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Testicular Development and Hematological Parameters of Male Broiler Breeders Under Subtropical Environment

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Abstract.- Testicular development of male broiler breeders were studied under different environmental temperatures. A total of 48 broiler breeder males of 18 weeks of age were procured from a company. Birds were divided in 3 equal groups (A, B and C). Birds of groups A were kept at 22 to 26°C in an environmentally controlled house and groups B and C were kept at 28 to 33 and 30 to 37°C, respectively in open sided naturally ventilated houses. Duration of the experiment was 8 weeks. Panting was observed in birds of groups C and B. Water consumption of group C was significantly higher compared to groups A and B. Body temperature of group C remained significantly higher in comparison to other two groups throughout the experiment. Erythrocyte counts, hemoglobin concentration and hematocrit were significantly higher in group A compared to group C. Testes weight, volume and comb area were significantly higher in group A. Histologically, many birds in group B did not have spermatozoa in the seminiferous tubules and necrotic cells were present along with round spermatids. In group C all the cells of spermatogenesis were present only in one bird, whereas spermatocytes and/or spermatids were seen in other birds.

Keywords: Environmental temperature, broiler breeders, hematological values, testes, histopathology of testes.

INTRODUCTION

Domestic fowl being homoeothermic can live comfortably only in a relatively narrow zone of thermo-neutrality ranging from 14.5 to 25.5°C. Any deviation from these temperatures depresses production performance of poultry birds resulting in decreased egg production, egg quality, growth rate, feed efficiency and increased mortality (Muiruri and Harrison, 1991; McDaniel *et al*., 2004).

A well known effect of high ambient temperature during summer months on male broiler breeders is a decrease in fertility (Kiers, 1982). Poultry birds kept in intensive conditions respond to elevated temperature and exhibited delayed maturity and lower fertility (Vo *et al*., 1980; McDaniel *et al*., 1996). Heat stress has been reported to produce changes in semen leading to increased infertility (Karaca *et al*., 2002). Most of the work on the effect of high ambient temperatures has been conducted in thermostatically controlled chambers. Accessible literature provides meager information about the effects of naturally occurring high temperatures and its diurnal variation upon onset of maturity and morphology of testes in growing chicken. The present study describes the effects of naturally occurring environmental temperatures of two different regions in comparison with conditions of environmentally controlled houses upon the onset of maturity and morphological patterns of testes in male broiler breeder birds approaching puberty.

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MATERIALS AND METHODS

Birds and feed

A total of 48 broiler breeder males (Starbro) of 18 wks of age originating from same grandparent flock were obtained from a broiler breeder company. Fresh water was provided to the birds *ad libitum*. Birds were given a weighed amount of daily ration (15% total protein) according to Parent Stock Breeder Guide provided by the company.

Experimental design

Birds divided in 3 groups (A-C) having 16 in each were placed in different locations at the beginning of wk 19 (d 133) of age. Birds of group A were kept at ambient temperature (22 to 26°C) in an environmentally controlled house at Kalarkahar area

District Khushab. Birds of group B were kept in an open sided naturally ventilated house in village Padhrar of district Khushab where environmental temperature varied from 28 to 33°C. The 3rd group (C) was kept at 30 to 37°C in an open sided naturally ventilated house at Faisalabad. Duration of the experiment was 8 weeks. The birds of all groups were kept day and night at the above mentioned temperatures throughout the experiment. Maximum and minimum environmental temperature (ºC) inside each house was recorded on daily basis during the course of the experiment.

Clinical evaluation

Body temperature of birds was measured daily in afternoon by insertion of a lubricated glass thermometer into cloaca. Water consumption of the birds was recorded on daily basis. The comb area of each bird was determined at the end of each week using a planimeter (Takeda, Osaka, Japan). Behavioral alterations were determined subjectively by observing panting, alertness, attraction to the feed and crowing.

Hematological parameters

Blood samples from randomly selected four birds from each group at wk 19 (133d) and from six birds at wk 22 (154d) and 26 (182d) of age were collected from wing vein with and without anticoagulant (Na_2EDTA). Blood samples collected with anticoagulant were subjected to determination of erythrocyte counts (Natt and Herrick, 1952). Packed cell volume was determined using microhematocrit capillary tubes and hemoglobin was determined spectrophotometrically by the cyanmethemoglobin method using Drabkin's solution (Nazifi *et al*., 2010).

Gross and histopathology

Four birds randomly selected from each group were killed humanely at wk 19 (133d) of age to determine different parameters. Afterward six birds selected randomly from each group were killed humanely at wk 22 (154d) and the remaining birds at wk 26 (182d) of age. Visceral organs were examined for the presence of gross lesions. Testes were weighed and their volume determined by volume displacement technique. Slices from the middle portion of testes from each bird were fixed in 10% neutral buffered formalin and processed for histopathological examination by routine paraffin embedding method. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (Khadiga *et al*., 2009).

Statistical analysis

Data thus collected from above experiments were subjected to analysis of variance. Different means were compared by Duncan's multiple range tests using Mstat-C software package. The level of significance was $P \leq 0.05$.

RESULTS

Environmental temperatures

Mean maximum and minimum environmental temperatures (ºC) of houses of all three groups for each week has been presented in Table I. Mean temperature (± SD) of groups A, B and C during the study period varied between 22.54±0.60 to 25.32±0.77, 28.17±2.27 to 33.18±2.20 and 30.96±2.11 and 36.86±2.49ºC, respectively.

Clinical parameters

Birds in all the three groups kept in different climatic locations remained active and consumed all the feed offered within 60 to 90 minutes. No panting was observed in birds of group A. Birds of groups B and C started panting when temperature reached 33°C. Panting became intense at 37°C. Birds in group C were keeping their feathers away from the body and most often found standing around the water pens with wet heads. Crowing in birds of group A started at age of 154d and on 176d all the birds were crowing. In group B crowing started at the age of 168d and at 182d all the birds were crowing. In group C crowing of birds did not start even at 182d of age.

The water consumption of birds of group C was significantly ($P<0.05$) higher than those of groups A and B (Table II). However, the water consumption of birds of group B was non significantly different from group A.

Mean cloacal temperature (Table II) of the birds of group C remained significantly ($P<0.05$) higher than those of group A and B throughout the

Table I.- Weekly environmental temperature (ºC) of poultry houses of different groups kept in different climatic regions (Mean±SD).

Week of experiment (Age in days)	Group A		Group B		Group C	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1 (133)	22.86±0.38	25.71±0.49	28.43±3.36	32.00±3.61	29.14±3.29	33.29±2.98
2 (140)	22.14±0.38	25.29±0.49	28.00±2.38	32.86±1.57	30.86±1.35	37.14±1.46
3 (147)	22.86±0.38	25.71±0.49	30.14±1.46	34.00±1.73	31.71±1.80	38.14±0.90
4 (154)	22.43±0.43	24.86±0.69	27.00±1.91	31.43±1.62	30.57±1.40	36.43±1.62
5 (161)	22.71±0.71	25.71±0.49	29.57±0.98	34.43±1.40	31.86±1.95	37.57±2.51
6 (168)	22.57±0.53	25.86±0.90	30.43±1.27	35.57±0.79	31.71±0.76	38.86±0.69
7 (175)	22.14±0.38	24.57±0.79	27.14±2.48	32.14±2.04	29.29±2.14	35.14±2.61
8 (182)	22.57±1.13	24.06±0.69	27.68±1.21	33.00±0.82	32.57±1.13	38.29±0.76
Average (wk 1-8)	22.54±0.60	25.32±0.77	28.17±2.27	33.18±2.20	30.96±2.11	36.86±2.49

Table II.- Effect of three different ambient temperatures upon the water consumption (ml/day) and body temperature (°C) of male broiler breeders (Mean±SD)

Age week (day)	Groups*		
	A (22-26 °C)	B (28-33°C)	C (30-37°C)
Water consumption (ml/day)			
19 (133)	590±42.4	590±42.4	590±42.4
20 (140)	620±57.0b	710±74.2b	1300±4a
21 (147)	610±65.2b	730±120.4b	1110±74.2a
22 (154)	620±57.0b	760±74.2b	1120±103.7a
23(161)	620±57.0b	680±57.0b	980±178.9a
24 (168)	570±57.0b	660±65.2b	1060±165.7a
25 (175)	610±65.2b	680±135.1b	1020±90.3a
26 (182)	640±89.4b	645±37.1b	1100±145.8a
Body temperature (°C)			
19 (133)	40.73±0.132	40.73±0.142	40.73±0.154
20 (140)	40.89±0.122b	41.58±0.185a	41.62±0.279a
21 (147)	41.11±0.182b	41.51±0.131b	41.98±0.362a
22 (154)	40.87±0.139b	41.60±0.178a	41.58±0.177a
23(161)	40.96±0.161c	41.47±0.236b	42.17±0.429a
24 (168)	40.89±0.122c	41.36±0.185b	41.93±0.402a
25 (175)	40.93±0.068c	41.42±0.186b	42.53±0.241a
26 (182)	40.91±0.151b	41.24±0.149b	42.73±0.203a

*Birds of groups A were kept at 22 to 26°C in an environmentally controlled house and groups B and C were kept at 28 to 33 and 30 to 37°C, respectively in an open sided naturally ventilated houses. Values in each row followed by different letters are statistically significant ($P \leq 0.05$).

experiment with the exception of day 140 and 154 of age when it was non significantly different from group B. The body temperature of the birds of group A remained significantly lower from that of group B throughout the duration of the experiment with the exception of day 147 and 182 when it was non-significant.

Hematological studies

The erythrocyte counts, hemoglobin concentrations and hematocrit values of birds of all groups at the beginning of experiment (18 weeks of age) were statistically non significant from each other (Table III). Hemoglobin concentration remained non significantly different among all groups at 22 weeks of age. At 22 and 26 weeks of age the erythrocyte counts and at 26 weeks the hemoglobin concentration in birds of group C were significantly lower than those of group A and B whereas the later two were non significantly different from each other. At 22 weeks of age the hematocrit values of birds of group A was significantly higher than those of group C but not from those of group B. At 26 week the hematocrit values of birds of group B and C were non significantly different from each other but significantly lower than those of group A (Table III).

Testes weight, volume and comb area

Mean relative weight (percent of body weight) and volume of testes of different groups have been presented in Table IV. Testes weight and volume of birds of all groups at the beginning of experiment (133d of age) was statistically non significant from each other. At 154d of age the testes weight and volume of group B and C differed non-significantly from each other, however, were

significantly (P<0.05) lower than that of group A. At 182d of age the testes weight and volume of groups A and B were differed non-significantly from each other but were significantly higher from that of group C.

Table III.- Effect of three different ambient temperatures upon the hematological parameters (Mean±SD) in male broiler breeders

Age week (day)	Groups* A	B	C
Erythrocyte counts (10^6/µl)			
19 (133)	4.88±0.29	5.00±0.16	4.94±0.08
22 (154)	6.41±1.23a	6.82±0.28a	5.28±0.16b
26 (182)	8.08±1.41a	7.32±1.51a	5.55±0.14b
Hemoglobin concentration (g/dl)			
19 (133)	7.01±0.50	7.28±0.55	7.13±0.67
22 (154)	9.01±1.33	9.45±0.59	8.30±0.37
26 (182)	10.71±1.6a	10.14±2.12a	7.16±0.18b
Hematocrit (%)			
19 (133)	32.20±2.17	31.80±2.17	31.60±0.55
22 (154)	39.80±3.11a	37.20±6.53ab	30.60±0.89b
26 (182)	41.80±1.30a	31.38±5.51b	32.80±3.19b

*See footnote in Table II.

Table IV.- Effect of three different ambient temperatures upon various testicular parameters (Mean±SD) in male broiler breeders.

Age week (day)	Groups* A	B	C
Relative testicular weight (% of body weight)			
19 (133)	0.352±0.119	0.352±0.118	0.408±0.038
22 (154)	1.048±0.219a	0.586±0.043b	0.439±0.077b
26 (182)	1.172±0.209a	0.948±0.240a	0.651±0.372b
Testes volume (ml)			
19 (133)	9.00±1.58	9.00±1.58	9.40±1.82
22 (154)	29.60±7.02a	20.00±1.581b	13.6±2.70b
26 (182)	42.60±11.8a	34.40±9.24a	17.40±11.59b
Comb area (cm^2)			
19 (133)	3.440±6.46	2.18±7.73	2.24±3.22
22 (154)	32.60±6.54a	27.60±2.97b	18.92±11.56c
26 (182)	40.60±10.6b	51.40±20.27a	31.60±18.57c

*See footnote in Table II.

Comb area at 133d of age of all groups was non-significantly different from each other. At 154d of age, comb area in all groups differed significantly (P<0.05) from each other where comb area values were the largest in group A followed by Group B and C. At 182d, all the groups differed significantly from each other; comb area being the largest in group B followed by group A and C.

Testosterone concentration

At the beginning of experiment (18 weeks), serum testosterone concentration of the birds was non-significantly different among three groups. At 22 weeks of age, serum testosterone concentration in birds of group B was significantly (P<0.05) more than in birds of group C, however, both groups were non-significantly different from group A. At 26 weeks of age, serum testosterone concentration in birds of all groups differed non-significantly (Table V).

Table V.- Effect of three different ambient temperatures upon testosterone concentration and seminiferous tubule diameter (Mean±SD) in male broiler breeders

Age week (day)	Groups* A	B	C
Testosterone (ng/ml)			
19 (133)	2.90±0.22	3.06±0.24	2.84±0.23
22 (154)	8.14±4.42ab	9.72±1.17a	5.04±1.56b
26 (182)	7.02±2.82	8.21±3.04	8.25±3.67
Seminiferous tubule diameter (µm)			
19 (133)	92.02±2.49	92.30±2.73	91.24±2.52
22 (154)	187.61±6.43a	154.45±8.69b	126.69±28.71c
26 (182)	194.98±6.94a	165.94±10.16b	144.80±14.77c

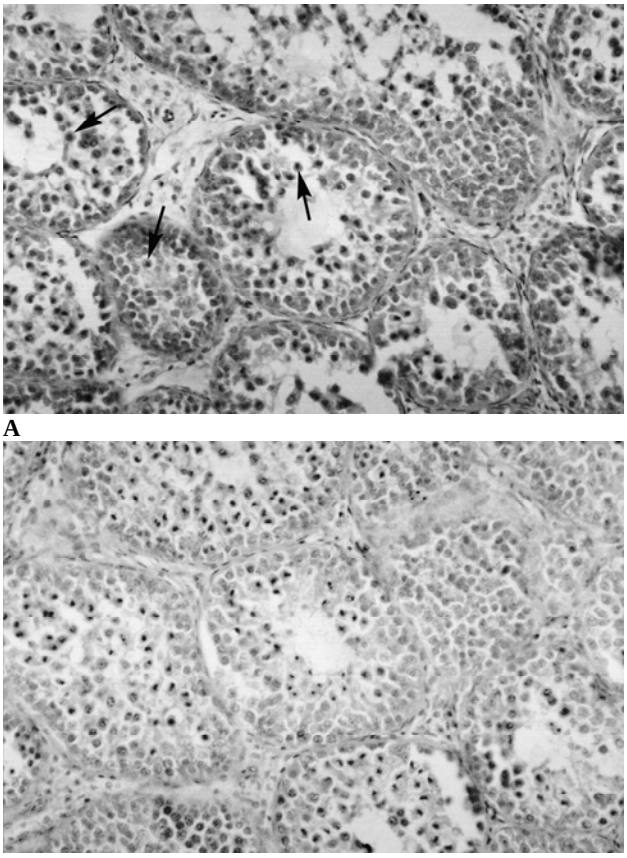
*See footnote in Table II.

Gross and histopathology

Birds of group A and B had larger and softer testes as compared to those of group C. The parenchyma of testes bulged out on incising in group C. The color, consistency and size of different visceral organs including liver, kidneys, spleen and intestine did not differ between the groups.

In group A seminiferous tubules of birds at 133d of age were lined by spermatogonia, spematocytes, round and elongated spermatids. Round spermatids out numbered the elongated spermatids in all tubules. Tubules of three of six birds, in addition to above cells also had bunches of

immature spermatozoa facing toward periphery of tubules. At 182d of age all the cells of spermatogenesis were present in seminiferous tubules. The immature spermatozoa were present in bunches facing towards the basement membrane of the tubules. In many tubules spermatozoa were present in the lumen.



A

B

Fig. 1. Histological structure of testes of a bird of group B (A) showing pyknotic nuclei of round spermatids (arrows) and group C (B) showing presence of spermatogonial cells, spermatocytes and no other maturing cells. H and E staining. X400.

In group B at 133d of age two birds showed all stages of spermatogenesis in some of the seminiferous tubules. In one bird stages of spermatogenesis were observed along with pyknotic nuclei among the round spermatids (Fig. 1A). In three birds tubules contained dark pyknotic rounded nuclei in place of round spermatids. In most of the tubules elongated spermatids were not present. In some tubules aggregates of cells resembling primary spermatocytes and spermatids were present in the lumen of seminiferous tubules. At 182d of age all cells of spermatogenesis were present in four birds. Lumen of some tubules contained mixture of spermatozoa and immature cells. In two birds only spermatogonia, spermatocytes and round spermatids were present. Round cells with darkly stained pyknotic nuclei were present among the round spermatids in many tubules. Multinucleated giant cells were also present in many tubules.

In group C at 133d of age lumen of the seminiferous tubules were lined only with spermatogonial cells and spermatocytes. Lumen of the tubules contained eosinophillic material and/or spermatocytes. In two birds the seminiferous tubules had only rounded spermatids. Multinucleated giant cells were also present in the lumen of some tubules. At 182d of age seminiferous tubules of three birds did not show spermatogenesis and only spermatogonia and spermatocyte were present (Fig.1B). In some tubules mitotic bodies were present in spermatocytes and few round spermatids were present. In two birds spermatogonia, spermatocytes and round and elongated spermatids were present in the tubules. Multinucleated cells were present in the lumen of some seminiferous tubules. In one bird, seminiferous tubules contained cells of all stages of spermatogenesis.

Diameter of seminiferous tubules

The diameter of seminiferous tubule of birds of all groups at the beginning of experiment (126d of age) differed non-significantly from each other. At 154 and 182d of age the diameter of tubules of birds in all three groups was significantly ($P<0.05$) different from each other being largest in group A followed by groups B and C (Table V).

DISCUSSION

Poultry birds are known to live comfortably in a temperature range of 14.5 to 25.5ºC (Muiruri and Harrison, 1991). Temperatures above and below this range are known to pose stress to the birds and adversely affect their performance (Saima *et al*., 2010). Temperature above 28^{o}C has reportedly been unfavorably high for the egg laying

type of birds (Arighi *et al*., 1987; Ahmad *et al*., 2010). In male broiler breeders adverse effects upon semen quality were observed at 27^{o}C (Kiers, 1982) and reduced fertility was observed at 29 and 32^{o}C (McDaniel *et al*., 1995). According to Daghir and Jones (1995), seasonal variation in sperm production is well documented and less sperms are produced during the summer months which reduce fertility. Part of this reduction in sperm production is associated with reduction in feed intake and thus nutrient intake. In the present study, environmental temperature of birds of group A constantly remained within comfortable range and hence did not suffered from heat stress. A diurnal variation in the environmental temperature of birds of group B suggested that the birds of this group constantly suffered from heat stress during day time while at night the heat stress was partially alleviated. Birds of group C always had environmental temperature above 28ºC and hence constantly lived in uncomfortable temperature.

Panting was observed in birds of group B and C accompanied by increased water intake was an attempt to alleviate the body heat with loss of water, such action to alleviate heat has been reported in hyperthermic birds (Whittow, 1986; Nishibri *et al*., 1989; Brackenbury and Amaku, 1990). Nesheim *et al*. (1979) concluded that there was direct effect of increase in ambient temperature to the body temperature. Elevated body temperature of the birds of group B and C despite panting and increased water intake suggested inability of these birds to reduce their body temperature.

In male fowl crowing is an androgen dependent behavior which develops among other factors with the pubertal increase of testosterone in blood circulation (Marler *et al*., 1992; Marx *et al*., 2004). A delayed onset of crowing in birds of group B and C indicated a delayed onset of puberty in these birds. Vo *et al*. (1980) also reported delayed sexual maturity of birds kept at higher ambient temperature. A non-significant difference of serum testosterone level, among the birds of three groups kept at different temperature regimes suggested no effect of elevated ambient temperature upon serum testosterone levels as has also been reported previously (Damber and Jansn, 1978; Hjollund *et al*., 2002).

In the present study, erythrocyte counts and hemoglobin concentration were significantly higher in group A and B birds as compared to group C birds, these findings show the deleterious effects of heat on these hematological parameters. Similar views have been expressed by Chaudhry and Sial (1973), as they stated that birds kept under continuous high temperature stress, had significantly lowered blood cell counts than those kept in cold temperature. Zimmerman *et al.* (1975) have reported that high environmental temperature decrease hemoglobin level in chickens. Hematocrit value was the highest in group A birds as these birds were kept as the lowest temperature in the present study. In this regard, contrary findings have been reported by Altan *et al.* (2000), as they did not observe effect of heat stress (38±1°C for 2 hours at 14 and 15d of age) on hematocrit values whereas Altan *et al*. (2003) reported decreased hematocrit values in birds exposed to heat stress (38±1°C for 3 hours at 36 and 37d of age).

A significant decrease in relative weight of testes of birds of group B and C compared with those of group A suggested an inhibitory effect of high ambient temperature upon gonadal development. Histologically, smaller diameter of seminiferous tubules, absence of cells beyond spermatids, pyknotic nuclei in spermatids and multinucleated cells in tubules observed in group B and C but not in testes of group A at 22 wk of age could be a consequence of heat induced stress. Huston (1975) reported a delayed spermatogenesis in maturing cockerels kept at 30°C. There is scanty information about histological alterations observed in the testes of the chicken kept at high ambient temperature. With these reasons the results of present study have been compared with testes of mammals exposed to high ambient temperature. Exposure of rats to high temperature resulted in testicular atrophy (Pucak *et al*., 1977). Boars kept at elevated ambient temperature resulted in reduced fertility, semen output, quality and inhibitory effect on spermatic maturation (Wettemann and Bazer, 1985). Abdominal and inguinal testes of horses exhibited arrested spermatogenesis and early spermatocytes and most mature stage of spermatogenic cells were not observed. These effects were attributed to the elevated temperature

of abdomen and inguinal canal (Al-Saffar and Rose, 2002). Gasinska and Hill (1990) reported that heating the mice scrotum to 43°C for 30 minutes resulted in decreased spermatids and smaller diameter of seminiferous tubules in the testes. Similarly, elevating one testis of mice into the abdomen resulted in decreased diameter of seminiferous tubules and less mature germinal cells (Salman *et al*., 1988). Miraglia and Hayashi (1993) reported giant cells and multinucleated cells in the tubules along with decrease in tubular diameter in rats after heating of testes to 43 to 45°C for short period from age of 60 to 90 d. Similarly, an increase in the scrotum temperature has been reported to induce a decrease in sperm counts and increased production of abnormal sperms in mice (Cairnie and Leach, 1980) and bulls (Wildeus and Entwistle, 1983). According to Daghir and Jones (2008), seasonal variation in sperm production is well documented and less sperms are produced during the summer months which reduce fertility. In their view, part of this reduction in sperm preproduction is associated with reduction in feed intake and thus nutrient intake. Histological findings of present study in male chicken exposed to high ambient temperatures resembled to those reported in different mammals exposed to high ambient temperatures or high scrotal temperatures.

At 26 wk of age appearance of all cells of spermatogenesis in testes of all birds of group B and only one of six birds in group C suggested that delay in onset of maturity was reduced in the birds intermittently exposed to heat stress and comfortable environmental temperatures as was the case in group B. An inverse relation between ambient temperature and age at onset of puberty has been described by some workers (Huston, 1975; Vo *et al*., 1980; McDaniel *et al*., 1995). The birds of groups C and B were exposed to higher light intensity and longer duration of the natural day length compared with those of group A kept which were kept in environmentally controlled house. These factors have been known to enhance the early onset of sexual maturity yet these birds attained sexual maturity later than those kept in controlled environment suggesting that environmental temperature alone might have been a major factor in delay of sexual maturity.

The results of the present study suggested that naturally high ambient temperatures delayed the onset of maturity, testicular development as suggested by decrease in testes weight, volume, smaller diameter of seminiferous tubules, absence of certain cells of spermatogenesis and presence of abnormal cells in the tubules. However, a diurnal variation resulting in comfortable temperature at night partially alleviated the effect of heat stress and reduced the delay in onset of maturity.

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