Effect of Supplemental Light on Growth, Prolactin, Progesterone and Luteinizing Hormone in Water Buffalo (Bubalus bubalis)

by

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(ABSTRACT)

Fifty Surti buffalo heifers between 17 and 42 mo of age (n = 24, < 24 mo; n = 26, > 24 mo) were used to study the changes in body weight, prolactin (Prl), progesterone (P_4) and luteinizing hormone (LH) as a result of supplemental lighting. Buffalo were randomly assigned to either natural day light + 4 hr supplemental light (S; n = 25) or natural day light (C; n = 25) groups on d 11 of the experiment. Data on individual body weights (BW) and ovarian structures were obtained weekly beginning from d 1 and d 14, respectively. Jugular vein blood samples were obtained on alternate days from d 1 to d 10, and twice weekly from d 11 to d 98 to determine serum Prl, P_4 and LH. Between d 99 and d 105 blood samples were obtained at -30, -15, 0, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min post gonadotropin releasing hormone (GnRH) administration (30 μg) to determine LH response. Meteorological data were recorded throughout the study. Data on conception of heifers were obtained within 6 mo of the end of the study.

Day-light ambient temperatures and relative humidity generally were >27 C and <70%, respectively. Heavy precipitation during the 11th and

12th wk interrupted the drought. S group animals had 5.8% heavier ($p < 0.01$) BW, 6.1 kg net BW gain ($p < 0.01$), higher mean Prl (42.6 ± 0.2 vs 40.1 ± 0.2 ng/ml; $p < 0.01$), a Prl increase (35.5 ± 2.0 ng/ml to 46.0 ± 1.8 ng/ml vs 41.7 ± 2.3 ng/ml to 42.8 ± 2.2 ng/ml; $p < 0.01$), higher P_4 (0.39 ± 0.02 vs 0.18 ± 0.02; $p < 0.07$), and higher mean LH (0.52 ± 0.01 vs 0.46 ± 0.01; $p < 0.06$) than C. Older heifers had 67% greater BW ($p < 0.01$) during the treatment period, but a net BW loss (-7.8 kg vs 4.6 kg; $p < 0.01$), higher mean Prl (49.0 ± 0.2 vs 33.7 ± 0.2; $p < 0.01$), P_4 (0.39 ± 0.02 vs 0.18 ± 0.02; $p < 0.07$) and LH (0.54 ± 0.01 vs 0.44 ± 0.01; $p < 0.01$) compared to younger heifers. Older light supplemented heifers had higher mean P_4 (0.39 ± 0.02 vs 0.18 ± 0.02; $p < 0.07$) than the other groups. Peak response to GnRH was reached at 30 min post GnRH. Older control heifers had highest peak response ($p < 0.01$) to GnRH.

These weight and hormonal changes suggest that 4 hr supplemental light can stimulate body weight gain, pituitary function and ovarian activity in peri-pubertal buffalo under the existing planes of nutrition. High rainfall and humidity adversely affected ($p < 0.01$) BW. The S heifers had a greater rate of weight loss at rainfall above 60 mm than did C heifers. Rainfall had a negative effect on Prl and LH. Further investigations should be conducted to evaluate the optimum age range and environmental characteristics which affect supplemental light administration.

DEDICATION

This dissertation is dedicated to my dearest parents

late and

with all my love and respect

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CHAPTER 1

INTRODUCTION

Water buffalo (Bubalus bubalis) have become an integral part of Asian agriculture because of their versatility as a work animal and ability to serve as a multi-purpose animal providing milk and meat. Despite its long history of serving mankind, very little attention has been paid to the improvement of this species. Nonetheless, the recent development programs of Asian countries have stressed the economic importance and unexploited potential of water buffalo to improve agricultural productivity.

Attempts to increase buffalo production require special attention to efficiency of reproduction. Indices of reproduction such as age at puberty, age at first calving, conception rate and calving interval provide a means of assessing reproductive efficiency. However, due to the very short history of water buffalo research, available information regarding such aspects of reproduction is of limited value and scarce as compared to that of cattle. This is especially true in the case of pubertal aspects of reproduction.

Available research indicates that buffalo take considerably more time to attain puberty than cattle. This is unfortunate because the age at puberty plays a pivotal role in determining the fertile life span of the animal. Hence, it is imperative to explore the means of hastening the onset of puberty of buffalo. Sufficient data are not available on the factors affecting the onset of puberty of water buffalo. Because of the

similarities in anatomy of reproductive tract, cyclic hormonal changes and postpartum hormonal profiles to those of cattle, it could be possible that factors which modify the pubertal onset in cattle could act the same way to affect sexual maturity of buffalo.

Nutrition and photoperiod are important regulators of onset of puberty in cattle. Under the current situation in many buffalo rearing countries such as Sri Lanka, an immediate improvement in the plane of nutrition for buffalo cannot be expected. Thus, the attempts to improve reproductive efficiency must emphasize other managerial aspects. Supplemental lighting (total day length 16 hr) improved the growth rate and hastened the sexual maturity of prepubertal Holsteins even under low planes of nutrition when the natural day length was less than 13 hr. In tropical countries such as Sri Lanka, where natural day length does not exceed 13 hr and plane of nutrition for livestock is low, such manipulation of photoperiod may be used as a management tool if proven to be beneficial. However, no attempt has been made to evaluate the influence of photoperiod and supplemental lighting on growth and puberty of buffalo. Hence, the prime objectives of this study were :

1. to assess the body weight response of pre-pubertal Surti buffalo heifers of different ages to 4 hr of supplemental lighting under tropical climatic conditions,

2. to characterize changes in circulating prolactin, progesterone and luteinizing hormone concentrations in response to supplemental lighting,

3. to evaluate the pituitary response to gonadotropin releasing hormone in pre-pubertal buffalo heifers of different ages under conditions of supplemental lighting, and

4. to relate the above mentioned changes to changes in ambient temperature, relative humidity and rainfall.

CHAPTER 2

LITERATURE REVIEW

2.1 CLASSIFICATION

Water buffalo (Bubalus bubalis) belong to the same family (Bovidae) and tribe (Bovini) as cattle, but appertain to a different category as the tribe of bovini is divided into groups based on the fundamental anatomical differences and inability of interbreeding among groups. Of the three groups of the bovini tribe, Bovina, cattle; Syncerina, African buffalo; Bubalina, Asian buffalo; only the last consists of the genus Bubalus. Species belonging to this genus include: arnee (Indian wild buffalo); anoa (Indonesian wild buffalo); bubalis (domesticated water buffalo); and tamarao (Phillipine wild buffalo). The bubalis is reported to have developed over the centuries from its wild ancestor arnee (Mason, 1977).

There are two general types of domesticated water buffalo, the swamp buffalo and the river buffalo (Macgregor, 1941). Even though, the diploid numbers of chromosomes are different in the two types, being 48 in the swamp buffalo and 50 in the river buffalo (Fisher and Ulbrich, 1968; Toll and Halnan, 1976; Ulbrich and Fisher, 1967), the amount of chromosomal material is similar. Thus, they can crossbreed to produce a fertile hybrid progeny having a diploid number of 49 chromosomes (Mason, 1974b).

The swamp buffalo are slate grey in color, stockily built with a large belly, and ox-like in appearance. Their horns grow outward in the plane of the forehead and then make a semi-circle. Average live weight of adult swamp buffalo ranges from 250 to 550 kg. They wallow in any water they can find or mud puddle they can make with their horns and are mainly distributed in the eastern half of Asia. Swamp buffalo are primarily employed as a work animal. Although they are not used as a commercial dairy animal, they are often milked and also used for meat purposes.

The river buffalo are black or dark grey in color, have a comparatively longer face and body, smaller girth and bigger limbs than swamp buffalo. Their horns grow downward and backward, then curve upward in a spiral. Average live weight of adult river buffalo ranges from 350 to 800 kg depending on the breed. They prefer to wallow in deep clean water and are mainly distributed in the western parts of Asia (Mason, 1974a). Several well defined dairy breeds have been evolved as a result of selection and breeding over generations in India and Pakistan. At present, there are 16 recognized breeds of river buffalo in south Asia and they are classified further into five major groups: Murrah; Gujarat; Uttar Pradesh; Central Indian; and South Indian. The different breeds belonging to each group are given below.

Group		Breeds
Murrah	------	Murrah, Nili-Ravi, Kundi
Gujarat	------	Surti, Mehsana, Jafarabadi

Uttar Pradesh ------ Bhadawari, Tarai

Central Indian ------ Nagpuri, Pandharpuri, Manda, Jerangi, Kalahandi, Sambalpur

South Indian ------ Toda, South Konara (Mason, 1974a)

However, it should be emphasized that despite of above mentioned breeds of pedigreed water buffalo, most are nondescript animals that have not been selected or bred for productivity.

2.2 DISTRIBUTION

2.2.1 World

The domesticated water buffalo numbers 150 million, approximately one-eighth the world population of cattle (Cockrill, 1979). Almost 95% of them are distributed in the tropical regions including India, which alone has approximately half (> 60 million) the world buffalo population. China has the second largest buffalo population (30 million) while, Pakistan has the third largest population of 10 million. Thailand and the Philippines have approximately 5 million each. The remaining buffalo are distributed in many countries such as Nepal (4 million), Indonesia (3 million), Vietnam (2 million), Egypt (2 million), and Burma (2 million) in numbers less than 5 million (Mahadevan, 1984). The western countries such as Bulgaria, Italy, Brazil, and the USSR have smaller population for special products like cheese. In countries where buffalo are already economically important they are increasing in numbers faster than cattle.

For example, during the period between 1961 and 1972 the buffalo population in India increased by 13.1% as compared with a 1.9% increase in the cattle population. Similarly, in Pakistan there was a 7% increase in the number of buffalo from 1972 to 1978 in comparison to a 1.5% increase in the cattle population (Mahadevan, 1984). A two percent increase in world buffalo population has been estimated between 1964 and 1974 (Cockrill, 1977).

2.2.2 Sri Lanka

Sri Lanka, a tropical country located south of India, has about 0.7 million buffalo representing 30% of the total livestock population. The majority of them are indigenous buffalo, commonly referred to as Lanka buffalo. They are smaller in size than buffalo indigenous to many other south-east Asian countries, rarely exceeding 320 kg in mature weight (Cockrill, 1977). They assume phenotypic characteristics of swamp buffalo, and have been categorized as belonging to the swamp type (Macgregor, 1941). However, the karyotype of Lanka buffalo was reported similar to that of river buffalo possesing 50 diploid number of chromosomes (Bongso et al., 1978). Thus, it was hypothesized that the Lanka buffalo was a descendant of river type, which changed phenotypically to assume characteristics of the swamp type (Bongso et al., 1978). Although the national buffalo herd is distributed throughout the country, it is more concentrated in the north-western, north-central, and eastern provinces (Figure 1).

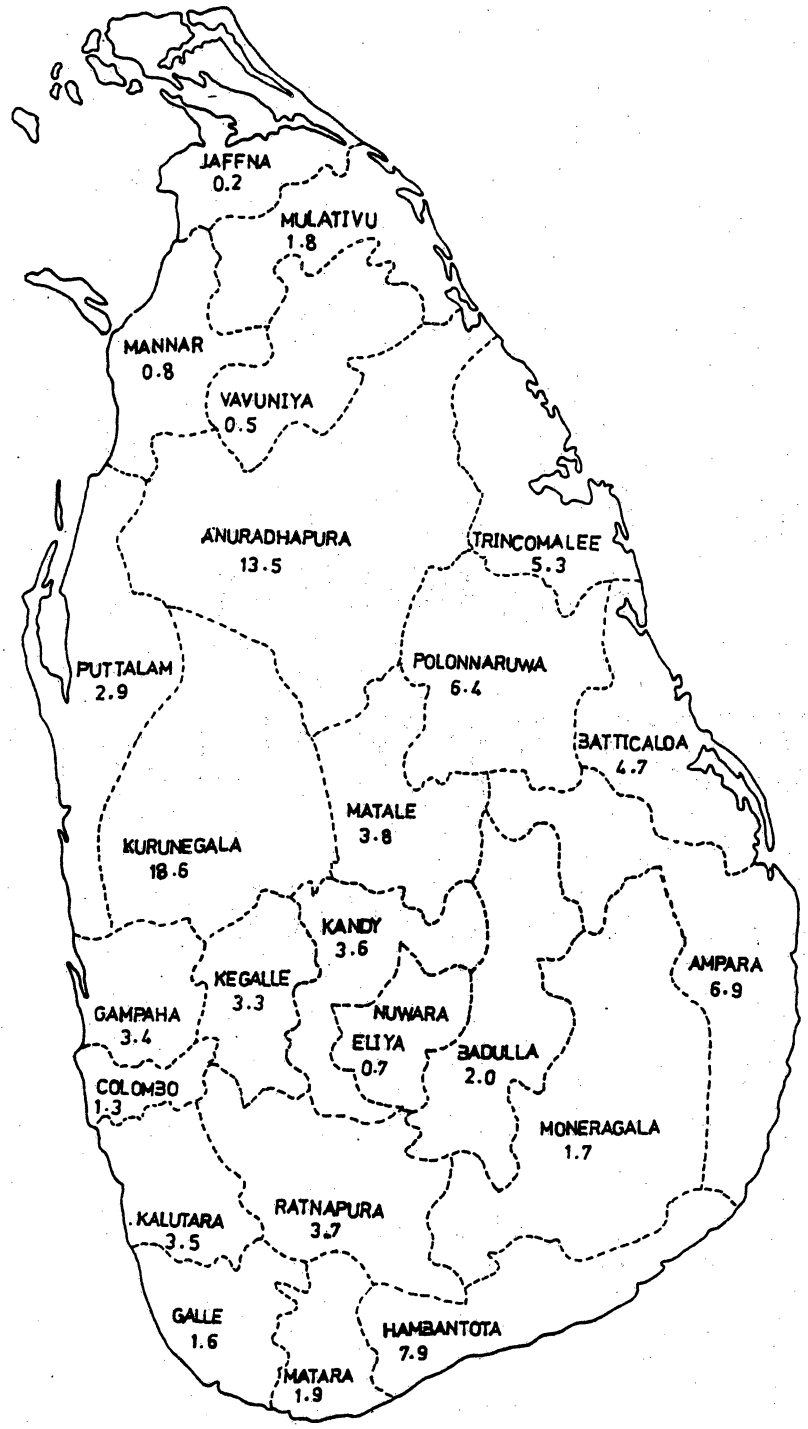

Figure 1. Distribtution of national buffalo population in Sri Lanka.

2.3 IMPORTANCE

2.3.1 Work

For several thousand years the water buffalo has been recognized as a domestic work animal. It is the classic work animal of Asia, an integral part of that continent's traditional village farming structure. Buffalo are probably the most versatile of all work animals as they are used for ploughing, harrowing, leveling of land, puddling, hauling, threshing grain, riding, milling, and packing purposes. In Sri Lanka with recently opened large new tracts of farmland in the valley of the Mahaweli River, the demand for such working animals is becoming increasingly important. Comparatively larger hooves, more flexible pastern and fetlock joints, slower ponderous gait and greater ability to work are the advantages of this species over cattle (De Silva et al., 1985).

The work capacity of buffalo has been estimated by several researchers. Lin (1975) reported that a mature buffalo was able to pull a load considerably greater than twice its body weight. An average buffalo was capable of ploughing 0.25 ha in a working day of 7 to 8 hr and could cope with 3 ha of paddy field in a cultivating season. Similar observations have been made by other researchers (Cockrill, 1974). A pair of buffalo weighing 600 kg or more could transport a load of 250 kg at a speed of 3 km/hr in a working day of 10 hr (Cockrill, 1981) which included 2 rest periods. Kumaratilaka and Buvanendran (1979) estimated the work capacity of Sri Lanka buffalo for ploughing, puddling, and

threshing of 0.1, 0.05, and 0.06 ha per buffalo per day, respectively. Siriwardena et al. (1982) observed that a pair of buffalo could plough an average of 0.2 ha per day.

Under utilization of buffalo for work has been reported by many workers (Cockrill, 1974). In Taiwan, an average buffalo worked about 53 d/yr (Lin, 1975) while, in Thailand the number of working days varied between 66 and 146 d/yr with an average of 122 d (Buranamanas, 1963). In Sri Lanka, buffalo were used for ploughing only over a period of 4 to 8 wk each cultivating season (Siriwardena et al., 1982) while, the actual work was performed between 6.5 and 7 hr per pair per week. The peak weekly average was 17 to 22 hr (Ryan et al., 1981).

2.3.2 Milk

In addition to serving mankind as a work animal, buffalo play an important role in the economy of several countries as a dairy animal. Two outstanding examples are India and Pakistan. In India, although the proportion of buffalo to cattle is 1:3, the buffalo contributes over 60 percent of the milk produced in the country (Cockrill, 1979). In Pakistan, about 70 percent of the milk produced comes from buffalo, although they are outnumbered by cattle by 30 percent. In Egypt, where buffalo and cattle are almost equal, buffalo contribute about 65 percent of the milk produced annually (Mahadevan, 1977).

Depending upon the breed, the environmental conditions, and the management practices the daily milk yield of lactating buffalo ranges from

a low of 1 to 4 liters produced by the actively working draft female to 16 or more litres from quality dairy animals (Cockrill, 1981). The length of lactation is usually 270 to 300 d (Cockrill, 1979), and the yield drops sharply after the third or fourth lactation (Cockrill, 1974). Persistence of milk yield, when measured using the ratio of the amount of milk produced in the second 100 days of lactation to that produced in the first 100 days of lactation, was 0.83 in the first lactation and 0.75 for subsequent lactations. The average figure for all lactations was 0.76 (Maymone and Malossini, 1961).

Several figures are available on the average daily milk yield of indigenous Lanka buffalo under existing management conditions (with once a day milking, calf reared with the dam, and no supplementary feeding apart from grazing). Wijeratne (1962) reported a daily milk yield of 1.5 kg and Kumaratilake and Buvanendran (1979) reported 2.4 liters, while De Silva et al. (1985) estimated 1.1 to 1.9 liters. This low production level was considered due to low genetic potential since no improvement was noticed under better management and feeding. However, a rapid gain in milk yield can be obtained by crossbreeding to dairy breeds such as Murrah and Surti (Frisch and Vercoe, 1984). Where this has been done, yields have increased from 350 kg for the swamp buffalo to about 1000 kg for the F_1 swamp x river generation (Guzman, 1980). In Sri Lanka the imported Murrah breeds yield daily milk of 4.6 kg as compared to 4.2 kg of locally bred Murrah (Rajamahendran et al., 1981). The increase in milk yield of crossbreeds appear to be progressive with the increase in Murrah blood to the $7/8^{th}$ level (Jalatge, 1980). The length

of lactation also increased from 180 to 200 d in the swamp type (Kumaratilake and Buvanendran, 1979) to about 270 d in the crossbreed.

Buffalo milk contains less water, more total solids, more fat, slightly more lactose, and more protein than cow milk (Table 1). It lacks carotene and thus is more whitish in color than European and Zebu cow milk. However, the vitamin A content is similar to that of European and Zebu cow milk. B complex vitamins (except riboflavin) and vitamin C content are also similar to cow milk (BOSTID, 1981). The richness of buffalo milk allows for a greater price over cow milk in countries where it is consumed. A great variety of butters, including the dehydrated clarified form known as ghee, and cheeses such as Italian mozzarella and ricotta, gemir of Iraq, cincho of Venezuela, and pecorino of Bulgaria are made from buffalo milk. The richness of buffalo milk makes it highly suitable for processing. To produce 1 kg of cheese usually 8 kg of cow milk is required whereas only 5 kg of buffalo milk will serve the same purpose. Similarly, 1 kg butter could be produced with 10 kg buffalo milk as compared to 14 kg of cow milk (BOSTID, 1981).

2.3.3 Meat

Besides its capability as a work and dairy animal, the water buffalo serves as a potential source of quality meat. For example, in India 60% of the meat obtained from cattle and buffalo come from buffalo (Mahadevan, 1977). However, the potential of buffalo as a meat animal has never been adequately investigated despite the long time awareness

Table 1. Comparison between buffalo milk and cow milk.

	Buffalo	European cow	Zebu cow
Fat%	7.64	3.9	4.97
Protein%	4.36	3.47	3.18
Lactose%	4.83	4.75	4.59
Total solids	17.96	12.82	13.45

Source: The water buffalo. FAO Animal production and health series: No 4

of its importance as a work and dairy animal. Because buffalo have been used as draft animals for centuries, they have evolved with exceptional muscular development. However, since most buffalo meat is derived from old animals slaughtered at the end of their productive life as dairy or work animals, the meat is of poor quality. When they are properly reared and fed, the meat is tender and palatable (Cockrill, 1979).

The advantages of developing buffalo for meat purposes are numerous. Buffalo are more efficient converters of coarse feed-stuff than cattle, due to the ability to digest more lignin and cellulose (Bhatia et al., 1979; Langar et al., 1984). A total crude fiber content of 25 to 26% in the oat-silage based ration has been recommended for optimum nutrient utilization (Chauhan et al., 1985).

Buffalo veal is an esteemed delicacy in Egypt and Italy, where it commands a comparatively high market price. In one veal production trial Egyptian buffalo calves had an average birth weight of 41 kg and were slaughtered at 30 to 40 d of age at an average weight of 61 kg. They showed an average daily gain of 0.59 kg and a dressing percentage over 65 while, Italian buffalo calves slaughtered at 4 mo age had average carcass yield of 55 to 60 percent (Cockrill, 1977).

Water buffalo are ideal animals for feedlots. When reared and fed for slaughter at 12 to 18 mo of age, a final live weight of 300 to 350 kg could be obtained at a lower cost than cattle (Cockrill, 1981).

A comparison of Murrah and Lanka breeds of buffalo and Friesian, Red Sindhi, and local cattle in the dry zone of Sri Lanka revealed a faster growth rate from 8 to 22 mo of age in buffalo than in cattle (Matsukawa

et al., 1976). Another study using Buffalo, Red Sindhi, and Friesian steers at 12 mo of age reported daily live weight gains of 0.51, 0.31, and 0.24 kg, respectively after fattening on Guinea grass and coconut meal concentrate for 26 wk. The dressing percentage was highest for buffalo (51) compared to Red Sindhi (46) and Friesian (43) breeds (Tilakaratne et al., 1976).

Buffalo meat is similar to beef in basic properties such as structure and physical characteristics, chemical composition, and palatability (Cockrill, 1977). The muscle pH (5.4), shrinkage on chilling (2%), moisture content (76.6%), protein (19%), and ash content (1%) were similar in beef and buffalo meat. The layer of subcutaneous fat is thinner on buffalo than on comparably fed cattle. Buffalo fat is always white and meat is darker in color. Buffalo with more than 25% fat are difficult to produce. Because average choice grade beef carcasses contain 35% fat, buffalo are considered lean animals (BOSTID, 1981).

Thus, it appears that buffalo are a multipurpose animal with qualities similar to cattle, but perform better than the cattle indigenous to many tropical countries. Their popularity is evidenced by the increase in numbers faster than cattle in countries where they are already economically important. Promotion of buffalo production as an aid to meet future demands for food supply has received considerable attention in recent development program in many buffalo rearing countries including Sri Lanka.

2.4 REPRODUCTION

Reproduction is a major factor to consider in an effort to increase buffalo production. Indices such as age at puberty, age at first calving, conception rate, and calving interval are used in assessing the efficiency of reproduction. However, for the purpose of this review, only the pubertal aspects of reproduction will be emphasized with special reference to buffalo and related species such as cattle and sheep.

2.4.1 Puberty

Puberty is the transitional period between the juvenile state and adulthood during which considerable growth related physical changes and neuroendocrine related physiological changes take place. Since these changes result in the achievement of fertility, age at which the onset of puberty occurs is of major economic importance in rearing domesticated animal species. The entire sequence of events leading to, and the numerous mechanisms controlling the initiation of puberty in the female are not understood completely. However, available data on several mammalian species such as cattle, sheep, rodents, and primates indicate that the control mechanism regulating mammalian reproduction is comprised of at least three components:

1. The arcuate nucleus of the medial basal hypothalamus and its transducer neurosecretory neurons. These translate neural signals into a hormonal signal via gonadotropin-releasing hormone (GnRH).

2. The pituitary gonadotropes which, in response to the GnRH signal release of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

3. The gonads which respond to gonadotropins in terms of maturation, hormone production, and ova production.

Modulating factors could exercise their effect at any of these loci.

GnRH input to the pituitary is obligatorily intermittent as evidenced by the results of continuous versus pulsatile GnRH infusion experiments (Nakai et al., 1978; Belchetz et al., 1978). This intermittent delivery of endogenous GnRH was suggested to be the consequence of a synchronous discharge of GnRH containing neurons in response to some neuronal signal generator or oscillator (Goodman and Karsch, 1981; Knobil, 1981). The rhythmic discharge of GnRH is essential for the release of gonadotropins from the pituitary as shown by suppression of gonadotropin secretion in ovariectomized rhesus monkeys in response to intravenous administration of antisera developed against synthetic GnRH (McCormack et al., 1974). Any small change in frequency of exogenous GnRH administration exerted profound effects on gonadotropin secretion in rhesus monkeys with hypothalamic lesions (Knobil, 1980), suggesting the requirement of a certain inherently fixed frequency of endogenous

GnRH discharge for the establishment of an appropriate pulsatile pattern of gonadotropin secretion. Immature ovarian follicles respond to gonadotropin pulses by increasing in size, and secreting quantities of estrogen, which in turn control the magnitude of pituitary response to GnRH pulses (Karsch et al., 1973) via negative feed back (Beck et al., 1976; Day et al., 1984; Gonzalez-Padilla et al., 1975a; Hobson and Hansel, 1972). In addition, progesterone acts synergistically with estrogens to inhibit gonadotropin release (Beck et al., 1976; Karsch et al., 1973) probably via endogenous opiate peptides (Quigley and Yen, 1980). This pattern of gonadotropin release, which is controlled by the reciprocal changes in circulating concentrations of sex steroids is called "basal" or "tonic" secretion. In the adult female, an additional secretory pattern called "cyclic" secretion exists. The latter is a result of positive or stimulatory feedback mechanisms of sex steroids at the level of the pituitary (Pohl and Knobil, 1982). When the circulating sex steroid concentration (especially estrogen) exceeds a certain threshold value for a certain period of time, the negative feedback is interrupted and steroids exert a stimulatory action on the pituitary resulting in a surge of gonadotropins (with no requirement of any increment in GnRH stimulus), which enables ovulation to take place (Peters and Lamming, 1983).

In the post-natal female, such cyclic secretion of gonadotropins does not exist and hence, ovulation does not occur. In addition, during the infantile period the tonic gonadotropin secretion is also infrequent and the gonadal steroid secretion is very low (Levasseur, 1977; Hafs and McCarthy, 1979). Puberty is associated with increased secretion of

gonadotropins, and secretion of sex steroids in response to gonadotropins (Lacroix and Pelletier, 1979; McCarthy et al., 1979; Levasseur, 1977). Changes in peripheral gonadotropins and sex steroid hormone concentrations take place from birth to puberty (Schams et al., 1981). The pituitary of male and female calves was able to release gonadotropins after GnRH treatment at any age even when basal gonadotropin secretion was low. However the response to GnRH treatment was greater among prepubertal calves than among younger animals (Schams et al., 1981). A change in the pituitary response to estrogen negative feed back was suggested as one reason for the observed changes in gonadotropin secretion (Schillo et al., 1982; Day et al., 1984). Fertile ovulation and hence puberty also could be induced in prepubertal heifers using sex steroids (Gonzalez-Padilla et al., 1975b; Short et al., 1976) and/or gonadotropins (Seidel et al., 1971). Exogenous gonadotropin releasing hormone also has been shown to stimulate gonadotropin secretion in prepubertal heifers (Barnes et al., 1980; McCleod et al., 1984). Thus, it appears that both the pituitary as well as the ovary are competent enough and are able to respond to specific stimuli long before puberty. Nevertheless, gonadotropin secretion in the young animal is infrequent and very low in comparison to the pre-pubertal animal. Evidently, the main limiting factor which regulates the gonadotropin secretion, and hence controls the timing of onset of puberty lies at the level of the hypothalamus (Amann and Walker, 1983).

Available evidence suggest that the activity of the neuronal oscillator is initiated with a low frequency (Amann and Walker, 1983) probably due

to hyper-sensitivity to negative feedback action (Foster et al., 1985). This favors an accumulation of FSH in circulation while LH pulses are of low frequency (Wildt et al., 1981). The low LH pulses, although able to stimulate follicular growth and increases in estradiol, are not sufficient for the development of pre-ovulatory follicle and the attendant estradiol signal for the pre-ovulatory gonadotropin surge (Foster et al., 1985). During the course of puberty the activity of the neural oscillator is accelerated probably as a result of reduced sensitivity to negative feedback action (Pohl and Knobil, 1982; Reiter and Grumbach, 1982). As a consequence, GnRH pulses are released at an inherently greater frequency and the pituitary gland produces the requisite pattern of circulating gonadotropins necessary for successful completion of the first follicular phase culminating in ovulation (Foster et al.,1985). Progesterone concentrations are very low and may be undetectable in the post-natal animal. This progesterone is probably of adrenal origin (Schams et al., 1981). Circulating progesterone also increases before first estrus (Gonzalez-Padilla et al., 1975a). This progesterone rise is of ovarian origin (Beradinelli et al., 1979), and probably plays a role in the establishment of a pulsatile pattern of a gonadotropin secretion appropriate for the development of an ovulatory surge (Gonzalez-Padilla et al., 1975c; Schams et al., 1981). Thus it appears that monitoring of circulating gonadotropins (especially LH) and sex steroid concentrations will provide an indication of the onset of puberty in the female animal.

Following ovulation, the releasable pool of LH was depleted (Hansel and Convey, 1983) while the pituitary becomes refractory to GnRH stim-

ulus (Convey et al., 1981). The pituitary pool of gonadotropins can be estimated by monitoring gonadotropin concentrations in serial blood samples drawn prior to and post GnRH administration.

Not much data are available on the maturation of the hypothalamic pituitary ovarian axis of the buffalo. However, there are similarities in the anatomy of the reproductive organs of buffalo and of cattle, and similarities in the reproductive cyclic changes (Dobson and Kamonpatana, 1986). Therefore, it is reasonable to assume that the maturational changes in buffalo also are likely to follow those of cattle. The following available data on the attainment of puberty of buffalo support this assumption.

2.4.2 Puberty in Buffalo:

Puberty is probably one of the least investigated aspects of buffalo reproduction because of technical difficulties in determining accurately the age at puberty. Many earlier studies assessed age at puberty based either on the age at first calving or on the age at first detected estrus. For example, age at puberty as assessed by first detected estrus among well nourished buffalo was between 15 to 18 mo and at 198 kg body weight, while under field conditions first estrus could not be detected until 24 to 36 mo (Bhattacharya, 1974). In Sri Lanka, the first calving of buffalo occurs at a body weight of about 480 kg and between 33 and 45 mo, although many do not calve until much later (Lundstrom et al., 1982). In comparing these reports to those of cattle which attain puberty

between 7 and 12 mo and at 250 to 300 kg body weight, and deliver their first calf between 24 and 36 mo of age, it appears that buffalo takes a longer time to start their fertile reproductive life.

Recent studies on puberty in buffalo were based on examination of growth of genitalia as well as monitoring of gonadotropins and sex steroids. Morphological and histological examination of genitalia of 16 Egyptian male buffalo calves of successive ages ranging from 38 to 58 wk revealed that sexual maturity was attained between 50 and 54 wk (i.e. between 12.5 and 13.5 mo) of age (Taha et al., 1980). Another long term study conducted in northern Australia including 1229 buffalo females and 891 males killed between 1963 and 1971 revealed that age at puberty, as assessed on the basis of tooth eruption and examination of internal genitalia, was between 14 and 19 mo (Tulloch and Grassia, 1981).

In another attempt to study growth of genitalia and age at attainment of puberty, data on rectal palpation and measurements on reproductive tracts were obtained from 161 pre-pubertal, pubertal non-pregnant and post-pubertal Murrah buffalo heifers aged between 17 and 37 mo and body weight between 180 and 500 kg. The results of this study indicated that both the uterus and cervix increased in size as the body weight of the heifers increased. Sixty one percent buffalo attained puberty at 320 kg body weight as opposed to 13.5% that exhibited estrus at 280 kg body weight (Singh and Singh, 1985).

Puberty of male Egyptian buffalo defined as the age when an ejaculate containing 10×10^6 spermatozoa per ml of which 10% or more are motile was obtained, took place at a mean age of 17 mo (Hemeida and Badawy,

1981). In a later attempt to study age at puberty using sex steroids and gonadotropins as indicators, 59 Egyptian buffalo bull calves were classified into 14 age groups 1 to 2 mo apart, and weekly blood samples were collected for 4 to 7 wk to monitor circulating hormones. Serum androstenedione and testosterone were low at birth (141.3 ± 33.5 pg/ml and 18.0 ± 2.9 pg/ml, respectively). Androstenedione concentrations gradually increased from birth (141.3 ± 33.5 pg/ml) until 8 mo of age and declined thereafter, whereas testosterone remained low (26.0-40.6 pg/ml) until 8 mo of age and significantly ($p < 0.05$) increased thereafter. LH concentrations were low (2.12 ± 0.47 ng/ml) at birth and increased between 6 and 15 mo of age (3.29-3.50 ng/ml). Based on these results, it was concluded that in Egyptian buffalo bull calves the pubertal period occurs between 8 and 15 mo of age (Hemeida et al., 1985).

A similar study conducted in India used 155 male buffalo calves aged between newborn to 48 mo and classified into 17 age groups. Blood samples were collected between 0800 hr and 1000 hr from March to July 1982, and sera were analyzed to quantify testosterone, progesterone and estradiol-17β. Testosterone concentrations were < 100 pg/ml from birth until 15 mo of age, and peak concentration of 422 ± 79 pg/ml was reached between 24 and 30 mo age. This was followed by another peak of 793 ± 193 pg/ml at 42 to 48 mo of age. The two peaks corresponded to puberty and maturity, respectively (Sharma et al., 1984).

The only available data on hormonal profiles of buffalo heifers comes from India. Heifers of various ages (birth to 42 mo and classified into 15 age groups) were used for the study. Jugular vein blood samples

were obtained daily up to 3 d after birth, and once during the latter part of life in different age groups. Once detected in estrus, blood samples were obtained in the morning and evening on the day of estrus and then on 1, 3, 5, 7, 9, 11, 13, 15 and daily from 16 to 22 days after estrus exhibition. The study lasted one year. Peripheral circulating concentrations of plasma progesterone, estradiol-17β and LH were quantified by radioimmunoassay procedures. The buffalo heifers exhibited estrus only after 30 mo of age and had high levels of LH and estradiol and low levels of progesterone on the day of estrus. Estradiol concentration was higher in heifers over 18 mo of age than in the younger ones. Progesterone concentration increased with advancing age with a further rise after 30 mo of age. LH concentration was greater during the neonatal period than during pre- and peri-pubertal periods. The increases in estradiol-17β and progesterone after 30 mo of age were probably indicative of the onset of puberty of buffalo, and pubertal transition commenced after a body weight of > 250 kg has been reached (Jain and Pandey, 1985). The reported LH trends with age did not agree with the results of a previous study which investigated LH profiles of pre-pubertal and post-pubertal buffalo (Galhorta et al., 1981).

On the basis of reports on onset of puberty in buffalo, it appears that certain similarities as well as differences exist in the endocrine aspects of puberty of buffalo and cattle. Buffalo tend to take a longer time to attain sexual maturity as compared to cattle. In an attempt to advance the age at puberty in buffalo, the factors affecting attainment of puberty need to be considered. However, due to lack of sufficient information

on this aspect with regard to buffalo, related studies on cattle and other species will be brought into focus.

2.5 FACTORS AFFECTING ONSET OF PUBERTY

Similar to many other phenotypical traits, onset of puberty is also subjected to the influence of various genetic, environmental, social and managerial variables. As the attainment of puberty is an event signalling the completion of a maturational process, any factor which has the ability to modify the involved transitional steps would alter the onset of puberty.

Age and body weight (Sorensen et al., 1959; Pritchard et al., 1972) have long been recognized as two important traits characterizing the onset of puberty. Breeds differ in age at puberty (Laster et al., 1972; 1976; 1979; Gregory et al., 1979) probably because rate of growth is a hereditary character (Laster et al., 1976) which has the ability to alter pubertal onset. Level of nutrition is an important managerial factor to be taken into consideration in raising young animals, as it could modify the daily weight gain and thereby affect the onset of puberty (Short and Bellows, 1971). Under-nutrition results in increased age at puberty, subnormal conception rate, and underdeveloped udders (Sorensen et al., 1959; Short and Bellows, 1971). However, over-feeding (particularly pre-pubertal) also is not desirable as it may result in weak estrus symptoms, decreased mammary parenchyma development (Sejrsen et al., 1982) , and lower subsequent milk production (Sejrsen, 1978). Other than such hereditary and managerial variables, the importance of olfactory

stimuli and social interactions on pubertal onset was demonstrated by the increased percentage of heifers reaching puberty after exposure to bull urine (Izard and Vandenbergh, 1982). Environment also has an impact on the sexual maturation process. The main theme of the next section of this review will be centered towards discussing the effects of photoperiod and other related environmental factors on puberty.

2.5.1 Photoperiod

Although cattle ovulate throughout the year, reproductive efficiency tends to fluctuate seasonally. In northern latitudes reproductive efficiency is comparatively lower in winter, while in equatorial regions it is lower in summer (Tucker and Oxender, 1980). Lower reproductive efficiency in summer has been reported for buffalo reared in tropical regions (Siddappa and Patil, 1979), while those reared in European countries do not exhibit seasonality in estrous activity (Vale et al., 1984). Such observed seasonal trends in reproductive activities in domesticated animals were frequently attributed to fluctuations in the environmental conditions. Among the environmental factors which affect reproduction, ambient temperature, relative humidity and photoperiod apparently are the variables that have received considerable attention during the past.

Ambient temperatures above 28 C reduced growth rates of cattle via effects on feed intake (Ragsdale et al., 1957). However, at ambient temperatures between -7 and 18 C growth could be stimulated by increasing day length (Tucker and Oxender, 1980). The earlier reports

on the effects of photoperiod on growth rate of pre-pubertal cattle were not conclusive even though a majority of them indicated supplemental light was beneficial. For example, faster rate of body weight gain was observed in heifers supplemented with light during the night and fed silage in comparison to the control heifers fed the same diet (Smith et al., 1964). In another study with cattle an average daily weight gain of 1.13 kg was reported for the supplemental light treatment group as oppose to 0.96 kg/d for the control group (Boren et al., 1965). Improved feed conversion efficiency and faster growth rate in response to supplemental lighting was detected in beef heifers and steers (Robertson and Lipper, 1964; Ray et al., 1975). In contrast, no beneficial effect on growth rate with supplemental lighting could be found in other experiments (Nelson et al., 1969; Lofgren and Parsons, 1973). Nevertheless, improved feed conversion efficiency had been determined in both these studies.

More recent evidence has accumulated favoring the hypothesis that supplemental lighting is beneficial for improving growth rate and hastening the onset of puberty in cattle. Holstein heifers, 3 to 6.5 mo of age increased heart girth from 112 to 141 cm during a 16 wk period when exposed to cool-white florescent light between 0600 and 2200 hr (total of 16 hr light daily). The heart girth gain (28.7 ± 1.1 cm) was significantly greater than the 24.8 ± 1.1 cm heart girth gain observed among control heifers of similar age and provided with the same feeding regimen during the study period. A similar experiment, conducted during a 22 wk period measuring weekly body weight instead of heart girth, indicated a 0.86 ± 0.02 kg daily weight gain for heifers receiving supplemental

light. This was significantly greater than the average daily weight gain of 0.78 ± 0.02 kg reported for the control group (Peters et al., 1978). This 10 to 15 percent increase in growth rate was achieved with no requirement of additional feed supply implying an increase in feed conversion efficiency under conditions of supplemental lighting. This stimulation of feed conversion efficiency and body weight gain in heifers by supplemental lighting could be detected even under conditions of low plane of nutrition (Petitclerc et al., 1983b). The latter finding implicates the possibility of manipulation of photoperiod as a useful management tool especially in less developed countries where the plane of nutrition for livestock is low.

An experiment designed to investigate the effects of various durations of light and dark exposures on body growth of Holstein heifers during autumn and winter revealed the requirement of an 8 hr period of darkness to achieve increases in weight gain (Peters et al., 1980). However, another study conducted during summer months using 16 hr light : 8 hr dark period as a treatment, when the natural photoperiod ranged between 13.6 and 15.3 hr of light, was not able to detect any significant difference between growth rates of treatment and control groups (Peters et al., 1978). Thus, it appears that beneficial effects of light on body growth are most likely to occur when natural day length was less than 13 hr per day (Tucker, 1981).

In addition to stimulation of body growth and feed conversion efficiency, supplemental lighting seems to have the ability to hasten the onset of puberty. This is not surprising as faster growth rates induce earlier

onset of estrous cyclicity in cattle. Although numbers of animals were too small to make a definitive judgement, 16 hr of light daily induced three out of ten heifers to undergo at least one estrous cycle (based on cyclic serum progesterone patterns) before 10.5 mo of age as compared to none in the control group which received 9 to 12 hr of natural day light (Peters and Tucker, 1978). In a later attempt to determine whether the exposure to supplemental lighting hastens the onset of puberty in pre-pubertal heifers, significant reduction in age at first estrus was observed among treated heifers (Hansen et al., 1983). A greater ovarian growth also could be detected among treated heifers as compared to the control group. In another study when serum progesterone concentrations were measured as an indicator of onset of puberty, beneficial effects of supplemental lighting on sexual maturation were demonstrated (Petitclerc et al., 1983b). In this experiment, supplemental lighting stimulated heifers to reach puberty at 3 to 5% lighter weight than animals exposed to short duration of photoperiods.

Based on such evidence it can be concluded that supplemental lighting of 16 hr per day has beneficial effects on the growth rate and sexual maturity of pre-pubertal cattle even under conditions of low planes of nutrition when the natural day length is less than 13 hr. Since buffalo are similar to cattle in many reproductive aspects, it can be expected that the pre-pubertal buffalo also would respond favourably to supplemental lighting. However, I am unaware of attempts to evaluate the influence of photoperiod and supplemental lighting on growth and puberty of buffalo.

2.5.2 Mechanism

Onset of puberty has long been recognized as a growth related, neuroendocrine involved maturational event. However, very little information is available on how growth is linked mechanistically to the neuroendocrine systems governing the pattern and level of hormones involved in the pubertal process. Much less is known about the mechanisms linking external influential factors such as nutrition and photoperiod to growth and puberty.

Of the hormones which respond to changes in photoperiod, prolactin appears to be the most susceptible, exhibiting a pronounced increase (Bourne and Tucker, 1975; Leining et al., 1979) within a week and achieving maximum response between 5 to 8 wk (Leining et al., 1979) at temperatures above 5 C (Peters and Tucker, 1978). Thus, circulating prolactin concentrations can be used as an indicator of photoperiodic response in long-term experiments. However, provision of a daily dark period is necessary in such experiments as secretion of prolactin is photosensitive (Petitclerc et al., 1983a). Since secretion of prolactin appears to participate in the maturational process that leads to onset of estrous cyclicity in the rat (Ojeda et al., 1980), and possibly serves as a homeorrhetic factor involved in the photoperiodic regulation of growth in cattle (Petitclerc et al., 1983) and sheep (Forbes et al., 1979), its involvement in the onset of puberty is frequently suggested but remains to be established in ruminants.

Evidence for regulation of gonadotropin secretion by photoperiodic changes comes from studies using seasonal breeders such as sheep. Adult ovariectomized ewes with estradiol implants showed seasonal fluctuations in LH, with concentrations highest during short days as compared to long days (Legan and Karsch, 1980; Platt et al., 1983). No seasonal pattern in tonic LH secretion could be detected in ovariectomized ewes without implants (Legan et al., 1977) suggesting the seasonal variation in estradiol negative feedback action. In the case of the prepubertal lamb reared in short days, exposure to artificial long days for about one week was necessary to initiate the puberty associated characteristic increase in LH secretion and cyclicity (Foster and Yellon, 1985; Yellon and Foster, 1985).

The involvement of endogenous opioid peptides such as β -endorphins in mediating the effects of photoperiod on LH and prolactin release was shown by the experiments with ovariectomized ewes. Intravenous infusions of naloxone (an opiate receptor antagonist) increased mean plasma concentrations of LH, while infusion of morphine (an opiate receptor agonist) decreased mean concentration of LH. These effects were attributed to changes in period of LH pulses. Naloxone did not alter prolactin release, while morphine increased it. These results demonstrated the ability of endogenous opiate peptides to inhibit the LH pulse generator in ovariectomized ewes and suggested the possibility of photoperiodic regulation of prolactin release via opiate neurons (Schillo et al., 1985).

The role of the pineal gland in receiving photic information, and transmitting it to the hypothalamic-pituitary-gonadal axis via pineal

melatonin has received great attention during the past years, although the precise physiological mechanisms are not known yet. Melatonin is synthesized from another pineal indolamine, serotonin. The levels of serotonin in the pineal are greater during the day time and lower in the dark (night), while melatonin concentration fluctuates in the opposite direction. This daily rhythm in the production of pineal hormones was common for all mammalian species thus far studied (Rollag, 1981). The effects of melatonin on reproduction were similar to that of photoperiod, and it affects gonadotropin concentrations similarly to photoperiod (Bittman et al., 1983; Bittman and Karsch, 1984). Thus, the effects of melatonin on gonadal function may be exerted by altering gonadotropin secretion (Hansen, 1985). However, attempts to relate melatonin as a coordinating signal of photic cues to the onset of puberty have not been successful (Tamarkin et al., 1985).

Based on the findings of previous experiments, a working hypothesis was proposed recently to account for the timing of puberty in the female sheep (Foster et al., 1985). According to this hypothesis, the activity of the GnRH pulse generator which dictates secretory patterns of gonadotropins from the pituitary is hypersensitive to the inhibitory feedback actions of ovarian estradiol during the juvenile state, and hence the resultant frequency of the pulse generator is low. As a consequence, LH pulses are produced at a low frequency, and such LH pulses are not sufficient for the development of the pre-ovulatory follicle. Growth related metabolic signals, as well as signals related to external determinants such as nutrition and photoperiod are monitored via internal cues such

as hormonal concentrations. These internal cues modulate the GnRH pulse generator such that the hypersensitivity to inhibitory feedback is removed once a sufficient physiological size (assessed by metabolic cues) is attained and the appropriate photoperiod sequence is experienced (as assessed by humoral signals). This allows the GnRH pulse frequency to be increased resulting in an appropriate LH pulse frequency to produce the pre-ovulatory follicle and ovarian estradiol sufficient to evoke gonadotropin surge and ovulation. Although various lines of evidence support this proposed scheme of events, it could be continually modified and existing gaps can be completed as new information accumulates. Whether or not the same sequence of events take place during the pubertal transition of buffalo remains to be investigated.

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 LOCATION

The study was conducted at the National Livestock Development Board Farm, Melsiripura, Sri Lanka, during the period between July 10 and October 27, 1984. Fifty non-pregnant Surti buffalo heifers aged 16 to 42 mo (n = 24, < 24 mo; n = 26, > 24 mo) were randomly selected for the experiment.

3.2 GENERAL MANAGEMENT

All the heifers were allowed day time grazing from 0930 hr to 1400 hr, wallowing between 1430 hr and 1630 hr, and night-time paddocking after 1730 hr. A concentrate mixture containing 65% coconut meal[1] and 35% rice bran[2] was provided at the level of 2.5 kg/animal/day between 0900 hr and 0930 hr, while similar amounts of available grass and/or hay mixture were left to be consumed during the night.

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1 Contains 89% dry matter, 11% crude protein and 18% crude fibre as a percentage of dry matter (FAO, 1981).

2 Contains 89% dry matter, 20% crude protein and 9% crude fibre as a percentage of dry matter (FAO, 1981).

3.3 TREATMENT

Beginning on the d 11 (July 21, 1984) to d 98 (October 20, 1984) of the experiment, 4 hr of supplemental light per day between 1800 hr and 2200 hr was provided to 25 heifers (n = 12, < 24 mo; n = 13, > 24 mo) randomly allotted as the treatment group. Two 60 W white florescent bulbs placed midway from the ends of the open housing were used to provide 16 hr light : 8 hr dark (16 L : 8 D) lighting regime. The remaining heifers (n = 12, < 24 mo; n = 13 > 24 mo) designated as the control group received natural photoperiod of 12 hr light : 12 hr dark (12 L : 12 D) cycle. The treatment group was housed separately in the open housing area during the night, while the night paddock of the control group was removed about 450 m.

3.4 DATA

Meteorological data including hourly ambient temperature, relative humidity, and daily rainfall were recorded using a hygrothermograph (model SL-8368-50, Cole-Parmer Instrument Co.) and a rain guage, respectively.

On d 1 (July 10, 1984) of the experiment, the individual ages and weights (BWT) were recorded. Weekly body weights were obtained thereafter throughout the study.

Rectal palpation was conducted on d 1 of the experiment to separate pregnant heifers from non-pregnant ones. The latter catagory was used

for the experiment. Weekly rectal palpations were conducted and information concerning ovarian structures was recorded beginning from d 14.

Follow up data on heifers that became pregnant during the 6 months following the end of the experiment was obtained.

Jugular vein blood samples were obtained using vacutainer tubes from all the heifers on alternate days from d 1 until d 10. Blood samples were then collected twice weekly until d 98 (October 19, 1984) of the experiment. These samples were stored and later assayed to determine serum prolactin (Prl), progesterone (P_4), and luteinizing hormone (LH) concentrations.

Between d 99 and d 106 each heifer was catheterized in the jugular vein and serial blood sampling was conducted on the following day. Fifteen ml blood samples were collected at -30, -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 min post gonadotropin releasing hormone (GnRH) administration (30 ug/heifer) via jugular cannulae. These blood samples were used to determine serum luteinizing hormone response to GnRH.

3.5 HORMONE DETERMINATION

All blood samples were centrifuged at 3000 g on the day after they were collected, and serum was stored at -4 C at Melsiripura. Later, serum samples were transferred to the Department of Animal Science of University of Peradeniya where, subsequent lab work was carried out.

Serum prolactin was quantified by a double antibody radioimmunoassay with bovine Prl antiserum. The specific antiserum was used at a

1:40,000 dilution and bound 39% of radiolabelled Prl in the absence of unlabelled hormone. All samples were assayed in duplicate following validation of the assay procedure for buffalo Prl. Intra- and interassay coefficients of variation calculated for serum pools were 9.5 and 13.2%, respectively.

The radioimmunoassay of LH was performed through a heterologous assay with specific antibovine LH serum. The specific antiserum was used at a 1:80,000 dilution and bound 27% of radiolabelled LH in the absence of unlabelled hormone. All samples were assayed in duplicate following validation of the assay procedure for buffalo serum LH. Intra- and interassay coefficients of variation calculated for serum pools were 8.6 and 14.7%, respectively.

To quantify serum progesterone, a Coat-a-Count RIA kit (Diagnostic Products Co., California) was used following validation for buffalo serum P_4, and all samples were assayed in duplicate. Intra- and interassay coefficients of variation calculated for serum pools were 7.3 and 11.8%, respectively.

(Assay procedures used in determining serum Prl, LH and progesterone and the results of validation procedures are in the appendix A-F).

3.6 METHOD OF ANALYSIS

Hormone and body weight data were analyzed using following complete basic statistical model:

$$Y_{abcde} = u + I + Tmt_a + AG_b + Tmt \times AG_{ab} + H_{(ab)c} + T_d$$

$$+ T \times Tmt_{ad} + T \times AG_{bd} + T \times Tmt \times AG_{abd} + e_{abcde}$$

where,

Y_{abcde} = dependant variable (BWT, Prl, P_4, LH),

u = mean of the parameter measured,

I = covariate (average of the pre-treatment values),

Tmt_a = effect of treatment,

AG_b = effect of age group (< 24 mo, > 24 mo)

$Tmt \times AG_{ab}$ = effect of treatment by age group interaction,

$H_{(ab)c}$ = effect of heifer within treatment by age group interaction,

T_d = effect of time (wk for BWT, twice/wk for Prl, P_4 and LH, and hr for LH response to GnRH),

$T \times Tmt_{ad}$ = effect of time by treatment interaction,

$T \times AG_{bd}$ = effect of time by age group interaction,

$T \times Tmt \times AG_{abd}$ = effect of time by treatment by age group interaction,

e_{abcde} = residual effect.

(Tmt_a, AG_b and $Tmt*AG_{ab}$ were tested by $H_{(ab)c}$, while all the other effects were tested by e_{abcde}).

The average estimate of each dependent variable during the pre-treatment period and/or pre-injection period was included as a covariate in the model to avoid misinterpretation of parameters tested by heifer variable. Further analysis was conducted using time as a continuous variable, with the covariate and without three-way interaction.

Whenever an interaction with time was significant in a later model, regression equations for the interaction were calculated and used to plot regression lines. Least squares means were superimposed on the graphs.

Body weight and hormone data were further analyzed as a function of average daily temperature, average daily relative humidity and daily rainfall as continuous variables (in place of time).

Chi square analysis was performed on follow-up data on number of heifers that became pregnant in each group.

The same basic model was used to analyze palpation data. However, the effect of age group and its interactions had to be eliminated due to insufficient number of heifers in the younger group. The covariate also was not included in the model due to unavailability of data.

Weekly averages of daily maximum temperature, daily minimum temperature, maximum humidity and minimum humidity were calculated. Total weekly rainfall was superimposed on the graphs.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 METEOROLOGICAL DATA

During the 14 wk treatment period, ambient temperatures generally fluctuated between 25 and 34 C, while on several days lower and upper extreme values of 21 and 37 C were reached. In general, atmospheric temperature was above 27 C during a large part of the day-light hours (between 1000 hr and 1800 hr). Daily minimum and maximum temperatures occurred at 0600 hr and 1400 hr, respectively (Figure 2). Weekly average daily maximum temperatures ranged between 31 and 37 C, exhibiting an upward trend until the 10th wk and a decline thereafter. Weekly average daily minimum temperature fluctuated between 23.8 and 27.4 C without any specific pattern, but assumed the lowest values during the 9th wk (Figure 3).

Diurnal fluctuations in relative humidity were (34% to 100%) of greater magnitude, and exhibited an opposite diurnal pattern compared to that of temperature, reaching minimum and maximum values between 1400 hr and 1500 hr and between 0700 hr and 0800 hr, respectively (Figure 4). Relative humidity was < 70% during most of the day-light hours (1000 hr to 1800 hr). Thus, the prevailing ambient conditions during the grazing period (0950 to 1400 hr) were characteristic of temperatures above 27 C and relative humidities below 70%. Weekly average daily

Figure 2. Diurnal pattern of ambient temperature at Melsiripura farm.

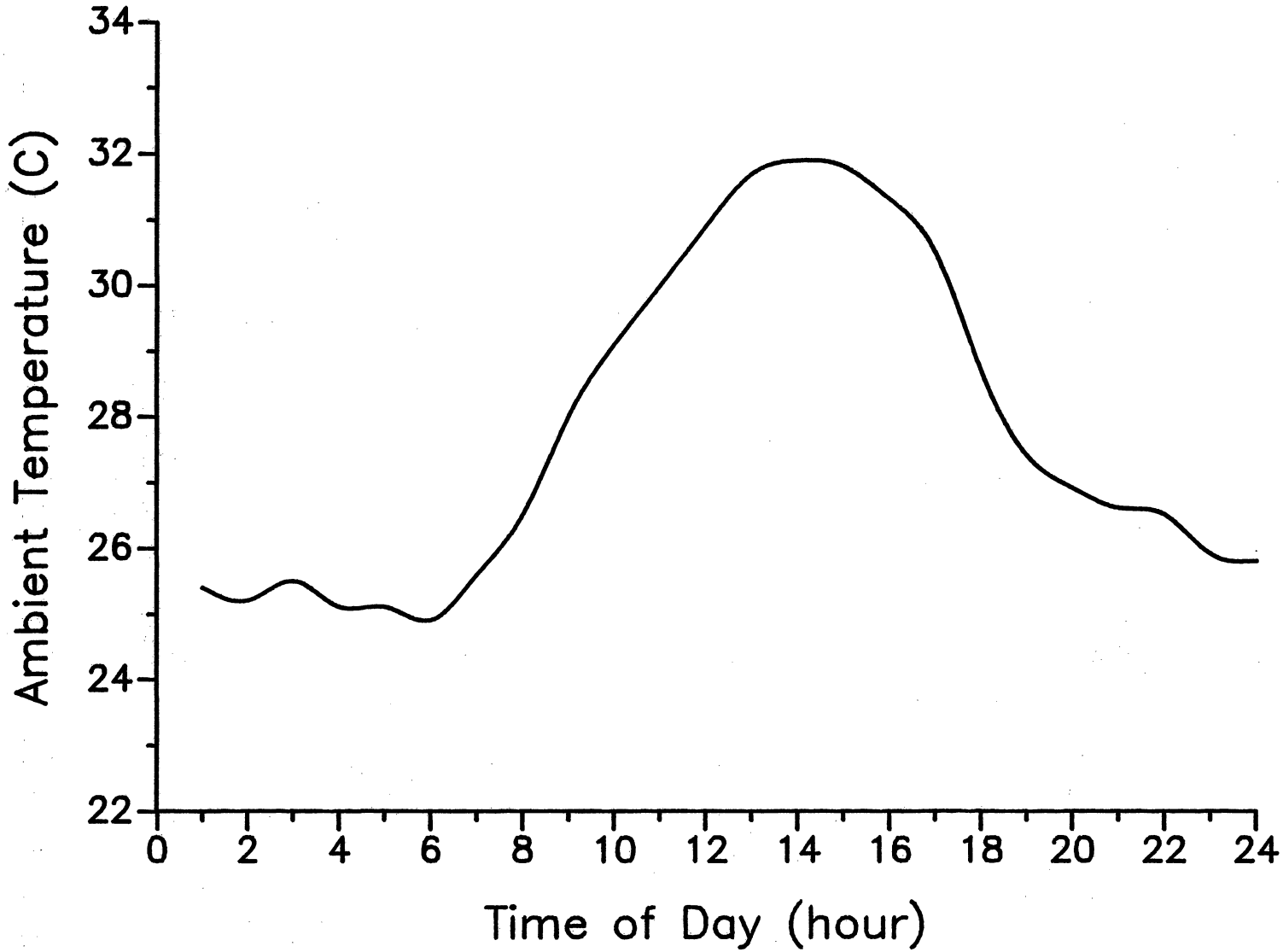

Ambient Temperature (C)
34
32
30
28
26
24
22
0
2
4
6
8
10
12
14
16
18
20
22
24
Time of Day (hour)

Figure 3. Mean weekly temperatures at Melsiripura farm.

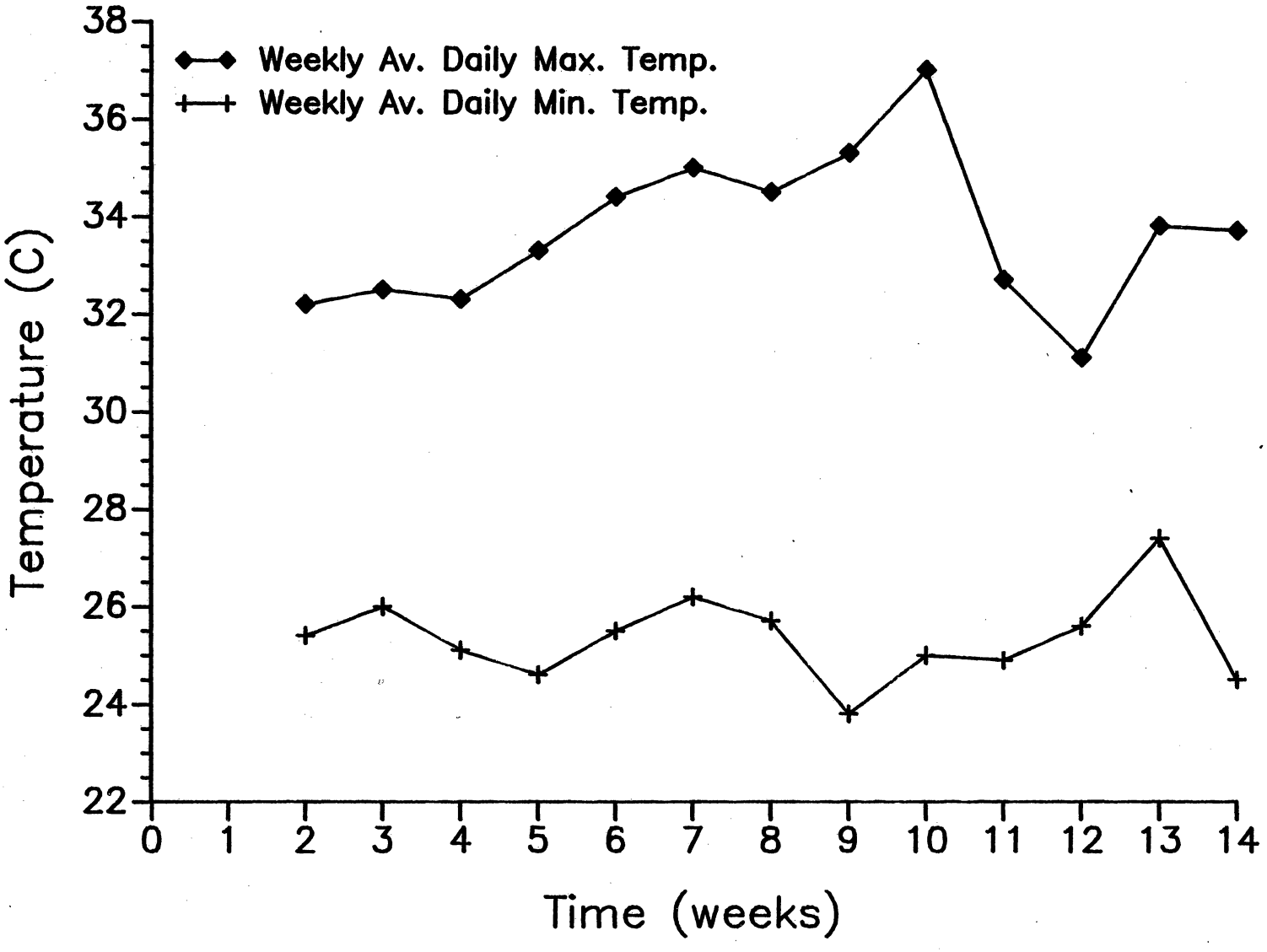
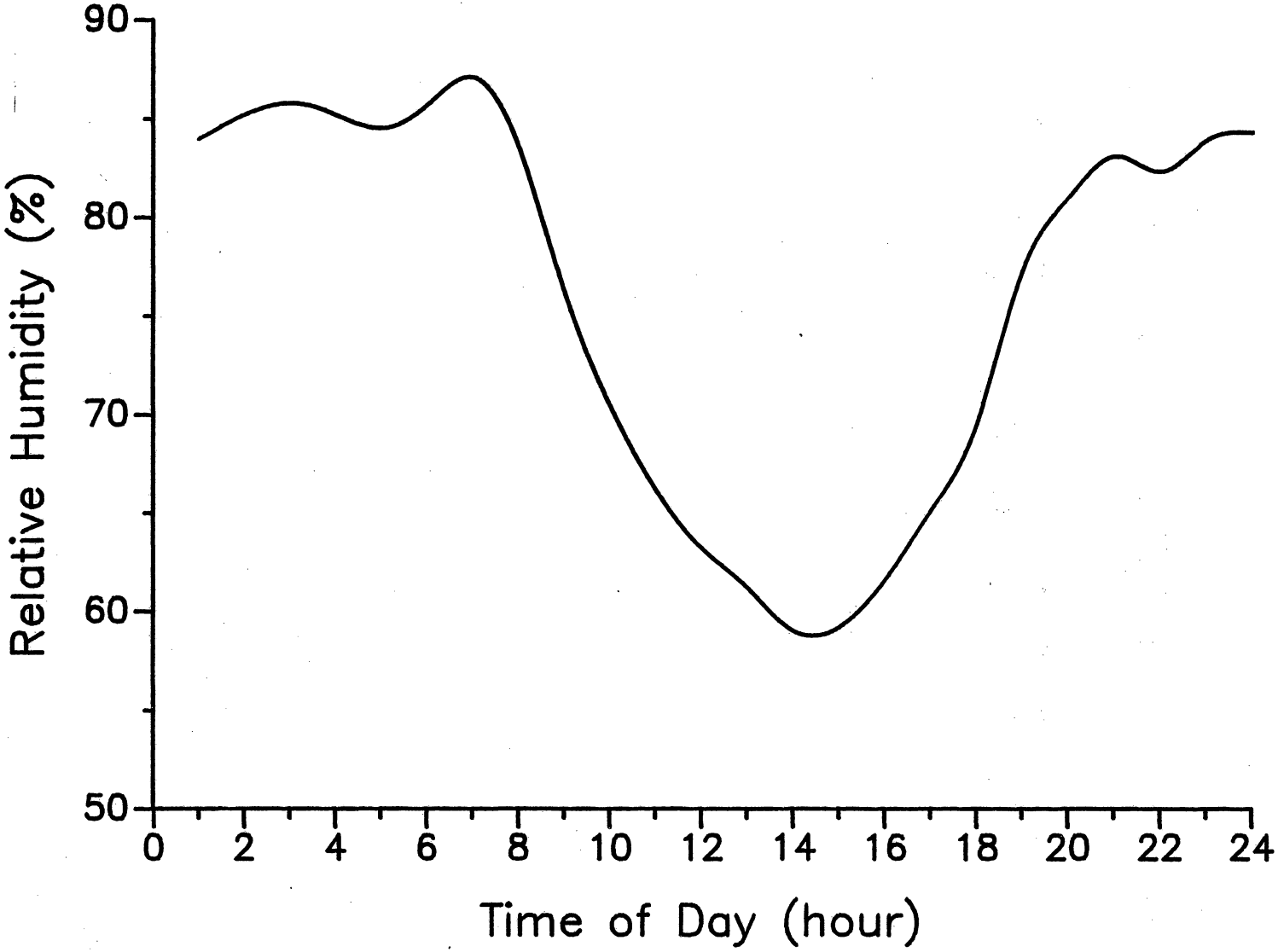

Weekly Av. Daily Max. Temp.
Weekly Av. Daily Min. Temp.
Temperature (C)
38
36
34
32
30
28
26
24
22
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Time (weeks)

Figure 4. Diurnal pattern of relative humidity at Melsiripura farm.


90
80
70
60
50
Relative Humidity (%)
0
2
4
6
8
10
12
14
16
18
20
22
24
Time of Day (hour)

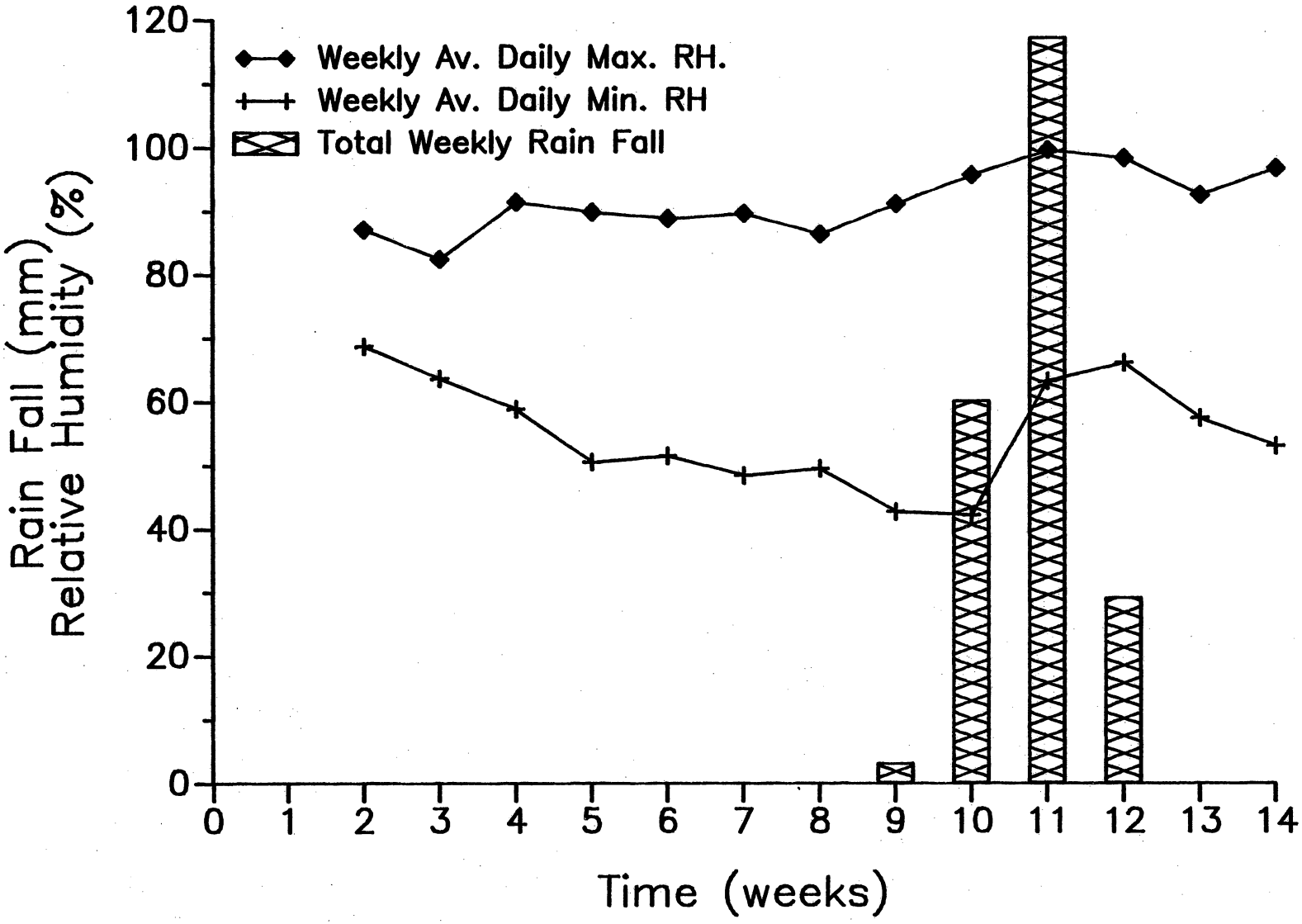
maximum relative humidity values ranged between 82.4 and 99.5% and assumed peak values during the 11th wk. Weekly average daily minimum relative humidity exhibited a declining trend from the second until the 10th wk and then increased (Figure 5). These fluctuations in the ambient temperature and relative humidity indicated the prevalence of a drought until the 10th wk.

Total weekly rainfall was the only environmental variable which experienced a considerable amount of fluctuation. Weekly rainfall varied between 0 and 117 mm, while the latter amount was received during the 11th wk (Figure 5). This explains the increase in relative humidity along with a sudden drop in ambient temperature during that week breaking the drought condition (Figures 3 and 5). Although 60 mm precipitation was received during the 10th wk, more than 90% (55 mm) was received during the last night of the 10th wk. Thus, the cooling effect of rainfall was not reflected in the 10th wk temperature values. Rainfall during the 12th wk (29 mm) was more evenly distributed over the week making ambient conditions cooler and humid (Figures 3 and 5).

4.2 BODY WEIGHT

During the experimental period, individual weekly body weights of the heifers varied between 106 kg and 371 kg. The model used to analyze these variations resulted in a R^2 value of 0.99 indicating that the variation caused by unknown variables was negligible (< 1%). Individual weekly body weights were significantly affected by treatment ($p < 0.01$),

Figure 5. Mean weekly relative humidity and total weekly rainfall at Melsiripura farm.


Weekly Av. Daily Max. RH.
Weekly Av. Daily Min. RH
Total Weekly Rain Fall
Rain Fall (mm)
Relative Humidity (%)
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (weeks)

age group ($p < 0.01$), pre-treatment body weight ($p < 0.01$), heifers ($p < 0.01$), effects of time ($p < 0.01$), time by treatment interaction ($p < 0.01$), and time by age group interaction ($p < 0.01$). Treatment by age group interaction and the three way interaction were not significant (Table 2). All these significant effects except for those of treatment and of age group remained unchanged when the analysis was conducted using time as a continuous variable. In addition, significant quadratic effects of time and its interactions with treatment and with age group were detected (Appendix G).

Least squares mean body weight of heifers that received supplemental lighting for the 14 wk period was 5.8% heavier (202.4 ± 0.3 kg) than that of control heifers (191.3 ± 0.3 kg). Beneficial effects of supplemental lighting on body growth have been reported for cattle (Peters et al., 1978; Petitclerc et al., 1983b). The significantly ($p < 0.01$) heavier body weight for the treatment group indicates supplemental lighting is beneficial even with buffalo. This assumption is further strengthened by the fact that there were no differences in planes of nutrition between two groups because all the management practices (except for provision of additional 4 hr of light) and food supply were similar for both the groups throughout the experimental period.

The least squares mean weekly body weight of older buffalo heifers (> 24 months) was 67% above (246.1 ± 0.2 kg) that of younger (< 24 months) heifers (147.6 ± 0.2 kg). This age associated increase in body weight was expected as buffalo heifers gain weight until about 4 yr, when a mature weight is attained.

Table 2. Analysis of variance of weekly body weights of buffalo heifers exposed to 4 hr supplemental lighting daily.

Source	df	MS	F
Treatment (Tmt)[a]	1	20121.57	26.81**
Age group (AG)[a]	1	1568591.81	2089.81**
Tmt * AG[a]	1	733.82	0.98
IBWT[b]	1	869028.48	49687.16**
Heifer (Tmt * AG)	45	750.59	42.92**
Time	12	1708.17	97.68**
Time * Tmt	12	189.56	10.84**
Time * AG	12	72.51	4.15**
Time * Tmt * AG	12	16.44	0.94
Residual	550	17.49	
Total	647		

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Initial body weight

Pre-treatment body weight of buffalo heifers (which is denoted as IBWT covariate) which ranged between 107 and 334 also significantly ($p < 0.01$) affected body weight (Table 2). Since the body weight is partly determined by age of the animal until a mature weight is attained, and these heifers were of a considerable age range (17 to 42 mo), such variation in bodyweights was expected even prior to the beginning of the experiment. Therefore, variation caused by pre-treatment body weights was excluded from other effects by considering it as a covariate in the model.

In addition to the variation due to pre-treatment body weights, weekly body weights during the treatment period were significantly ($p < 0.01$) affected by the individual heifer variation (Table 2). Body weight is characteristic of the individual animal, and change in individual weight is a function of variation in multiple factors such as age, feed intake and physiological status of the animal.

Weekly body weights of the heifers varied significantly ($p < 0.01$) over time (Table 2). Both linear as well as quadratic time effects were significant ($p < 0.01$; appendix G). Least squares mean body weight of the 50 heifers fluctuated slightly, but assumed an upward trend until the 10th wk and declined thereafter (Table 3). This could be partly due to variation in feed availability. Both day-time grazing as well as forage supply for the night-time was entirely dependant upon pasture availability within the farm, which in turn was dependant upon climatic variables (especially rainfall). In addition, day-time grazing was interrupted by heavy continuous precipitation during grazing periods toward the end of

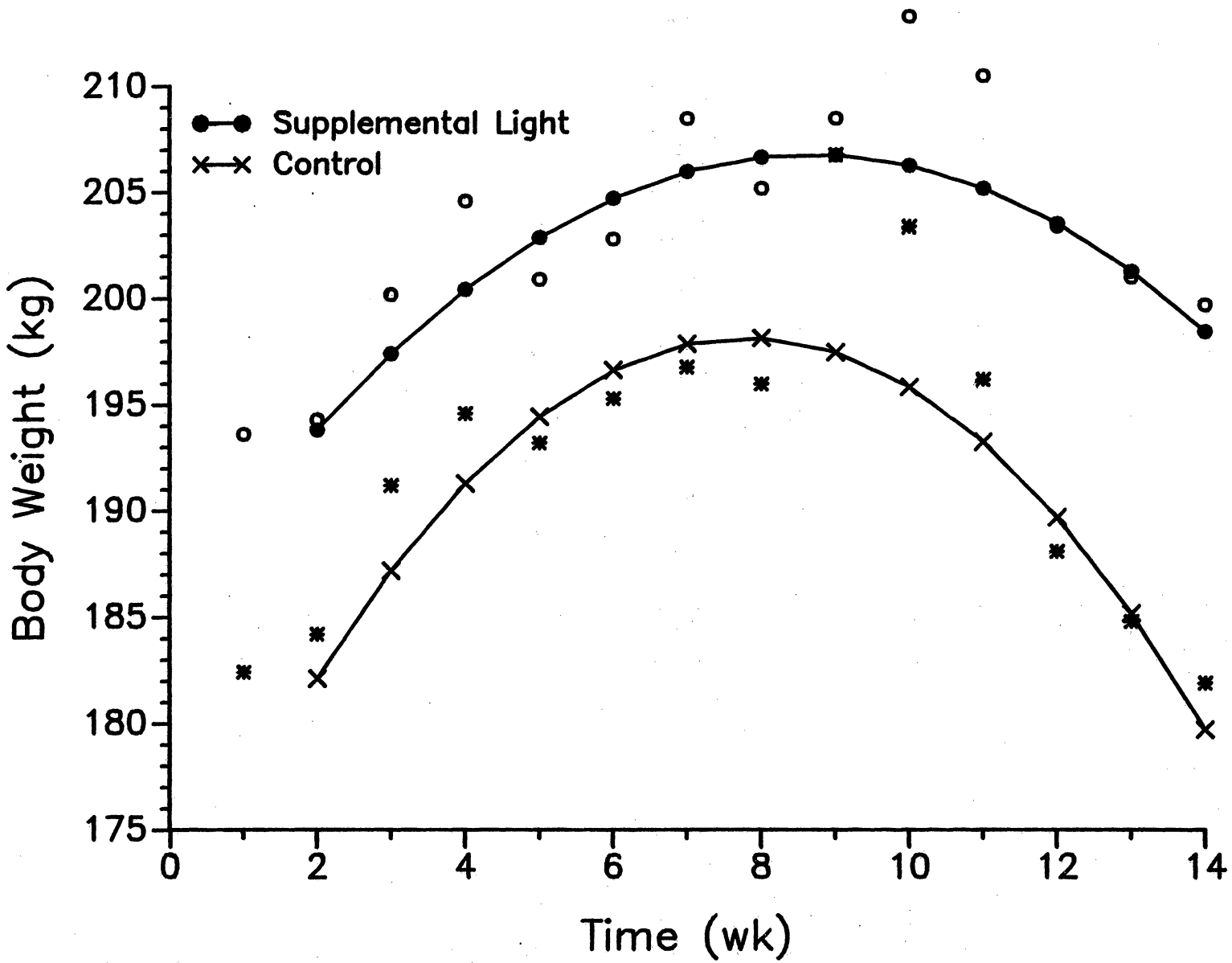
Table 3. Least squares mean (± SE) of weekly body weight (kg) for buffalo heifers exposed to 4 hr supplemental light daily.

Week	Body weight	
	Actual	Adjusted
1	186.1 ± 0.7	
2	188.6 ± 0.7	190.4 ± 1.2
3	193.7 ± 0.7	195.4 ± 1.2
4	197.6 ± 0.7	199.3 ± 1.2
5	195.1 ± 0.7	196.7 ± 1.2
6	197.1 ± 0.7	198.7 ± 1.2
7	200.7 ± 0.7	202.3 ± 1.2
8	198.6 ± 0.7	200.3 ± 1.2
9	207.0 ± 0.7	208.8 ± 1.2
10	206.2 ± 0.7	207.8 ± 1.2
11	201.3 ± 0.7	203.0 ± 1.2
12	193.8 ± 0.7	195.5 ± 1.2
13	191.0 ± 0.7	192.6 ± 1.2
14	189.0 ± 0.7	190.6 ± 1.2

the study. The sudden drop in body weights after the 10th wk was the result of possible interruption of grazing by rainfall. Additional analysis (Appendix H) revealed that rainfall and relative humidity had a negative effect on body weight. The adverse effect of rainfall and relative humidity on body weight could be overcome by provision of forages in a sheltered area during the rainy season.

In addition to significant effects exerted by treatment and time, treatment by time interaction on body weight also was significant ($p < 0.01$; Table 2). Mean body weights of both the supplemental light group and control group changed in a quadratic manner over the 14 wk period (Figure 6, Appendix I). Heifers in the supplemental light treated group gained weight until the 8th wk, maintained the same body weight during the 9th and 10th wk and then experienced a loss during the following weeks (Figure 6). Amount of precipitation increased dramatically after the 10th wk. Interruption of grazing by continuous day-time rainfall occured during the 11th and 12th wk. The observed loss in bodyweight following the 10th wk was due in part to high humidity and rainfall (Appendix H). Control group heifers also had an upward trend in weekly body weights until about the 7th wk of the experiment, plateaued between the 7th and 9th wk and declined thereafter (Figure 6). Forage supply within the farm was adversely affected by the drought situation especially after the 6th wk of the experiment and by rainfall after the 10th wk. Apparently the consequences of the depletion of quantity and quality of feed supply was reflected more dramatically among the control group heifers. However, when rainfall changes were assessed in relation to

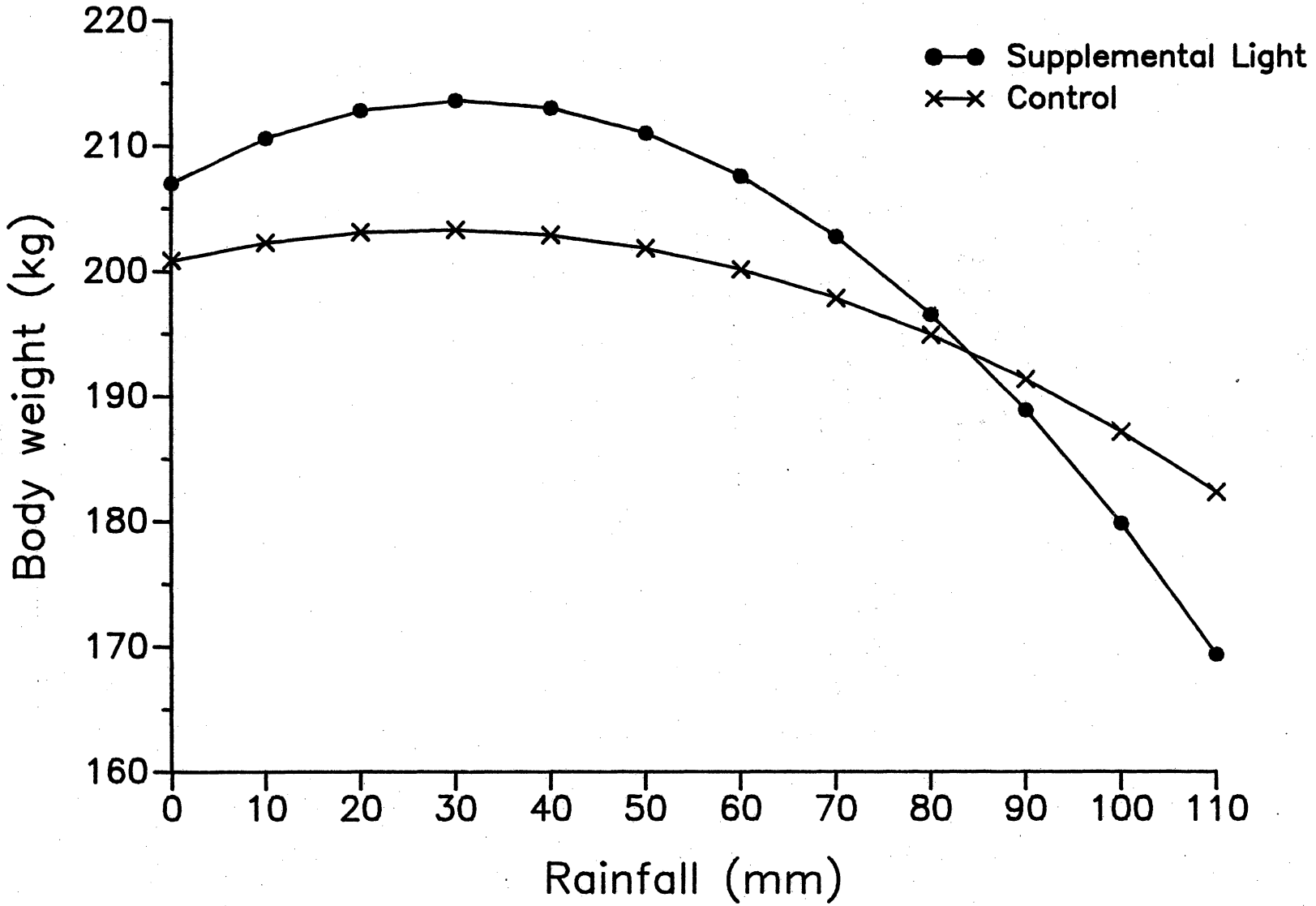
Figure 6. Weekly body weights of buffalo heifers by treatment group.


Supplemental Light
Control
Body Weight (kg)
210
205
200
195
190
185
180
175
0
2
4
6
8
10
12
14
Time (wk)

body weight the supplemental light treated heifers had a more rapid decline in body weight at rainfall above 60 mm (Figure 7). The ability of the treatment group heifers to maintain the upward trend in body weight between 7th and 10th wk (Figure 6) despite the declining plane of nutrition as a result of no rain might be due to the stimulating effect of supplemental lighting because plane of nutrition was similar for both groups. Such stimulation of body weight gain under low plane of nutrition via supplemental lighting has been observed among Holstein heifers (Petitclerc et al., 1983b). However, supplemental lighting alone cannot stimulate weight gain below a certain threshold plane of nutrition and in the face of heavy rainfall as evidenced by the greater loss in body weight by treatment group following the 10th wk (Figure 6).

As a result of these fluctuations, the control group heifers ended up with the same pre-treatment body weight (Appendix I) while light treated heifers had a mean net weight gain of 6.1 kg at the end of the experiment (Figure 6). Additionally, when body weight was adjusted for pre-treatment body weights supplemental light treated heifers gained 2.6 kg over the 13 wk treatment period as opposed to 2.2 kg weight loss for control heifers (Appendix I). The actual net gain averages to 0.5 per wk per animal, and is much lower than the estimated daily weight gain in cattle in response to supplemental lighting (Peters et al., 1978; Petitclerc et al., 1983b). Differences in genetic, environmental, nutritional and management conditions among the reported experiments and this experiment might have contributed to the observed differences in net gain. However, the results of this experiment indicate supplemental

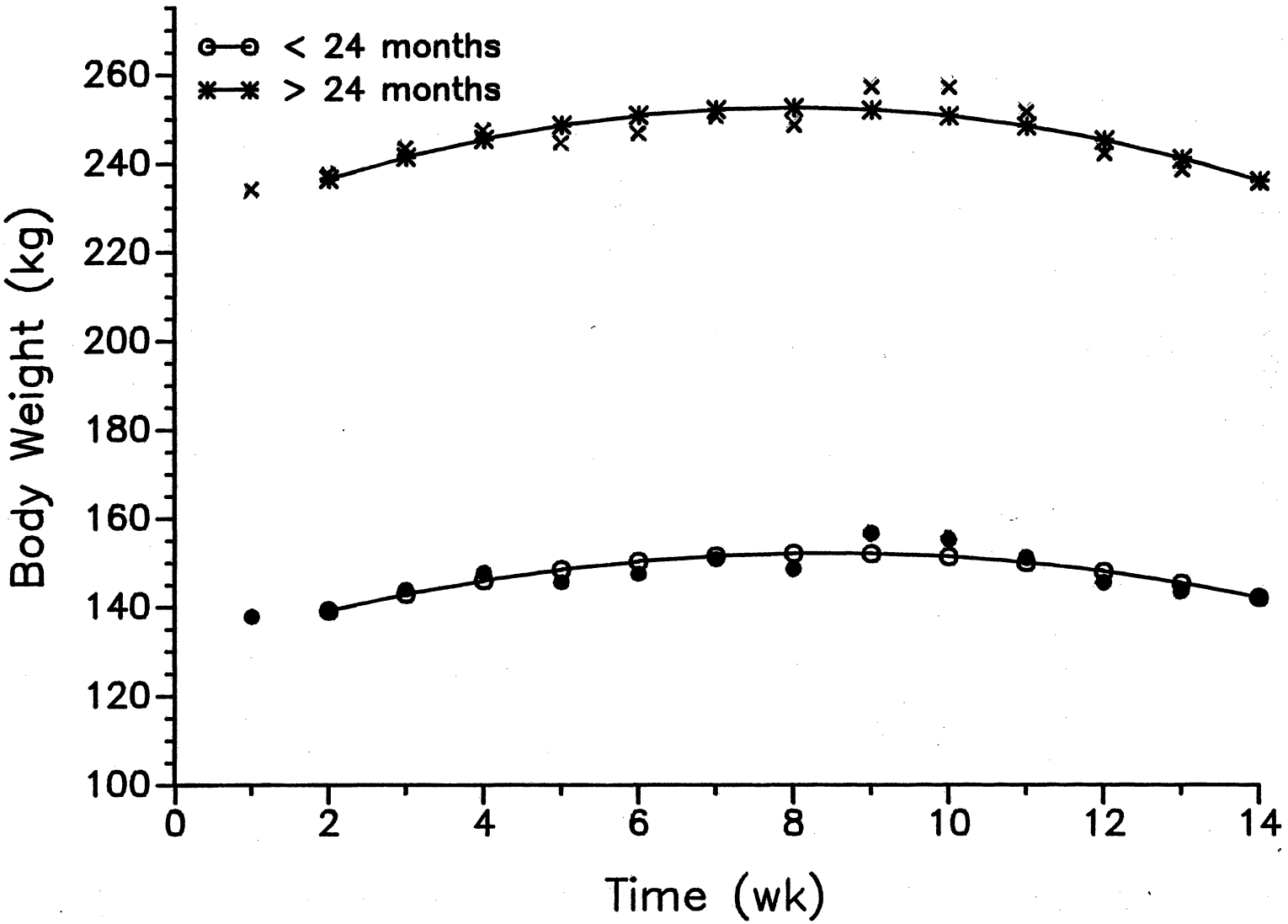
Figure 7. Effect of rainfall on body weight of buffalo heifers exposed to 4 hr supplemental lighting daily.


Supplemental Light
Control
Body weight (kg)
220
210
200
190
180
170
160
0
10
20
30
40
50
60
70
80
90
100
110
Rainfall (mm)

lighting as being stimulating to body growth in buffalo heifers even under the existing plane of nutrition and conditions of management. The use of 4 hr of supplemental lighting to increase body weight gain in buffalo heifers in Sri Lanka requires further investigation before any recommendations are made since this was the first experiment of this kind and the experiment was limited to one location and to a 14 wk period during one part of the year.

Body weights of heifers in the two age groups also exhibited a significant ($p < 0.01$) age by time interaction (Table 2; Appendix G). Weights of both the age groups varied in a quadratic manner. Weight of older heifers increased up to the 10th wk and declined thereafter. Body weight of younger heifers also assumed an upward trend until the 9th wk, maintained that weight until the 10th wk and declined thereafter (Figure 8; Appendix J). These variations also reflect combined effects of drought and interruptive rainfall. During the 14 wk period younger animals had a net weight gain of 4.6 kg, while the net weight loss of older animals was 7.8 kg (Appendix J). When body weight was adjusted for the pre-treatment weight, younger heifers gained 2.8 kg during the treatment period compared to a 2.4 kg loss for older heifers (Appendix J). Actual net gain younger heifers averaged approximately 0.36 kg net weight gain per week. This estimated value for net weight gain for younger animals is smaller comparatively to the estimated net gain values for calves (Peters et al., 1978). Nonetheless, it appears that supplemental lighting was more beneficial for younger animals than for older heifers in terms of body growth. Further investigations are required

Figure 8. Weekly body weights of buffalo heifers exposed to 4 hr supplemental lighting daily by age group.


< 24 months
> 24 months
Body Weight (kg)
260
240
220
200
180
160
140
120
100
0
2
4
6
8
10
12
14
Time (wk)

to determine the optimum age group to provide supplemental lighting to achieve maximal body growth.

4.3 HORMONES

Circulating concentrations of prolactin (Prl), progesterone (P_4) and luteinizing hormone (LH) in twice weekly blood samples differed in their trends, variation and responses. Results of analyses of data on each hormone will be discussed separately in the following sections.

4.3.1 Prolactin (Prl)

Serum Prl fluctuated between 20.1 and 69.7 ng/ml with a mean value of 41.5 ± 0.2 ng/ml in twice weekly samples. The model used to test independent variables resulted in a R^2 value of 0.84, implying the appropriateness of the model (Table 4).

Supplemental light treated heifers (42.6 ± 0.2 ng/ml) had a significantly ($p < 0.01$) greater Prl concentrations compared to the control heifers (40.1 ± 0.2 ng/ml). Elevation in circulating Prl concentrations in response to supplemental lighting (Bourne and Tucker, 1975; Leining et al., 1979) at temperatures above 5 C (Peters and Tucker, 1978) has been observed in cattle. Although similar data are not available for buffalo, the significantly greater Prl in the treatment group implies the susceptibility of this hormone in buffalo heifers to provisions of supplemental light. In addition to the differences between the treatment

Table 4. Analysis of variance for serum prolactin in buffalo heifers exposed to 4 hr supplemental light daily.

Source	df	MS	F
Treatment (Tmt)[a]	1	1972.42	10.99**
Age group (AG)[a]	1	73329.66	408.73**
Tmt * AG[a]	1	217.80	1.21
IPRL[b]	1	21902.67	1160.71**
Heifer (Tmt * AG)	45	179.41	9.51**
Time	25	157.99	8.37**
Time * Tmt	25	107.46	5.70**
Time * AG	25	18.37	0.97
Time * Tmt * AG	25	27.21	1.44
Residual	1106	18.87	
Total	1255		

** $p < 0.01$

[a] Tested by heifer (Tmt * AG)

[b] Pre-treatment Prl

groups, circulating concentrations of Prl in both the groups were (41.5 ng/ml) were comparable to circulating Prl concentrations in Holstein cattle during spring and summer seasons (Perera et al., 1985).

Least squares mean Prl in older animals (49.0 ± 0.2 ng/ml) was significantly ($p < 0.01$, Table 4) greater than that of younger animals (33.7 ± 0.2 ng/ml). Such changes of Prl associated with age have been observed among cattle (McCarthy et al., 1979) and sheep (Morrison et al., 1981). The consequences of the age associated elevation in Prl are not clear except for the involvement of Prl in mammary differentiation and lactogenesis (Akers et al., 1981a; 1981b).

Pre-treatment Prl concentrations (basal circulating Prl) ranged between 24.1 and 52.1 ng/ml with a mean value of 35.6 ± 0.2 ng/ml, and significantly ($p < 0.01$; Table 4) affected Prl variation over the sampling period. Circulating Prl fluctuates depending on factors that are characteristic to the animal such as age and physiological state (Koprowski and Tucker, 1973; Pahwa and Pandey, 1984). The observed individual variation in circulating Prl was probably a result of differences in such characteristic variables.

Significant ($p < 0.01$) heifer variation also was evidenced in the analysis (Table 4). In addition to age and physiological state, factors which cause basal Prl changes could include external disturbances and time of day. Although an attempt was taken to collect jugular blood samples between 0800 hr and 0930 hr of the day with minimum evident stress to the animal, the heifers required considerable time to become

accustomed to handling. Variables responsible to handling likely contributed to the observed individual variation in Prl.

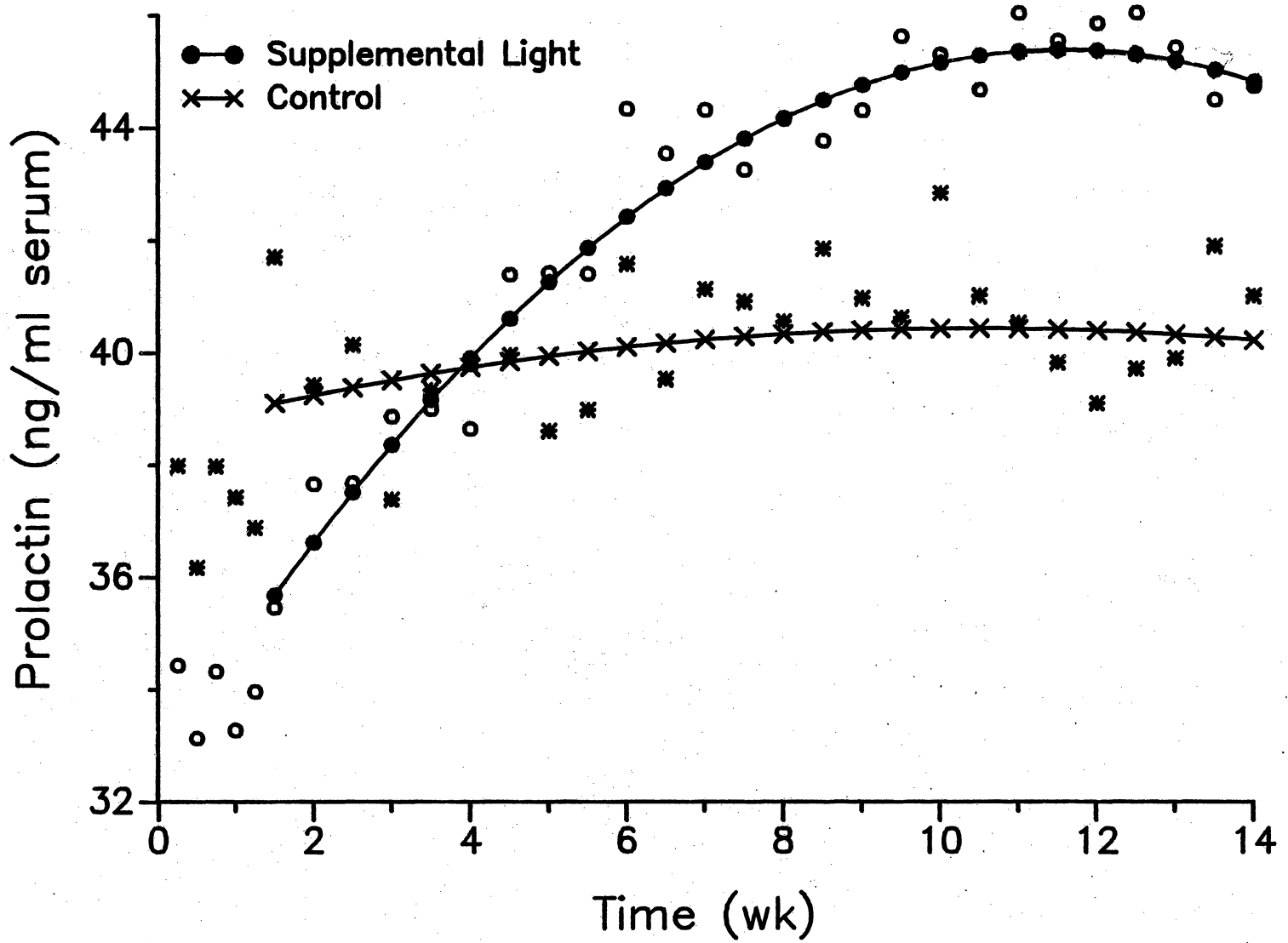
Twice weekly Prl concentrations significantly ($p < 0.01$) varied over the 14 wk experimental period (Table 4 ;Appendix K) increasing until the 10th wk (Table 5). Circulating Prl fluctuates parallel to changes in ambient temperature (Tucker and Wettemann, 1976; Wettemann and Tucker, 1974). Temperature as well as photoperiod act as regulators of prolactin secretion from the pituitary (Tucker, 1982). Temperature had a significant linear effect on Prl while, rainfall during the study period significantly affected Prl (Appendix L) in a negative manner.

Twice weekly Prl concentrations were significantly ($p < 0.01$) affected by time by treatment interaction (Table 4). Both the treatment group and control group serum Prl varied in quadratic manner with time (Figure 9; Appendix M). Both average daily temperature (linear effect) and rainfall significantly affected serum Prl (Appendix L). Change in Prl concentrations in the control group heifers were minimal as compared to those of the supplemental light treated group heifers. The latter group Prl concentration increased from 35.5 ± 2.0 ng/ml to a maximum of 46.0 ± 1.7 ng/ml during treatment period while the control group changed from 41.7 ± 2.3 ng/ml to 42.8 ± 2.2 ng/ml (Appendix M) during the treatment period. In addition to the differences in Prl during the treatment period, treatment group heifers appeared to have a lower Prl during the pre-treatment period. The latter group was haltered in the housing area during the night from d 1 of the study. They might have been less excited than the control group during blood sampling during the following

Table 5. Mean ± SE of Prolactin concentrations (ng/ml) before and after exposure to 4 hr daily supplemental lighting.

Time (wk)	Prolactin (ng/ml)
0.25	36.2 ± 1.3
0.50	34.6 ± 1.2
0.75	36.1 ± 1.2
1.00	35.3 ± 1.2
1.25	35.4 ± 1.2
1.50	38.6 ± 1.6
2.00	38.5 ± 1.5
2.50	38.9 ± 1.5
3.00	38.1 ± 1.5
3.50	39.1 ± 1.5
4.00	39.2 ± 1.4
4.50	40.7 ± 1.4
5.00	40.1 ± 1.5
5.50	40.2 ± 1.2
6.00	41.9 ± 1.4
6.50	41.5 ± 1.6
7.00	42.7 ± 1.5
7.50	42.1 ± 1.4
8.00	42.5 ± 1.6
8.50	42.8 ± 1.6
9.00	42.7 ± 1.4
9.50	43.2 ± 1.5
10.00	44.0 ± 1.5
10.50	42.8 ± 1.4
11.00	43.5 ± 1.5
11.50	42.6 ± 1.4
12.00	42.5 ± 1.5
12.50	42.9 ± 1.5
13.00	42.6 ± 1.4
13.50	43.2 ± 1.2
14.00	42.9 ± 1.3

Figure 9. Weekly prolactin concentration in buffalo heifers exposed to 4 hr supplemental lighting daily.


Supplemental Light
Control
Prolactin (ng/ml serum)
44
40
36
32
0
2
4
6
8
10
12
14
Time (wk)

morning. This could be a factor contributing to lower Prl during pre-treatment period in the treatment group.

The slight increase in Prl in the control group heifers was partly a reaction to increasing ambient temperatures until the 10th wk. Apparently the weekly mean Prl concentrations of that group increased until the 10th wk, and then declined at the 11th and 12th wk when temperatures dropped as a result of precipitation. At the 13th and 14th wk when temperatures started to rise again (Figure 3), a second increase in Prl concentrations was apparent supporting the positive relation between Prl and temperature. In contrast, the supplemental light treated heifers maintained their elevated Prl concentrations despite the rainfall implying an effect of supplemental lighting on circulating Prl. Whether this elevated Prl had any beneficial effects on maintaining body weight of the treatment group by alleviating thermal stress during the 7th, 8th and 9th wk of the study warrants further investigation.

4.3.2 Progesterone.

Serum progesterone concentrations varied between 0.10 ng/ml and 5.13 ng/ml with a mean value of 0.30 ± 0.02 during the study period. The model used to analyze these fluctuations resulted in a R^2 value of 0.72 implying that the unexplained variation amounted to only 28%.

The supplemental light treated heifers had a mean progesterone concentration of 0.39 ± 0.02 ng/ml, which was significantly ($p < 0.07$; Table 6) greater than the mean value of control heifers (0.18 ± 0.02 ng/ml).

Table 6. Analysis of variance for weekly progesterone of buffalo heifers exposed to 4 hr supplemental lighting daily.

Source	df	MS	F
Treatment (Tmt)[a]	1	10.45	3.82*
Age group (AG)[a]	1	10.87	3.97*
Tmt * AG[a]	1	9.83	3.59*
IPRG[b]	1	195.69	1223.06**
Heifer (Tmt * AG)	42	2.74	17.12**
Time	21	0.32	1.99**
Time * Tmt	21	0.14	0.88
Time * AG	21	0.13	0.80
Time * Tmt * AG	21	0.16	1.01
Residual	896	0.16	
Total	1026		

** $p < 0.01$

* $p < 0.07$

[a] Tested by heifer (Tmt * AG)

[b] Pre-treatment progesterone concentrations

Circulating progesterone values exceeding 1 ng/ml in cattle are an indication of attainment of puberty and/or existence of functioning corpora lutea, hence occurance of cyclic activity (Hansel et al., 1973). While mean progesterone did not exceed 1 ng/ml, the higher mean progesterone concentrations detected in the supplemental light treated heifers indicate the presence of more cycling heifers in that group as compared to the control group.

In addition to the effect of treatment, circulating progesterone concentrations were significantly ($p < 0.07$) affected by age group (Table 6). Heifers older than 24 mo had a mean progesterone concentration of 0.39 ± 0.02 ng/ml compared to the mean concentration of 0.18 ± 0.02 ng/ml in heifers younger than 24 mo. Heifers become cyclic as they become older, and have higher mean progesterone concentrations during luteal phase of the cycle (Arora and Pandey, 1982; Hansel et al., 1973; Kanai and Shimizu, 1984). The greater mean serum progesterone in the older group suggests existence of more cycling animals in that group.

Treatment by age group interaction also was significant ($p < 0.07$) for progesterone (Table 6). Younger heifers of light treated group had significantly lower progesterone concentrations (0.19 ± 0.02 ng/ml than older supplemental light treated heifers (0.59 ± 0.02 ng/ml) indicating the effect of age. In contrast, the mean progesterone concentration of older animals in the control group (0.19 ± 0.02 ng/ml) was not greater than that of the younger heifers (0.18 ± 0.02 ng/ml). The higher mean progesterone values in the older heifers of the light treated group above all the other groups demonstrates the beneficial effects of light treatment

on attainment of puberty and/or persistance of cyclic activity among heifers older than 24 mo.

Initial pre-treatment progesterone concentrations varied significantly ($p < 0.01$) between 0.10 ng/ml and 3.79 ng/ml serum having a mean value of 0.35 ± 0.02 ng/ml during the first 10 d of experiment(Table 6). Heifer variation also significantly ($p < 0.01$) affected the weekly progesterone variation (Table 6). Circulating progesterone varies with age and physiological status of the animal (Jain and Pandey, 1985; Kanai and Shimizu, 1984). As the heifers used for this experiment were of a wide age range (17 to 42 mo) and would have been in different physiological states at the begining of the experiment, such differences in basal progesterone concentrations could be expected.

Serum progesterone concentrations significantly ($p < 0.01$) varied over time (Tables 6 and 7; Appendix N). In the cycling female, serum progesterone increases during the luteal phase to achieve maximum concentrations prior to luteal regression and then reaches a minimum prior to ovulation remaining low during the follicular phase (Arora and Pandey, 1982; Hansel et al., 1973; Kanai and Shimizu, 1984). In addition to these cyclic fluctuations, serum progesterone in buffalo has been shown to be lower at high ambient temperature conditions (Rao and Pandey, 1982). Most cycling buffalo females become anestrous under hot environmental conditions and under low planes of nutrition (Kaur and Arora, 1982; 1984). The natural pasture growth was retarded resulting in a decline in the quantity as well as the quality of available pasture within the farm due to the drought. In addition, there was a significant ($p < 0.05$)

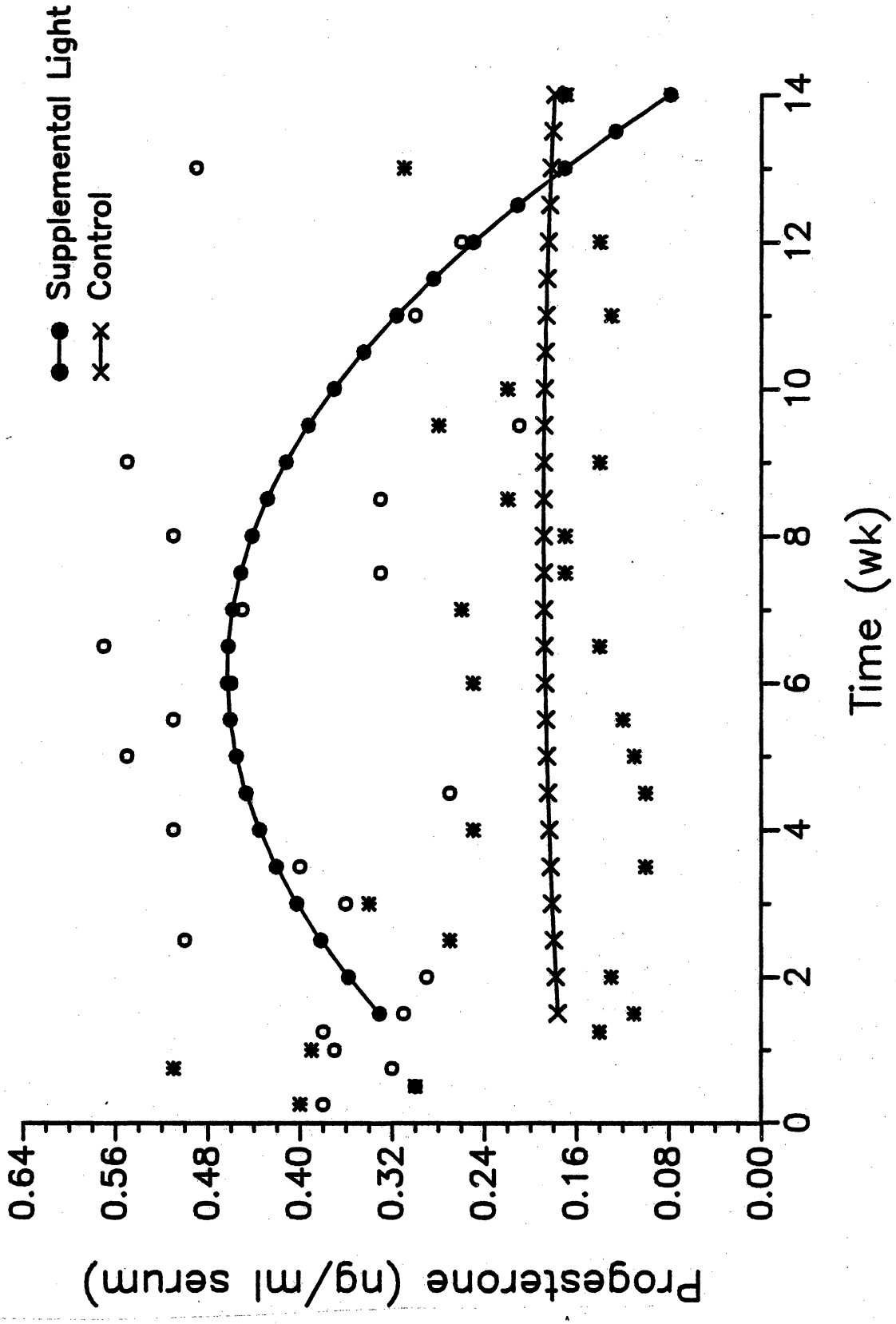
Table 7. Mean ± SE of progesterone (ng/ml) before and after exposure to 4 hrsupplemental light daily.

Time(wk)	Progesterone (ng/ml)
0.25	0.38 ± 0.09
0.50	0.29 ± 0.11
0.75	0.41 ± 0.13
1.00	0.37 ± 0.13
1.25	0.27 ± 0.09
1.50	0.22 ± 0.10
2.00	0.22 ± 0.09
2.50	0.39 ± 0.09
3.00	0.33 ± 0.14
3.50	0.27 ± 0.11
4.00	0.39 ± 0.10
4.50	0.19 ± 0.09
5.00	0.37 ± 0.14
5.50	0.38 ± 0.12
6.00	0.31 ± 0.11
6.50	0.43 ± 0.12
7.00	0.32 ± 0.10
7.50	0.26 ± 0.09
8.00	0.37 ± 0.12
8.50	0.24 ± 0.09
9.00	0.42 ± 0.11
9.50	0.22 ± 0.04
10.00	0.26 ± 0.08
11.00	0.21 ± 0.08
12.00	0.20 ± 0.06
13.00	0.41 ± 0.01
14.00	0.17 ± 0.03

negative effect of relative humidity on serum progesterone (Appendix O). These factors might have been responsible for variation in serum progesterone concentrations over time.

A significant ($p < 0.05$) treatment by time interaction resulted when supplemental light period progesterone concentrations were analyzed using an alternative model considering time as a continuous variable (Appendix N). The graph plotted using the regression coefficients derived from this alternative model (Figure 10) exhibits significant variations of progesterone concentrations of the treatment group as compared to the control group. In the control group heifers progesterone values remained near 0.18 ng/ml throughout the study. This further confirms the least squares mean progesterone concentrations estimated for control group heifers and for treatment by age interaction. In contrast, supplemental light treated heifers had a rise in serum progesterone until about the 8th wk and a decline thereafter (Figure 10; Appendix P). Circulating progesterone increases during the luteal phase (Hansel et al., 1973). The upward trend in progesterone until the 8th wk suggests an increase in the number of cycling heifers in the treatment group. Cycling buffalo become anestrous under conditions of high ambient temperatures and low plane of nutrition as discussed in the previous section. Thus, the following decline might be a consequence of adverse effects of humidity and low nutrition and/or more heifers becoming anestrous.

Figure 10. Progesterone concentrations of buffalo heifers exposed to 4 hr supplemental lighting daily.


Supplemental Light
Control
Progesterone (ng/ml serum)
0.64
0.56
0.48
0.40
0.32
0.24
0.16
0.08
0.00
0
2
4
6
8
10
12
14
Time (wk)

4.3.3 Luteinizing Hormone (LH)

4.3.3.1 Concentrations During Treatment Period

Concentrations of LH during the supplemental light period fluctuated between 0.10 ng/ml and 5.44 ng/ml having a mean value of 0.49 ± 0.01 ng/ml serum. The model used to analyze this variation had a R^2 value of 0.33 (Table 8) indicating that the variation caused by unknown variables was approximately 67%. Nonetheless the use of above mentioned model and an alternative model with time as a continuous variable (Appendix Q) was continued because other influential variables were not identified.

Least squares mean concentration of LH in the supplemental light treated heifers (0.52 ± 0.01 ng/ml) was significantly ($p < 0.06$; Table 8) greater than that of the control group (0.46 ± 0.01 ng/ml). Gonadotropins are secreted in greater amounts during prepubertal period (Schams et al., 1981; Schillo et al., 1983) with cycling females having higher circulating LH compared to noncycling ones. Hence, the significantly higher LH in the treatment group can be inferred as being an indication of having more prepubertal and/or cycling heifers in that group. This further supports the higher mean progesterone concentrations detected among treatment group heifers.

Age group affected serum LH concentrations significantly ($p < 0.01$; Table 8). Heifers older than 24 mo had a mean LH concentration of 0.54 ± 0.01 ng/ml as compared to the 0.44 ± 0.01 ng/ml for younger heifers.

Table 8. Analysis of variance for weekly luteinizing hormone of buffalo heifers exposed to 4 hr supplemental lighting daily.

Source	df	MS	F
Treatment (Tmt)[a]	1	0.85	4.05*
Age group (AG)[a]	1	2.82	13.43**
Tmt * AG[a]	1	0.22	1.05
ILH[b]	1	2.75	30.55**
Heifer (Tmt * AG)	42	0.21	2.33**
Time	25	0.94	10.35**
Time * Tmt	25	0.12	1.30
Time * AG	25	0.10	1.13
Time * Tmt * AG	25	0.06	0.70
Residual	1049	0.09	
Total	1195		

** $p < 0.01$

* $p < 0.06$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment LH concentrations

Significant elevations in circulating gonadotropin concentrations with age during the pre-pubertal period have been observed among cattle (Schillo et al., 1981; 1983). Higher mean LH concentrations in the older group likely indicate the presence of more cycling heifers.

Pre-treatment LH concentrations varied significantly ($p < 0.01$; Table 8) between 0.19 ng/ml and 1.04 ng/ml with a mean of 0.43 ± 0.16 ng/ml during the first 10 days before supplemental lighting was provided. Heifer variation also was highly significant ($p < 0.01$; Table 8). Basal LH concentration is a function of age (Schams et al., 1981), physiological stage and stage of the estrous cycle (Kanai and Shimizu, 1984). Since the heifers used for this experiment were of a wide age range and might have been in various physiological situations, existance of such individual variation is reasonable.

Mean LH concentrations during the supplemental light period significantly ($p < 0.01$; Tables 8 and 9; Appendix Q) varied over time. Gonadotropins exhibit characteristic pulsatile patterns depending on GnRH input (Knobil, 1980) and feedback action of sex steroids (Beck et al., 1976; Karsch et al., 1973). Without obtaining frequent serial blood samples, tonic secretory patterns could not be detected even though pulses may exist. Mean LH increases dramatically during the preovulatory gonadotropin surge (Arora and Pandey, 1982; Hansel and Convey, 1983; Kanai and Shimizu, 1984). Seasonal variations in LH concentrations depending on climatic variations also have been reported for buffalo (Rao and Pandey, 1983). Temperature and rainfall significantly affected serum LH (Appendix R). Involvement of Prl for

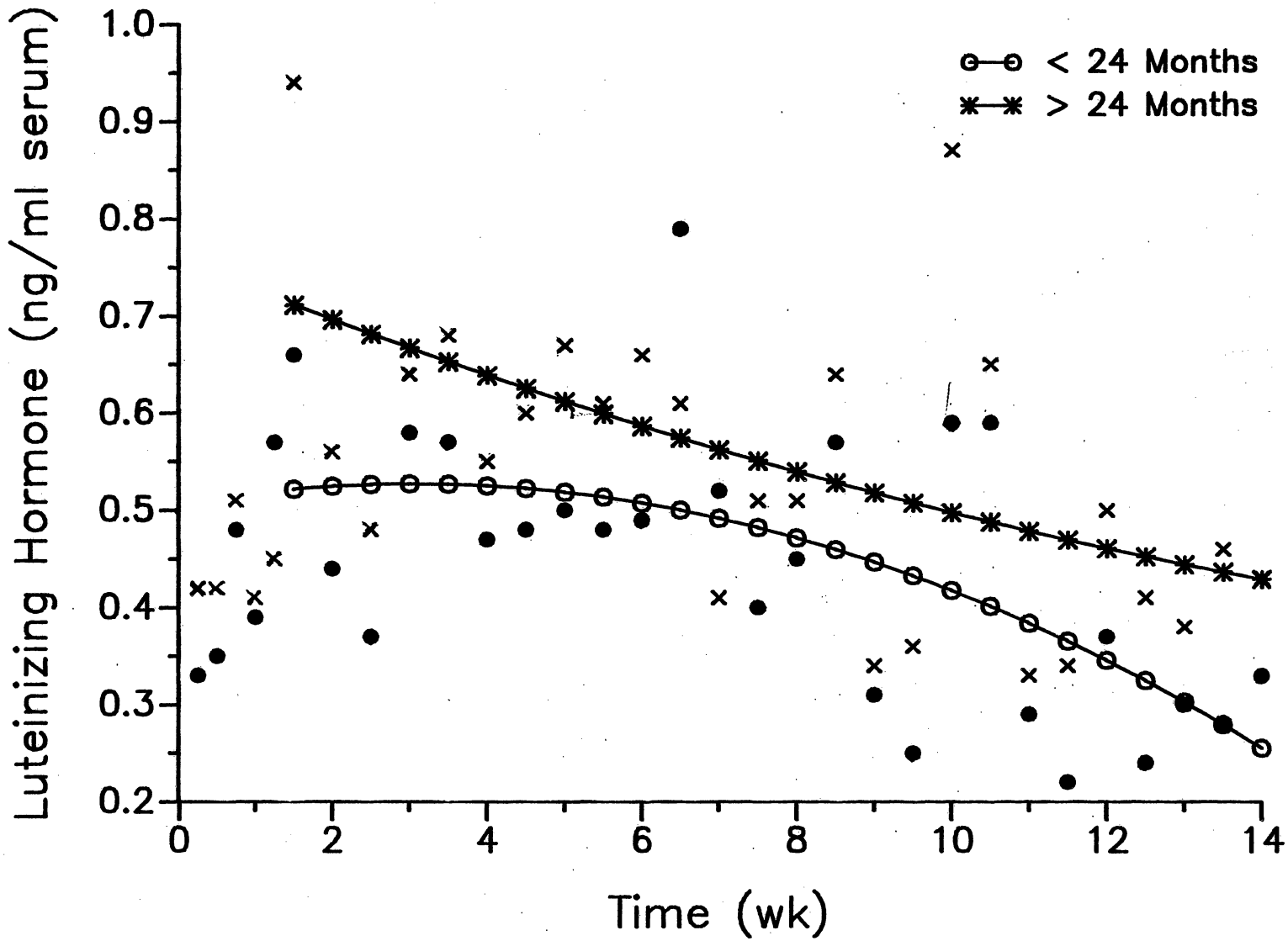
Table 9. Means ± SE of LH (ng/ml) of buffalo heifers exposed to 4 hr supplemental lighting daily.

Time(wk)	LH (ng/ml)
0.25	0.37 ± 0.02
0.50	0.38 ± 0.02
0.75	0.49 ± 0.03
1.00	0.39 ± 0.03
1.25	0.50 ± 0.03
1.50	0.79 ± 0.08
2.00	0.50 ± 0.02
2.50	0.42 ± 0.03
3.00	0.60 ± 0.02
3.50	0.62 ± 0.03
4.00	0.50 ± 0.02
4.50	0.54 ± 0.02
5.00	0.59 ± 0.03
5.50	0.54 ± 0.03
6.00	0.57 ± 0.03
6.50	0.70 ± 0.10
7.00	0.46 ± 0.06
7.50	0.45 ± 0.02
8.00	0.48 ± 0.03
8.50	0.60 ± 0.04
9.00	0.32 ± 0.02
9.50	0.30 ± 0.02
10.00	0.72 ± 0.07
10.50	0.61 ± 0.05
11.00	0.31 ± 0.02
11.50	0.28 ± 0.03
12.00	0.43 ± 0.04
12.50	0.32 ± 0.04
13.00	0.33 ± 0.04
13.50	0.37 ± 0.03
14.00	0.38 ± 0.03

inhibition of ovarian function by acting at the hypothalmic-pituitary level has been suggested (McNeily, 1979; 1980). However, no information is available on such a relationship in buffalo. The observed variation of LH during the treatment period might have been caused by the distinct phase of the tonic LH secretion the non-cycling heifers were in, the stage of the ovarian cycle the cycling heifers were experiencing at the time of blood sampling and the changes in temperature, relative humidity and rainfall.

A significant ($p < 0.05$) quadratic time by age interaction was evident when analyzing time as a continuous variable (Appendix Q). Mean LH concentrations of older animals were greater than those of younger heifers at the beginning of the experiment implicating the age related increase in circulating LH. This difference was maintained throughout the experimental period. The LH values of older heifers declined gradually to 0.43 ± 0.04 ng/ml at the 14th wk while LH of younger heifers remained around 0.5 ng/ml until the 6th wk and declined in the following weeks to reach a 0.33 ± 0.05 ng/ml concentration at the 14th wk (Figure 11; Appendix S). Adverse effects of low plane of nutrition and of high ambient temperatures (Rao and Pandey, 1983) have been reported on circulating gonadotropins of buffalo. As discussed earlier during the first 10 wk of the experiment a drought condition prevailed and after the 6th wk the plane of nutrition was adversely affected. The observed downward trend of LH in this experiment probably was a result of decline in plane of nutrition and the prevailing drought conditions.

Figure 11. Luteinizing hormone concentration of buffalo heifers exposed to 4 hr supplemental light daily by age group.


Luteinizing Hormone (ng/ml serum)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
2
4
6
8
10
12
14
Time (wk)
< 24 Months
> 24 Months

4.3.3.2 LH response to GnRH

Luteinizing hormone concentrations varied significantly between 0.32 ng/ml and 36.44 ng/ml with a mean value of 3.46 ± 0.17 ng/ml following intravenous administration of 30 μg GnRH per heifer. The model used to analyze this variation resulted in a R^2 value of 0.70 indicating the appropriateness of the considered independent variables. Neither the treatment nor the age group effect and their interaction exerted any significant influence on LH variation (Table 10).

Pre-injection LH concentrations which varied between 0.17 ng/ml and 1.82 ng/ml with a mean value of 0.41 ± 0.01 ng/ml significantly ($p < 0.01$) affected the observed variation of LH (Table 10).

Heifer variation also significantly ($p < 0.01$) affected LH response to GnRH (Table 10). A fixed GnRH dose (30 μg/heifer) was administered regardless of body weight or stage of the estrous cycle of the heifers. The response to GnRH varied depending on maturation of hypothalamic pituitary axis and hence age, dose of GnRH (McLeod et al., 1984) and stage of the ovarian cycle in the cycling animal (Convey et al., 1981). In addition to the variables which affected basal LH concentrations these factors could have contributed to the observed significant variation in LH response to GnRH among heifers.

Luteinizing hormone increased dramatically in response to GnRH administration ($p < 0.01$; Tables 10 and 11). Basal LH values significantly increased from 0.41 ± 0.03 ng/ml prior to GnRH to a maximum of 7.11 ± 0.88 ng/ml at 30 min following GnRH administration and gradually de-

Table 10. Analysis of variance of LH response to GnRH in buffalo heifers exposed to 4 hr supplemental lighting daily.

Source	df	MS	F
Treatment (Tmt)[a]	1	13.63	0.35
Age group (AG)[a]	1	23.12	0.60
Tmt * AG[a]	1	48.26	1.26
ILH[b]	1	174.48	36.20**
Heifer (Tmt * AG)	42	38.33	7.95**
Time	8	239.32	49.63**
Time * Tmt	8	4.36	0.90
Time * AG	8	7.52	1.56
Time * Tmt * AG	8	10.13	2.10*
Residual	343	4.82	
Total	421		

* $p < 0.05$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-injection LH

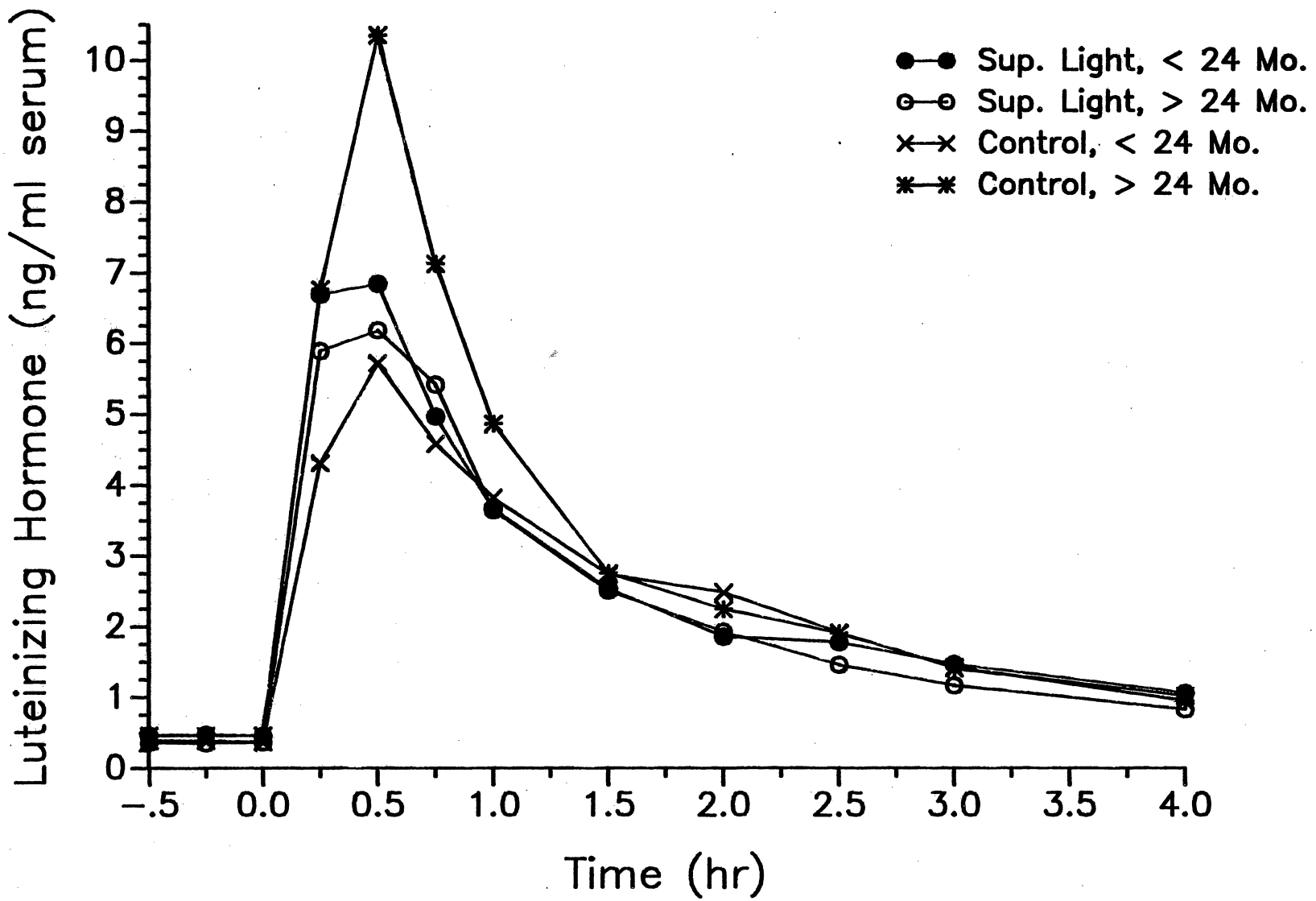
Table 11. Means ± SE of LH (ng/ml) of buffalo heifers before and after GnRH administration.

Time (Hr)	LH (ng/ml)
-0.50	0.41 ± 0.03
-0.25	0.41 ± 0.03
0.00	0.41 ± 0.03
0.25	5.87 ± 0.57
0.50	7.11 ± 0.88
0.75	5.44 ± 0.56
1.00	3.95 ± 0.40
1.50	2.62 ± 0.23
2.00	2.11 ± 0.18
2.50	1.74 ± 0.13
3.00	1.35 ± 0.09
4.00	0.95 ± 0.06

clined thereafter. However, pre-injection values were not reached during the sample collection period (4 hr post GnRH; Table 11). GnRH stimulates gonadotrophs to release their releasable pool of LH if the pituitary is not refractory (Convey et al., 1981). The immediate increase in LH following administration of GnRH might have been caused by stimulation of pituitary gonadotrophs to release their readily releasable pool of gonadotropins by exogenous GnRH. Circulating concentrations of LH declined following this release probably as a result of reduced secretion due to depletion of the releasable LH pool, down regulation of pituitary GnRH receptors and metabolic clearance of LH. Similar responses in LH to GnRH over time has been reported for buffalo heifers (Aboul-Ela et al., 1983).

Neither the interactions between time and treatment nor between time and age group were significant. Nonetheless, the time by treatment by age group three way interaction significantly ($p < 0.01$) affected the variation in LH (Table 10). Basal mean LH ranged between 0.35 ± 0.12 and 0.47 ± 0.12 ng/ml serum. All four groups of heifers responded to GnRH and reached maximum LH concentrations at 30 min following GnRH administration. Heifers > 24 mo in the control group exhibited greater peak response to GnRH than the other three groups (Figure 12; Appendix T). Older heifers were expected to exert a greater response to GnRH (McLeod et al., 1984). However, the response to GnRH varies depending on dose and physiological state (Convey et al., 1981). Older heifers of supplemental light treated group were comparatively heavier than the heifers of other treatment groups. But the analysis using body weight

Figure 12. Luteinizing hormone response to gonadotropin releasing hormone in buffalo heifers exposed to 4 hr supplemental light daily.


Luteinizing Hormone (ng/ml serum)
0
1
2
3
4
5
6
7
8
9
10
−.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Time (hr)
Sup. Light, < 24 Mo.
Sup. Light, > 24 Mo.
Control, < 24 Mo.
Control, > 24 Mo.

as a covariate did not show an association of body weight with the LH response (Appendix U).

4.4 OVARIAN STRUCTURES

Data obtained from weekly palpation of ovarian structures indicated that only 3 heifers of < 24 mo group had any palpable structures. Due to insufficient numbers for comparison, the age group parameter and its interactions were excluded from the basic model used to analyze the data. Coefficients of determination of the models varied between 0.20 and 0.26. Pre-treatment data were not available because the initial palpation was conducted only to separate pregnant from the non-pregnant heifers.

None of the independent variables tested (except for the effect of heifer) affected the number of follicles on the left ovary, number of follicles on the right ovary, number of corpora lutea on the right ovary, number of corpora lutea on the left ovary, and number of follicles on both the ovaries combined at the time of palpation (Tables 12, 13 and 14). The number of corpora lutea on both the ovaries combined was the only parameter which was affected significantly by weeks following initiation of treatment ($p < 0.02$). Both the number of follicles and number of corpora lutea had opposite directional cyclic changes in the cycling animals (Figure 13). The cyclic changes should have a duration equal to the duration of estrous cycle. Mature follicles can be misinterpreted as corpora lutea and visa versa during rectal palpation (Figure 13). It was extremely difficult to conduct palpation in these heifers because they

Table 12. Analyses of variance of Number of Follicles on the Right Ovary (FOLRO) and on the Left Ovary (FOLLO).

Source	df	FOLRO MS	F	FOLLO MS	F
Treatment (Tmt)[a]	1	0.00	0.00	0.24	0 38
Heifer (Tmt)	19	0.09	1.26	0.29	2.57**
DPT[b]	12	0.09	1.23	0.15	1.35
DPT * Tmt	12	0.05	0.61	0.14	1.23
Residual	228	0.07		0.11	
Total	272				

** $p < 0.01$

[a] Tested by Heifer (Tmt)

[b] Weeks post treatment

Table 13. Analyses of variance of Number of Corpora Lutea on the Right Ovary (CLRO) and on the Left Ovary (CLLO).

Source	df	CLRO MS	F	CLLO MS	F
Treatment (Tmt)[a]	1	0.04	0.23	0.12	0.73
Heifer (Tmt)	19	0.19	2.52**	0.17	1.85*
DPT[b]	12	0.10	1.42	0.14	1.53
DPT * Tmt	12	0.06	0.80	0.06	0.72
Residual	228	0.07		0.09	
Total	272				

** $p < 0.01$

* $p < 0.02$

[a] Tested by Heifer (Tmt)

[b] Weeks post treatment

Table 14. Analyses of variance of Number of Follicles (FOL) and Number of Corpora Lutea (CL) on both ovaries.

Source	df	FOLO MS	F	CLO MS	F
Treatment (Tmt)[a]	1	0.22	0.62	0.31	1.09
Heifer (Tmt)	19	0.36	2.18**	0.28	2.01*
DPT[b]	12	0.20	1.20	0.31	2.21*
DPT * Tmt	12	0.19	1.17	0.13	0.95
Residual	228	0.16		0.14	
Total	272				

** $p < 0.01$

* $p < 0.02$

[a] Tested by Heifer (Tmt)

[b] Weeks post treatment

were not accustomed to it. Thus, there was a possibility of obtaining incorrect information via rectal palpation.

4.5 CONCEPTION DATA

Chi-square analysis of number of heifers in each group which became pregnant following the treatment revealed that 32% of the treatment group heifers conceived compared to 20% in the control group. This difference was not significant. Because the light treatment was limited to only 14 wk a conclusive statement cannot be drawn on effects of photoperiod on conception. When chi-square analysis was performed between age groups, the results indicated a significantly ($p < 0.05$) lower conception rate in younger heifers (12.5%) as compared to older heifers (38.5%). The ability to conceive depends on sexual maturity of the heifer, physiological condition, nutritional status (Bhalaru et al., 1981; Kaur and Arora, 1982), climatic conditions (Ahmad et al., 1981; Lundstrom et al., 1982) and fertility of semen. Sexual maturity partially depends upon age (Pritchard et al., 1972; Sorensen et al ., 1959). This is confirmed by this significant ($p < 0.05$; Table 15) difference in conception between age groups.

Figure 13. Mean Numbers of Follicles and Corpora Lutea on both ovaries between 2nd and 14th wk.

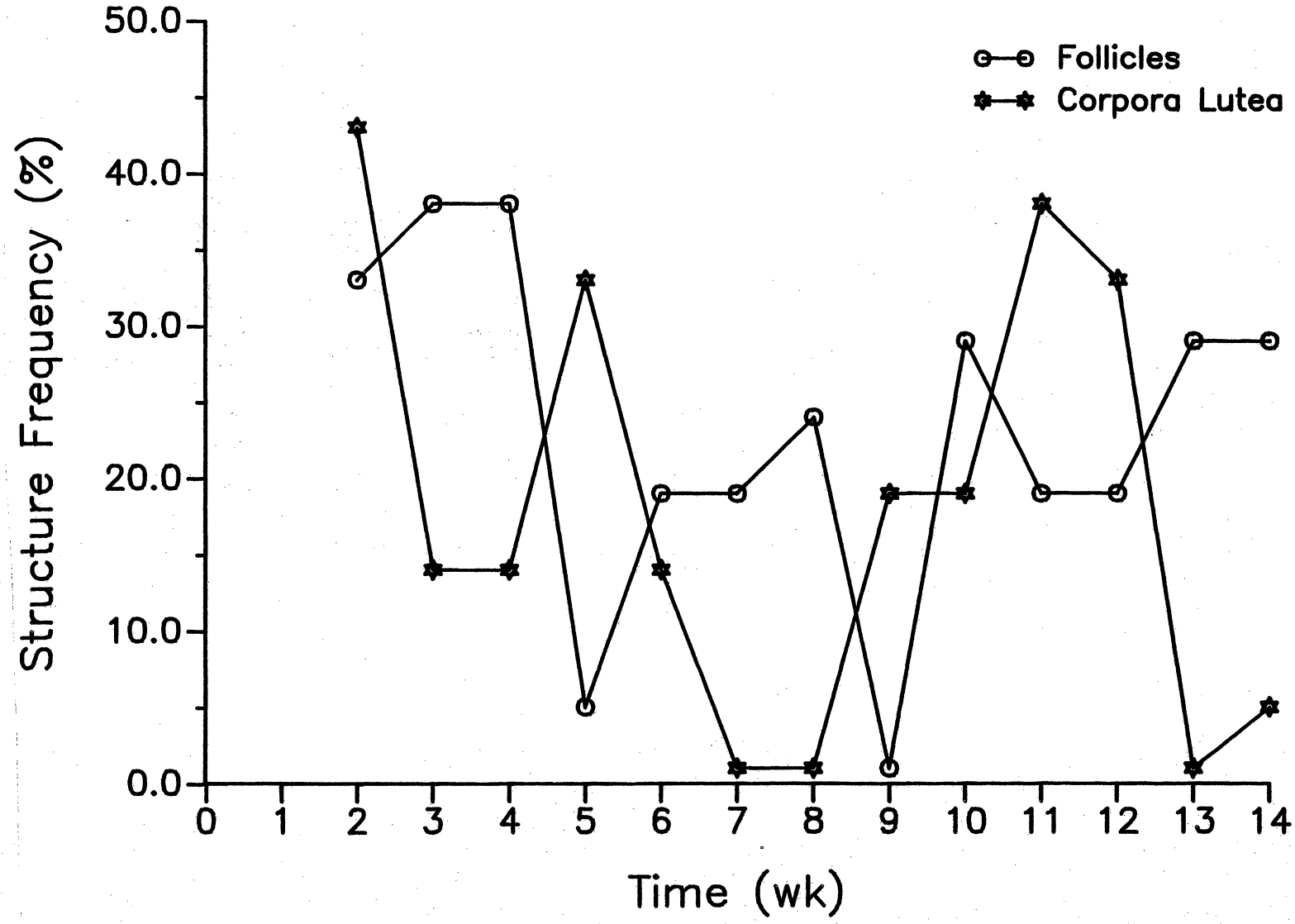

Follicles
Corpora Lutea
Structure Frequency (%)
50.0
40.0
30.0
20.0
10.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Time (wk)

Table 15. Results of Chi-Square analysis on number of heifers became pregnant within 6 mo following the experiment.

	> 24 mo	< 24 mo
Number	10	3
Percent	38.5[a]	12.5[b]

[a], [b] Differ at $p < 0.05$

Chi-Square = 4.37; $p < 0.05$; df = 1

CHAPTER 5

SUMMARY AND CONCLUSIONS.

Daily ambient temperature fluctuated between 25 and 34 C but was above 27 C during most of the day-light hours. Daily minimum and maximum temperatures occured at 0600 hr and 1400 hr, respectively. Weekly average of daily maximum temperatures increased until the 10th wk while, weekly average daily minimum temperatures reached the lowest values during the 9th wk.

Daily relative humidity assumed extreme values of 34% and 100% and exhibited an opposite diurnal pattern to that of temperature. During the day-light hours relative humidity was < 70%. Weekly average daily maximum relative humidity values peaked during the 11th wk.

Total weekly rainfall varied between 0 and 117 mm. Precipitation started at the end of the 10th wk, reached a peak at the 11th wk, and continued through the 12th wk. Sudden changes in ambient temperature and relative humidity resulted from this rainfall. These weather variables clearly indicated a drought condition prevailed during the experimental period until the 10th wk.

During the experimental period, individual weekly body weights of the heifers varied between 106 kg and 371 kg. Least squares mean body weight of heifers that recieved supplemental lighting was 5.8% heavier ($p < 0.01$) than that of control heifers. Since the plane of nutrition and management conditions was similar for both the groups, the heavier

body weights were obtained with supplemental lighting. Least squares mean weekly body weights of older buffalo heifers (> 24 mo) were 67% above ($p < 0.01$) those of younger (< 24 mo) buffalo due to age associated increases in weight.

Pre-treatment body weight of buffalo heifers as well as individual weight of heifers varied significantly ($p < 0.01$) probably as a result of differences among individual's age and feed intake. Both linear and quadratic effects of time for body weight were significant ($p < 0.01$). Mean body weights of 50 heifers increased until the 10th wk and declined thereafter. The variation in quantity and quality of available pasture over time due to changes in ambient temperature, relative humidity and rainfall could have caused these variations.

Treatment group heifers experienced a net weight gain of 6.1 kg (0.5 kg/wk/heifer; $p < 0.01$) during the experiment as compared to 0.5 kg net weight loss in the control group implicating supplemental lighting as being beneficial for body growth of buffalo.

Younger animals had a net weight gain of approximately 4.6 kg (0.36 kg/wk/heifer; $p < 0.01$) while the older animals ended up with 7.8 kg (0.6 kg/wk/heifer) net weight loss. These results implicate age associated changes in weight gain and possibility of supplemental lighting being more beneficial for younger animals.

Serum Prl fluctuated between 20.1 and 69.7 ng/ml with a mean value of 41.5 ng/ml in weekly samples. Mean Prl concentration of the light treated group (42.6 ± 0.2 ng/ml) was significantly ($p < 0.01$) greater

than that of the control group (40.1 ± 0.2 ng/ml) indicating a small photoperiodic stimulation of Prl release in buffalo.

Mean serum Prl in older animals (49.0 ± 0.2 ng/ml) was significantly ($p < 0.01$) greater than that of younger animals (33.7 ± 0.2 ng/ml) suggesting age associated changes in circulating prolactin concentrations. Significant variation observed in basal pre-treatment concentrations (range 24.1 to 52.1 ng/ml; mean 35.6 ng/ml) was primarily related to age and individual animal variation ($p < 0.01$).

Prl concentrations significantly ($p < 0.01$) varied over the 14 wk experimental period. Changes in ambient temperature conditions, stress caused by restraining of heifers for blood sampling, and changes in physiological conditions of heifers during the experimental period might have caused these variations. Prolactin concentrations were generally unchanged over time among control group heifers but treatment group heifers exhibited increased Prl concentration (35.5 ± 0.2 ng/ml to 46.0 ± 0.2 ng/ml). Supplemental lighting thus increased concentration of serum Prl ($p < 0.01$).

Serum progesterone concentrations varied between 0.10 ng/ml and 5.13 ng/ml with a mean value of 0.30 ± 0.02 during the study period. Mean circulating progesterone concentration was significantly greater ($p < 0.07$) among treatment group heifers (0.39 ± 0.02 ng/ml vs 0.18 ± 0.02 ng/ml) suggesting the existence of greater numbers of cyclic heifers in that group.

Heifers older than 24 mo had a mean serum progesterone concentration of 0.39 ± 0.02, which was significantly greater ($p < 0.07$) than the mean

concentration of younger heifers (0.18 ± 0.02 ng/ml). Age related changes in sex steroids reflect this difference.

Older heifers of the light treated group had significantly greater ($p < 0.07$) progesterone concentrations (0.39 ± 0.02 ng/ml) than the other groups (0.18 ± 0.02 ng/ml). The beneficial effects of light treatment on attainment of puberty and/or on persistance of cyclic activity are indicated by this treatment by age interaction.

Pre-treatment progesterone concentration, and heifer variation significantly ($p < 0.01$) affected serum progesterone during the supplemental light period. Differences in circulating progesterone associated with age, physiological status of the animal and light response could be responsible for these changes.

Serum progesterone concentrations significantly ($p < 0.01$) varied over time possibly as a result of changes in relative humidity, plane of nutrition and estrous cyclicity. Supplemental light treated heifers had an increase in mean progesterone until about the 8th wk and progesterone declined thereafter while the control group heifers maintained a stable mean progesterone throughout. These significant ($p < 0.01$) changes in trends in progesterone profiles as well as concentrations suggest the possible effects of supplemental lighting and changes in plane of nutrition during the experimental period on cyclicity.

Weekly serum concentrations of LH fluctuated between 0.10 ng/ml and 5.44 ng/ml with a mean value of 0.49 ± 0.01 ng/ml. Mean LH concentration of the treatment group heifers (0.52 ± 0.01 ng/ml) was significantly ($p < 0.06$) greater than that of the control group (0.46 ±0.01

ng/ml), probably as a result of having more prepubertal and/or cycling heifers in that group also indicated by increased serum progesterone.

Heifers older than 24 mo had a mean LH concentrations of 0.54 ± 0.01 ng/ml as compared to 0.44 ± 0.01 ng/ml of the younger heifers. implicating the existence of more cycling heifers in that group.

Weekly mean LH concentrations significantly ($p < 0.01$) varied over time. Both the linear and quadratic time by age interactions were significant ($p < 0.05$). The LH values of older heifers declined from 0.94 ± 0.16 ng/ml gradually to assume 0.43 ± 0.04 ng/ml at the 14th wk while LH of younger heifers remained around 0.50 ng/ml until about the 6th wk and declined in the following weeks to reach 0.33 ng/ml serum at the 14th wk. The observed decrease in LH partly was a result of the decline in plane of nutrition and changes in ambient conditions especially in rainfall.

Luteinizing hormone concentrations significantly varied between 0.32 ng/ml and 36.44 ng/ml with a mean value of 3.46 ± 0.17 ng/ml following intravenous administration of 30 μg GnRH per heifer. Pre-injection LH concentrations varied between 0.17 ng/ml and 1.82 ng/ml with a mean value of 0.41 ± 0.01 ng/ml ($p < 0.01$). Heifer variation significantly ($p < 0.01$) affected the LH response to GnRH. Differences in age, physiological condition, and treatment experienced during the previous 14 wk period could be responsible for these differences.

A significant ($p < 0.01$) time effect was evident in serum LH concentrations following GnRH administration. Mean circulating LH increased from 0.41 ± 0.03 ng/ml to a maximum of 7.11 ± 0.88 ng/ml at 30 min

following GnRH administration and gradually declined thereafter. However, basal values were not reached during the sampling period (4 hr post GnRH).

Treatment by age group by time interaction was significant ($p < 0.01$). Basal mean LH ranged between 0.35 ± 0.12 and 0.47 ± 0.12 ng/ml among groups. All four groups of heifers responded to GnRH and reached maximum LH concentrations at 30 min following GnRH administration. Heifers > 24 mo in the control group had a greater response to GnRH than the other three groups.

Data obtained from weekly palpation of ovarian structures indicated that only 3 heifers of < 24 mo had any palpable structures. All the tested variables except number of follicles on the right ovary exhibited significant heifer variation ($p < 0.02$). Number of corpora lutea detected on both the ovaries varied significantly ($p < 0.02$) over weeks post-treatment indicating existence of cyclicity.

Thirty-two percent of heifers which received light treatment conceived within 6 mo following the end of the study as compared to 20% in the control group. Whether this was a result of growth and reproductive function stimulated by light treatment cannot be concluded because the treatment was not continued. A significantly ($p < 0.05$) greater percent of older heifers (38.5%) conceived as compared to 12.5% of younger heifers, indicating age related differences in the ability to conceive.

Based on the results of this short term experiment, it appears that supplemental lighting is beneficial for body growth of buffalo heifers even under the existing plane of nutrition and management conditions. Pro-

vision of 4 hr of extra light stimulated ovarian activity as indicated by elevated serum gonadotropin and progesterone concentrations. However, the beneficial effects of light apparently cannot be exerted below a certain threshold plane of nutrition. Rainfall during grazing period adversely affected body growth by interrupting and decreasing grazing time. Provision of feed under a sheltered area during the rainy season might alleviate adverse effects of rainfall on feed intake and body growth and thereby enhance the attainment of puberty in the young growing buffalo. Arrangement for extra hours of grazing during rainy season if possible also is worthy of consideration. Prior to making any recommendations for the use of supplemental light to stimulate body growth, ovarian activity and onset of puberty for buffalo heifers in Sri Lanka more detailed long term experiments using more age groups and different duration and/or intensity of light need to be conducted.

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APPENDIX A
RADIOIMMUNOASSAY PROCEDURES USED FOR PRL AND/OR LH.

1. Label 12x75 mm borosilicate glass tubes as TC (total counts) and NSB (non specific binding) tubes in duplicate. Label additional tubes necessary for each standard, control and unknown sample in duplicate.

2. Pipette 0, 5, 10, 20, 40, 80 and 160 ul of standard hormone (0.025 ng/ml for Prl and 0.05 ng/ml for LH) to correspondingly labelled tubes. Pippette 25 ul of each control and unknown sera for the Prl assay and/or 300 ul unknown sera for the LH assay in duplicate into tubes labelled for unknown samples. (Note: The above mentioned quantities of unknown samples were estimated to be optimum sample size based on the results of validation procedure (Appendix B,C) conducted using different quantities of unknowns.

3. Add 1% bovine serum albumin in double distilled phosphate buffered saline (Appendix D) to all tubes except TC to make total volume in the tube equal to 500 ul.

4. Add 100 ul of first antibody (Appendix D) to all tubes except TC and NSB. Add 100 ul of water to NSB tubes.

5. Add 100 ul radiolabelled hormone (approximately 17,000 cpm/100 ul for Prl; 30,000 cpm/100 ul for LH) to all tubes.

6. Incubate the tubes at room temperature for 24 hr.

7. Add 100 ul of second antibody to all tubes except TC.

8. Incubate the tubes in the cold (4 C) for 24 hr.

9. Add 3 ml of cold phosphate buffered saline (Appendix D) to all tubes except TC and centrifuge at 2,500 g for 30 min. (Addition of saline should be done immediately before centrifugation).

10. Decant the supernatant liquid (except TC) and allow the tubes to be drained until dry (approximately 1 hr).

11. Blot dry the inside walls of the tubes (except TC) and count on a gamma counter for 1 min. (Detailed description of reagents used and results of validation procedures are in appendix B-D).

APPENDIX B
RESULTS OF PROLACTIN VALIDATION

Different dilutions of the standard stock solution (0.025 ng/ul) in BSA-PBS (standard curve), various aliquotes of buffalo control serum (dose response curve) and 25 ul control serum + dilutions of standard hormone (displacement curve) included for validation. Means of 4 replicates are given below.

Standard Curve

Concentration (ng/tube)	Counts/Minute	cv
TC	16620	0.56
NSB	425	1.26
0.00	6532	7.83
0.125	5543	10.69
0.25	4722	8.08
0.50	4017	18.07
1.00	3337	11.67
2.00	2277	8.79
4.00	1742	7.12

Dose Response Curve

Serum (ul/tube)	Prl content (ng/ml)	cv
5	162.9	50.2
10	129.3	24.5
15	102.2	31.0
20	85.6	35.7
25	76.7	31.8
30	76.4	6.8
40	70.2	28.7
50	66.4	4.7
60	71.8	20.5
70	64.3	0.6
80	63.9	1.3
90	67.2	27.2
100	62.9	1.6
125	65.2	1.5
150	64.1	12.6
175	56.4	35.7
200	57.5	12.1
225	61.7	12.9
250	67.3	11.1
275	59.1	20.1
300	61.7	6.7
325	67.2	27.2
350	75.6	6.2
375	85.6	3.4
400	88.0	2.1
425	102.2	10.8
450	111.4	35.7
475	123.9	27.8
500	135.0	12.07

Displacement curve

Standard (ng) + Control (ul)	Concentration added (ng)	Concentration recovered (ng)
0.00 + 25	1.88	1.88
0.125 + 25	2.11	1.96
0.25 + 25	2.13	2.30
0.50 + 25	2.38	2.40
1.00 + 25	2.88	2.75
2.00 + 25	3.88	4.01
4.00 + 25	5.88	5.53

APPENDIX C

RESULTS OF LUTEINIZING HORMONE VALIDATION

Different dilutions of the standard stock solution (0.050 ng/ul) in BSA-PBS (standard curve), various aliquotes of buffalo control serum (dose response curve) and 300 ul control serum + dilutions of standard hormone (displacement curve) included for validation. Means of 4 replicates are given below.

Standard Curve

Concentration (ng/tube)	Counts/Minute	cv
TC	29430	0.10
NSB	390	1.33
0.00	7873	3.32
0.25	4915	16.82
0.50	4243	10.75
1.00	3309	22.15
2.00	1677	2.80
4.00	1089	3.45
8.00	776	1.36

Dose Response Curve

Serum (ul/tube)	LH content (ng/ml)	cv
5	non detectable	
10	non detectable	
15	non detectable	
20	non detectable	
25	non detectable	
30	non detectable	
40	0.95	52.5
50	0.78	127.6
60	0.67	104.9
70	0.50	49.3
80	0.53	96.9
90	0.31	60.9
100	0.49	88.4
125	0.66	25.0
150	0.75	14.0
175	0.62	23.5
200	0.63	14.1
225	0.59	8.7
250	0.58	10.1
275	0.66	59.2
300	0.42	45.3
325	0.62	7.4
350	0.60	7.3
375	0.58	5.5
400	0.57	10.6
425	0.42	22.5
450	0.40	18.4
475	0.51	5.7
500	0.55	12.2

Displacement curve

Standard (ng) + Control (ul)	Concentration added (ng)	Concentration recovered (ng)
0.00 + 300	0.60	0.58
0.125 + 300	0.85	1.00
0.25 + 300	1.10	1.20
0.50 + 300	1.60	1.80
1.00 + 300	2.60	2.50
2.00 + 300	4.60	4.50
4.00 + 300	8.60	8.60

APPENDIX D
REAGENTS FOR PROLACTIN AND LUTEINIZING HORMONE ASSAYS

1. Double Distilled Phosphate Buffered Saline (DD-PBS) is used as the starting solvent for all the other reagents used through out the assay. This should be stored in a cold place (4 C). DD-PBS contains 0.01 M sodium phosphate (mixture of monobasic and dibasic), 0.14 M sodium chloride containing 0.1% thimerosal, 0.2% sodium azide, and 9.14 mg phenol red/liter and prepared in double distilled water. The pH is adjusted to 7.5.
2. 1% Bovine Serum Albumin in DD-PBS (BSA-PBS) is made by adding bovine serum albumin to DD-PBS to be equal to 1% and adjusting the pH to 7.5. BSA-PBS is used to dilute standards, radio-labelled hormones, and unknowns in the assay.
3. Ethylenediaminetetraacetate disodium salt (EDTA) is added to DD-PBS to equal 0.05 M concentration of EDTA to make 0.05 M EDTA-PBS. The pH is adjusted to 7.5 EDTA-PBS is used to prepare dilutions of second antibody and to make control sera (Guinea pig control serum for prolactin assay, and Rabbit control serum for luteinizing hormone assay).
4. Control serum diluted to 1:250 (i.e. 1 + 249) in 0.05 M EDTA-PBS is used to dilute first antibody for the use of the assays (1 : 40000 for Prl; 1 : 80000 for LH).
5. First antibody : Guinea pig antibovine prolactin is diluted to desired concentration using guinea pig control serum, and rabbit anti-bovine luteinizing hormone is diluted using rabbit control

serum prepared as in step 4. When preparing the antibodies, place a portion of the diluent in the container before adding the antibody to minimize binding of the antibody to the container. Also, save some of the diluent to rinse the antibody storage vial and pippette tips to recover all of the antibody.

6. Radiolabelled hormone : For use in the assay a portion of the iodinated stock solution is diluted in 1% DD-PBS and stored at 4 C.

7. Second antibody : For the use in the assay the desired antibody (sheep serum containing antibodies to guinea pig globulin for Prl assay, and anti rabbit gamma globulin for LH) is diluted in EDTA-PBS to optimum concentrations (1 : 20 for Prl; 1 : 10 for LH).

8. Hormone standards : To prepare standards, purified hormone is obtained from the National Institute of Health in the lyophilized form. This is then weighed and dissolved and diluted to yield a final concentration of 0.025 ng/ul in 1% BSA-PBS. The standard curve runs from 0.1 ng/tube to 4.0 ng/tube with a total of 7 standards in duplicate for Prl assay, and 0.5 ng/tube to 8.0 ng/tube with a total of 7 standards in duplicate for LH assay.

9. Standard sera: Sera collected from several buffalo heifers before the experiment started (as a test bleeding) were pooled, and was used as the control in each assay to estimate intra-assay and inter-assay variation.

APPENDIX E

RADIOIMMUNOASSAY PROCEDURE FOR PROGESTERONE.

1. Label four uncoated[a] 12x75 mm polypropylene tubes as TC (total counts) and NSB (nonspecific binding) tubes in duplicate. Label fourteen progesterone antibody-coated tubes[b], A through G in duplicate for standards[c]. Label additional antibody-coated tubes, also in duplicate, for each control and unknown sample.

2. Pipette 100 ul of the zero standard A into the NSB and A tubes, and 100 ul each of the standards B through G into correspondingly labelled tubes. Pipette 100 ul of each control and unknown sample into the tubes prepared. Pipette all samples directly to the bottoms of the tubes.

3. Add 1.0 ml of buffered (^{125}I) progesterone[d] to every tube. Vortex briefly and gently. Make sure that the tracer is dispensed within 10 min to all tubes assayed at a time.

4. Incubate at room temperature for 3 hr. The incubation time may be extended to 6 hr at room temperature with no significant effect on the assay results.

5. Decant or aspirate the contents of all tubes except the TC tubes. Allow them to drain for at least 3 min. The tubes may be left

inverted for hours until dry without loss of antibody-bound material.

6. Count tubes for 1 min in a gamma counter.

a, b, c, d: Supplied in the Coat – a – Count^{R} no extraction progesterone solid phase RIA kit (Diagnostic Products Corporation, California) (Result of validation procedure for progesterone are in Appendix F).

APPENDIX F

RESULTS OF PROGESTERONE VALIDATION

Different concentrations of standard hormone (standard curve), various aliquots of buffalo control serum (dose response curve) and 50 ul control serum + 50 ul standard hormone (displacement curve) included for validation. Means of 4 replicates are given below.

Standard Curve

Concentration (ng/tube)	Counts/Minute	cv
TC	51769	0.10
NSB	140	0.02
0.00	20679	2.32
0.10	18836	18.75
0.50	15467	8.28
2.00	9644	2.07
10.00	4319	1.13
20.00	2551	4.30
40.00	1587	4.40

Dose Response Curve

Serum (ul/tube)	P_4 Content (ng/ml)	cv
5	1.83	63.8
10	0.45	38.9
15	0.59	19.7
20	0.50	38.6
25	0.43	14.1
30	0.48	9.8
40	0.49	8.6
50	0.43	1.3
60	0.33	7.5
70	0.33	8.6
80	0.33	1.2
90	0.30	7.8
100	0.33	1.6
125	0.34	1.5
150	0.33	6.4
175	0.36	19.5
200	0.34	16.0
225	0.33	5.1

Displacement curve

Standard(ng) + Control (ul)	Concentration added (ng)	Concentration recovered (ng)
0.00 + 50	0.16	0.15
0.05 + 50	0.21	0.23
0.25 + 50	0.41	0.43
1.00 + 50	1.16	1.18
5.00 + 50	5.16	4.68
10.00 + 50	10.16	11.07
20.00 + 50	20.16	22.07

APPENDIX G
ANALYSIS OF VARIANCE OF WEEKLY BODY WEIGHTS OF BUFFALO HEIFERS (TIME AS A CONTINUOUS VARIABLE).

Source	df	MS	F
Treatment (Tmt)[a]	1	1854.83	2.47
Age group (AG)[a]	1	65399.69	86.94**
Tmt * AG[a]	1	725.03	0.96
IBWT[b]	1	868879.60	31288.43**
Heifer (Tmt * AG)	45	752.22	27.09**
Time	1	14635.67	527.01**
$Time^2$	1	14837.10	534.26**
Time * Tmt	1	542.70	19.54**
$Time^2$ * Tmt	1	872.39	31.41**
Time * AG	1	312.40	11.25**
$Time^2$ * AG	1	443.06	15.95**
Residual	592	27.77	
Total	647		

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Initial body weights

Analysis of variance of weekly body weights of buffalo heifers (time as a continuous variable).

APPENDIX H
ANALYSIS OF VARIANCE OF WEEKLY BODY WEIGHTS (TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL AS CONTINUOUS VARIABLES).

Source	df	MS	F
Treatment (Tmt)[a]	1	29.12	0.00
Age group (AG)[a]	1	62.08	0.01
Tmt * AG[a]	1	4055.08	0.82
IBWT[b]	1	5560197.53	213936.03**
Heifer (Tmt * AG)	45	4941.99	190.15**
Avg. temperature (AVGT)	1	0.25	0.01
$AVGT^2$	1	0.77	0.03
Avg. relative humidity (AVGH)	1	687.54	26.45**
$AVGH^2$	1	198.55	7.64**
Rainfall (RF)	1	2002.77	77.04**
RF^2	1	1330.54	51.18**
AVGT * Tmt	1	46.41	1.78
AVGT2 * Tmt	1	64.21	2.47
AVGT * AG	1	7.27	0.28
$AVGT^2$ * AG	1	5.50	0.21
AVGH * Tmt	1	0.23	0.01
$AVGH^2$ * Tmt	1	7.74	0.30
AVGH * AG	1	17.32	0.67
$AVGH^2$ * AG	1	40.57	1.56
RF * Tmt	1	337.04	12.96**
RF^2 * Tmt	1	186.74	7.18**
RF * AG	1	0.06	0.00
RF^2 * AG	1	0.42	0.02
Residual	4063	25.99	
Total	4130		

* $p < 0.05$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment body weights (Note: Daily body weight trends were estimated using weekly body weight values for this analysis).

APPENDIX I
LEAST SQUARES MEAN (± SE) WEEKLY BODY WEIGHTS OF BUFFALO HEIFERS BY TREATMENT GROUP.

Week	Supplemental Light		Control	
	Actual	Adjusted	Actual	Adjusted
1	193.6 ± 1.2		182.4 ± 1.2	
2	194.3 ± 1.2	191.3 ± 1.7	184.2 ± 1.2	189.5 ± 1.7
3	200.2 ± 1.2	194.2 ± 1.7	191.2 ± 1.2	196.5 ± 1.7
4	204.6 ± 1.2	198.6 ± 1.7	194.6 ± 1.2	199.9 ± 1.7
5	200.9 ± 1.2	195.0 ± 1.7	193.2 ± 1.2	198.4 ± 1.7
6	202.8 ± 1.2	196.8 ± 1.7	195.3 ± 1.2	200.6 ± 1.7
7	208.5 ± 1.2	202.6 ± 1.7	196.8 ± 1.2	202.1 ± 1.7
8	205.2 ± 1.2	199.3 ± 1.7	196.0 ± 1.2	201.3 ± 1.7
9	208.5 ± 1.2	205.5 ± 1.7	206.8 ± 1.2	212.1 ± 1.7
10	213.3 ± 1.2	207.3 ± 1.7	203.4 ± 1.2	208.3 ± 1.7
11	210.5 ± 1.2	204.6 ± 1.7	196.2 ± 1.2	201.4 ± 1.7
12	203.4 ± 1.2	197.5 ± 1.7	188.1 ± 1.2	193.4 ± 1.7
13	201.0 ± 1.2	195.2 ± 1.7	184.8 ± 1.2	190.1 ± 1.7
14	199.7 ± 1.2	193.9 ± 1.7	181.9 ± 1.2	187.3 ± 1.7

Least squares mean (± SE) weekly body weights of buffalo heifers by treatment group.

APPENDIX J
LEAST SQUARES MEAN OF WEEKLY BODY WEIGHTS (KG) OF BUFFALO HEIFERS BY AGE GROUP.

Week	< 24 Months		> 24 Months	
	Actual	Adjusted	Actual	Adjusted
1	137.8 ± 0.9		243.3 ± 0.8	
2	139.6 ± 0.9	189.7 ± 1.8	237.6 ± 0.8	191.1 ± 1.7
3	143.9 ± 0.9	194.1 ± 1.8	243.4 ± 0.8	196.6 ± 1.7
4	147.8 ± 0.9	198.0 ± 1.8	247.4 ± 0.8	200.6 ± 1.7
5	145.6 ± 0.9	195.8 ± 1.8	244.4 ± 0.8	197.6 ± 1.7
6	147.5 ± 0.9	197.7 ± 1.8	246.6 ± 0.8	199.8 ± 1.7
7	150.8 ± 0.9	201.0 ± 1.8	250.4 ± 0.8	203.6 ± 1.7
8	148.7 ± 0.9	198.9 ± 1.8	248.5 ± 0.8	201.7 ± 1.7
9	156.8 ± 0.9	206.9 ± 1.8	257.1 ± 0.8	210.6 ± 1.7
10	155.3 ± 0.9	205.5 ± 1.8	257.0 ± 0.8	210.2 ± 1.7
11	151.2 ± 0.9	201.4 ± 1.8	251.4 ± 0.8	204.6. ± 1.7
12	145.6 ± 0.9	195.8 ± 1.8	242.0 ± 0.8	195.2. ± 1.7
13	143.6 ± 0.9	193.8 ± 1.8	238.3 ± 0.8	191.5 ± 1.7
14	142.4 ± 0.9	192.5 ± 1.8	235.5 ± 0.8	188.7 ± 1.7

Least squares mean of weekly body weights (kg) of buffalo heifers by age group.

APPENDIX K
ANALYSIS OF VARIANCE OF WEEKLY PROLACTIN (TIME AS A CONTINUOUS VARIABLE).

Source	df	MS	F
Treatment (Tmt)[a]	1	651.58	3.63
Age group (AG)[a]	1	1550.27	8.65**
Tmt * AG[a]	1	215.27	1.20
IPRL[b]	1	21939.02	1145.04**
Heifer (Tmt * AG)	45	179.27	9.36**
Time	1	1186.47	61.93**
Time^2	1	634.75	33.13**
Time * Tmt	1	621.59	32.45**
Time^2 * Tmt	1	314.33	16.41**
Time * AG	1	19.78	1.03
Time^2 * AG	1	23.50	1.23
Residual	1201	19.16	
Total	1255		

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment prolactin Concentrations

Analysis of variance of weekly prolactin (time as a continuous variable).

APPENDIX L
ANALYSIS OF VARIANCE OF WEEKLY PROLACTIN (TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL AS CONTINUOUS VARIABLES).

Source	df	MS	F
Treatment (Tmt)[a]	1	3.26	0.02
Age group (AG)[a]	1	14.07	0.08
Tmt * AG[a]	1	203.26	1.13
IPRL[b]	1	22031.71	993.76**
Heifer (Tmt * AG)	45	180.22	8.13**
Avg. temperature (AVGT)	1	0.13	0.01
$AVGT^2$	1	0.00	0.00
Avg. relative humidity (AVGH)	1	0.39	0.02
$AVGH^2$	1	0.04	0.00
Rainfall (RF)	1	249.31	11.25**
RF^2	1	15.32	0.69
AVGT * Tmt	1	13.41	0.61
AVGT2 * Tmt	1	10.39	0.47
AVGT2 * AG	1	7.37	0.33
$AVGT^2$ * AG	1	8.09	0.36
AVGH * Tmt	1	39.02	1.76
$AVGH^2$ * Tmt	1	36.16	1.63
AVGH * AG	1	5.33	0.24
$AVGH^2$ * AG	1	5.62	0.25
RF * Tmt	1	138.71	6.26*
RF^2 * Tmt	1	49.82	2.25
RF * AG	1	5.80	0.26
RF^2 * AG	1	5.85	0.26
Residual	1191	22.17	
Total	1255		

* $p < 0.05$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment prolactin Concentrations

APPENDIX M
MEAN ± SE OF PROLACTIN CONCENTRATIONS (NG/ML) IN TWO TREATMENT GROUPS DURING 14 WK.

Time(wk)	Supplemental Light	Control
0.25	34.4 ± 1.6	38.0 ± 2.0
0.50	33.1 ± 1.6	36.2 ± 1.6
0.75	34.3 ± 1.6	38.0 ± 1.8
1.00	33.3 ± 1.7	37.4 ± 1.7
1.25	34.0 ± 1.5	36.9 ± 1.8
1.50	35.5 ± 2.0	41.7 ± 2.3
2.00	37.7 ± 2.2	39.4 ± 2.3
2.50	37.7 ± 2.1	40.1 ± 2.2
3.00	38.9 ± 2.5	37.4 ± 1.9
3.50	39.0 ± 2.1	39.3 ± 2.1
4.00	38.6 ± 2.0	39.8 ± 2.1
4.50	41.4 ± 2.3	39.9 ± 1.7
5.00	41.4 ± 2.2	38.6 ± 2.0
5.50	41.4 ± 1.9	39.0 ± 1.4
6.00	44.3 ± 2.2	41.6 ± 1.9
6.50	43.5 ± 2.4	39.5 ± 2.1
7.00	44.3 ± 2.2	41.1 ± 2.1
7.50	43.3 ± 2.1	40.9 ± 1.8
8.00	44.1 ± 2.1	40.5 ± 2.6
8.50	43.8 ± 2.1	41.8 ± 2.5
9.00	44.3 ± 2.1	40.9 ± 1.8
9.50	45.6 ± 2.0	40.6 ± 2.2
10.00	45.3 ± 2.1	42.8 ± 2.2
10.50	44.7 ± 1.9	41.0 ± 2.1
11.00	46.0 ± 1.7	40.5 ± 2.3
11.50	45.6 ± 1.8	39.8 ± 2.1
12.00	45.8 ± 1.8	39.1 ± 2.2
12.50	46.0 ± 1.7	39.7 ± 2.3
13.00	45.4 ± 1.9	39.9 ± 1.8
13.50	44.5 ± 1.7	41.9 ± 1.8
14.00	44.7 ± 1.8	41.0 ± 1.8

Mean ± SE of Prolactin concentrations (ng/ml) in two treatment groups during 14 wk.

APPENDIX N
ANALYSIS OF VARIANCE OF WEEKLY PROGESTERONE (TIME AS A CONTINUOUS VARIABLE).

Source	df	MS	F
Treatment (Tmt)[b]	1	0.02	0.01
Age group (AG)[b]	1	0.01	0.00
Tmt * AG[b]	1	9.83	3.59[a]
IPRG[c]	1	195.53	1222.06**
Heifer (Tmt * AG)	42	2.74	17.13**
Time	1	0.77	4.77*
Time^2	1	0.92	5.68*
Time * Tmt	1	0.57	3.53[a]
Time^2 * Tmt	1	0.70	4.33*
Time * AG	1	0.43	2.67
Time^2 * AG	1	0.47	2.93
Residual	974	0.16	
Total	1026		

[a] $p < 0.07$

* $p < 0.05$

** $p < 0.01$

[b] Tested by Heifer (Tmt * AG).

[c] Pre-treatment progesterone Concentrations.

Analysis of variance of weekly progesterone (time as a continuous variable).

APPENDIX O
ANALYSIS OF VARIANCE OF WEEKLY PROGESTERONE (TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL AS CONTINUOUS VARIABLES).

Source	df	MS	F
Treatment (Tmt)[a]	1	0.07	0.02
Age group (AG)[a]	1	0.09	0.03
Tmt * AG[a]	1	9.78	3.58*
IPRG[b]	1	195.67	1222.93**
Heifer (Tmt * AG)	42	2.73	17.06**
Avg. temperature (AVGT)	1	0.01	0.06
$AVGT^2$	1	0.01	0.05
Avg. relative humidity (AVGH)	1	0.63	3.85*
$AVGH^2$	1	0.62	3.83*
Rainfall (RF)	1	0.02	0.12
RF^2	1	0.29	1.77
AVGT * Tmt	1	0.00	0.04
AVGT2 * Tmt	1	0.00	0.03
AVGT * AG	1	0.03	0.21
$AVGT^2$ * AG	1	0.03	0.22
AVGH * Tmt	1	0.26	1.63
$AVGH^2$ * Tmt	1	0.26	1.63
AVGH * AG	1	0.11	0.68
$AVGH^2$ * AG	1	0.12	0.74
RF * Tmt	1	0.00	0.00
RF^2 * Tmt	1	0.07	0.43
RF * AG	1	0.00	0.00
RF^2 * AG	1	0.06	0.38
Residual	962	0.16	
Total	1026		

* $p < 0.08$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment progesterone Concentrations

Analysis of variance of weekly progesterone (temperature, relative humidity and rainfall as continuous variables).

APPENDIX P
MEAN ± SE OF PROGESTERONE (NG/ML) IN BUFFALO HEIFERS BY TREATMENT GROUP.

Time(wk)	Supplemental Light	Control
0.25	0.38 ± 0.12	0.38 ± 0.15
0.50	0.31 ± 0.12	0.28 ± 0.17
0.75	0.32 ± 0.16	0.50 ± 0.22
1.00	0.38 ± 0.19	0.37 ± 0.19
1.25	0.38 ± 0.17	0.14 ± 0.02
1.50	0.32 ± 0.19	0.11 ± 0.01
2.00	0.30 ± 0.18	0.12 ± 0.01
2.50	0.50 ± 0.16	0.27 ± 0.03
3.00	0.31 ± 0.18	0.35 ± 0.22
3.50	0.41 ± 0.20	0.10 ± 0.00
4.00	0.52 ± 0.19	0.25 ± 0.04
4.50	0.27 ± 0.17	0.10 ± 0.00
5.00	0.56 ± 0.26	0.12 ± 0.01
5.50	0.51 ± 0.21	0.24 ± 0.10
6.00	0.46 ± 0.21	0.14 ± 0.01
6.50	0.58 ± 0.22	0.25 ± 0.03
7.00	0.45 ± 0.19	0.16 ± 0.03
7.50	0.33 ± 0.18	0.17 ± 0.02
8.00	0.51 ± 0.21	0.20 ± 0.06
8.50	0.33 ± 0.17	0.13 ± 0.01
9.00	0.55 ± 0.20	0.28 ± 0.06
9.50	0.21 ± 0.08	0.22 ± 0.04
10.00	0.37 ± 0.15	0.13 ± 0.02
11.00	0.30 ± 0.16	0.10 ± 0.00
12.00	0.26 ± 0.12	0.14 ± 0.01
13.00	0.49 ± 0.18	0.31 ± 0.03
14.00	0.17 ± 0.05	0.16 ± 0.04

Mean ± SE of progesterone (ng/ml) in buffalo heifers by treatment group.

APPENDIX Q
ANALYSIS OF VARIANCE OF WEEKLY LH (TIME AS A CONTINUOUS VARIABLE).

Source	df	MS	F
Treatment (Tmt)[a]	1	0.24	1.16
Age group (AG)[a]	1	0.77	3.64
Tmt * AG[a]	1	0.20	0.91
ILH[b]	1	2.69	25.37**
Heifer (Tmt * AG)	42	0.21	2.12**
Time	1	0.03	0.29
Time^2	1	0.11	1.09
Time * Tmt	1	0.27	2.66
Time^2 * Tmt	1	0.16	1.56
Time * AG	1	0.42	4.10*
Time^2 * AG	1	0.42	4.10*
Residual	1143	0.10	
Total	1195		

* $p < 0.05$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment LH Concentrations

APPENDIX R
ANALYSIS OF VARIANCE OF WEEKLY LH (TEMPERATURE, RELATIVE HUMIDITY AND RAINFALL AS CONTINUOUS VARIABLES).

Source	df	MS	F
Treatment (Tmt)[a]	1	0.03	0.14
Age group (AG)[a]	1	0.25	1.19
Tmt * AG[a]	1	0.21	1.00
ILH[b]	1	2.72	27.20**
Heifer (Tmt * AG)	42	0.21	2.12**
Avg. temperature (AVGT)	1	0.09	0.88
$AVGT^2$	1	0.10	1.02
Avg. relative humidity (AVGH)	1	0.17	1.68
$AVGH^2$	1	0.15	1.52
Rainfall (RF)	1	0.21	11.59**
RF^2	1	2.00	19.25**
AVGT * Tmt	1	0.01	0.11
AVGT2 * Tmt	1	0.01	0.13
AVGT * AG	1	0.30	2.95
$AVGT^2$ * AG	1	0.32	3.08*
AVGH * Tmt	1	0.13	1.25
$AVGH^2$ * Tmt	1	0.12	1.19
AVGH * AG	1	0.00	0.00
$AVGH^2$ * AG	1	0.00	0.00
RF * Tmt	1	0.03	0.28
RF^2 * Tmt	1	0.11	1.12
RF * AG	1	0.27	2.58
RF^2 * AG	1	0.33	3.17*
Residual	1131	0.10	
Total	1195		

* $p < 0.08$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-treatment LH Concentrations

APPENDIX S
MEAN ± SE OF LH (NG/ML) OF BUFFALO HEIFERS EXPOSED TO 4 HR SUPPLEMENTAL LIGHTING DAILY BY AGE GROUP.

Time(wk)	< 24 months	> 24 months
0.25	0.33 ± 0.03	0.42 ± 0.04
0.50	0.35 ± 0.03	0.42 ± 0.04
0.75	0.48 ± 0.04	0.50 ± 0.04
1.00	0.38 ± 0.04	0.40 ± 0.05
1.25	0.56 ± 0.06	0.44 ± 0.04
1.50	0.66 ± 0.07	0.94 ± 0.16
2.00	0.44 ± 0.02	0.56 ± 0.05
2.50	0.37 ± 0.02	0.48 ± 0.06
3.00	0.58 ± 0.04	0.63 ± 0.02
3.50	0.57 ± 0.04	0.68 ± 0.05
4.00	0.47 ± 0.02	0.54 ± 0.04
4.50	0.48 ± 0.02	0.59 ± 0.04
5.00	0.50 ± 0.03	0.67 ± 0.06
5.50	0.48 ± 0.03	0.61 ± 0.06
6.00	0.49 ± 0.02	0.66 ± 0.06
6.50	0.79 ± 0.20	0.61 ± 0.05
7.00	0.53 ± 0.12	0.40 ± 0.04
7.50	0.40 ± 0.03	0.51 ± 0.03
8.00	0.44 ± 0.04	0.51 ± 0.05
8.50	0.57 ± 0.06	0.64 ± 0.06
9.00	0.31 ± 0.03	0.34 ± 0.03
9.50	0.25 ± 0.02	0.36 ± 0.04
10.00	0.58 ± 0.09	0.86 ± 0.11
10.50	0.59 ± 0.08	0.64 ± 0.07
11.00	0.29 ± 0.02	0.33 ± 0.03
11.50	0.22 ± 0.01	0.34 ± 0.07
12.00	0.36 ± 0.05	0.50 ± 0.07
12.50	0.24 ± 0.01	0.40 ± 0.07
13.00	0.29 ± 0.04	0.37 ± 0.07
13.50	0.28 ± 0.02	0.46 ± 0.06
14.00	0.33 ± 0.05	0.43 ± 0.04

Mean ± SE of LH (ng/ml) of buffalo heifers exposed to 4 hr supplemental lighting daily by age group.

APPENDIX T
MEANS ± SE OF LH (NG/ML) RESPONSE TO GNRH BY TREATMENT BY AGE GROUP BY TIME INTERACTION.

Time (Hr)	Supp. Light		Control	
	24 < mo.	24 > mo.	24 < mo.	24> mo
-0.50	0.46 ± 0.13	0.36 ± 0.03	0.38 ± 0.03	0.46 ± 0.06
-0.25	0.47 ± 0.13	0.35 ± 0.02	0.38 ± 0.04	0.46 ± 0.06
0.00	0.45 ± 0.12	0.36 ± 0.02	0.36 ± 0.05	0.46 ± 0.06
0.25	6.69 ± 1.34	5.89 ± 1.14	4.31 ± 0.55	6.77 ± 1.42
0.50	6.84 ± 1.40	6.18 ± 0.62	5.72 ± 1.00	10.35 ± 3.47
0.75	4.96 ± 0.88	5.41 ± 0.61	4.58 ± 0.89	7.12 ± 2.09
1.00	3.66 ± 0.63	3.64 ± 0.37	3.82 ± 0.85	4.86 ± 1.36
1.50	2.53 ± 0.38	2.50 ± 0.33	2.73 ± 0.58	2.75 ± 0.68
2.00	1.85 ± 0.30	1.92 ± 0.28	2.48 ± 0.39	2.24 ± 0.51
2.50	1.77 ± 0.23	1.45 ± 0.18	1.91 ± 0.29	1.90 ± 0.41
3.00	1.46 ± 0.17	1.16 ± 0.14	1.40 ± 0.15	1.42 ± 0.31
4.00	1.05 ± 0.12	0.82 ± 0.07	1.01 ± 0.11	0.94 ± 0.18

Means ± SE of LH (ng/ml) response to GnRH by treatment by age group by time interaction.

APPENDIX U
ANALYSIS OF VARIANCE OF LH RESPONSE TO GNRH (BODY WEIGHT AND PRE-INJECTION LH AS COVARIATES).

Source	df	MS	F
Treatment (Tmt)[a]	1	17.90	0.48
Age group (AG)[a]	1	15.00	0.40
Tmt * AG[a]	1	44.21	1.18
ILH[b]	1	170.96	35.46**
BWT[c]	1	2.60	0.54
Heifer (Tmt * AG)	43	37.52	7.78**
Time	8	239.55	49.65**
Time * Tmt	8	4.70	0.97
Time * AG	8	7.38	1.53
Time * Tmt * AG	8	10.48	2.17*
Residual	341	4.82	
Total	421		

* $p < 0.05$

** $p < 0.01$

[a] Tested by Heifer (Tmt * AG)

[b] Pre-injection LH concentrations

[c] Bodyweight prior to GnRH administration

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