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PROFILE OF GONADAL HORMONES IN THE MALE AND FEMALE HOUBARA BUSTARD DURING THE YEAR

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Abstract. The present study was undertaken to estimate the levels of reproductive hormones i. e: testosterone, estradiol and progesterone, to investigate the breeding biology of Houbara Bustard. The male and female birds were used from Houbara Research and Rehabilitation Center (HRRC) Rahim Yar Khan. The plasma blood samples of 5 male and 5 female birds were collected during non-breeding and breeding seasons. Then testosterone, estradiol and progesterone were measured by RIA (Radioimmunoassay). The results showed that there was no significant difference in the levels of testosterone during non-breeding and breeding seasons. The level of estradiol was lower during non-breeding season (9.70 ± 0.72 pg/ml) and higher during breeding season (14.35 ± 0.77 pg/ml) and there was a significant difference in the levels during non-breeding and breeding seasons ($p < 0.01$). There level of progesterone was higher during non-breeding season (0.72 ± 0.10 ng/ml) while it was lower during the breeding season (0.55 ± 0.10 ng/ml). Thus there was a significant difference in the values of progesterone during non-breeding and breeding seasons ($p < 0.001$).

Keywords: Pakistan, Houbara Bustard, *Chlamydotis undulata,* physiology, reproductive hormones.

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Профиль половых гормонов у самцов и самок джека в течение года. - Т. Махмуд, М.М. Ахмед, М.С. Надим. - Беркут. 13 (1). 2004. - Уровни половых гормонов – тестостерона, эстрадиола и прогестерона – изучались в Центре по исследованию и реабилитации джека в Рахим Яр Хане (Пакистан). Плазма крови 5 самцов и 5 самок бралась во время гнездового и негнездового периодов. Уровень гормонов измерялся при помощи радиоиммунного анализа. Достоверных различий уровня тестостерона в гнездовой и внегнездовой периоды не обнаружено. Уровень эстрадиола был ниже во внегнездовой период ($p < 0,01$), а прогестерона – в гнездовой ($p < 0,001$).

INTRODUCTION

Houbara bustard (*Chlamydotis undulata*) belongs to the bustard family Otididae. The family is world wide in distribution, chiefly inhabiting the open plains and semidesert regions. There are three sub-species of Houbara bustard viz. *Ch. u. macqueenii, Ch. u. undulata* and *Ch. u. furtaventurae*.

Chlamydotis undulata macqueenii is mainly winter visitor in Pakistan. Its breeding season starts from February and continues till the end of June. Winter visitors from Central Asia form the greater part of Pakistani Houbara populations. Schwabl (1992) showed in migratory European Robins (*Erithacus rubecula*) that the males defending winter territories (non-breeding) had low while males defending breeding territories had elevated levels of LH and testosterone. Similarly in females, LH and estradiol levels were low in winter and elevated in spring (breeding season). Steimer et al. (1981) showed that estradiol 17-b formed from testosterone in the brain is thought to be involved in the hormonal control of male sexual behaviour in some mammalian species. Brenowitz et al. (1998) have shown that exposing wild-caught White-crowned Sparrows (*Zonotrichia leucophrys*) to long day photoperiods in the laboratory may not induce circulating concentrations of testosterone as high as those seen in the wild. Changes in circulating testosterone are primarily responsible for the seasonal morphological changes in the song nuclei. Testis size and circulating T concentrations are greater in spring than in fall birds.

Keeping in view the importance of breeding biology Houbara, the present study was undertaken to estimate the levels of testosterone, estradiol and progesterone during the non-breeding and breeding seasons, to understand the reproductive behaviour of this species.

MATERIALS AND METHODS

The present study was undertaken at HRRC (Houbara Research and Rehabilitation Center) Rahim Yar Khan, Pakistan, with the collaboration of Houbara Foundation International Pakistan. The Center is 90 km away from Rahim Yar Khan in the Cholistan desert located 28^0 32 N and 70^0 55 E. The temperature of the area was recorded from July 2001 to June 2002. The blood samples of 5 male and 5 female birds were collected regularly during non-breeding (from September 2001 to January 2002) and breeding (from February 2002 to June 2002) seasons. Each time 0.5 – 1.0 ml blood was obtained from the brachial vein of each bird. The blood was centrifuged at 3000 rpm for 10–15 minutes in the field laboratory of HRRC. In this way plasma was obtained, sealed and stored in the freezer. Later on, the levels of testosterone, estrogen and progesterone were estimated using Radioimmunoassay (RIA).

Protocol for testosterone RIA

All the reagents and plasma samples were allowed to thaw. 50 ml of standard, control and sample were successfully added to the rifles tubes. Then 500 ml of testosterone tracer was added to each of the tube. All the tubes were shaken well and thoroughly mixed. The tubes were incubated for 3 hours at 37°C in a water bath. Then all the free radioactive material was decanted in a radioactive sink. After 10 minutes the bound radioactivity was measured in a 16-chamber gamma counter. The data was analyzed by Student's t-test.

Protocol for estradiol RIA

100 ml of standard, control and sample were successfully added to the antibody-coated tubes. Then 500 ml of tracer was added to each of the tube. The tubes were shaken for thorough mixing on a vortex mixer. Then the tubes were incubated for 3 hours at 18–5°C with shaking (350 rpm). Then all the free radioactive material was decanted into a radioactive sink, the tubes were placed in an inverted position. After 10 minutes the bound radioactivity was counted in a 16 channels gamma counter. The results were analyzed by Student's t-test.

Protocol for progesterone RIA

100 ml of the zero standard "A" was pipetted into the NSB (non-specific binding) and "A" tubes. Then 100 ml of each of the standards i.e., from "B" through "G" were pipetted into the corresponding tubes. After that 100 ml of each control and sample were pipetted into the corresponding tubes. Then 1 ml of 125-I progesterone tracer was added to each of the tube. All the tubes were shaken on a vortex mixer and incubated for 3 hours at 15–28°C. The free radioactive material was decanted into a radioactive sink and the tubes were placed in an inverted position for 10 minutes. The bound radioactivity was measured in a gamma counter and the results were analyzed by Student's t-test.

RESULTS

1. TESTOSTERONE

Non-breeding season

During the month of September, the plasma testosterone level was 0.23 ± 0.11 ng/ml, with a slight increase in December and January; and the values were 0.24 ± 0.14 ng/ml and 0.26 ± 0.14 ng/ml, respectively.

Breeding Season

During the month of February, the plasma testosterone level was 0.30 ± 0.08 ng/ml. It slightly increased during March and then slightly decreased during April. The values were 0.33 ± 0.08 ng/ml and 0.30 ± 0.05 ng/ml, respectively. During the month of May there was again a slight increase in the testosterone level and then slight decrease during June. The values were 0.32 ± 0.05 ng/ml and 0.31 ± 0.02 ng/ml, respectively (Table, Fig. 1). Thus there was no significant difference in the levels of testosterone during non-breeding and breeding seasons.

Average levels of Testosterone (ng/ml) in male, Estradiol (pg/ml) and Progesterone (ng/ml) during non-breeding (from September 2001 to January 2002) and breeding (from February to June 2002) seasons in female Houbara Bustard

Средние уровни тестостерона (нг/мл) у самцов, эстрадиола (пг/мл) и прогестерона (нг/мл) во время негнездового (сентябрь 2001 г. – январь 2002 г.) и гнездового (февраль–июнь 2002 г.) сезонов у самок джека

Hormone	Sep.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Testosterone	0.23±0.11	0.24±0.14	0.26±0.14	0.30±0.08	0.33±0.08	0.30±0.05	0.32±0.05	0.31±0.02
Estradiol	8.50±0.98	9.70±1.00	10.80±0.86	9.50±0.92	10.20±0.93	14.20±0.60	15.60±0.75	21.99±2.40
Progesterone	1.62±0.08	2.80±0.22	0.74±0.16	0.39±0.04	0.41±0.04	0.61±0.04	0.76±0.05	0.60±0.04

2. ESTRADIOL LEVELS

Non-breeding season

During the month of September, the level of estradiol was 8.5 ± 0.98 pg/ml. It gradually increased during December and January and the values were 9.7 ± 1.00 pg/ml and 10.86 ± 0.86 pg/ml, respectively.

Breeding season

During February the plasma estradiol level was 9.5 ± 0.92 pg/ml. It increased to 10.2 ± 0.93 pg/ml during March. Further gradual increase was observed during April and May , and the values were 14.2 ± 0.60 pg/ml and 15.6 ± 0.75 pg/ml, respectively. Then during the month of June the estradiol level inclined to 21.99 ± 2.40 pg/ml (Table, Fig. 2). Thus during the non-breeding season the average estradiol level was comparatively low (9.70 ± 0.72 pg/ml) while during the breeding season the plasma estradiol level was comparatively high (14.35 ± 0.77 pg/ml). Therefore, there was a significant difference in the values during non-breeding and breeding seasons ($P < 0.01$)

3. PROGESTERONE LEVELS

Non-breeding season

During the month of September, the plasma progesterone level was 1.62 ± 0.08 ng/ml. It increased during December up to 2.80 ± 0.22

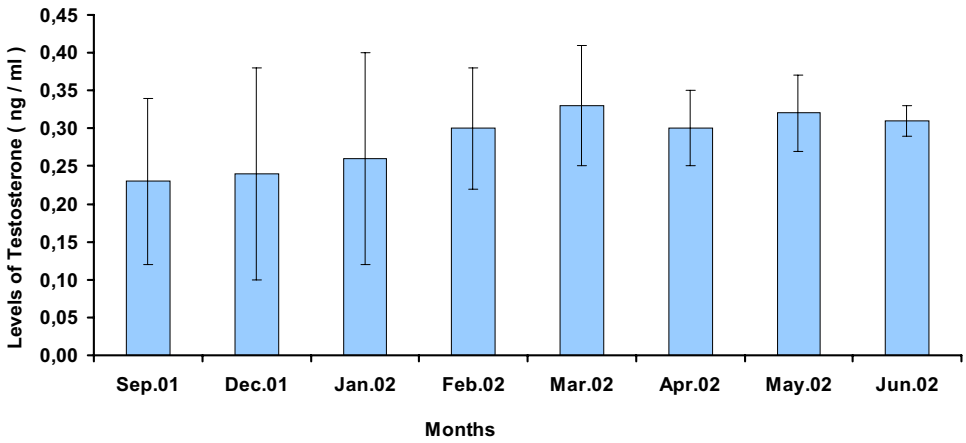

Fig.1 . Levels of testosterone (ng/ml) during non-breeding and breeding seasons in male Houbara Bustard.

Рис. 1. Уровни тестостерона (нг/мл) во время негнездового и гнездового периодов у самцов джека.

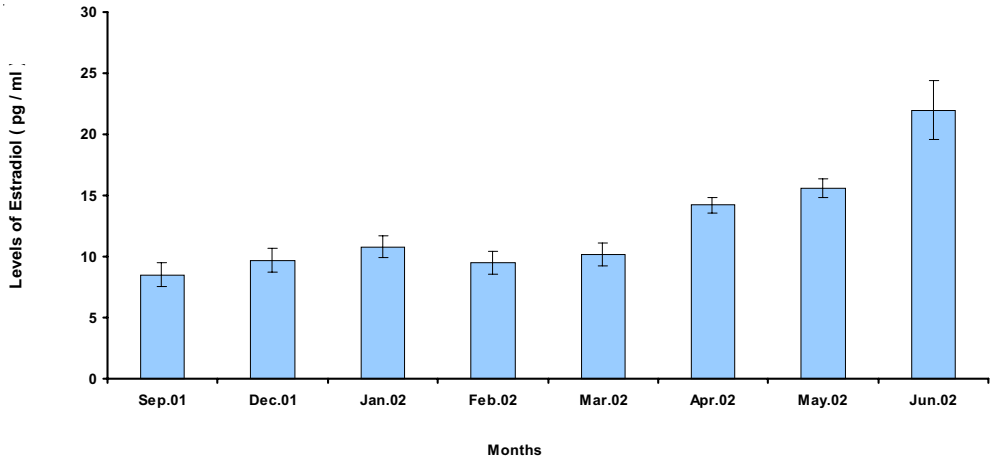

Fig. 2. Levels of estradiol (pg/ml) during non-breeding and breeding seasons.
Рис. 2. Уровни эстрадиола (пг/мл) во время гнездового и негнездового сезонов.

ng/ml. However, during January the progesterone level decreased and its value was 0.74 ± 0.16 ng/ml.

Breeding season

During February the progesterone level was 0.39 ± 0.04 ng/ml. It gradually increased during March, April and May. The values were 0.41 ± 0.04 ng/ml, 0.61 ± 0.04 ng/ml and 0.76 ± 0.05 ng/ml, respectively. However, during June it decreased to 0.60 ± 0.04 ng/ml (Table, Fig. 3). Overall during non-breeding season the level of progesterone was found to be high (0.72 ± 0.10 ng/ml) when compared with that of breeding season (0.55 ± 0.03 ng/ml). Thus there was a significant difference in the values during non-breeding and breeding seasons.

DISCUSSION

The temperature of the area showed a great fluctuation. It was lowest in January 2002 while maximum in June 2002 (Table 4). There was no significant difference in the levels of test-

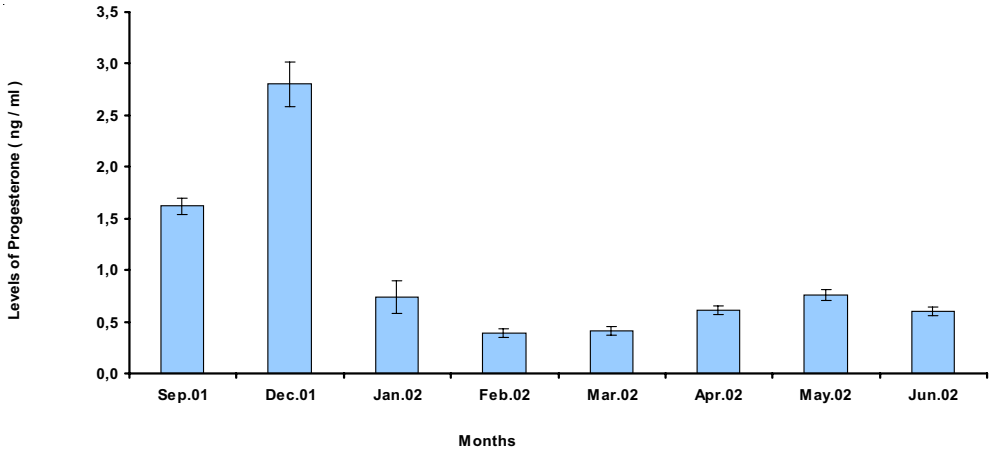

Fig. 3. Levels of progesterone (ng/ml) during non-breeding and breeding seasons.
Рис. 3. Уровни прогестерона (нг/мл) во время гнездового и негнездового сезонов.

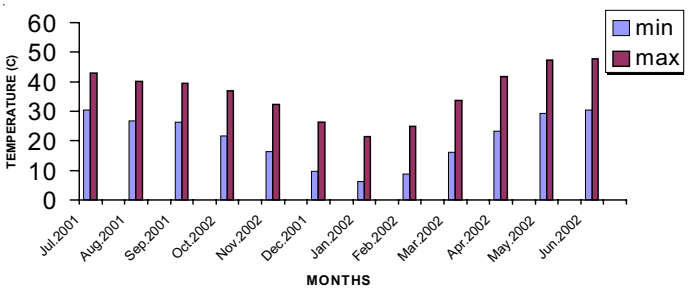

Fig.4. Temperature record (mean minimum and maximum) at HRRC, Rahim Yar Khan from July 2001 to June 2002.
Рис. 4. Температуры (средний минимум и максимум) в Центре по исследованию и реабилитации джека в Рахим Яр Хане с июля 2001 г. до июня 2002 г.

osterone during non-breeding and breeding seasons. This may be due to the fact that the birds were in captive conditions rather than in wild and so there was no expression of territorial behavior, which is mostly noted in the other birds. However, the male Houbara Bustard did display their plumage during the breeding season, which is the indicator of their sexual maturity. These results are in accordance with Brenowitz et al. (1998) who showed that exposing wild-caught white crowned sparrows to long day photoperiods in the laboratory may not induce circulating concentrations of testosterone as high as those seen in the wild. Testis size and circulating concentrations of testosterone are greater in spring than fall birds. According to Hunt et al. (1995) three passerines, the White-crowned Sparrow, American Tree Sparrow (*Spizella arborea)* and Savannah Sparrow (*Passerculus sandwichensis*) show patterns of territorial aggression typical of species. Well-defined territories are defended for several weeks, during which there is a prolonged peak in plasma concentrations of testosterone. Similarly Schwabl (1992) showed that in migratory European Robins, the male defending winter territories (non-breeding) had low while males defending breeding territories had elevated levels of LH and testosterone.

There was a significant difference in the levels of estradiol during non-breeding and breeding seasons in female Houbara Bustard. The estradiol levels were high during breeding while low during non-breeding season. These results are in accordance with the findings of Schwabl (1992) who showed that in female migratory European Robins, the LH and estradiol levels were low in winter (non-breeding season) and elevated in spring (breeding).

The level of progesterone was high during non-breeding season and low during the breeding season. This is because during the breeding season the females were fertile. Licht et al. (1982) showed that in the Olive Ridley sea turtle (*Lepidochelys olivacea*) the levels of LH and progesterone increase more than an order of magnitude within a day after oviposition.

Further research work is needed to understand the complete breeding biology of Houbara Bustard, keeping in view some other parameters like LH, FSH, and gonadal size etc. in both wild and captive birds.

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