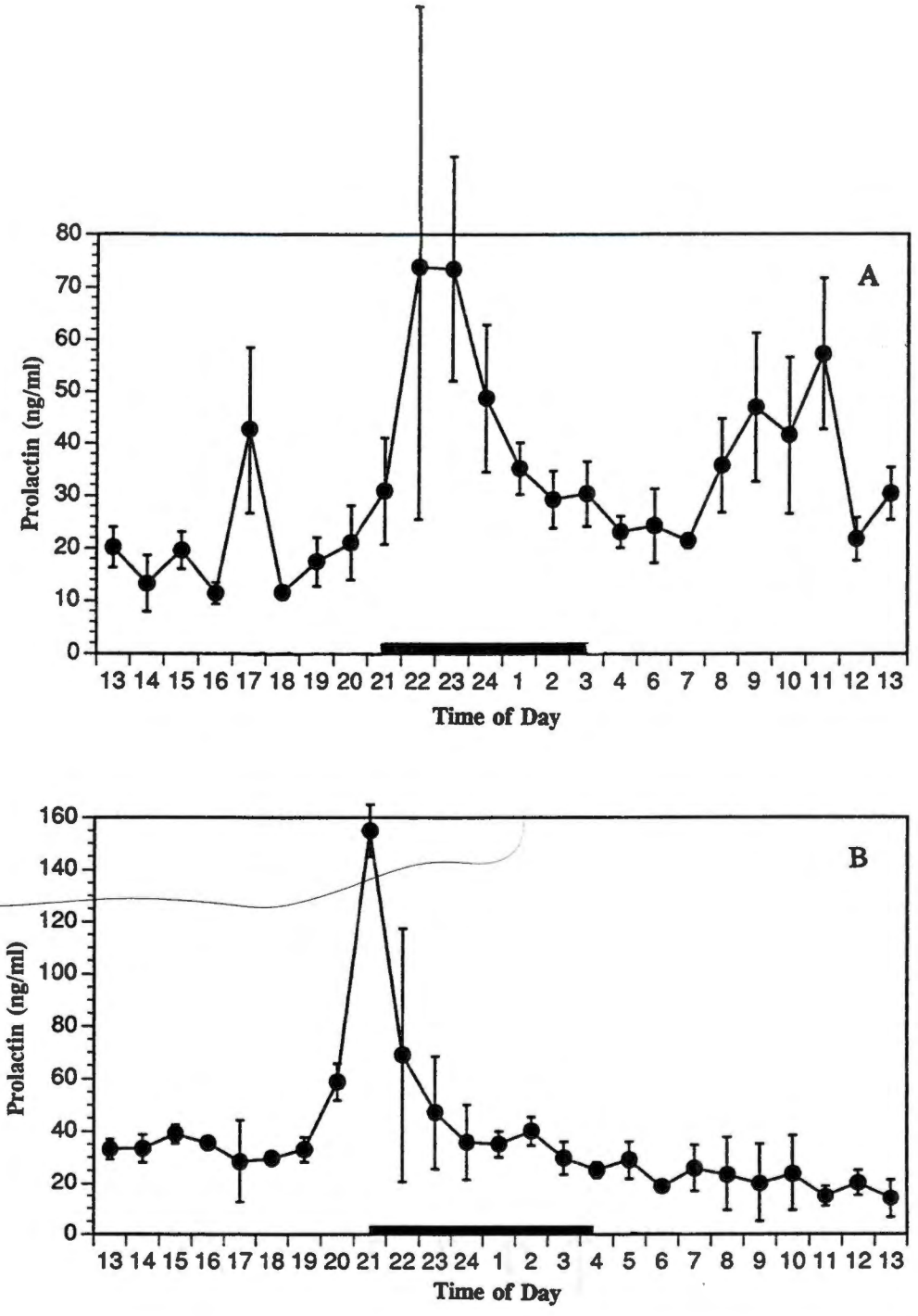


Figure 2-1. Group (continuous or skeletal) mean serum prolactin (PRL) concentrations by hour of blood sampling (n = 5 for both groups). (A) Pre-treatment mean serum PRL concentrations of Group 1 (continuous). (B) Pre-treatment mean serum PRL concentrations of Group 2 (skeletal). Vertical lines indicate standard error. Horizontal black lines over x-axis indicate dark time.

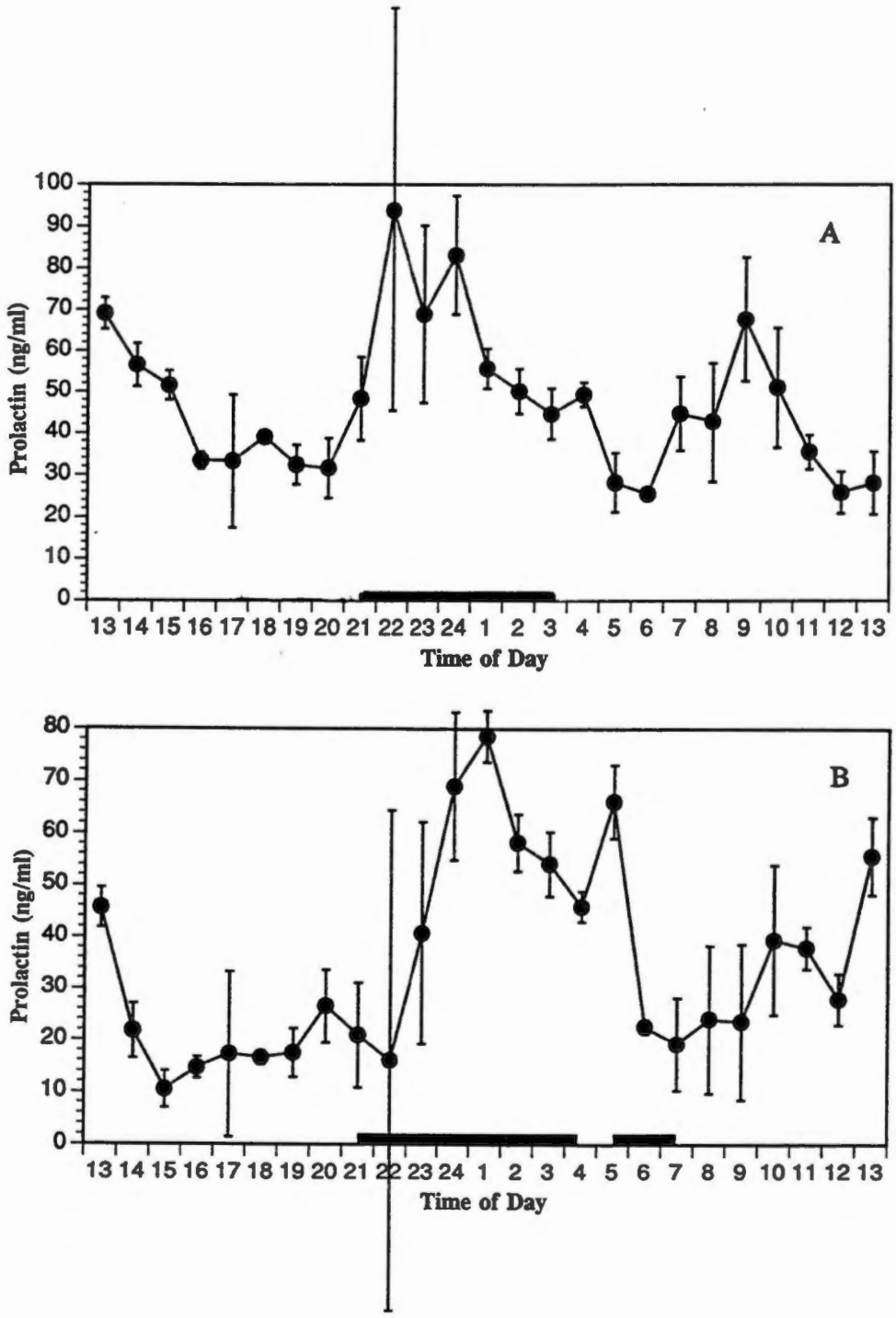
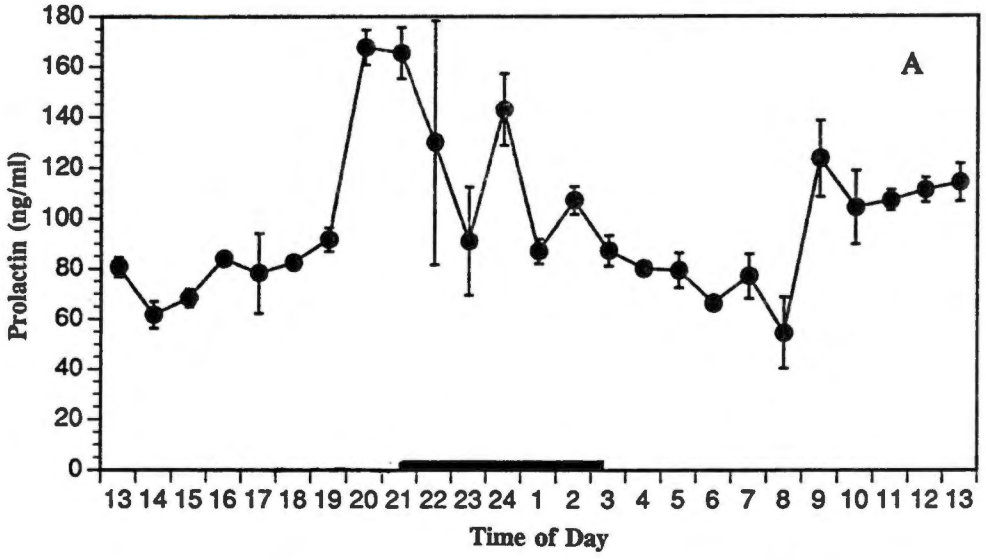


Figure 2-2. Group (continuous or skeletal) mean serum (PRL) concentrations by hour of blood sampling (n = 5 for both groups). (A) Week 6 mean serum PRL concentrations of Group 1 (continuous). (B) Week 6 mean serum PRL concentrations of Group 2 (skeletal). Vertical lines indicate standard error. Horizontal black lines over x-axis indicate dark time.



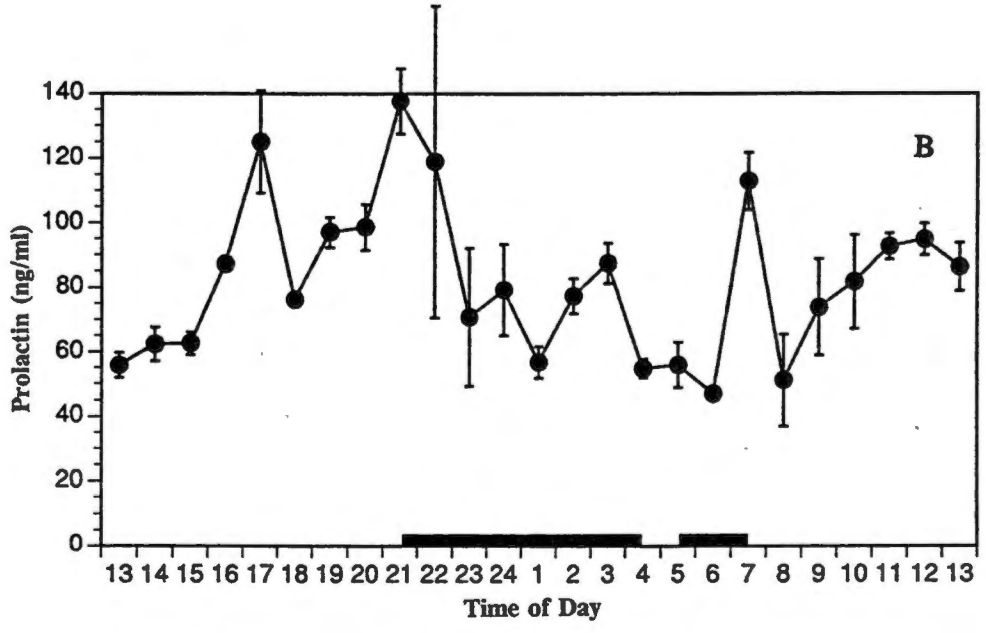


Figure 2-3. Group (continuous or skeletal) mean serum (PRL) concentrations by hour of blood sampling (n = 5 for both groups). (A) Week 14 mean serum PRL concentrations of Group 1 (continuous). (B) Week 14 mean serum PRL concentrations of Group 2 (skeletal). Vertical lines indicate standard error. Horizontal black lines over x-axis indicate dark time.

described by Fulkerson et al. (1980) would aptly describe results of the present study. Serum PRL concentrations are known to be affected directly by increasing ambient temperatures (Koprowski and Tucker, 1973; Wettemann and Tucker, 1976; and Wettemann et al., 1983). Temperature ($P < 0.0002$) and time of sampling ($P < 0.0099$) were significant when added to the statistical model used to analyze PRL data.

Stanisiewski et al. (1988) established that length of light exposure affects plasma prolactin in cattle. Bulls exposed to 16 h light had significantly greater plasma PRL levels than bulls exposed to 8 h light. Petitclerc et al. (1983) compared effects of two lighting periods: 6L:8D:2L:8D to 6L:14D:2L:2D and concluded that the concentration of prolactin in bulls given 6L:8D:2L:8D was greater ($P < 0.05$) than for bulls maintained on 6L:14D:2L:2D. It appears cattle possess a photosensitive rhythm for secretion of prolactin. This photosensitive rhythm was triggered equally well by cool-white fluorescent, Vita-Lite fluorescent, incandescent, high pressure sodium or mercury vapor lamps (Stanisiewski et al., 1984).

Behavioral observations made on five multiparous cows in each treatment group are summarized in Tables 2-8 and 2-9. Mean daily time spent eating and standing and the frequency of eating, drinking, and standing is reported by cow within group. No significant differences between behaviors in the two groups were detected. In this study, cows that received less light time tended to stand more. During week 6, Group 2 (skeletal) cows stood an average of 9.4 h/d, while Group 1 (continuous) cows stood 6.84 h/d. Phillips and Schofield (1989) also found cows who received more light stood less.

TABLE 2-8. Group 1 (continuous) individual cow 2-day averaged daily time spent eating and standing and frequency of eating, drinking, and standing up.

	Eating time (h)	Eating frequency	Drinking frequency	Standing time (h)	Standing frequency
Cow 11					
Pretreatment	5.39	14.5	19.5	9.78	10.5
Week 6	4.07	8.0	15.0	7.78	9.0
Week 13	4.60	9.5	17.5	7.31	11.0
Cow 12					
Pretreatment	5.30	9.5	12.5	6.34	8.5
Week 6	4.05	9.0	10.5	6.20	9.5
Week 13	4.33	7.5	14.5	6.52	13.5
Cow 13					
Pretreatment	7.25	14.5	22.0	8.08	6.5
Week 6	5.38	7.5	19.5	7.66	8.0
Week 13	4.74	12.5	23.0	8.51	7.5
Cow 14					
Pretreatment	6.10	17.5	18.0	11.67	9.0
Week 6	3.30	8.5	10.5	7.34	17.0
Week 13	3.88	9.0	12.0	7.28	21.5
Cow 15					
Pretreatment	5.60	14.5	22.0	7.48	14.5
Week 6	3.02	8.5	21.5	5.19	14.0
Week 13	4.26	10.0	14.0	6.90	10.5
Group 1 (5 cow avg.)					
Pretreatment	5.93	14.1	18.8	8.67	9.8
Week 6	3.96	8.3	15.4	6.84	11.5
Week 13	4.36	9.7	16.2	7.30	12.8

TABLE 2-9. Group 2 (skeletal) individual cow 2-day averaged daily time spent eating and standing and frequency of eating, drinking, and standing up.

	Eating time (h)	Eating frequency	Drinking frequency	Standing time (h)	Standing frequency
Cow 21					
Pre-treatment	5.55	10.0	11.0	10.46	9.5
Week 6	4.88	8.5	6.0	8.58	11.0
Week 13	4.59	8.5	6.0	8.77	8.0
Cow 22					
Pre-treatment	6.8	15.0	19.0	12.82	12.5
Week 6	4.48	9.5	14.0	9.15	11.5
Week 13	3.92	9.0	9.5	9.14	11.0
Cow 23					
Pre-treatment	6.3	16.0	24.0	9.55	10.5
Week 6	3.66	10.0	14.5	7.45	10.5
Week 13	4.74	12.5	23.0	8.51	7.5
Cow 24					
Pre-treatment	5.23	17.0	23.5	9.7	14.0
Week 6	4.73	9.5	13.0	8.4	14.0
Week 13	4.36	12.0	11.5	8.62	13.0
Cow 25					
Pre-treatment	5.32	15.5	23.5	11.48	7.0
Week 6	4.51	12.0	15.5	13.42	5.5
Week 13	4.26	9.0	14.5	9.64	5.5
Group 2 (5 cow average)					
Pre-treatment	5.85	14.7	20.2	10.80	10.7
Week 6	4.45	9.9	12.6	9.40	10.5
Week 13	3.85	9.5	10.1	8.79	9.2

Mean minutes spent eating each hour for the five cows in each group are presented in Figures 2-4, 2-5, and 2-6. Average daily eating time for Group 1 cows on week 13 was 262 min in 9.7 eating bouts. Group 2 cows ate an average of 231 min in 9.5 eating bouts. Tanida et al. (1984) determined similar overall means of total eating time were 270 to 280 min/d spent in 10 to 12 eating bouts.

Mean minutes standing each hour for the 5 cows in each group are shown in Figures 2-7, 2-8, and 2-9. Pretreatment lighting was the same for both groups. Although not significantly different, cows in Group 2 with the longer dark time stood an average of 2.5 h more per day on week 6 of the experiment than cows in Group 1. By week 13, cows under Group 1 lighting were standing and lying down an average of 2.6 more times per day than cows in Group 2. All cows were fed silage and concentrate rations at 0700, 1100, and 1600 h, with hay fed after the 0700 and 1100 feedings. A corresponding peak in eating followed these feedings, especially the 1600 feeding. By week 13, the highest eating peak for Group 2 cows was at 2000 h, right before dark time began at 2100 h. This peak was not nearly so noticeable at week 6. Lighting regimes of 24-h light versus 18L:6D have not been found to affect eating behavior or milk production (Tanida et al., 1984 and Marcek and Swanson, 1984).

Evans and Hacker (1989b) postulated that a skeletal lighting photoperiod was as effective as a long continuously lighted period if light was provided 13 to 15 h after subjective dawn. Within that 13 to 15 h window seems to be a photosensitive phase for a circadian rhythm which affects increased milk production (Evans and Hacker, 1989a; and Bilodeau et al., 1989); calving synchronization (Evans and Hacker, 1989b);

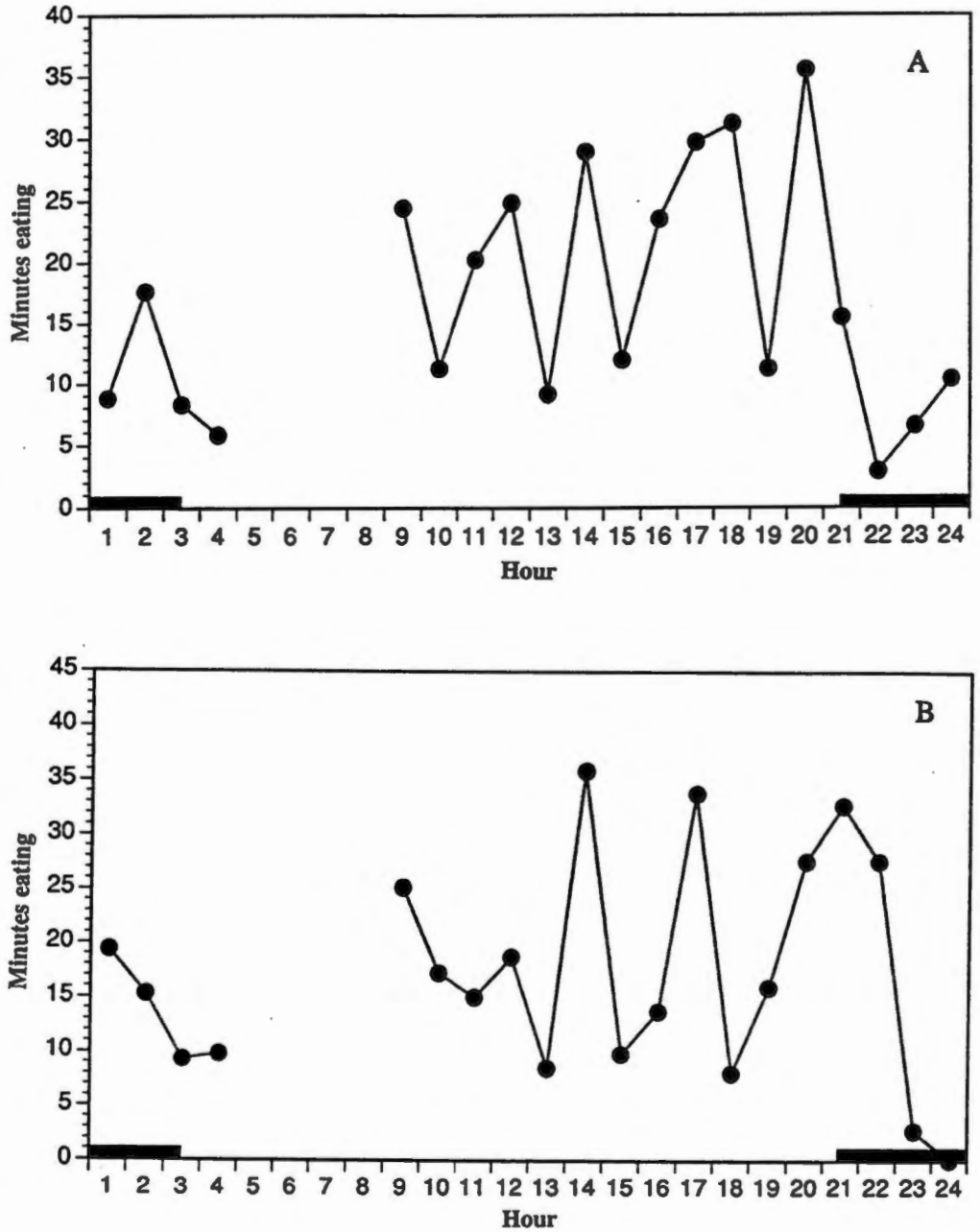
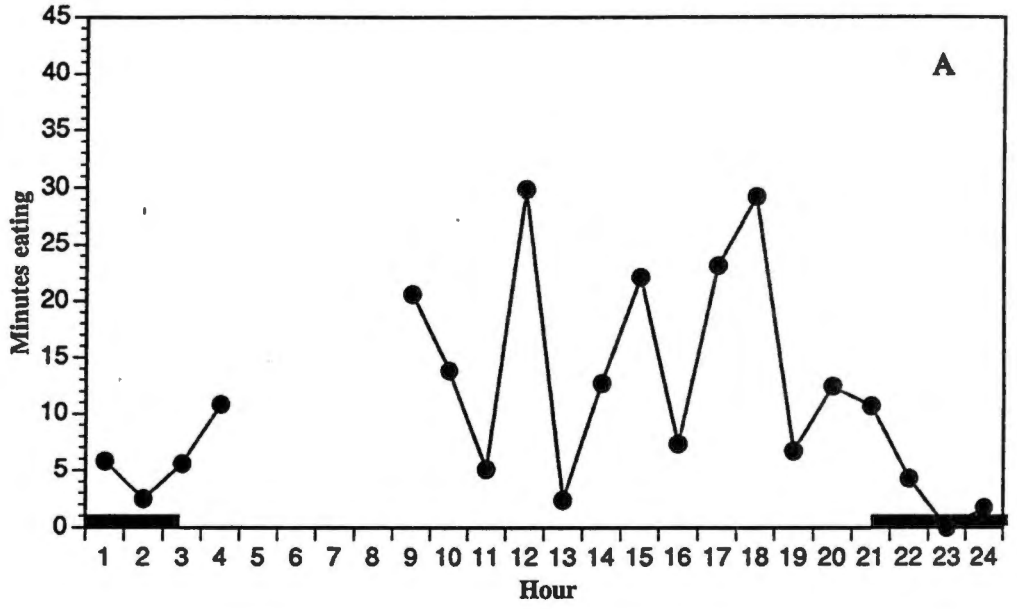


Figure 2-4. Group mean time (min) eating by hour. (A) Group 1 (continuous) during pre-treatment period. (B) Group 2 (skeletal) during pre-treatment period with same light exposure as Group 1. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.



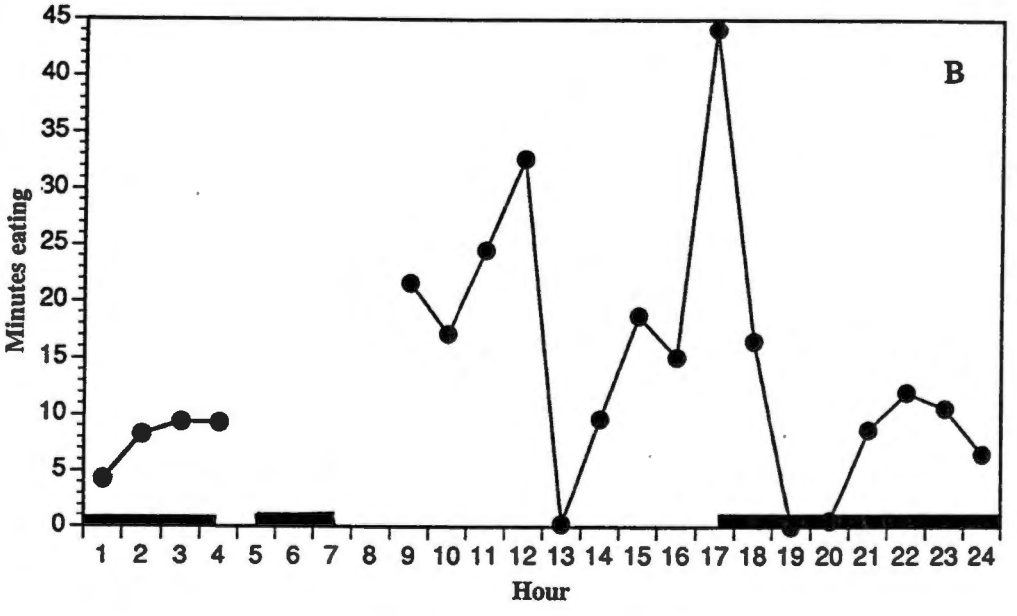


Figure 2-5. Group mean time (min) eating by hour. (A) Group 1 during treatment week 6. (B) Group 2 during treatment week 6. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.

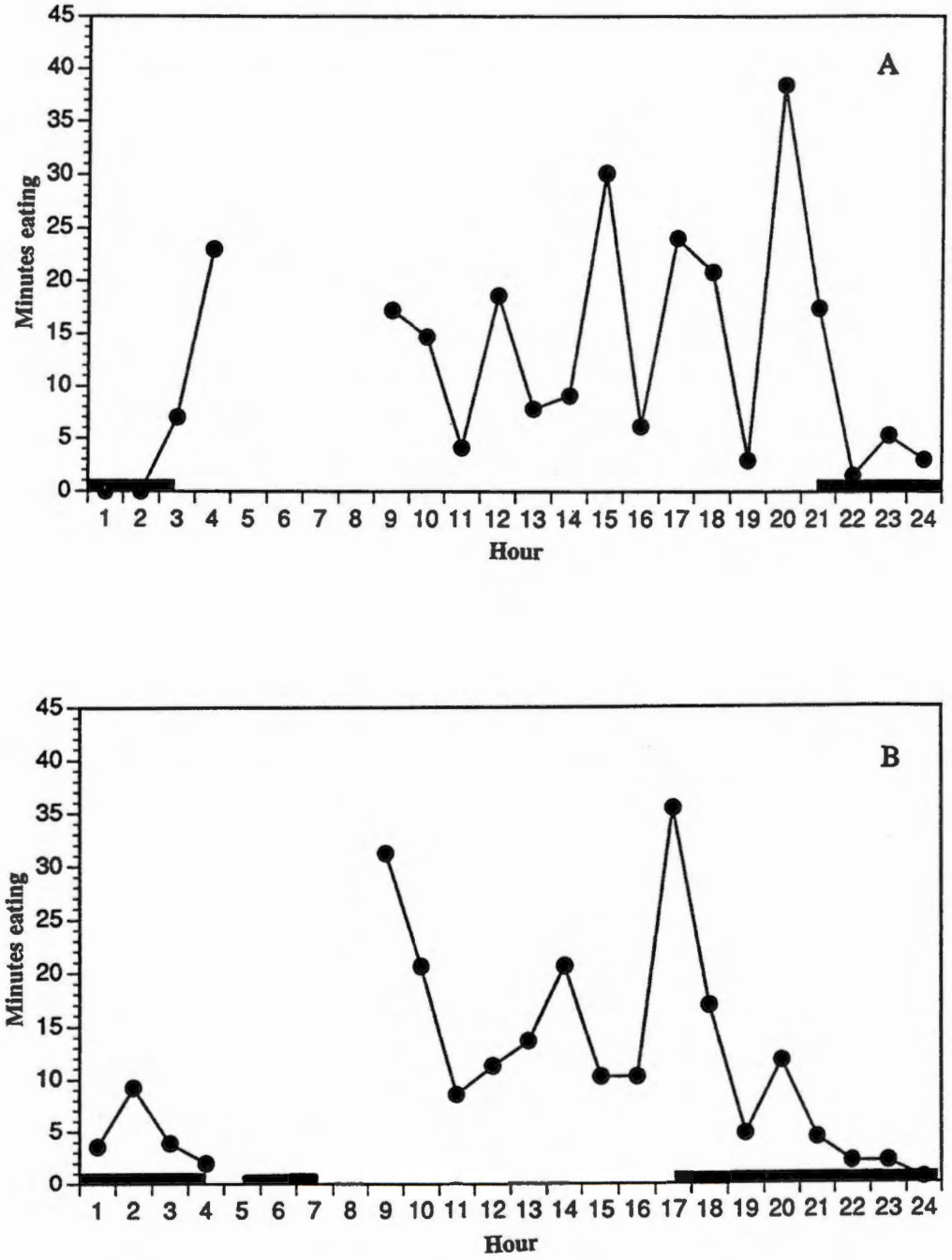
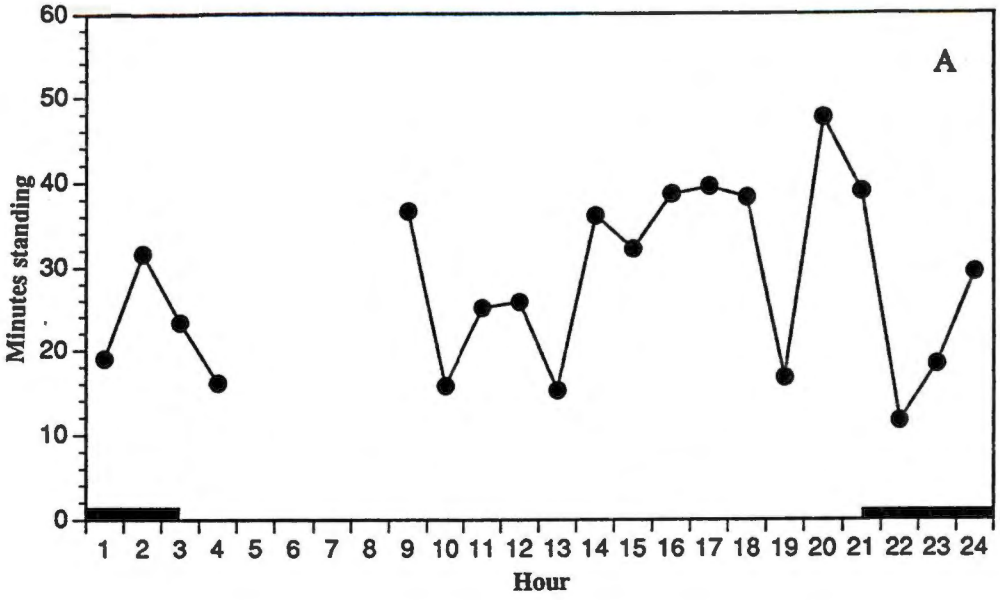


Figure 2-6. Group mean time (min) eating by hour. (A) Group 1 during treatment week 13. (B) Group 2 during treatment week 13. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.



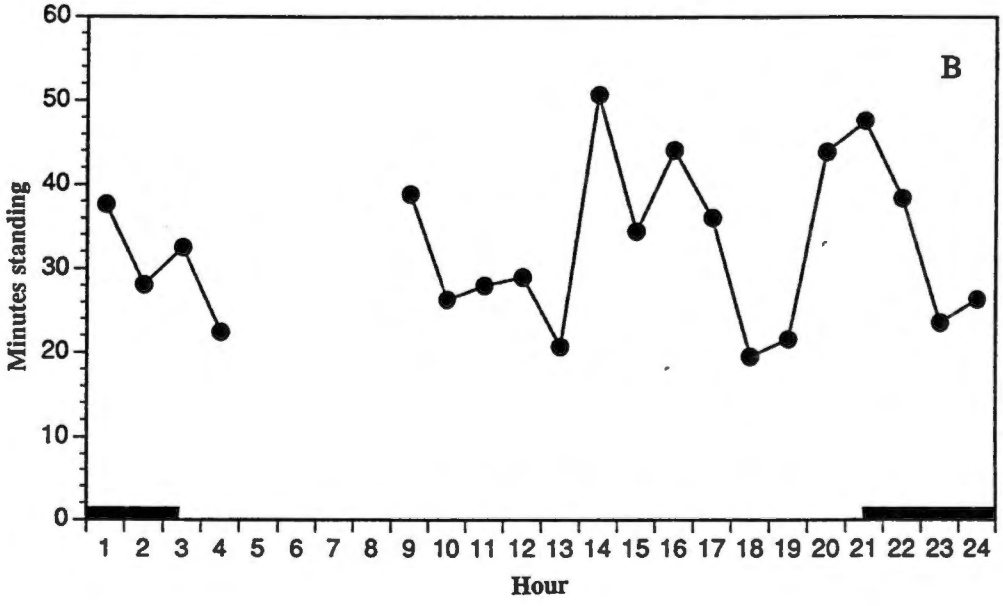


Figure 2-7. Group mean time (min) of standing by hour. (A) Group 1 (continuous) during pre-treatment period. (B) Group 2 (skeletal) during pre-treatment period with same light exposure as Group 1. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.

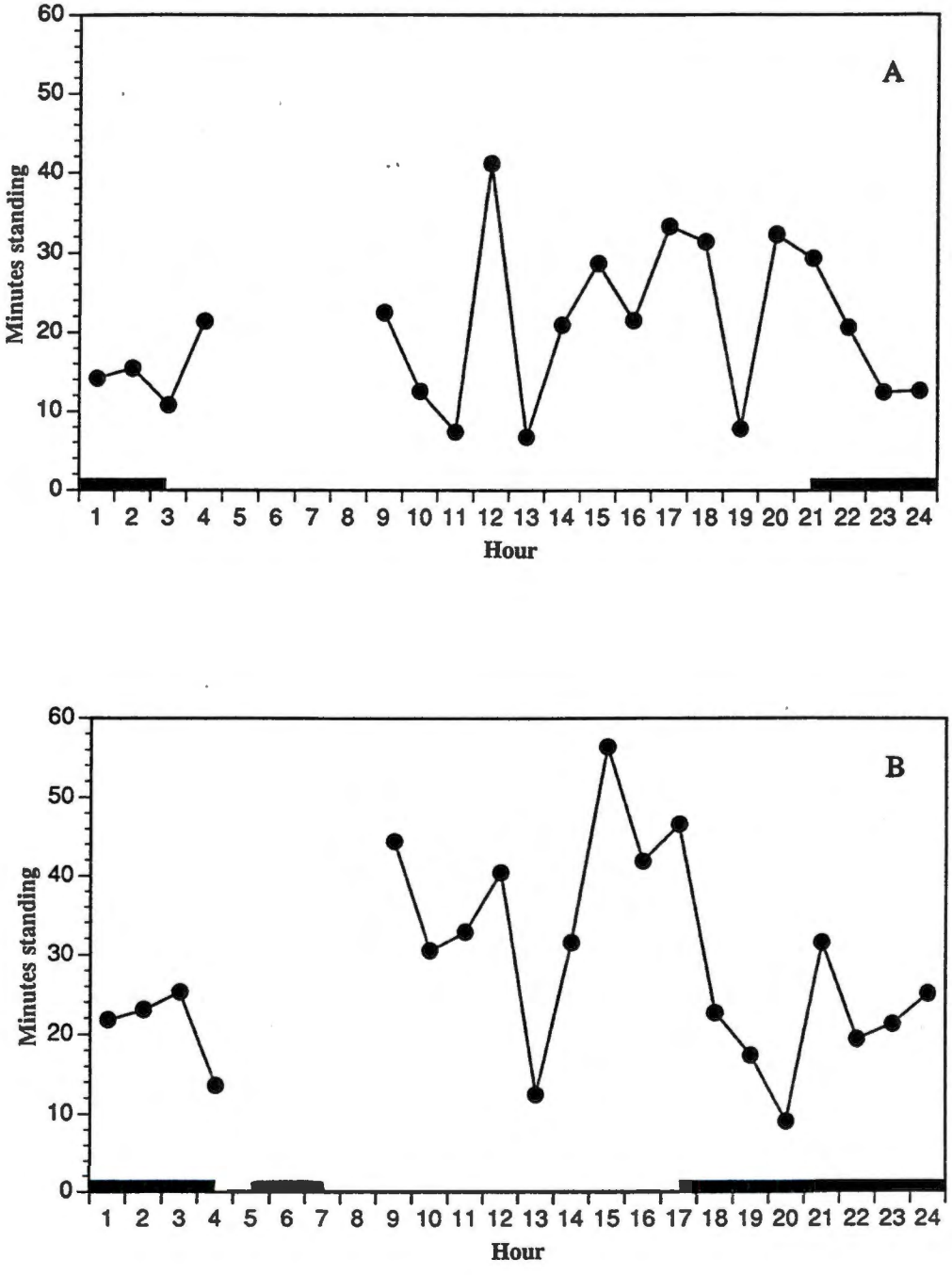
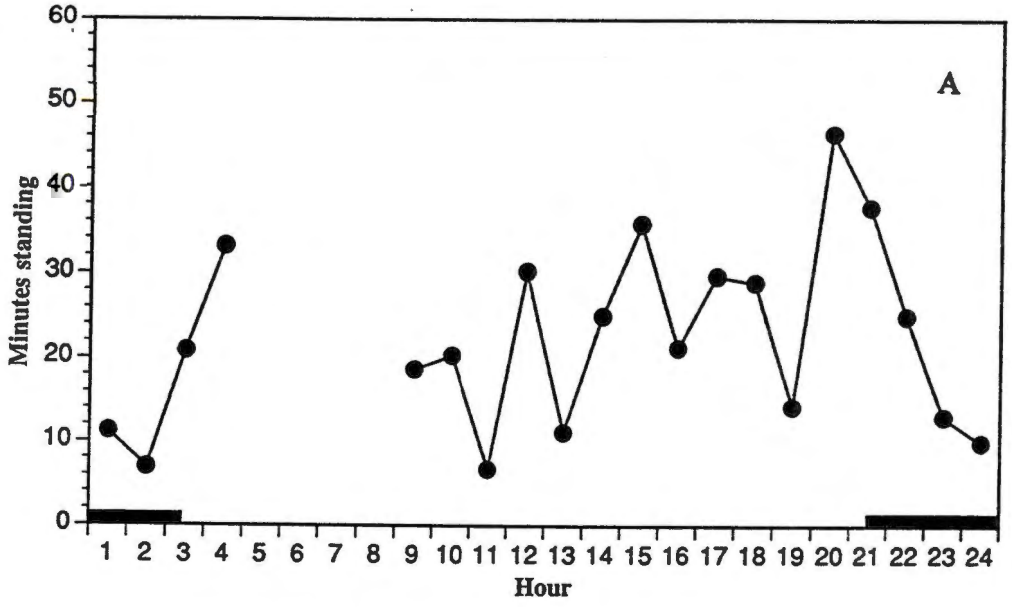


Figure 2-8. Group mean time (min) of standing by hour. (A) Group 1 (continuous) during treatment week 6. (B) Group 2 (skeletal) during treatment week 6. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.



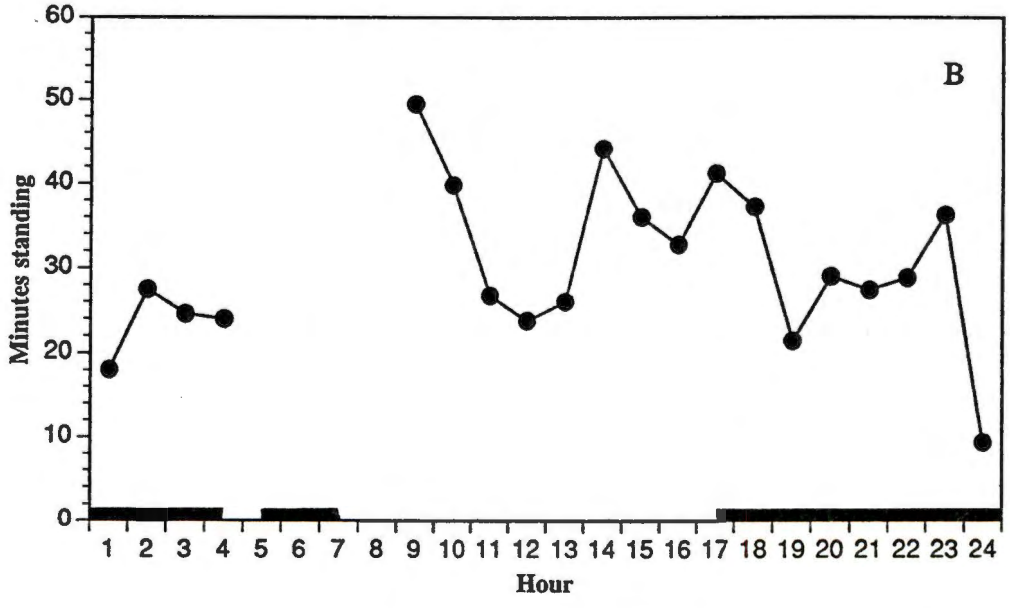


FIGURE 2-9. Group mean time (min) of standing by hour. (A) Group 1 (continuous) during treatment week 13. (B) Group 2 (skeletal) during treatment week 13. Black horizontal bar on x-axis indicates dark time. Cows were being milked or exercised between 0400 and 0800 h.

an additional feeding bout (Evans and Hacker, 1989b) and increased plasma prolactin levels (Petitclerc et al., 1983).

Other factors are presently known to compound and confound the 13 to 15 h light effect. Lighting systems which simulate dawn and dusk have been shown preferable to simply turning all lights on and off (Zinn et al., 1986). Previous lighting history may enhance or negate photoperiod effects (Gustafson, 1994; and Marcek and Swanson, 1984). A related factor is whether a photorefractory period occurs in cows (Marcek and Swanson, 1984; Petitclerc et al., 1985).

Determining the beginning of subjective dawn is difficult. Pittendrigh and Minis (1964) determined asymmetric skeletons involving a main photoperiod of 8 h or more can simulate a long photoperiod in two ways: with the night interruption as terminator of the skeleton or with the night interruption as initiator of the skeleton. Simulation of long photoperiods is better when the interruption functions as initiator, rather than terminator, of the long skeleton.

Schanbacher and Crouse (1981) found a photoperiod of 7L:9D:1L:7D was as effective as 16L:8D in stimulating secretion of PRL in adult rams and ewes. Insertion of a one hour pulse of light at any other time during the scotoperiod (dark period) was ineffective in stimulating secretion of PRL. Normally sheep have a marked diurnal increase in secretion of PRL at the beginning of the scotoperiod. If sheep also have a photosensitive period 13 to 15 h after subjective dawn, subjective dawn began with the one-h skeletal light period, which added to the 7 h of dark and 7 more h of light would

have resulted in lights on 13 to 15 h after subjective dawn, rather than off if subjective dawn started at the beginning of the 7 h of light.

The present experiment had a 10L:11D:1L:2D photoperiod. If subjective dawn began with the 10 h light period, cows would have been in the dark at 13 to 15 h after subjective dawn. However, milk yield and hormone concentrations of Group 2 cows were comparable to cows given 18 h of light. If activating a photosensitive phase period accounted for the equivalent milk yield of both groups, subjective dawn began with the 1 h skeletal period and was added to the 2D and 10L h to equal 13 which is within the photosensitive phase period hypothesized by Evans and Hacker (1989a).

Thirty-two 100 watt incandescent bulbs maintained light at 100 lx in both stanchion barns used for this experiment. One 100 watt bulb in East Tennessee will use one kilowatt of electricity in 10 hours which costs 5.45 cents (Knoxville Utilities Board rates, May 1996). In this experiment, lights were on 18 h daily in one barn and 11 h total in the other barn with comparable milk yields. In one year, the barn with lights on 11 hours would save $444 compared to the barn where lights were on 18 hours.

CONCLUSION

Manipulation of photoperiod could prove to be one of the easiest and least expensive ways to improve milk yield. However, much is still unknown. Recommendations to keep lights on 16 h a day to increase milk yield do not take into consideration possible photorefractory effects, the potential for greater yields with a dawn and dusk-like rheostat, or the potential to decrease electrical expense by using well-timed skeletal photoperiods.

In this study, cows exposed to a 10 h light period, plus a 1 h skeletal light period, produced comparable milk yields. In addition, first lactation cows had similar serum concentrations of T_3, PRL, and cortisol; and multiparous cows behaved similarly in eating, drinking, standing, and lying as cows exposed to a 18L:6D photoperiod. Results suggest that by using a skeletal photoperiod, electrical costs could be lowered without decreasing production.

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PART III.

EFFECTS OF FEEDING FREQUENCY AND INGREDIENT ORDER ON BEHAVIOR OF DAIRY COWS

ABSTRACT

To detect trends in behavioral feeding preference, 48 lactating cows were observed 72 continuous h during five different feed management regimes. Treatments were: hay and silage fed simultaneously at 0830, 1300, and 1630 h; hay fed at 0730, 1145, and 1530 h and silage fed at 0830, 1300, and 1630 h; silage fed at 0730, 1145, 1530 h and hay fed at 0830, 1300, and 1630 h; hay and silage fed simultaneously at 0700, 1000, 1300, and 1600 h; and hay and silage fed simultaneously at 0700 and 1630 h. Binomial *z*-scores indicated that cows had definite eating patterns which went across all treatments. However, behavioral differences between treatments were not detected. Strongest feeding preferences were to eat grain, then silage, drink water, and then eat hay. On average, cows ate silage 9.51 times/d, 18.45 min each time; hay 5.59 times/d, 10.91 min each time; grain 7.33 times/d; and drank water 4.68 times daily.

INTRODUCTION

Much attention has been given to balancing rations to meet nutritional needs of lactating dairy cows. Although many dairies now feed total mixed rations, or a combination of grazing and grain, for others, conventionally feeding hay, silage, and grain separately is still the most practical method.

Increased frequency of feeding cattle has been shown to improve milk yield (Nocek and Brand, 1985; and Campbell and Merilan, 1961) and milk fat concentrations (Sutton, et al., 1985 and 1986; and Campbell and Merilan, 1961). However other studies (Stanley and Morita, 1967; and French et al., 1990) have not verified similar benefits. Production benefits will only occur if increased feeding leads to increased dry

matter intake or greater rumen microbial growth (Hungate et al., 1966). Rumen microbes grow best with a steady supply of balanced nutrients to prevent major decreases in pH and meet nutritional needs (Hungate et al., 1966).

Much can be learned by watching cows to understand activity patterns. Objectives of this experiment were to observe the behavioral patterns of cows fed silage and hay at different frequencies and in different orders and to relate those patterns to optimizing feed intake.

MATERIALS AND METHODS

Animals

Forty-eight mid-lactation Holstein cows from The University of Tennessee Cherokee Dairy Research herd were painted with large identifying numbers, and housed, fed, and milked together. All cows had calved during the prior 6 mo and were in various reproductive stages. Over half the cows were in their second lactation (mean 2.7 lactations), but varied from first to sixth lactation. Mean age of cows at last calving was 52.1 mo, average 305 day mature equivalent (ME) milk yield was 10,528 kg, and average 305 day ME fat was 343.1 kg.

Housing

Cows were confined to a spacious concrete drylot area which included a free stall barn with adequate stalls for all cows, a water trough, four computerized grain feeders, a 21 m hay mow, and a 27 m silage trough. The drylot adjoined the milking parlor holding pen which the cows entered at approximately 0115 and 1315 h each day to be milked. A mercury vapor lamp lit the drylot at night providing light for cows to easily

see the hay manger, silage trough, water trough, and grain feeders. In addition, a row of incandescent lights above the silage trough provided nightly light at trough level. Lights were on in the free stall area only when cows were moved to the milking parlor at night.

Data Collection

All cows were exposed to five feeding regimes for a minimum of one week before a 72 h continuous observation period was begun by 18 students. Generally, two students were on observation duty at a time, one in a lookout over the hay mow observing cows at the silage trough, and another in the hay mow overlooking the hay racks, water trough, and grain feeders.

Observers noted the time a cow started eating silage or hay and the time when eating ceased. If a cow backed away from the silage trough or hay rack, but resumed eating within one min, it was considered one continuous eating period. Each time a cow entered a grain feeder, or took a drink, it was recorded. Observers were present for 3 d prior to the first observation day to acclimate the cows to the observers' presence and give the observers practice to minimize observer variation. A nearby research weather station provided temperature and relative humidity information (Table 3-1).

Treatments

Cows were fed a pretreatment diet of corn silage and alfalfa or grass hay at 0700 h and as needed throughout the day, supplemented with 18% crude protein grain fed in computerized feeders. Cows received grain to meet National Research Council (1988) recommendations based on individual production and stage of lactation.

TABLE 3-1. Average daily ambient temperature and relative humidity during each treatment.

Treatment	Day	Average temperature (°C)	Average relative humidity (%)
	1	7.0	71.7
1[a]	2	7.5	90.5
	3	10.7	69.4
	1	17.0	63.0
2[b]	2	16.8	65.9
	3	19.9	52.5
	1	6.3	53.6
3[c]	2	7.6	47.6
	3	12.5	52.5
	1	11.0	48.0
4[d]	2	12.9	48.9
	3	2.9	74.1
	1	17.0	54.6
5[e]	2	6.7	65.9
	3	10.2	48.2

[a] Treatment 1 - Hay and silage fed 3x simultaneously each day.
[b] Treatment 2 - Hay and silage fed 3x each day, hay an hour prior to silage.
[c] Treatment 3 - Hay and silage fed 3x each day, silage an hour prior to hay.
[d] Treatment 4 - Hay and silage fed 4x each day simultaneously.
[e] Treatment 5 - Hay and silage fed 2x each day simultaneously.

During the five treatments, alfalfa hay came from only one source. However, silage source varied between some of the treatments. Analysis of hay and silage fed during each treatment is found in Table 3-2.

Cows were observed, beginning in early March through late April, after 7 d exposure to the following feeding regimes:

Treatment 1 - Hay and silage fed simultaneously at 0830, 1300, and 1630 h (3x).

Treatment 2 - Hay fed at 0730, 1145, and 1530 h, and silage fed at 0830, 1300, and 1630 h (3x-hay first).

Treatment 3 - Silage fed at 0730, 1145, and 1530 h, and hay fed at 0830, 1300, and 1630 h (3x-silage first).

Treatment 4 - Hay and silage fed simultaneously at 0700, 1000, 1300, and 1600 h (4x).

Treatment 5 - Hay and silage fed simultaneously at 0700 and 1630 h (2x).

Statistical Analysis

Behavioral observations for each cow taken over the five 72 h observation periods were analyzed. Frequency and duration of hay and silage eating, along with frequency of grain eating and water drinking were calculated by treatment. In addition, PC Elag (Bakeman, 1986) was used to calculate binomial *z*-score tests which gauged the extent to which observed behavioral values exceeded the expected values.

TABLE 3-2. Alfalfa hay and corn[1] or corn-wheat[2] silage analysis of feeds fed during each treatment (crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF)).

Feed	Treatment 1[1a]	Treatment 2[2b]	Treatment 3[2c]	Treatment 4[1d]	Treatment 5[1e]
Alfalfa hay					
Dry matter %	84.85	86.66	85.73	86.51	86.51
CP %	14.60	14.45	13.38	16.89	18.85
ADF %	44.87	44.63	47.83	42.12	40.88
NDF %	64.27	65.32	68.79	58.05	54.61
Silage					
Dry matter %	89.80	88.90	88.26	87.51	88.42
CP %	6.62	9.11	6.84	10.21	7.14
ADF %	36.52	30.10	30.87	28.55	27.93
NDF %	63.79	51.72	57.22	50.29	52.96

[a] Treatment 1 - Hay and silage fed 3x simultaneously each day.
[b] Treatment 2 - Hay and silage fed 3x each day, hay an hour prior to silage.
[c] Treatment 3 - Hay and silage fed 3x each day, silage an hour prior to hay.
[d] Treatment 4 - Hay and silage fed 4x each day simultaneously.
[e] Treatment 5 - Hay and silage fed 2x each day simultaneously.

To analyze treatment effects, cows were subdivided into 5 groups of nine or ten animals each balanced for stage of lactation, lactation number, sire and ME milk. An analysis of covariance, using temperature as a covariate, was performed on the groups of 9 or 10 cows with SAS general linear model procedure (SAS, 1985).

In an effort to analyze treatment differences on silage eating time, an analysis of covariance, using temperature as a covariate, was performed on the groups of 9 or 10 cows with SAS general linear model procedure (SAS, 1985). A natural log transformation of silage eating time was used to normalize the distribution.

RESULTS AND DISCUSSION

Average duration and frequency of silage and hay eating times, and frequencies of water drinking, and grain eating are reported in Table 3-3. Cows spent an average of 2.92 h/d eating silage, averaging 9.51 times for 18.45 min. Just over 1 h/d was spent eating hay, 5.59 times for 10.91 min. Tanida et al. (1984), reported very similar values of 4.5 h total daily eating time divided into 10 to 12 bouts of 24 to 27 min. These times are somewhat less than the six hours of combined hay and silage eating time noted by Webb et al. (1963).

Quality of the alfalfa hay fed in the present experiment was poor, very coarse with low crude protein, especially during the first three treatments (see Table 3-1). Different silages were fed during different treatments. During treatments 2 and 3 when cows ate silage for the shortest durations, corn-wheat silage was being fed. Cows seemed to make up for the shortened bouts with more frequent stops at the silage trough, however. Holstein cows given an ad libitum choice of corn silage and alfalfa hay

TABLE 3-3. By treatment and overall average duration, frequency, and total time of daily hay and silage eating and frequency of grain eating and water drinking.

	Treatment					
Parameter	1[a]	2[b]	3[c]	4[d]	5[e]	All Trt. Mean
Silage eating time/bout (min)	21.40	17.43	17.15	17.82	18.45	18.45
Silage eating frequency/d	9.28	10.45	9.77	9.61	8.46	9.51
Total time eating silage/d (h)	3.31	3.03	2.79	2.85	2.60	2.92
Hay eating time/bout (min)	11.05	11.60	10.07	10.69	11.13	10.91
Hay eating frequency/d	7.21	5.67	4.62	5.27	5.22	5.59
Total time eating hay/d (h)	1.33	1.10	0.77	0.94	0.97	1.02
Grain eating frequency/d	8.03	6.74	7.70	6.60	7.59	7.33
Water drinking frequency/d	4.40	5.40	4.28	4.85	4.46	4.68

[a] Treatment 1 - Hay and silage fed 3x simultaneously each day.
[b] Treatment 2 - Hay and silage fed 3x each day, hay an hour prior to silage.
[c] Treatment 3 - Hay and silage fed 3x each day, silage an hour prior to hay.
[d] Treatment 4 - Hay and silage fed 4x each day simultaneously.
[e] Treatment 5 - Hay and silage fed 2x each day simultaneously.

expressed a wide variation in their dry matter intake (from 23.6 to 77.7% corn silage DM) and showed no indication of switching to less silage as lactations progressed (Coppeck et al., 1974).

Feeding hay an hour ahead of silage or vice versa did not increase the number of trips to the hay mow or silage trough, but did lead to the highest amount of time eating hay per feeding bout (11.6 min) and the second highest overall amount of time eating hay (1.1 h). Time spent eating both hay (1.33 h) and silage (3.31 h) was greatest during treatment 1 when cows were fed 3 times a day simultaneously. Ambient temperature was also the lowest during treatment 1 (average 8.4° C).

Feeding four times a day did not increase frequency or duration of eating compared to the other treatments. Feeding twice a day, with adequate feed for cows to eat ad libitum throughout the day, led to the lowest amount of time eating silage per day (156 min) compared to 199; 182; 167; and 171 min, for treatments 1 through 4, respectively. Vasilatos and Wangsness (1980) found confined cows fed ad libitum had very similar eating patterns; averaging 12.1 meals/d, 20.9 min in duration.

Drinking frequency per day varied from 4.28 to 5.4, average 4.68. Least drinks per day occurred during the two coldest treatment periods (1 and 3). Highest drinks/d occurred during treatment 2 which was the warmest treatment period (averaging 17.9° C). Factors known to influence drinking behavior include eating pattern, water temperature, whether water is given in a bowl or a trough, flow rates into water bowls, animal dominance, stray voltage, temperature, humidity, dry matter intake, nature of diet, and milk production (Murphy, 1992).

Computerized grain feeders at the Cherokee Research Station are designed to allocate one quarter of each cow's daily grain needs into 1.09 kg meals every six hours and record amounts of grain eaten by each cow. From the observers' vantage point, it was impossible to tell whether a cow actually received grain whenever entering a grain feeder. However, the grain feeders were well used both day and night, particularly right after milking, and the cows were generally eating their full allocated amounts.

By assigning nine or ten cows to five groups, an analysis of covarience was performed on silage and natural log silage to test differences between groups. Temperature was not a significant covariate and no significant differences were found between groups.

PC Elag, a computer program designed to determine frequencies, probabilities, and likelihood-ratios of event-sequence data, was used to analyze behavioral differences between the five treatments (Bakeman, 1986, and Bakeman and Gottman, 1986). Table 3-4 lists the average behavioral frequency and probability estimates of eating silage, hay, and grain by treatment. Over the 72 h treatment 1 observation period, each cow averaged 16.7 visits to the silage trough to eat. During the 3 observation days, 32.5% of all observed "events" (silage, hay and grain eating and water drinking) were silage eating. Between the first three treatments, which all involved 3x feeding, the probability of silage eating went up both when hay and when silage were fed first. The trend for less water drinking during the two colder treatments (1 and 3) again was noticeable.

Z-scores indicate likelihood ratios of one behavioral event (given code) happening before another (target code). Scores over 1.96 absolute value indicate a

TABLE 3-4. Average behavioral frequency and probability estimates by treatment of eating silage, hay, and grain, and drinking water.

Treatment	Silage	Hay	Water	Grain
1[a]				
Frequency	16.7	12.6	7.8	14.3
Probability	32.5	24.6	15.3	27.7
2[b]				
Frequency	24.2	13.2	12.7	15.9
Probability	36.4	20.1	19.3	24.2
3[c]				
Frequency	23.0	10.9	9.8	17.9
Probability	37.4	17.9	16.0	28.7
4[d]				
Frequency	21.8	12.1	10.9	15.4
Probability	36.1	20.3	18.3	25.3
5[e]				
Frequency	20.0	11.7	10.6	17.3
Probability	33.6	19.6	18.1	28.7

[a]Based on data from 44 cows over 72 h with an average of 51.3 events per cow.
[b]Based on data from 47 cows over 72 h with an average of 66.1 events per cow.
[c]Based on data from 46 cows over 72 h with an average of 61.6 events per cow.
[d]Based on data from 48 cows over 72 h with an average of 60.2 events per cow.
[e]Based on data from 47 cows over 72 h with an average of 59.6 events per cow.

behavioral transition happens significantly more or less often than expected ($P > 0.05$) (Bakeman and Gottman, 1986). Table 3-5 shows z-scores by given code for each treatment. When the given code was silage, and the target code was silage, the large negative z-scores indicate cows seldom eat silage, stop eating silage, and resume eating silage, without eating hay, or grain, or drinking water first. Cows also seldom went from eating silage to eating hay. However, cows about equally went from eating silage, to eating grain or drinking water, except when the temperature was higher during the second treatment and there was a strong preference for water.

Z-scores also indicated cows generally preferred to eat grain, or silage, after eating hay. Cows particularly went for grain after hay during treatment 5 (2x). After a drink of water, cows typically headed for the hay mow during all treatments. During treatment 2 (3x-hay first) and treatment 4 (4x), cows seemed to have a stronger preference for silage. Following a stop at the grain feeder, cows generally headed for the silage trough. Second choice, particularly during treatment 2, was the hay mow.

Dry matter intake and water consumption of four cows fed a total mixed ration 1,2, 4, and 8 times daily was observed by Nocek and Braund (1985). Peak hourly water consumptions were associated with peak hourly intakes of dry matter. Given the opportunity, cows tended to consume feed and water alternately, which was also evident in this study.

Table 3-6 shows the first and second sequence event order cows followed after morning and evening milkings during treatment 1. Many cows had third, fourth, and more events following milking which are not indicated in the figure. After morning

TABLE 3-5. *Z*-scores for behavioral sequencing from a given behavior to a target behavior by treatment. Scores over 1.96 (absolute value) indicate a behavioral transition occurred significantly more often than expected ($P < 0.05$).

Given code	Target codes			
Silage	Silage	Hay	Water	Grain
Trt. 1 - 3x	-9.07	-1.93	5.71	6.45
Trt. 2 - 3x, hay first	-11.79	-4.04	10.16	7.67
Trt. 3 - 3x, silage first	-10.08	-3.19	7.56	7.24
Trt. 4 - 4x	-11.04	-1.96	7.31	7.41
Trt. 5 - 2x	-10.70	-1.95	7.81	5.95

Given code	Target codes			
Hay	Silage	Hay	Water	Grain
Trt. 1 - 3x	0.83	-4.24	-.43	3.74
Trt. 2 - 3x, hay first	2.65	-4.36	-2.72	3.50
Trt. 3 - 3x, silage first	2.04	-5.12	-1.23	3.29
Trt. 4 - 4x	1.47	-5.30	0.59	2.82
Trt. 5 - 2x	2.19	-6.45	-2.14	5.18

Given code	Target codes			
Water	Silage	Hay	Water	Grain
Trt. 1 - 3x	1.63	8.49	-5.70	-5.32
Trt. 2 - 3x, hay first	4.47	7.09	-6.39	-5.77
Trt. 3 - 3x, silage first	2.80	8.66	-6.68	-5.24
Trt. 4 - 4x	4.32	7.61	-7.44	-5.32
Trt. 5 - 2x	2.43	8.16	-6.77	-3.91

Given code	Target codes			
Grain	Silage	Hay	Water	Grain
Trt. 1 - 3x	7.41	-0.10	-1.27	-6.72
Trt. 2 - 3x, hay first	7.70	2.50	-4.31	-7.19
Trt. 3 - 3x, silage first	7.35	1.18	-2.44	-6.79
Trt. 4 - 4x	7.80	0.92	-3.05	-6.76
Trt. 5 - 2x	7.59	1.00	-1.06	-7.90

TABLE 3-6. Three day average morning (A) and evening (B) event sequence frequencies after cows left milking parlor in treatment 1. Given event is the first event to occur. Target event is the second sequential event.

A. Event sequence after morning milking.

Given Event	Target Event: Silage	Hay	Grain	Water
Silage	5.0 to silage only	2.0	4.3	2.7
Hay	0.7	0.3 to hay only	0.7	0.3
Grain	9.3	1.3	5.3 to grain only	1.3
Water	1.6	1.0	1.0	1.0 to water only

B. Event sequence after evening milking.

Given Event	Target Event: Silage	Hay	Grain	Water
Silage	0.7 to silage only	1.7	5.3	2.0
Hay	2.3	0.7 to hay only	1.7	0.0
Grain	6.7	1.0	4.7 to grain only	1.0
Water	3.0	1.3	0.3	1.0 to water only

milking, cows most often went to eat grain first, followed by silage. Eating grain or silage only before heading for the freestalls were the second and third most popular post-milking activities. Going first to silage, then to grain was fourth. An average of 4.3 cows/d neither ate nor drank immediately after milking; 10.7 ceased eating and drinking within 15 min. Cows who chose to eat and drink after milking averaged 38.0 ± 31.4 min before ceasing that activity. Despite there being no fresh feed in the bunk during the early morning hours, out of the 42 cows considered in this survey, over half spent over 0.5 h eating or drinking after milking, decreasing the chance of immediately lying in a soiled stall and permitting mastitis-causing bacteria from entering the teat sphincter.

Upon completion of the afternoon milking, eating grain followed by silage, and silage followed by grain, were the two most popular event sequences. More cows headed for water in the afternoon. Average time spent eating and drinking was 45.6 ± 36.4 min with an average of 7.6 cows spending < 15 min. Over the three days, an average of 6.3 cows/d did not eat or drink following milking and presumably headed straight for the freestalls.

During this study, silage was permitted to be "cleaned up" every night. By morning, many cows would head to the feed trough whenever they heard the silage unloader start up. Throughout the rest of the day, regardless of treatment, relatively fresh feed was always available. In this study, cows did not appear to change eating habits to different feeding regimes. Perhaps a 7 d pre-observation period was not sufficient for cows to adjust, but more likely with feed nearly always available, they found no need to adjust.

CONCLUSION

Behavioral trends were quite evident from *z*-score analyses of the five treatments in this study, but little variation was found between treatments. Cows showed an obvious preference for drinking water, or eating grain, after eating silage. Following hay eating, cows typically headed for the grain feeder or silage trough. After a drink of water, cows were usually ready to eat hay or silage. Eating silage was their preference after grain.

Computerized grain feeders forced cows to ration grain intake throughout the day. *Z*-scores indicated cows rotated between consuming grain, silage, water, and hay throughout the day presumably providing rumen microbes a balanced mix of nutrients.

A survey of cows as they left the milking parlor during Treatment 1 indicated 10 to 15% of the cows did not stop to eat or drink before potentially lying down. Cows who stopped to eat or drink averaged 38.0 $\pm$ 31.4 min of standing activity following the morning milking and 45.6 $\pm$ 36.4 min following afternoon milking. Evidence indicates the teat sphincter muscle takes over an hour to close tightly (McDonald, 1975). Most of the cows in the herd were possibly at risk of environmental mastitis when ceasing to stand eating and drinking and proceeding to lie down in freestalls.

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APPENDICES

APPENDIX A

Radioimmunoassay Techniques (RIA) Techniques

Table A-1. Protocol for Prolactin **(PRL).** (Bolt and Rollins, 1983, modified.)

Sequence		1	2	3	4	5
Tubes	Tube #	RIA buffer	Sample	1st Ab	Tracer	Vortex
TC	1,2,3				100 μl	
NSB	5,6,7	600 μl			100 μl	
Bo	7,8,9	400 μl		200 μl	100 μl	
Stds*	10-39	200 μl	200 μl	200 μl	100 μl	
QC	40-43	300 μl	100 μl	200 μl	100 μl	
Samples	44-	**		200 μl	100 μl	

* PRL Standard Protocol: 204.8 mg/800μl is the starting point of the serial dilution 25.6 mg, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05.

** Sample volume (sample + buffer) must equal 400 μl. (Therefore, if 100 μl of sample is used, then add 300 μl of buffer or if 50 μl of sample is used, add 350 μl of buffer).

TC = Total count or the total amount (counts per minute) of tracer added to each tube.

NSB = Nonspecific binding, amount of interference of impurities in the assay tubes, buffer, etc.

Bo = Total binding (%) capacity of the working dilution of tracer and antibody to be used as the basis for determining hormone concentrations.

Standards = Known amounts of hormone used to construct the standard reference curve.

QC = Quality control, a standard unknown plasma sample used in assay to control intra- and interassay variation.

Sample = Unknown amount of the hormone that is to be measured.

PRL First Antibody (Ab) Recipe

$$\frac{x}{300} = \frac{\text{total volume}}{150{,}000} = \text{volume of stock 1st Ab.}$$

Total volume = [# of tubes in assay + 30 (for error)] x 200 μl (amount/tube).

Total volume - volume of 1st Ab = amount of Ab buffer required.

PRL Tracer Recipe

1. [# of tubes in assay + 30 (for error)] x 1000 counts per minute (cpm)/tube = total cpm required for assay.
2. Total CPM ÷ stock cpm/μl (take 10 μl of stock and count to get actual stock cpm/μl) - volume of stock tracer required.
3. [# of tubes in assay + 30 (for error)] x 100 μl (amount/tube) = total volume.
4. Total volume - volume of stock tracer = amount of RIA buffer required.
5. After preparing tracer solution, check 100 μl in gamma counter to see if it is reading 10,000.

Table A-2. Protocol for prolactin RIA continued.

Sequence	6	7	8	9	10	11
Tubes		NRS*	2nd Ab	PEG**	Vortex	
TC	Incubate					
NSB	for	100 μl	100 μl	500 μl		Incubate
Bo	48 h	100 μl	100 μl	500 μl		tubes at
Stds	at	100 μl	100 μl	500 μl		room
QC	room	100 μl	100 μl	500 μl		temperature
Samples	temp.	100 μl	100 μl	500 μl		for 5 to 6 h.

* Normal Rabbit Serum **(NRS)**.

** Polyethylene glycol **(PEG)**.

PRL Second Ab Recipe

1. [# of tubes + 30 (error)] x 100 μl = total volume.
2. Total volume ÷ 12 = volume of stock 2nd Ab.
3. Total volume - volume of 2nd Ab = amount of RIA buffer required.

Stepwise Procedure

1. Add RIA buffer to numbered assay tubes as indicated in Table A-1 column 1.
2. Add sample or standard to assay tubes as indicated in Table A-1 column 2.
3. Add 200 μl of anti-PRL (1st) Ab to all tubes except TC and NSB.
4. Add 100 μl of PRL-I^{125}.
5. Vortex.

6. Incubate all tubes for 48 h at room temperature.

7. Add 100 μl normal rabbit serum (NRS) (Miles Scientific, Inc.) to all tubes except TC.

8. Add 100 μl 2nd Ab to all tubes except TC.

9. Add 500 μl PEG (Sigma Chemical Co.) to all tubes except TC.

10. Vortex.

11. Incubate tubes 5 to 6 h at room temperature.

12. Centrifuge tubes at 3000 rpm for 20 min.

13. Decant supernatant and discard properly.

14. Let tubes drain for 10 min.

15. Count remaining tube radioactivity on the gamma counter.

See Appendix B for RIA buffer, Ab buffer, (ethylenedinitrilo)-tetraacetic acid (EDTA), NRS, and PEG buffer recipes.

APPENDIX B

BUFFERS AND REAGENTS

Reagents for Prolactin Radioimmunoassay (RIA)

1. Phosphate Buffer Solution (PBS): Use 0.5 M sodium phosphates and dilute with double distilled water to obtain desired molarity of buffer.

   NOTE: To adjust pH use 1M, 3M or 10M NaOH (base) or 5'sulfosalicylic acid (acid).

2. Basic RIA Buffer: Use a volumetric flask. Mix solution using 0.01 M PBS with a pH of 7.5; readjust pH after mixing. Add material to flask before adding liquid; fill only about halfway with PBS and dissolve materials before completing the mixture.

MATERIALS	LITER	½ LITER
0.1% Bovine Serum Albumin	1 gm	0.5 gm
0.135 M NaCl	7.89 gm	3.95 gm
0.005 M $NaHCO_3$	0.420 gm	0.21 gm
0.01% Thimerosal	100 mg	50 mg

3. (Ethylenedinitrilo)-tetraacetic acid **(EDTA)**: Use a volumetric flask. Mix an 0.1 M solution using double distilled water and adjust the pH to 7.5 $\pm$ 0.5.

MATERIALS	LITER	½ LITER
(Di)NaEDTA	37.22 gm	18.6 gm
0.1% Thimerosal	100 mg	50 mg

4. 2% Normal Rabbit Serum (NRS): Mix using a 0.01 M PBS with a pH of 7.5; readjust pH after mixing solution.

MATERIALS	LITER	½ LITER
Normal Rabbit Serum	20 ml	10 ml
0.01% Thimerosal	100 mg	50 mg

5. Polyethylene glycol (PEG): Mix a 5% solution in 0.01 M PBS with pH of 7.5; readjust after mixing.

MATERIALS	LITER	½ LITER
PEG	50 gm	25 gm
0.01% Thimerosal	100 mg	50 mg

6. First Ab buffer: Use a volumetric flask. Mix using a 0.1 M PBS with a pH of 7.5; readjust the pH after mixing.

MATERIALS	LITER	½ LITER
0.135 M NaCl	7.89 gm	3.95 gm
0.005 M $NaHCO_3$	0.420 gm	0.21 gm
0.05 M (Di)NaEDTA	18.6 gm	9.3 gm
1:300 Normal rabbit serum	3.3 ml	1.65 ml
0.01% Thimerosal	100 mg	50 mg

VITA

Mary Elizabeth Sowerby, daughter of Merton B. and Frances Morgan Sowerby of Warrenton, Virginia, was born in Beaufort, South Carolina, on January 24, 1953. She grew up on a dairy farm near Princeton, New Jersey, and graduated from Montgomery High School, Skillman, New Jersey, in June 1971.

In September, 1971, the author entered Virginia Polytechnic Institute and State University at Blacksburg, Virginia. In December, 1974, she completed her Bachelor of Science degree in Dairy Science with a second major in Biology. Continuing at Virginia Tech, she finished a Master of Science degree in Dairy Science in February, 1977.

During the following six months the author participated in a Young Agricultural Specialist Exchange Program to the former Soviet Union. Upon returning to the United States she was employed by Sire Power, Inc., Tunkhannock, Pennsylvania, as Communications Manager for a year. In January, 1979, she moved to south central Wisconsin to become Assistant Manager of the Genetic Mating Service program of American Breeders Service in DeForest, Wisconsin.

In May of 1981 the author came to East Tennessee to do sales and dairy evaluation work for American Breeders Service. She also worked part-time for the Tennessee Dairy Herd Improvement program. She began work on a Doctor of Philosophy degree in 1987 in Animal Science at the University of Tennessee, Knoxville, which was completed May, 1996.

Since 1992 the author has been employed by the University of Florida Cooperative Extension Service as a Multi-county Dairy Agent in Central Florida.

Impact of the Maturity of Corn for Use as Silage in the Diets of Dairy Cows on Intake, Digestion, and Milk Production

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ABSTRACT

Whole-plant corn was harvested at early dent, quarter milkline, two-thirds milkline, and black layer stages to evaluate the effects of maturity on intake, digestion, and milk production when corn was fed as silage in the diet. Twenty multiparous Holstein cows were used in a replicated experiment with a 4 × 4 Latin square design with 28-d periods. Diets containing 50% forage (67% corn silage and 33% alfalfa silage) and 50% concentrate (dry matter basis) were fed as total mixed rations. Moisture contents were 69.9, 67.6, 64.9, and 58.0% for silages from corn harvested at early dent, quarter milkline, two-thirds milkline, and black layer stages, respectively. Intakes of dry matter were similar across the four treatments and ranged from 3.73 to 3.79% of body weight. Milk production was highest (33.4 kg/d) for cows fed silage from corn harvested at the two-thirds milkline stage and lowest (32.4 kg/d) for cows fed silage from corn harvested at the early dent stage. Milk protein production was highest for cows fed silage from corn harvested at the two-thirds milkline stage (1.17 vs. 1.12 to 1.13 kg/d). Apparent total tract digestion of dry matter, organic matter, crude protein, acid detergent fiber, and starch was lowest for cows fed silage from corn harvested at the black layer stage. Although starch intake was similar for cows fed silage from corn harvested at the two-thirds milkline stage and for cows fed silage from corn harvested at the black layer stage (9 kg/d), intake of digestible starch was 0.4 kg/d lower for cows fed silage from corn harvested at the black layer stage. The optimum stage for corn that was ensiled was two-thirds milkline with some flexibility between quarter and two-thirds milkline.

(**Key words**: corn silage, intake, digestion, milk production)

Abbreviation key: **BL** = black layer, **ED** = early dent, **LAB** = lactic acid bacteria, **1/4 ML** = quarter milkline, **2/3 ML** = two-thirds milkline, **WPC** = whole-plant corn.

INTRODUCTION

Achieving high DM yield from whole-plant corn (**WPC**) and high milk production from cows fed WPC depends on the harvesting of the corn at the proper stage of maturity. Agronomic trials (7) have shown that DM yields of WPC are maximized by harvesting at two-thirds milkline (**2/3 ML**) to black layer (**BL**) stages.

At an immature stage of harvest, fiber concentrations are highest, which lowers the energy density of WPC (13). At a mature stage of harvest, digestibility of the stover is reduced (26), which may lower the energy density of WPC. Additionally, harvest of WPC at a mature stage may increase whole kernel passage and lower starch digestibility (10), resulting in lower energy density. Neither stover nor starch digestibility is considered in most equations that predict the energy value of silage from WPC from ADF concentration (15).

Moisture content of WPC is inversely related to stage of maturity at harvest (26). Whole-plant corn harvested at an immature or mature stage may be either too wet or too dry, respectively, for good silage preservation. Studies are limited on the feeding value of WPC harvested at varying stages of maturity for use as silage in the diets of lactating dairy cows. Huber et al. (12) reported increases in silage DMI and in milk production of cows as the maturity of WPC at harvest advanced from the soft stage to the hard dough stage. Harrison et al. (10) found higher milk production and total tract starch digestion for cows fed silage from WPC harvested at the one-half milkline stage versus milk production and starch digestion for cows fed silage from WPC harvested at the BL stage.

The objective of this study was to evaluate the effect of harvesting WPC at four stages of maturity for use as silage in the diets of dairy cows on DMI, total tract nutrient digestion, and milk production and composition.

Received October 2, 1996.
Accepted March 31, 1997.
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MATERIALS AND METHODS

At 110 d of relative maturity, a corn hybrid (4277; Cargill, Minneapolis, MN) selected for high grain yield was planted on a 5-ha plot at the University of Wisconsin, Arlington Experimental Station (Arlington). At harvest, the plot was divided into quadrants. Equal quantities of DM were removed from each of the four quadrants during harvest at each of the four stages of maturity. The harvest time was based on visual assessment of kernel milkline positioning. Harvest of WPC was at early dent (approximately half of kernels dented) (**ED**), quarter milkline (**1/4 ML**), 2/3 ML, and BL stages. After harvest at the ED stage in late August 1994, harvest of corn at 1/4 ML, 2/3 ML, and the BL stages was at 13-, 10-, and 20-d intervals, respectively. Whole-plant corn was harvested using a Gehl 8 knife chopper (model 860; Gehl, West Bend, WI) set at a 0.64-cm theoretical length of cut. Approximately 15 tonne of DM from each of the four maturities were stored in individual silo bags. Fermentation was for at least 1 mo before the bags were opened to start the feeding trial.

Twenty multiparous Holstein cows averaging 75 DIM at trial initiation were randomly assigned to treatment in a replicated 4 × 4 Latin square design with 28-d periods. The first 14 d of each experimental period were for diet adaptation; sampling was during d 15 to 28 of each period. Diets containing 50% forage and 50% concentrate (DM basis) were fed as a TMR once daily. The forage portion of the diet consisted of 67% corn silage and 33% alfalfa silage (DM basis). Treatment diets contained silage from corn harvested at the ED, 1/4 ML, 2/3 ML, or BL stages. Corn silages were removed from the silo bags and hauled to the University of Wisconsin, Madison Dairy Cattle Center every 3rd d. Upon delivery, dry buffered propionic acid (Myco Curb®; Kemin Inc., Des Moines, IA) was mixed by hand with each silage at the rate of 0.5% (as-fed basis) to inhibit aerobic deterioration during feedout. All cows received the same grain mix (Table 3), which was formulated to provide 18% CP (DM basis) in the diet and to meet or exceed NRC (19) allowances for minerals and vitamins.

Cows were milked twice daily, and production was recorded at each milking. Milk weights recorded during d 15 to 28 of each period were used for data analysis. Milk fat and protein concentrations were determined on a.m. and p.m. samples obtained on 3 consecutive d during the last week of each period by infrared analysis (Wisconsin DHI Laboratory, Appleton). Mean daily milk composition was an average of a.m. and p.m. samples using the proportion of daily production at each milking as a weighting factor. Body weight was recorded at the same time after the a.m. milking on 3 consecutive d at the start of the trial and on d 26 to 28 of each period. Amounts of feed offered and orts were recorded daily.

Corn silage and alfalfa silage DM were measured weekly using toluene distillation (4) for adjustment of the diet. Alfalfa silage, corn silages, and concentrate were sampled weekly during the last 2 wk of each period and composited by treatment within period for nutrient analyses. Orts were sampled on d 26 to 28 of each period and composited by cow within period. Samples were placed in a 60°C forced-air oven for 48 h and then ground through a Wiley mill (2-mm screen; Arthur H. Thomas, Philadelphia, PA). Feed and ort composites were analyzed for DM, OM, CP (2), ADF (8), sulfuric acid lignin (25), and NDF (25) using α-amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite. Measurement of starch and free glucose on feed and ort samples was by endoamylase and exoglucosidase incubation prior to the use of a glucose oxidase assay (11).

Corn silages were sampled upon delivery to the Dairy Cattle Center during the last 2 wk of each period, composited by period, and then analyzed for pH, lactic acid, VFA, and ethanol. Silage pH was determined as follows: approximately 50 g of duplicate samples were diluted with distilled water to 200 g in a blender jar. Samples were macerated for 30 s, macerated samples were filtered through two layers of cheesecloth, and pH was measured using a glass electrode pH meter (Corning no. 150; Corning Science Products, Corning, NY). Aliquots of the filtered extract (30 ml) were centrifuged at 25,000 × g for 30 min. Collected supernatants were frozen at −20°C until analyzed for organic acids and ethanol by HPLC (Varian Instrument Group, Walnut Creek, CA) as described by Muck and Dickerson (17).

Chopped fresh WPC samples (400 g) were obtained from the second, fourth, and sixth loads of each maturity stage as they were delivered to the bagger. Lactic acid bacteria (**LAB**) counts were determined immediately on 10 g of chopped fresh material taken from a composite of the three samples from each stage of maturity. Test material was placed in a sterilized blender jar, diluted with autoclaved distilled water, and then blended for 30 s. A 0.1% peptone solution was used for duplicate sets of serial dilutions from each sample. A pour-plate technique was used for LAB counts with Rogosa SL agar (Difco no. 0480; Difco Laboratories, Detroit, MI). Duplicate plates were used at each 10× dilution between 10^1 and 10^7 so that there were four plates per dilution from each sample. Plates were incubated in an 85% N_2, 10%

TABLE 1. Chemical composition of corn silages.

Item	Stage of maturity[1]			
	ED	1/4 ML	2/3 ML	BL
	(% of DM)			
Moisture	69.9	67.6	64.9	58.0
CP	7.5	7.3	7.1	7.0
NDF	52.0	44.4	40.5	41.3
ADF	32.0	27.1	23.9	24.2
Lignin	3.3	2.8	2.9	2.7
Starch	18.2	28.7	37.2	37.4

[1]Silages are designated by stage of maturity of whole-plant corn at harvest: ED = early dent, 1/4 ML = quarter milkline, 2/3 ML = two-thirds milkline, and BL = black layer.

CO_2, and 5% H_2 anaerobic environment at 30°C for 48 h.

Apparent total tract digestibilities of DM, OM, CP, ADF, and starch were determined using Yb as an external marker. A Yb solution (23) was sprayed onto wheat middlings. Each cow received 90 g of marked wheat middlings in the diet on d 21 to 28 to provide approximately 35 ppm in the ration DM. Fecal samples were collected daily at 1000 and 2200 h during the last 3 d of each period. Fecal samples were dried in a forced-air oven at 60°C for 72 h and then ground through a Wiley mill (2-mm screen). A fecal composite was made for each cow within period and analyzed for DM, OM, CP, ADF, and starch as previously described. The concentration of Yb in duplicate fecal samples was determined by direct current plasma emission spectroscopy (3) after dry-ashing at 500°C for 16 h. Apparent nutrient digestibilities in the total tract were calculated using Yb and nutrient concentrations in diet, ort, and fecal samples.

Performance and digestibility data were analyzed using the general linear models procedure of SAS (21) for a replicated Latin square design. All mean comparisons were by the least significant difference method after a significant ($P < 0.05$) treatment effect. Significance of effects was designated at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Chemical compositions of treatment silages are presented in Table 1. Moisture content declined from 69.9 to 58.0% as maturity of the corn advanced from the ED stage to the BL stage. This trend was also reported by Hunt et al. (13) and is related to kernel development (1).

Concentrations of NDF and ADF declined from 52.0 to 41.3% and from 32.0 to 24.2%, respectively, as maturity advanced from the ED stage to the BL stage. This decline was related to the increase in the proportion of grain in WPC as it matured (1). The paradox of corn silage is that, although the fiber content of the stover increases as maturity advances, the fiber content of WPC declines because the proportion of grain in WPC increases (6). No further decline in NDF or ADF was detected as maturity increased from the 2/3 ML stage to the BL stage, probably because increased fiber content of the stover offset any increase in the proportion of the grain after the 2/3 ML stage. Similar trends for NDF and ADF have been reported by others (26, 27). Lignin content was highest for silage from corn harvested at the ED stage and was not different for silage from corn harvested at the 1/4 ML, 2/3 ML, or BL stages. Higher lignin content of the silage from corn harvested at the ED stage was likely due to a lower proportion of grain in WPC. Starch content increased as maturity progressed from the ED stage to the 2/3 ML stage, but there was no difference between the 2/3 ML stage and the BL stage. This result agreed with the trends observed for NDF and ADF and was likely related to changes in the proportion of grain in WPC.

Silage pH and organic acid concentrations are presented in Table 2. Silage pH was lower for silage from corn harvested at the ED stage than that for silage from corn harvested at the 2/3 ML or BL stages. Lower pH for high moisture silages was expected because of higher concentrations of water-soluble carbohydrates and more extensive fermentation (5, 16). Lactate concentrations increased as moisture content increased. Lactate concentration was higher for silage from corn harvested at the ED stage than for silage from corn harvested at 2/3 ML or the BL stage. This result reflects silage pH differences and was expected because of higher concentrations of water-soluble carbohydrates (5, 16). Silage pH values and lactate concentrations were indicative of adequate preservation (16, 20). Differences in lac-

TABLE 2. pH and organic acid concentrations of corn silages.

	Stage of maturity[1]			
	ED	1/4 ML	2/3 ML	BL
pH	3.73	3.98	4.11	4.10
Organic acids, % of DM				
Lactate	5.55	4.67	4.15	3.95
Acetate	1.24	0.92	0.85	1.12
Propionate	0.22	0.40	0.44	0.47
Succinate	0.21	0.22	0.21	0.14
Ethanol	0.87	0.23	0.14	0.17

[1]Silages are designated by stage of maturity of whole-plant corn at harvest: ED = early dent, 1/4 ML = quarter milkline, 2/3 ML = two-thirds milkline, and BL = black layer.

TABLE 3. Ingredient and nutrient composition of the diet.

Ingredient				
				(% of DM)
Forage				
Corn silage				33.5
Alfalfa silage[1]				16.5
Concentrate[2]				
Shelled corn				26.5
Soybean meal (44% CP)				18.6
Meat meal				1.7
Urea				0.2
Dicalcium phosphate				0.4
Sodium bicarbonate				0.8
Trace-mineralized salt[3]				0.4
Limestone				0.8
Dynamate®[4]				0.2
Magnesium oxide				0.2
Vitamin premix[5]				0.2
	Stage of maturity[6]			
Nutrient	ED	1/4 ML	2/3 ML	BL
	(% of DM)			
OM	91.5	91.6	91.6	91.7
CP	18.2	18.1	18.0	18.0
NDF	29.1	26.5	25.2	25.5
ADF	18.7	17.1	16.1	16.1
Starch and free glucose	28.8	32.3	35.1	35.2

[1]Contained 21.8% CP and 31.1% ADF (DM basis).

[2]Contained 24.0% CP and 5.8% ADF (DM basis).

[3]Contained 0.55% Mn, 0.55% Zn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, and 0.002% Co.

[4]Pitman-Moore, Inc. (Mundelein, IL).

[5]Contained 2665 IU/g of vitamin A, 900 IU/g of vitamin D, and 3.52 IU/g of vitamin E.

[6]Silages are designated by stage of maturity of whole-plant corn at harvest: ED = early dent, 1/4 ML = quarter milkline, 2/3 ML = two-thirds milkline, and BL = black layer.

tate concentrations between silages were not related to LAB counts in the fresh WPC at ensiling. The LAB counts for silages from corn harvested at the ED, 1/4 ML, 2/3 ML, and BL stages were 5.91, 5.59, 6.57, and 5.94 $\log_{10}$ cfu/g of wet crop, respectively (data not presented).

Silage pH and lactate concentrations varied little across periods for corn harvested at the ED and BL stages. However, a higher pH coinciding with a lower lactate concentration was observed in period 2 for silages from corn harvested at the 1/4 ML and 2/3 ML stages. This increase was particularly apparent for silage from corn harvested at the 2/3 ML stage when pH reached 4.5 as lactate concentration declined to 2.3% of DM, which coincided with a winter warming trend during period 2 that might have affected aerobic stability. Reduced aerobic stability for silage from corn harvested at the 2/3 ML stage might have been caused by the bursting of the silo bag during the 1st wk of the ensiling process. The bag was resealed immediately, but the introduction of oxygen into the bag might possibly have made this silage more prone to aerobic instability (18). This aerobic instability was only apparent during the warming trend of period 2, and pH decreased, and lactate concentrations increased, to their original levels as observed during period 1 levels for periods 3 and 4. Despite problems with aerobic stability during period 2 for silage from corn harvested at the 2/3 milkline stage, DMI for cows fed all treatments were high, averaging 3.76% of BW (Table 4).

The ingredient and nutrient composition of experimental diets is presented in Table 3. Dietary CP concentration was similar across the four diets ranging from 18.0 to 18.2% (DM basis). Dietary NDF and ADF concentrations decreased, and starch concentration increased, as corn maturity advanced from the ED stage to the BL stage. These nutrients followed similar trends in the diets as in the silages. Concentrations reached a plateau at the 2/3 ML stage, and no further changes were detected as maturity advanced to the BL stage. Concentrations of NDF and ADF in the diet containing silage from corn harvested at the ED stage were similar to NRC (19) recommendations, but these concentrations were below NRC (19) recommendations for concentrations of NDF and ADF in diets containing silage from corn harvested at the 1/4 ML, 2/3 ML, and BL stages, which reflected the constant inclusion of corn silage in all diets and decreasing NDF and ADF concentrations as maturity advanced.

Body weight, DMI, and milk production data are presented in Table 4. Body weight and DMI were similar across the four treatments, ranging from 676 to 688 kg and from 3.73 to 3.79% of BW, respectively. Huber et al. (12) reported silage DMI at 1.88, 2.02, and 2.16% of BW for 25.4, 30.3, and 33.3% DM corn silages, respectively. Those results suggest the potential for lower DMI of high moisture corn silages, possibly related to their lower pH (22). However, Shaver et al. (22) reported higher DMI of corn silage that was partially neutralized with sodium bicarbonate prior to feeding. In our trial, the addition of sodium bicarbonate to the diet and the lower inclusion rate of corn silage [33% of dietary DM vs. 60% of dietary DM in the study of Huber et al. (12)] could have possibly alleviated the intake depression associated with the inclusion of high moisture corn silages in the diet.

Milk production was highest for cows fed the silage from corn harvested at the 2/3 ML stage and lowest for cows fed the silage from corn harvested at the ED stage ($P < 0.07$). Milk production was numerically

TABLE 4. Effect of corn maturity for use as silage in the diets of dairy cows on DMI, BW, and milk and milk components.

Item	Stage of maturity[1]				SEM
	ED	1/4 ML	2/3 ML	BL	
DMI					
kg/d	25.5	25.7	25.7	25.6	0.4
% of BW	3.75	3.73	3.77	3.79	0.04
BW, kg	683	688	683	676	3
Production, kg/d					
Milk	32.4[b]	32.6[ab]	33.4[a]	32.7[ab]	0.4
4% FCM	30.5	30.1	30.5	30.4	0.5
Milk fat	1.17	1.14	1.14	1.15	0.02
Milk protein	1.12[d]	1.12[d]	1.17[c]	1.13[d]	0.01
Composition, %					
Milk fat	3.60	3.54	3.43	3.52	0.05
Milk protein	3.49	3.48	3.50	3.48	0.02

[a,b]Means in the same row with different superscripts differ ($P < 0.07$).

[c,d]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Silages are designated by stage of maturity of whole-plant corn at harvest: ED = early dent, 1/4 ML = quarter milkline, 2/3 ML = two-thirds milkline, and BL = black layer.

(0.7 to 0.8 kg/d), but not statistically ($P > 0.10$) higher for cows fed silage from corn harvested at the 2/3 ML stage than for cows fed silage from corn harvested at the 1/4 ML or BL stages. Huber et al. (12) reported increased milk production as maturity of WPC advanced from the soft dough stage to the hard dough stage; silage DM concentrations of 25.4, 30.3, and 33.3% coincided with increases in DMI. Harrison et al. (10) reported higher milk production for cows fed WPC harvested at the one-half ML stage and fed as silage versus WPC harvested at the BL stage and fed as silage. There were no differences in milk fat percentage or production across the four treatments. Milk protein production was highest ($P < 0.05$) for cows fed silage from corn harvested at the 2/3 ML stage, possibly because of higher starch content of this silage than that of silage from corn harvested at the ED and 1/4 ML stages (Table 3) and the higher starch digestibility of silage from corn harvested at the 2/3 ML stage than that of silage from corn harvested at the BL stage (Table 5).

Apparent total tract nutrient digestibilities are presented in Table 5. Digestibilities of DM and OM were similar for cows fed silages from corn harvested at the ED, 1/4 ML, and 2/3 ML stages. This result is somewhat surprising because dietary ADF content decreased, and starch content increased, as corn maturity advanced from the ED stage to the 2/3 ML stage. However, this relationship can be explained by the decline ($P < 0.05$) in ADF and starch digestibilities as corn maturity advanced. The decline in ADF digestibility could be related to negative associative effects of higher starch diets on ruminal fiber digestion (9) or lower digestibility of stover as WPC matured (26). The decline in starch digestibility could be related to lower efficiency of postruminal starch

TABLE 5. Effect of corn maturity for use as silage in the diets of dairy cows on apparent total tract nutrient digestibilities.

Item	Stage of maturity[1]				SEM
	ED	1/4 ML	2/3 ML	BL	
	(%)				
DM	61.8[a]	62.1[a]	61.4[a]	58.5[b]	0.6
OM	65.2[a]	64.9[a]	63.8[a]	60.4[b]	0.7
CP	64.9[a]	63.8[a]	62.5[a]	56.1[b]	1
ADF	45.7[a]	38.3[b]	33.6[c]	29.4[d]	1.4
Starch	94.1[a]	92.9[ab]	92.2[b]	87.7[c]	0.6

[a,b,c,d]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Silages are designated by stage of maturity of whole-plant corn at harvest: ED = early dent, 1/4 ML = quarter milkline, 2/3 ML = two-thirds milkline, and BL = black layer.

digestion for cows fed higher starch diets (24) or more whole kernel passage from the lower moisture corn silages (10). Digestibilities of DM and OM were lowest ($P < 0.05$) for cows fed silage from corn harvested at the BL stage, which was related to lower ($P < 0.05$) digestibilities of CP, ADF, and starch for this treatment. Lower ADF and starch digestibilities for the silage from corn harvested at the BL stage relative to the silage from corn harvested at the 2/3 ML stage might be related to lower stover digestibility (26) and greater whole kernel passage (10), respectively, because dietary ADF and starch concentrations were similar. Dietary starch digestibility declined 6 percentage units between the ED stage and the BL stage. Calculated by difference, this decline represents a 20 percentage unit drop in starch digestibility for silage from corn harvested at the BL stage. Mechanical processing of corn silage prior to ensiling has been shown to increase milk production and reduce whole kernel passage (14) and would likely have improved performance of the silage from corn harvested at the BL stage. Intakes of digestible starch were 6.9, 7.7, 8.3, and 7.9 kg/d for cows fed silages from corn harvested at the ED, 1/4 ML, 2/3 ML, and BL stages, respectively (data not presented). Although starch intakes were similar for silages from corn harvested at the 2/3 ML and BL stages (9 kg/d), intake of digestible starch was 0.4 kg/d lower for cows fed silage from corn harvested at the BL stage.

CONCLUSIONS

Milk and milk protein production were, respectively, 1 and 0.05 kg/d higher for cows fed silage from corn harvested at the 2/3 ML stage than for cows fed silage from corn harvested at the ED stage. There were no differences in milk production among cows fed silages from corn harvested at the 1/4 ML, 2/3 ML, and BL stages. This result suggests that there is some flexibility in harvesting corn between the 1/4 ML and BL stages. However, milk protein production was 0.04 to 0.05 kg/d higher for cows fed silage from corn harvested at the 2/3 ML stage relative to those fed silage from corn harvested at the 1/4 ML and BL stages. Also, apparent total tract starch digestibility and digestible starch intake were lowest for cows fed silage from corn harvested at the BL stage, which could translate into lower milk production or BW gain in a longer term feeding trial or in a trial with higher producing cows. Our data suggest that 2/3 ML (65% moisture) was the optimum maturity stage for harvesting corn for use as silage in the diets of lactating dairy cows when the diets were formulated to have a fixed forage to concentrate ratio. Some flexibility did exist between 1/4 ML and 2/3 ML (65 to 68% moisture).

ACKNOWLEDGMENTS

The authors thank Dwight Mueller for supervision of corn production and harvest, Leland Danz for the feeding and care of the cows, and Sandra Bertics for technical support in the laboratory. Appreciation is extended to R. E. Muck for assistance with organic acid analysis and microbial counts of the silage.

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