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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 125-127

www.thepharmajournal.com
Received: 01-01-2023
Accepted: 04-02-2023

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Plasma concentrations of testosterone and estrogen in emus during breeding season

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DOI: https://doi.org/10.22271/tpi.2023.v12.i3b.19496

Abstract
Emus (*Dromaius novaehollandiae*) are flightless birds that belong to the order ratite. They are not only seasonal breeders but also monogamous. It is important to understand the reproductive cycle and the hormones that regulate their reproductive patterns of the emus to control the breeding activity of the emu when reared under farmed conditions. Hence, an attempt was made to study the hormonal profile of the emus during the pre-breeding, breeding and post breeding seasons. Blood samples were collected from thirty apparently healthy emus (15 male and 15 female) of 5 to 6 years of age during the different the pre-breeding, breeding and post breeding seasons. Plasma was separated by centrifugation and stored in aliquots at - 20 °C till further analysis. Hormones such as testosterone and estradiol were analysed using the commercially available kits. The data was subjected to statistical analysis by one way Anova. The results indicated a significantly higher (p<0.01) testosterone and estradiol levels during the breeding season in the male and female birds respectively. This study serves to understand the physiology behind the reproductive functions in the emus.

Keywords: Pre-breeding, breeding, post breeding, seasons, emu, estradiol, testosterone

1. Introduction
Emus (*Dromaius novaehollandiae*) are flightless and exotic birds belonging to the order ratite. They are reared for their meat, leather and oil which are reported to have high economic value. The anatomical and physiological features of emu birds are found to be suitable for rearing under both temperate and tropical climatic conditions.
Emu is a prolific breeder and the reproductive cycle is annual wherein, egg laying and sperm production starts in the autumn and last until spring (Malecki *et al.*, 1998) [8]. The breeding season of emu varies with the latitude in a pattern that is consistent with photoperiodic control (Blache *et al.*, 2001a) [2], as the sexual behaviour disappears with photo refractoriness. They also exhibit a peculiar reproductive cycle in that the male incubates the egg and also influences hatchability. They are monogamous and seasonal breeders. As hormones are known to influence production and reproduction in emus it is essential and necessary to understand their annual cycle of reproductive functions for controlling the breeding activity of the emus when reared under intensive system. Hence, a pioneer study was conducted to evaluate the concentrations of reproductive hormones such as testosterone and estradiol during the pre-breeding, breeding and post-breeding seasons. The breeding season of the emus in India starts during the month of October and ends in March.

2. Materials and Methods
The study was conducted in emu birds maintained at a commercial farm in Tamil Nadu, India. The birds were reared under intensive system with standard management practices. Blood samples were collected from apparently healthy thirty emu birds (15 male and 15 female) of 5 to 6 years of age in heparinised vacutainers from right jugular vein one month before the breeding season (pre-breeding season), during the breeding season and one month after breeding season (post-breeding season). Plasma was separated by centrifugation and stored in aliquots at - 20 °C till further analysis. The reproductive hormones analyzed were testosterone in the male emus and estradiol in the female emus using the ELISA kit obtained from Agappe Diagnostics Limited.

2.1 Statistical analysis
The data were statistically analyzed by one - way analysis of variance (ANOVA) and post hoc analysis were carried out using Duncan's test for multiple comparisons using SPSS software

version 20 for windows.

3. Results and Discussion

Plasma testosterone and estradiol concentrations during pre-breeding, breeding and post breeding seasons are presented in Table 1.

Table 1: Plasma testosterone and estradiol concentrations during breeding season in emu

Seasons	Testosterone (ng/ml) Mean±SE	Estradiol (pg/ml) Mean±SE
Pre-breeding (n = 15)	7.56±0.14 ^{b}	7.26±0.62 ^{b}
Breeding (n = 15)	10.37±0.34 ^{c**}	11.08±0.28 ^{c**}
Post-breeding (n = 15)	6.34±0.46 ^{a}	5.69±0.56 ^{a}
Pooled Mean (n = 45)	8.09±0.32	8.01±0.44

**- Highly significant (p<0.01)

Mean values having same superscript within a column do not differ significantly

3.1 Plasma testosterone concentration

The overall mean testosterone concentration observed during pre-breeding, breeding and post breeding seasons in emu birds was 8.09±0.32 ng/ml. The testosterone concentration was significantly higher (p<0.01) during breeding season than during the pre-breeding and post-breeding seasons.

The testosterone concentration recorded in this study was significantly increased during the pre-breeding and breeding seasons and then decreased during the post-breeding season. The highest concentration was observed during the breeding season which could be due to the participation of testosterone to trigger the behavioural events that lead to selection of partners before the start of physiological breeding season, where actually production of gametes, mating and preparatory mechanism to care the offspring occurs (Blache *et al*., 2001a) [2]. Malecki *et al.* (1997) [7] observed that the plasma testosterone concentration changed seasonally in the male emus, with the highest concentration in winter and the lowest during spring and summer. In the emu, increased plasma LH and testosterone concentrations during autumn and winter served as endocrine markers for the timing of breeding season. Malecki *et al*., (1998) [8] reported that plasma LH and testosterone concentrations increased in late summer during pair formation and remained constant throughout the autumn and winter when the females were laying eggs. The increase in LH secretion stimulates the steroidogenic activity of the Leydig cells resulting in increased plasma testicular concentrations that in turn stimulate sexual behaviour and further promote spermatogenesis. They also reported an abrupt fall in plasma testosterone concentrations between July and August. In the male emu, after the dissipation of reproductive refractoriness, testosterone or other inhibitory steroids produced by the testis were insufficient to suppress LH secretion to baseline values. As a result, plasma testosterone concentrations increased and the birds come into full breeding condition (Blache *et al*., 2001b) [3].

Valdez *et al.* (2014) [11] reported that the testosterone levels remained high throughout the reproductive period in the rhea and opined that monogamous species exhibit high testosterone levels only during terrestrial establishment and mate selection, with low levels during incubation and parental care when compared to polygamous species.

The decrease in the plasma testosterone concentration observed in the present study during the post-breeding season may be due to increase in the prolactin concentration at the end of the breeding season (Suganya and Leela, 2023) [10]. Bar (2006) [1] reported that the prolactin is gonadoinhibitory and higher concentration of prolactin induces photorefractoriness and causes direct suppression of gonadotropic hormones. Emus show a unique behaviour pattern during reproduction. One of the peculiarity of emu birds with respect to breeding is that the male adults are responsible for brooding which in turn causes a concomitant increase in prolactin that culminates the breeding season. Physiology provides scope for adaptation due to domestication and managemental practices, as there is no inherent method of brooding practiced, as the eggs laid are removed without allowing actual brooding, the participation of testosterone in parental care is uncertain. If allowed to brood, the clear picture of role of testosterone along with prolactin would have been elucidated.

The decrease in plasma testosterone during post-breeding season may also be due to increased thyroid hormones that causes testicular regression and decreased hypothalamic gonadotropin releasing hormone in male emus, indicating that thyroid hormones mimics the effects of long day length.

3.2 Plasma estradiol concentration

The overall mean estradiol concentration observed during pre-breeding, breeding and post breeding seasons in emu birds was 8.01±0.44 pg/ml. Estradiol (11.08±0.28 pg/ml) concentration was significantly higher (p<0.01) during breeding season than during the pre-breeding and post-breeding seasons.

In the present study, the plasma estradiol concentration in the emu was found to be significantly increased during the breeding season. The concentration started to increase during the pre-breeding season; the highest value was recorded during the breeding season and then declined during the post-breeding season which may be due to the fact that metabolic preparations for breeding precede sexual activity (Hegner and Wingfield, 1990) [6]. Similar observations were recorded by Degen *et al.* (1994) [5] in the female ostriches, wherein, the plasma estradiol 17 β concentrations increased at the start of egg production season, peaked when egg production was maximum and remained elevated throughout the rest of the egg production season. Bronneberg *et al.* (2007) [4] observed that the estradiol concentration was increased as soon as the egg production season started and decreased with or following the last oviposition of the egg production season in the ostrich hens.

In the avian seasonal breeders, the breeding season is initiated by photoperiodism which triggers a cascade of morphological, physiological and endocrinological reproductive changes. The hypothalamus starts to secrete GnRH, which in turn stimulates FSH and LH into the circulatory system. Rise in the circulating LH concentrations stimulates the bird's ovary and cause the growth of the small ovarian follicles. Under the influence of the stimulatory effects of LH, the production and secretion of steroids from the follicles rises, eventually leading to increased plasma concentrations of these hormones such as estradiol β (Bronneberg *et al*., 2007) [4].

Ovarian estrogens in the birds have a variety of additional functions related to reproduction, including the regulation of calcium metabolism for shell formation, induction of its own receptor in the oviduct and induction of progesterone receptor in the ovary and reproductive tract, enhance the growth of the oviduct and promote the formation of tubular secretory glands and epithelial differentiation. Estradiol also induces the

synthesis of ovalbumin and lysozyme in the oviduct and vitellogenin in the liver (Sturkie, 1998) [9]. The vitellogenins are incorporated into the egg yolk, they bind to calcium and their production is followed by a rise in serum calcium levels. This estrogen-controlled hypercalcemic effect is unique to avian species due to the need to produce large calcified eggs, requiring a rapidly mobilized source of calcium. So, the peak increase in estradiol during breeding season recorded in the present study could have been due to the above physiological role discussed.

The decline in the estradiol concentration at the end of breeding season may be due to increase in the prolactin concentrations above a critical threshold level that indirectly depresses LH activity which in turn terminates the breeding season. This mechanism is known as photo refractoriness, which within few weeks time may cause regression of the ovary and subsequently cease egg production season (Bronneberg *et al*., 2007) [4]. A decrease in circulating estrogen also is associated with the initiation of molting.

4. Conclusion

This pioneer study helps in understanding the functions of testosterone and estradiol during reproduction in the emu birds. Exploration of the endocrinology during periods of reproduction in the emu needs to be studied further in future.

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