

Effect of Photoperiod Length on Some Reproductive Traits and Hormonal Profiles in Buffalo Heifers

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Abstract: The present work aimed to study the effects of photoperiod length on some reproductive traits, blood components and melatonin hormone level (MLT) and its relationship with some sexual hormones during estrous period. Fourteen buffalo heifers at 11.1±3.3 months and 204.1±3.4 kg body weight were divided randomly into two similar groups (7 each). The first group(G1) was exposed to artificial light (16 hours light and 8 hours darkness /day) during autumn and winter seasons. The second group(G2) was exposed to natural day light (8 hours light and 16 hours darkness/day) during the same later seasons. The results obtained shows that body weight at puberty (208±11.2 vs. 201.1±1.2 Kg) was not significant between treatments. The same trend was observed in body weight at first ovulation 248.6±11.6 kg (G1), 239.8±10.2 Kg (G2), however, age at first ovulation decreased (P<0.05) when day light increase, 12.6±0.1 (G1) and 13.3±0.2 (G2) months, respectively. Total protein and albumin were not significant but differ among treatments groups. Globulin, T3, T4, glucose and triglycerides significantly increased (P<0.05)by increasing photoperiod. Melatonin (MLT) hormone concentration recorded sharp decline (P<0.05)when heifers exposed to long photoperiod during autumn and winter seasons. In G1, melatonin concentration level was significantly declined (P<0.05), while estradiol -17β (E2) and prostaglandin (PGF₂α) hormone concentrations were increased (P<0.05) throughout the estrous hours. However, progesterone (P4) concentration was not differ in G1, while the opposite was true in G2. Positive correlation (r= +0.85) was observed between MLT and PGF₂α throughout estrous hours and the opposite was observed with heifers in G2. Positive correlation (r= +0.57) between E2 and PGF₂α during ovulation with heifers in G1 and G2. Heifers in G1 was a good estrous signs than heifers in G2. The present study recommended supplementing and increasing of artificial light during autumn and winter seasons after sun set to 4 hours to induce strong and clear estrous, increase P4, E2, PGF₂α and decline MLT in buffalo heifers.

Key words: Buffalo heifers • Photoperiod • Estrous • Sex hormones

INTRODUCTION

In Egypt, buffaloes are the major milk producing animals besides its contribution as draught power and meat production. The animals however suffer from inherent reproductive problems such as poor oestrous expression and long calving intervals which limits its lifetime production especially in summer season [1], which associates with the long photoperiod. Such studies indicated that the incidence of silent heat was low in winter and very high in summer months [2, 3]. The later authors reported that the maximum reproductive activity in buffalo is accompanied with the green fodders availability, ambient temperature and shorter day-light. This report emphasizes that one of these factors or all of them deleteriously affected the ovarian and oestrous activities.

Moreover, it was indicated that melatonin has a positive effect on the reproduction of many species [3-5]. The long-day light also causes a remark decline of melatonin secretion and increased sex hormones secretion [6-8]. However, its role on reproduction control is still unclear. Little information is available on the timing of estrus and its relation to hormonal profile in buffalo heifers within the long photoperiod during summer and short photoperiod in winter season.

The present study aimed to define the effect of photoperiod length on some reproductive traits in relation to hormonal profiles during estrous hours.

MATERIALS AND METHODS

This work was carried out in Mehallet Moussa Experimental Research Station, Animal Production

Research Institute (APRI), during the period from October, 2004 to March, 2005.

Experimental animal: Fourteen buffalo heifers (204.1±3.4 kg and 11.1±3.3 months old) were randomly assigned to two groups (7 animals each). The first group (G1) consisted of long photoperiod in which heifers were exposed daily 16 hours of light and 8 hours darkness per day. The second group (G2) consisted of natural photoperiod 8 hours of light and 16 hours darkness daily.

Feeding: Heifers were fed the same ration of concentrate feed mixture (CFM), berseem and rice straw (RS). The heifers were fed according to Animal Production Research Institute [9]. Feed allowances were based on the animal body weight and age. The concentrate feed mixture (CFM) consisted of 30 % undecorticated cottonseed meal, 35% wheat bran, 16 % yellow corn, 10 % rice bran, 5% vines, 3% limestone and 1% common salt.

Housing: The buffalo heifers were housed in a tie stall barn that was modified to control light exposure. Supplemental lighting was provided to heifers under the long photoperiod length treatment by tungsten lamps mounted at highest of 2.5 meter. The artificial lighting period was 4 hrs starting after sun-set and the intensity of light was 162 lux. Intensity of light was measured using lux-meter (Gossen, Ponlux Electronic, Germany). To determine light intensity the lux-meter was put near the animals eye.

Blood components: Blood samples were collected from jugular vein catheters (inserted 3 days before the expected days of estrous or starting of estrous signs). At the beginning of the estrous signs, blood samples were collected venipunctur for each animal every 2h for 48 h then centrifuged at 3000 r.p.m. for 20 minutes. Serum samples were harvested and stored frozen -20°C until determined of melatonin hormone concentrations according to Stanisiewski *et al.* [10].

Melatonin was assayed through two main steps as follows:

A- Extraction: According to the procedure outlined by (Buhlmann Laboratories AG. Switzerland, 1995). Extraction of melatonin included many sub-steps (e.g., column preparation and conditioning, evaporation and reconstitution of extract).

B- Determination: Following completion of the previous steps, melatonin concentration was assayed by RIA Kits based on a double-antibody technique (Buhlmann Laboratories AG. Switzerland, 1995).

According to the manufacturer's information, the cross- reaction of the melatonin antiserum (at 50 binding percent) was 100% with melatonin, while it was <0.03% with each of serotonin, 5-hydroxy -indoleacetic acid, N- acetylserotonin 5-methoxytryptamine, 5-methoxy tryptophon, 6-sulfatoxy melatonin and 5-methoxy tryptophol for each. The melatonin standard curve ranged between 0.5 and 50 pg/ ml. Sensitivity value when assaying the minimum detectable concentration of melatonin in 400 ml unextracted samples was calculated to be 0.3 pg / ml. The intra and inter assay coefficients of variation were 8.5 and 13.4%, respectively.

Progesterone (p4) concentration (ng/ ml) was measured using antibody coated tube Kits (coat -A-count, Dpc, los angeles, USA) by radioimmunoassay technique. Estradiol 17β (E₂) were determined according to Perry *et al.* [11]. (sensitivity = 0.12 ±0.01pg/ml in for assays). Prostaglandin F₂α (PGF₂α) concentration was measured using correlate - EIA assay (A competitive immunoassay Kit, Assay designs Inc., USA). Age at puberty were determined according to progesterone (P4) concentration level in plasma (1= ng/ml or excessive). Age at first ovulation when the P4 level began to regular cyclists.

Puberty was determined by subtracting from the date at which plasma progesterone level reached 1.0 ng / ml as the method described by Kassim [3]. Ovulation was accompanied by displaying sexual behaviour. At the end of heat day was considered the date of ovulation (ovulatory estrus) providing that the progesterone level: 1.0 ng/ml according to Kassim [3] within the hours of ovulation. Zero time was determined when the progesterone and melatonin concentrations/reached the lowest values, while estradiol and PGF₂α were the highest values.

Signs of oestrous: Starting at puberty, all heifers were observed for signs of oestrous in the exercise lot in accordance with farm management program. In addition, heifers which reached its first oestrous were observed for signs for 2 hours between 8.00 to 10.00 am and 17.00 to 19.00 pm daily in a separate lot. Behaviour of oestrus become restless, twitched and raised her tail frequently. The vulva of the estrous female is sniffed by other mates, urination occurred frequently. Homosexual activity

(mounting other females or being mounted by others). Buffaloes in heat lose their eagerness to eat.

The data obtained were subjected to statistical analysis according to SAS [12] $Y_{ijk} = \mu + T_i + e_{ijk}$ wheres: Y_{ijk} = is observation taken on the animal, μ = overall mean, T_i = effect of photoperiod, e_{ijk} = random error.

Differences among means were tested for significant by using Duncan New Multiple Range test [13].

RESULTS AND DISCUSSION

Feed intake and growth performance: Table1 shows that buffalo heifers exposed to long photoperiod (16h light) slightly increased feed intake and body weight compared with that exposed to natural photoperiods (8 h light) during autumn and winter seasons. These results are in agreement with those reported by Dahi and Piticlerc [14] who reported that photoperiod management can be used to improve heifers growth. Dry matter intake did not significantly differant between the experimental groups (G₁ and G₂). Animals in G₁ had higher values in live body weight than that in G₂ at puberty and first ovulation. The opposite was true for age at puberty as heifers in G₁ had lower than in G₂. Age at puberty differed significantly (P<0.05) between the two experimental groups The same trend was observed in age and weight at first ovulation (Table1). The increased values observed in heifers at G₁ may be due to long photoperiod. Similar trends was reported by Afify *et al.* [5], Stanisiewski *et al.* [10], Dahi and Peticlerc[14] and Hassan *et al.* [15].

Generally, buffalo heifers which exposed to long photoperiod showed increased weight at first ovulation, decreased age at first ovulation and feed intake. Age at puberty is a major determinant of lifetime reproduction efficiency of buffalo heifers and related to nutrition. The plane of nutrition and feed intake had affect on sexual maturation involves effects on timing of the prepubertal, increase in LH secretion and seems to the LH generating system located in the hypothalamus. Long photoperiod increased LH secretion and reflecting metabolic status. In addition to changes in the reproductive system and sexual behavior there are a number of other behavioral and metabolic changes which accompany alterations in the photoperiod. Some species, there is no concomitant changes in feed intake, which suggests that there is an alteration in metabolic processes due to the alteration in day length [16].

Table 1: Effect of photoperiod on dry matter intake (DMI), total digestible nutrition (TDN), age and weight at puberty and first ovulation

Items	Experimental groups	
	G1	G2
DM intake (kg)	13.2±0.3	12.8±0.2
TDN intake (kg)	7.1±0.1	7±0.1
Age at puberty(month)	10.5±0.2b	11.7±0.4a
Weight at puberty (kg)	208.0±11.2	201.1±10.2
Age at first ovulation (month)	12.6±0.1b	13.3±0.2a
Weight at first ovulation (kg)	248.6±11.6	239.8±10.2

a and b means with different superscripts in the same row are different significantly (p<0.05)

Table 2: Effect of photoperiod on some blood parameters during estrous

Items	Experimental Groups	
	G1	G2
Total protein (g/dl)	6.24±0.1	5.99±0.1
Albumin (g/dl)	3.25±0.1	3.31±0.07
Globulin (g/dl)	2.99±0.02a	2.68±0.07b
A/G	1.1	1.2
T3 (ng/ml)	133.4±0.6a	116.6±0.1b
T4 (ng/ml)	36.9±1.1	32.2±1.2
Glucose (mg/100ml)	65.7±0.6a	55.5±0.4b
Triglycerides (mg/100ml)	60.5±0.5a	52.3±0.2b

a and b Means with different superscripts in the same row are different significantly (P<0.05)

Effect of photoperiod on some blood components during estrous: As shown in Table 2, total protein and albumin did not show significant differences between G₁ and G₂. However, significant differences (P<0.05) were found among globulin, T₃, T₄, glucose and triglyceride. Animals in G₁ showed recorded higher values than those in G₂, which may be due to that animals in G₁ exposed to long photoperiods aggregated metabolites than animals exposed to natural photoperiod (G₂). These trends may be due to that animals in G₁ consumed feed in the excessive light during this period than animals in G₂. The anabolic effects of increased duration of photoperiod in buffalo heifers are dependent of the gonads. Consistent increase in average gain of heifers G₁ in response to longer duration photoperiods have always been achieved (Tables 1, 2). The hormonal signals that mediate the anabolic effects of increasing exposure to light are associated with changes in thyroxin, glucose and triglycerides concentrations in blood. Increasing daily light to 16 h/day hastens the increase in concentrations of sex hormones of pubertal heifers. Thus, change in

Table 3: Effect of photoperiod length on P4, E2, MLT and PGF_{2α} hormone concentrations during, before and after ovulation hours

Hormones		Times						
		6	4	2	0	2	4	6
Progesterone	G1	0.63±0.02	0.59±0.02	0.54±0.02	0.47±0.02	0.52±0.02	0.59±0.03	0.69±0.02
	G2	0.7±0.01	0.6±0.01	0.6±0.01	0.5±0.02	0.6±0.02	0.7±0.02	0.7±0.02
Estradiol 17 _β	G1	10.5±0.5 ^a	11.0±0.4 ^a	13.3±0.3 ^a	13.8±0.3 ^a	7.7±0.1 ^a	4.3±0.1 ^a	2.8±0.1 ^a
	G2	7.3±0.2 ^b	7.4±0.1	8.3±0.1 ^b	10.5±0.2 ^b	7.2±0.2	3.4±0.1	1.7±0.1 ^b
Melatonin	G1	8.8±0.6 ^a	8.5±0.6 ^a	8.3±0.6 ^a	8.0±0.6 ^a	8.3±0.6 ^a	8.5±0.6	8.8±0.6 ^a
	G2	16.4±1.0 ^b	16.1±1.0 ^b	15.8±1.0 ^b	15.6±1.0 ^b	15.8±1.0 ^b	15.9±1.0 ^b	16.3±1.0 ^b
Prostaglandin F2 _α	G1	31.7±1.5 ^a	32.8±1.2 ^a	33.7±1.2 ^a	34.8±1.2 ^a	33.7±1.2 ^a	32.0±1.2 ^a	28.4±1.4 ^a
	G2	25.8±1.1 ^b	26.0±1.1 ^b	26.5±1.1 ^b	26.9±1.0 ^b	25.5±1.2 ^b	24.3±1.3 ^b	22.7±1.4 ^b

a and b: Means with different superscripts in the same column are different significantly (P<0.05)

secretion of reproductive hormones in perpubertal and pubertal heifers may be associated with anabolic efficiency and consistent with gonad dependency. Serum total protein, thyroxin, glucose and triglycerides concentrations were increased in animals G1 as duration of light increases. These results are in agreement with those reported by Afify *et al.* [5], Dahi and Pititclerc [14] and Hassan *et al.* [15].

Effect of photoperiod length on P4, E2, MLT and PGF_{2α}:

The results obtained in Table 3 showed that the effect of photoperiod on the serum P4, E2, MLT and PGF_{2α} concentrations of the buffalo heifers before and after ovulations was observed (Figs. 1 and 2) whereas animals in G1 being exposed to long photoperiod and G2 that exposed to natural photoperiod. The effect of photoperiod length on P4, E2, MLT and PGF_{2α} hormone concentrations before and after ovulation was observed in Figs. 3-6. Serum P4 concentration as a mean for seven animals before and after 6 hours of ovulation was higher in G2 compared to G1. The lowest values was obtained at zero time in both groups. It was observed that the P4 hormone concentration is clearness in long photoperiod, while E2 hormone concentration increased in long photoperiods and the highest values was recorded at zero time for both groups. P4 hormone concentration parallel decline in both experimental groups animals at all estrous hrs.

Estradiol-17_β (E2) hormone levels was differ significantly (P<0.05) between animals in G1 and G2 at hours of estrous. But, E2 hormone concentrations tended to be increase during estrous hours in both experimental groups. Similar results were reported by El-Wardani [17] and Anwar and Megahed [18].

Table 3 and Fig. 5 indicate that the long day light decreased the MLT hormone concentration in G1 compared with G2. On the other hand, PGF_{2α} hormone

concentration increased during long photoperiods. The same trend was noticed in MLT and PGF_{2α} hormone concentrations. This means that the highest concentration of E2 and PGF_{2α} hormone concentrations and lowest values of P4 hormone concentrations during estrous hours induction the ovulation. Whereas PGF_{2α} hormone concentrations induce the corpus leutium disappear. These results are in agreement with the results obtained by Kassim [3] and Anwar and Megahed [18]. According to these results the circulating MLT hormone concentration may differently modified throughout of the estrous time when heifers exposed to long light. In the present study minimizing of MLT hormone concentration was observed in (G1), while elevating of MLT hormone concentration was observed in (G2) during estrous. Photoperiodic control of gonadal function is probably a result of changes in gonadotropin secretion. In both long-day and short-day breeding species, exposure to inhibitory photoperiods caused a decline in pituitary and blood levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), while exposure to stimulatory day lengths caused opposite effects. Light exposure can regulate gonadotropin secretion by altering responsiveness of the hypothalamic-pituitary axis to the negative feedback actions of gonadal steroids. Photic-induced suppression of reproduction appears to be mediated by the suprachiasmatic nucleus of the hypothalamus in both long-day and short-day breeding species. In addition, in many female mammalian exposure to a nonstimulatory light cycle induces acyclicity which is accompanied by a cessation of ovulation and marked changes in serum estrogen and progesterone levels [19,20]. Photic-induced inhibition of reproductive physiology is also associated with a reduction in sexual behaviour [21], while this decline in reproductive behavior is probably due at least in part, to a decline in circulating steroid levels [22], it appears that an exposure at non-

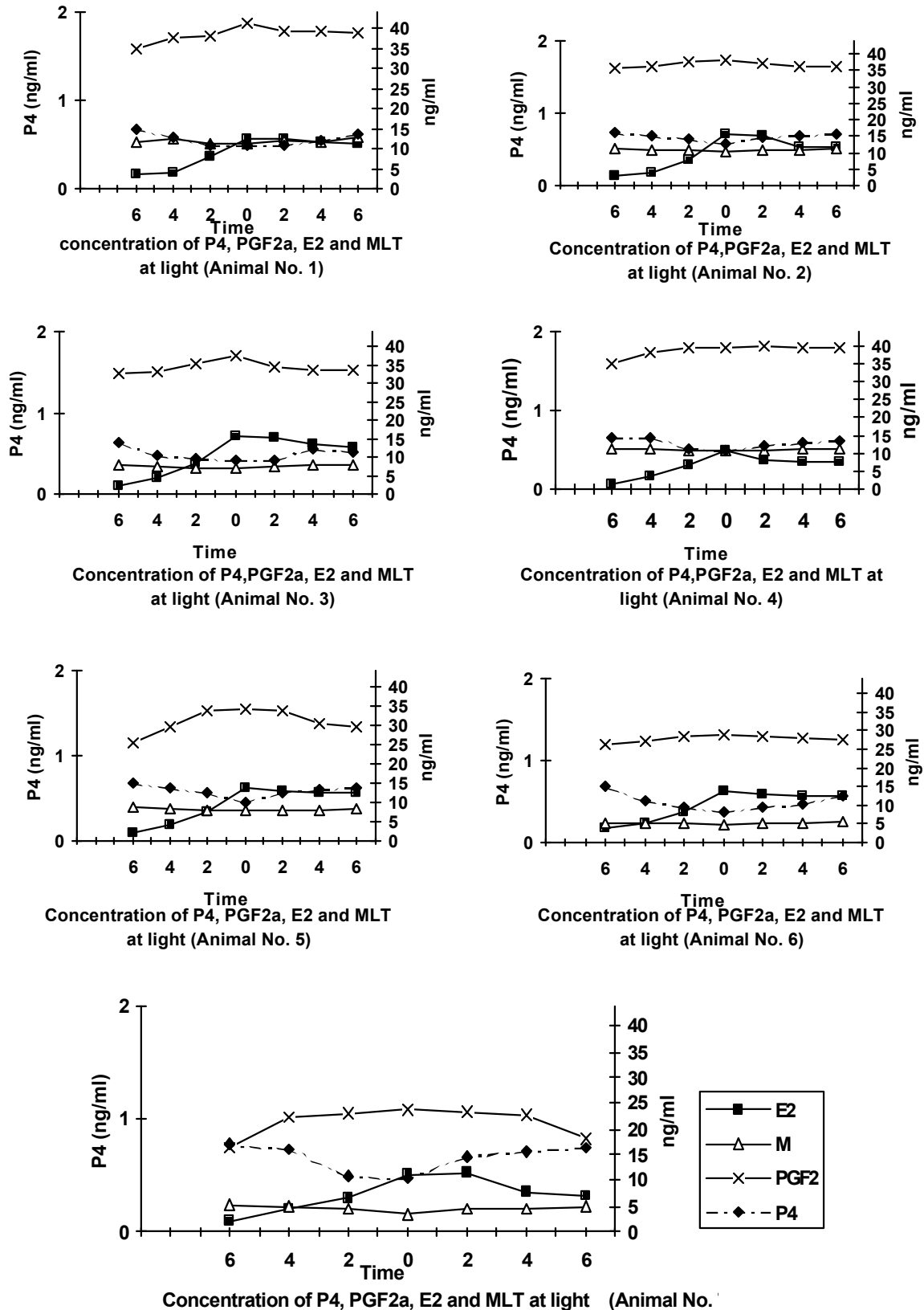


Fig. 1: Effect of long photoperiod on P4, E2, MLT and PGF2a concentration during estrus time

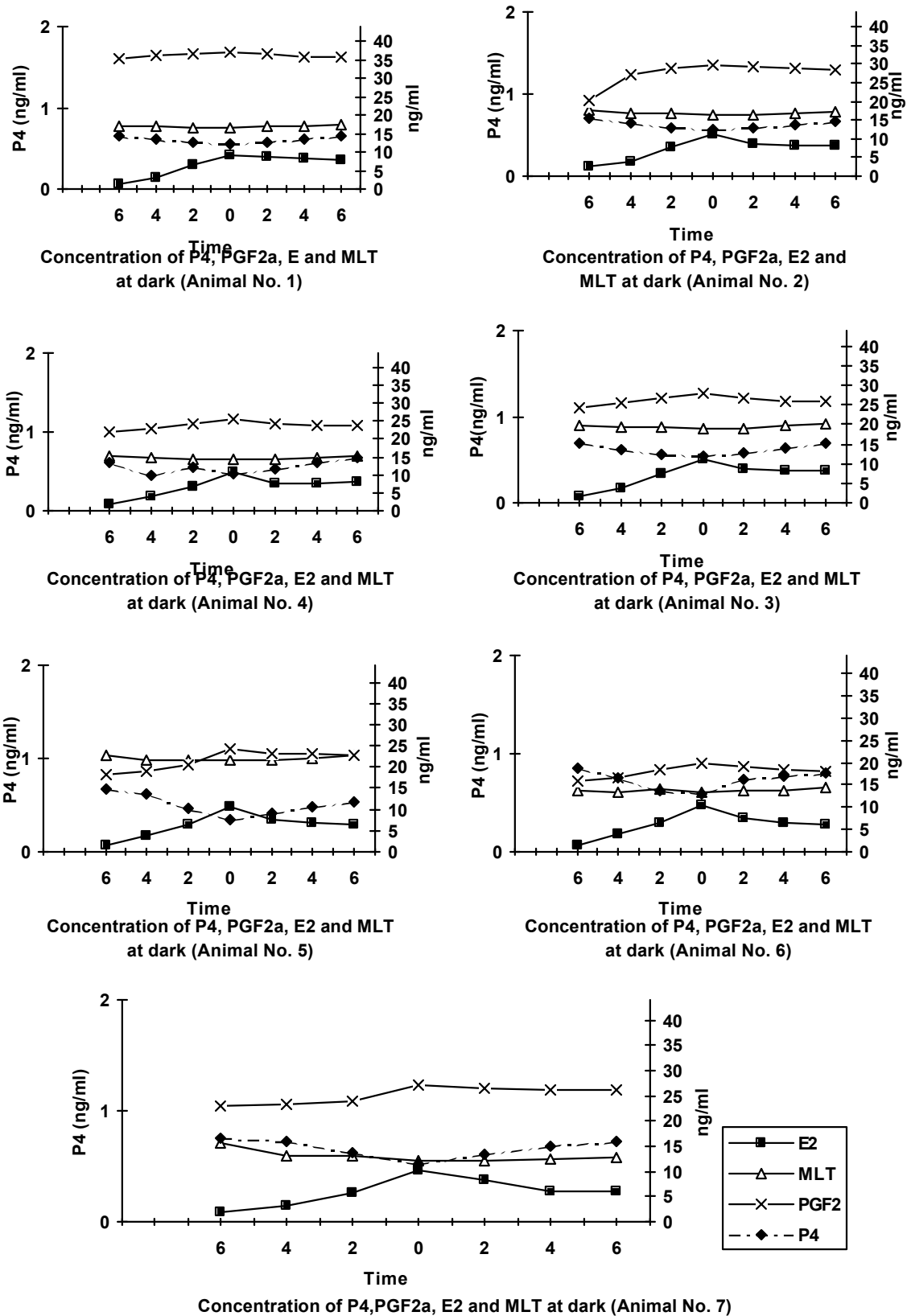


Fig. 2: Concentration of P4, PGF2a, E2 and MLT during estrus at dark season

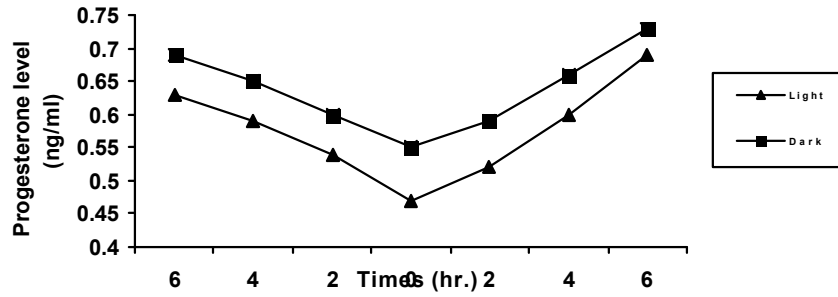


Fig. 3: Effect of photoperiod length on progesterone (P4) level during estrous

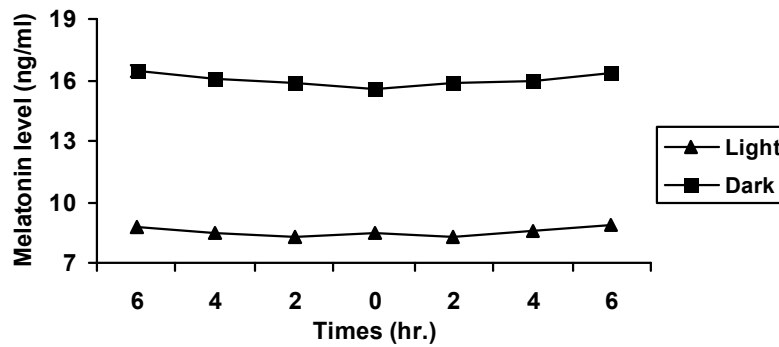


Fig. 4: Effect of photoperiod length on melatonin (MLT) concentration during estrous

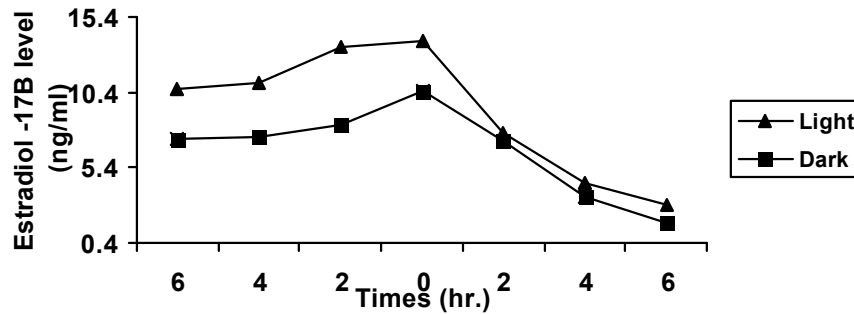


Fig. 5: Effect of photoperiod length on estradiol-17 B (E2) during estrous

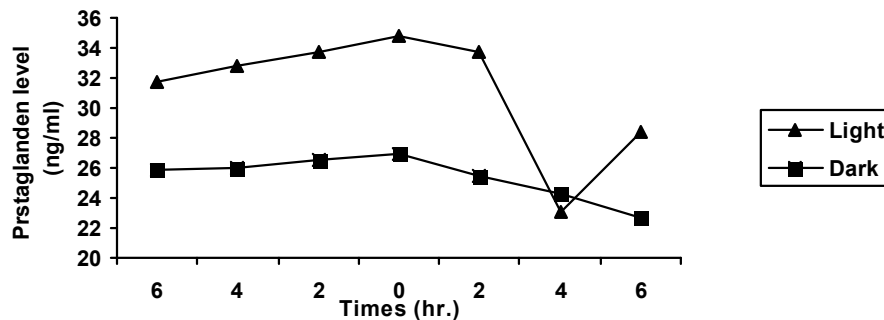


Fig. 6: Effect of photoperiod length on prostaglandin concentration (PGF2a) during estrous

Table 4: Correlation coefficient (±) among MLT,P4,E2 and PGF2α hormone before, during and after ovulation hours

Hormones		Pre ovulation				Ovulation				Post ovulation			
		P4	E2	MLT	PGF2α	P4	E2	MLT	PGF2α	P4	E2	MLT	PGF2α
Progesterone	G1	-	*-0.36	0.08	-0.15	-	0.02	0.31	0.15	-	**0.57	-0.02	-0.40
	G2	-	*-0.50	-0.07	-0.40	-	-0.18	0.03	-0.28	-	-0.05	-0.30	**0.50
Estradiol 17β	G1	0.36*	-	0.02	0.26	0.02	-	0.26	**0.57	**0.55	-	-0.06	0.40
	G2	-0.50*	-	0.20	0.52	-0.2	-	0.09	*-0.30	**0.57	-	0.01	0.20
Melatonin	G1	0.08	-0.02	-	0.85**	0.31	0.25	-	**0.85	-0.02	-0.06	-	**0.70
	G2	-0.07	*0.20	-	0.14	0.03	0.09	-	0.07	-0.30	-0.01	-	0.20
Prostaglandin F2α	G1	-0.15	0.26	**0.85	-	0.15	*0.57	**0.85	-	-0.40	0.38	**0.70	-
	G2	-0.40**	0.50	0.14	-	0.30	0.30	0.07	-	**0.50	0.22	0.20	-

*:Correlation coefficient **:Highly correlation coefficient

stimulating photoperiod can also decrease the behavioral responsiveness of seasonally breeding animals to the stimulating effects of gonadal hormone [21].

These results indicated that the treatment with long day photoperiod length corrected the hypogonadal conditions and stimulated follicular growth of ovaries. Moreover the sex hormones activity was stimulated by reducing of MLT levels and increased sex hormones which stimulated estrus activity.

Negative correlation ($r = -0.07$) between MLT and P4 concentrations in animals which not exposed to long photoperiod before ovulation was indicated in Table 4. The same trend was also observed between P4 and E2 ($r = -0.18$) at ovulation. The opposite was true for the correlation between P4 and E2 ($r = -0.57$) post ovulation. In addition, negatively correlation ($r = -0.4$, $r = -0.07$ and 0.5) was found between P4 and PGF_{2α}, MLT, E2 preovulation, respectively. Also, there was a negative correlation ($r = -0.28$, 0.03 and -0.18) between P4 and other hormones at ovulation in G2. At post ovulation a positive correlation ($r = 0.5$) between P4, PGF_{2α} and a negative correlation between P4 and MLT, E2 ($r = -0.3$ and $r = -0.05$) was observed respectively. As indicated in a highly positive correlation ($r = 0.85$) between MLT and PGF_{2α} at all phases of ovulation in animals exposed to long photoperiod was observed, correlation coefficient between E2 and PGF_{2α} was highly positive ($r = 0.57$) at ovulation and ($r = 0.57$) E2 and P4 postovulation in animals at G1. In animals of G2, the correlation between E2 and P4 pre and post ovulation was 0.5 which exposed to natural day length, but at ovulation a negative correlation ($r = -0.2$) was found between E2 and P4. Highly positive correlation ($r = 0.85$, 0.85 and 0.7) was found at all ovulation phases in animals exposed to long photoperiod (G1) between PGF_{2α} and MLT in Table 4. On the contrary it was observed in animals exposed to

natural day length (G2) (Table 4). Results of Reiter[23] and Miyauchi *et al.*[24] indicated that there was a negative relationship between gonadotropins and melatonin. This means that the increase of MLT concentration during winter months lead to poor reproductive in buffaloes, in agreement with the findings of the present study, it indicated a high concentration of P4 and E2 at estrous stimulated by decline of MLT. Also, Auchtung *et al.*[25] and Rius *et al.* [26] show that treatment of buffaloes and other ruminant by extending light during autumn and winter (dark season) decrease serum concentration of MLT, particularly after puberty and increase the serum concentrations levels of P4, E2 and PGF_{2α}. These results indicated that the treatment with long day photoperiod length corrected the hypogonadal conditions and stimulated follicular growth of ovaries. Moreover the sex hormones activity was stimulated by reducing of MLT levels and increased sex hormones which stimulated estrus activity. Oestrus or heat is a specific period of reproductive function when the female becomes receptive. Estrus in buffaloes is manifested by changes in the reproductive system and behavior. It usually lasts about 24 hours, but duration varies and may range from 11 to 12 hours. It occurs on an average of 21 day cycles. Determination of when a buffalo cow is in estrus is difficult because often the animals shows few external signs of heat. This increases the chances of missing a cycle, especially for artificial insemination. The intensity of estrus behavior in Egyptian buffaloes has been found to be much less than cows 48a. The usual weak during the hot months of summer.

A total number of 42 experimental buffaloes heifers were observed with estrous period. Frequencies of percentage and duration (hrs) of different estrous behaviour signs of buffalo heifers are presented in Table 5. Length of duration from the beginning to the end

Table 5: Effect of photoperiod on signs of estrous

Estrous signs	Experimental groups			
	G1		G2	
	F%	D	F%	D
Bellowing	67.2	7.0±.3 ^a	56.3	6.7±0.4 ^b
Tail raising	35.0	3.0±0.2	29.0	3.1±0.3
Restlessness	87.0	6.0±0.2	76.3	7.0±0.4
Isolation	90.0	7.0±0.6 ^a	82.0	5.4±0.4 ^b
Walking along side the wall	82.0	7.4±0.8 ^a	73.3	4.0±0.6 ^b
Frequent urination	33.3	3.3±0.2 ^a	60.0	2.0±0.5 ^b
Mounting others	31.3	7.4±0.3 ^a	20.0	6.7±0.3 ^b
Standing behaviour	34.6	13.0±0.8 ^a	25.9	9.1±1.1 ^b

F%: Frequency of sings percentage –D: duration (hrs)

a,b: means with different superscript in the same row are different significantly ($p < 0.05$)

of estrous signs appearance in Table 5 which reflected that the highest percentage was recorded for restlessness, isolation and walking along side the wall in all animals, but, the highest percentage was recorded for G1 (87, 90 and 82%). Similar results were obtained by Barkawi *et al.*[27] and Kassim [3] who reported that 80% and 70% of bellowing occurred in the buffaloes. It clear from the present study that bellowing as a sign of estrous may be less (67.2%) in heifers than in buffaloes. In winter during supplementing light after the sunset through estrous, heifers showed a higher ovarian activity when compared to that natural day. Extended day length inhibited MLT concentration in the blood, increasing sexual hormones during estrous and have a good signs of estrous than others. Tail raising has been observed in 29% to 35% of total estrous periods in the present study. These results disagree with Affify *et al.* [28] who indicated that buffaloes heifers did not show a uniform behaviour of raising the tail since 8% only of buffaloes heifers exhibited elevated tail and 12% with laterally deviated tail during estrous period. Frequent urination was detected a high value for G1 supplemented lighting after sunset followed by G2 exposed to natural day. Frequent urination is a major sign of estrous for heifers percentage estrous females. Following other male and female and sniffing the female vulva represented in Table 5. It revealed a lower values for G2 and higher values for G1. These phenomena of estrous signs were agreement with Affify *et al.* [28]. Stading behaviour signs of G1 was higher than G2.

It could be concluded from the present study that restlessness, bellowing, standing behaviour and frequent urination considered the most reliable signs of estrous in

buffalo heifers. The decline of MLT concentration in blood and increased P4, E2 and $\text{PGF}_2\alpha$ reflected the estrous signs. Animals in G1 was more detectable of estrous signs than G2 which may be related to the concentration of sex hormones.

In conclusion, long photoperiod was impressed their feed intake and reproductive performance of buffalo heifers. Long day light length during autumn and winter seasons decreased melatonin concentration in blood flow during optical gland which modified that to inhibit MLT secretion. Decreased MLT secretion which related to increase sexual hormones during estrous hours. Long photoperiod during estrous period specially during autumn and winter considered more beneficial in ruminant. It can be recommended to supplement and increase of artificial light during autumn and winter seasons after sun set to 4 hours to induce strong and clear estrous in buffalo heifers.

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