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# Estimation of the Changes in Hormone and Blood Parameters during Post-natal Development in Male Pati Duck

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## ABSTRACT

**Background:** The study of hormone and blood parameters in male Pati duck was carried out to investigate the changes that occur in these parameters during the post-natal development as better understanding of physiology is necessary for improving the productivity.

**Methods:** The blood and serum sample were collected from Pati duck at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks age groups. For each age group 6 representative samples were collected from the jugular vein. Hormones (testosterone, cortisol, triiodothyronine and thyroxine) were estimated using Radioimmunoassay (RIA) and haematological parameters were studied using digital analyser.

**Result:** Age related change observed with Testosterone hormone with maximum level observed in 40 weeks age group. T3 and T4 hormone were higher in younger age; T3 was highest in 20 weeks while T4 was highest in 1 month age group. Cortisol was higher in older groups and maximum level observed in 40 weeks age group. Among the haematological parameters significant changes was observed in packed cell volume (PCV), white blood cells (WBC), monocyte and neutrophils.

**Key words:** Avian, Cortisol, Haematology, Hormone, Testosterone, Thyroxine, Triiodothyronine.

## INTRODUCTION

Improving the productivity of any animal necessitates the understanding of its physiology including haematological characteristics to establish diagnostic baselines of blood characteristics for routine management practices (Orji *et al.* 1986). The determination of haematological and plasma metabolite levels may provide valuable information on the physiological state and form cornerstone of the medical diagnosis of diseases (Hauptmanova *et al.,* 2006; Harr, 2002). Haematological constituents usually reflect the physiological responsiveness of the animal to its external and internal environments and thus serve as a veritable tool for monitoring animal health (Pascalonpekelniczky *et al.,* 1994).

Testicular secretions are necessary for the normal expression of aggressive behavior. During breeding not only does testosterone increase aggression, but aggressive interactions also increase plasma testosterone levels (Soma, 2006). Elevated testosterone is associated with development of secondary sex organ and sexual behaviors (Balthazart, 1983) however the hormone also dramatically alters aggression and paternal care in male and some avian species (Lynn, 2008). Major functions of many steroid hormones, including testosterone, are conserved among vertebrates, but circulating concentrations nonetheless vary widely across species (Hau, 2010). Physiological process like growth, reproduction and energy metabolism in avian are mediated by steroid hormones (testosterone, cortisol and corticosterone), but systemic elevations of the hormones compromise fitness as they mobilize stored energy in response to crisis (Lee *et al*., 2012). In vertebrates, the primary neural system responsible for regulating reproduction consists of hypothalamic neurons that secrete gonadotropin-releasing hormone (Deviche *et al*., 2011). Of the three GnRH forms identified in birds so far (King and

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Millar, 1982; Miyamoto *et al*., 1984; Berghman *et al*., 2000), GnRH-I is considered the primary secretogogue of the gonadotropins-follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Kuenzel, 2000). Several studies have also demonstrated an important role of thyroid hormone in regulating seasonality of the male reproductive system (Fried *et al.* 1964; Goldsmith and Nicholls, 1984; Lien and Siopes, 1991; Wilson and Rienert, 1993; Deviche *et al*., 2011). The thyroid secretion in avian consists predominantly of T4 (Decuypere *et al*., 2005) while the concentration of 3, 3′, 5′-triiodothyronine T3 increased rapidly towards the end of hatching (Kühn *et al*., 1984). Thyroid hormones influence spermatogenesis and steroidogenesis (Wagner *et al*., 2008) thus hypothyroidism may be associated with impaired testicular development (Palmero *et al*., 1989; Francavilla *et al.,* 1991).

## MATERIALS AND METHODS

The experiment was conducted in the Radioimmunoassay (RIA) Laboratory, Department of Veterinary Physiology and Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Sciences, Assam Agricultural University, Khanapara, Guwahati. The experiment was carried out from February, 2018 to January, 2021.

Serum samples for hormone analysis were collected from whole blood using clot activator and kept in room temperature for 4 hours after which serum was separated using micropipette in a cryovial and stored at -20°C. The samples were processed in the Radio Immuno Assay (RIA) Laboratory. Estimation of hormone level in serum was conducted using commercially available hormone kit.

### T3 (Tri-iodothyronine) and T4 (Thyroxin)

Serum T3 concentrations was estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using 125 I - labeled T3 tracer and anti T3 monoclonal antibody coated tubes. Serum T4 concentrations using 125 I - labeled T4 tracer and anti T4 monoclonal antibody coated tubes.

| | |
|---|---|
| Step 1 Additions | To antibody coated tubes, add successively: 20 µl of calibrator, control or sample and 500 µl of tracer. Mix |
| Step 2 Incubation | Incubate 1 hour at 18-25°C with shaking (>280 rpm) |
| Step 3 Counting | Aspirate carefully the contents of the tubes (except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min. |

*Add 500 µl of tracer to additional tubes to obtain total cpm.

### Cortisol and testosterone

Serum cortisol concentrations was estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using 125 I - labeled cortisol tracer and anti-cortisol antibody coated tubes. Serum testosterone concentrations was estimated using RIA Kit (IMMUNOTECH, Czech Republic) using 125 I - labeled testosterone tracer and anti-testosterone antibody coated tubes.

| | |
|---|---|
| Step 1- Additions | To antibody coated tubes, add successively: 50 µl of calibrator, control or sample and 300 µl of tracer. Mix |
| Step 2- Incubation | Incubate 1 hour at 18-25°C with shaking (≥400 rpm) |
| Step 3- Counting | Aspirate carefully the contents of the tubes (Except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min |

*Add 500 µl of tracer to additional tubes to obtain total cpm.

Whole blood collected in EDTA stored at 4°C was used to estimate hematological parameters. Digital analyzer in the laboratory of TVCC, College of Veterinary Science, AAU, Khanapara was used for the study. Red Blood Cells, White Blood Cells, lymphocyte, monocyte, neutrophil, eosinophil, basophil and hemoglobin value of different age group were recorded.

### Statistical analysis

The data were analysed as per the method described by Snedecor and Cochran (1994) and were presented accordingly.

## RESULTS AND DISCUSSION

### Hormones

Triiodothyronine (T3) hormone in male Pati duck have no significant difference among the different age group (Table 1). There was a gradual non-significant decrease in the hormone concentration with age but suddenly hiked at 20 weeks of age. Biswas *et al.* (2010) also observed a decrease in T3 concentrations from 6 weeks to 30 weeks in White Leghorn, Kadakhnath and Aseel. Sinurat *et al.* (1987) observed a daily decrease in the T3 level in broiler after 20 days. A decrease in T3 with age indicated that the hormone plays an important role in growth and development of birds (Biswas *et al*., 2010) since T3 is metabolically more active than T4 in avian (Klandorf *et al*., 1981).

Thyroxine (T4) hormone showed no significant difference among the different age group in male Pati duck (Table 1). There was a non-significant decrease in the hormone concentration at early post-natal stages from 1 month to 20 weeks of age, with a slight increased at 30 weeks and later decline at 40 weeks. The finding was comparable with observations by Biswas *et al.* (2010) in White Leghorn where T4 concentration decreased from 6 weeks to 30 weeks of age. Harvey *et al.* (1980) recorded T4 concentration of during dark period and during light period in domestic duck which was in the same range recorded in Pati duck at 1 month and 6-8 weeks. Sinurat *et al.* (1987) observed a daily decrease in the T4 level in broiler with initial mean value of after 20 days. The higher concentration of hormone during early age may be attributed to an increased metabolic rate, especially to energy production as well as

to their involvement in the growth and development of the bird at early age of life. (Biswas *et al*., 2010).

The mean values of testosterone hormone in male Pati duck showed significant difference (P<0.05) among the age groups (Table 1). The minimum level recorded in 1 month age group increased with age till 40 weeks of age. The concentration observed at different stages of development was comparatively lower compared to earlier report by Abdul Rahman (2018) during development of Guinea cock. Hau *et al.* (2010) reported a testosterone level in Tropical birds which were in the same range observed in the present study in 30- and 40-weeks old Pati duck. Whereas Tanabe *et al.* (1983) reported a testosterone level in 14 days old duck which was comparatively higher than the findings in the present study. Simoes *et al*. (2017) observed highest testosterone concentrations during peak production in domestic duck. The difference in the findings may be attributed to sample collection time as some avian have gonadally active period (breeding season) and in-active period which may affect the hormone level (Wang *et al*., 2022). Testosterone levels showed seasonality, with the highest levels observed during peak reproduction and at the beginning of quiescence in domestic duck (Simoes *et al*., 2017).

Cortisol hormones in male Pati duck reached maximum level at 40 weeks which was significantly higher than the other age groups (Table 1). Flament *et al.* (2012) reported cortisol level of 4.25±1.36 ng/ml in 8 weeks old hybrid duck which was lower than the findings in 6-8 weeks old Pati duck. A cortisol level of 0.56±0.17 ng/ml in 14 days old chick by Tanabe *et al.* (1983). Schmidt and Soma (2008) observed a decreasing trend of cortisol concentration with age in songbird. Cortisol is the preferred Glucocorticoids for immune regulation during development in birds (Schmidt and Soma, 2008 and Breuner, 2008). Sarkar *et al.* (2013) observed an increased in the hormone due to transportation and handling stress in broiler which could be attributable to the high level observed in the study.

**Hematology**

The blood parameters (Hemoglobin, packed cell volume (PCV), red blood cells (TEC), white blood cells (WBC), lymphocyte, monocyte, neutrophil, eosinophil and basophil count observed during development in male Pati duck were presented in Table 2.

Red blood cell count showed no significant changes among the different age groups, thus no age-related change in the RBC count in male Pati duck of Assam. The finding was comparable to the records by Mostaghni *et al*. (2005) in Flamingo and Black-headed gull. Olayemi *et al*. (2006) also recorded the RBC count in male Nigerian laughing dove (2.78±0.44 × $10^{12}$/L) and Nigerian duck (2.43±0.58 × $10^{12}$/L). The value observed in the present study was lower than those earlier determined by Kecici and Col (2011) in male pheasants chick while it was similar with adult. The finding was also lower than the value recorded by Okeudo *et al*. (2003) in Southern Nigerian Duck. The difference in findings can be attributed to difference in species and breed. Difference in environment may also have some effect on the count as Niedojadlo *et al*. (2018) reported a 10% lower RBC count in cold acclimated birds. The male sex hormone, testosterone, was implicated to be responsible for the higher

**Table 1:** Average hormonal parameters of Pati duck during post-natal development.

| Hormone | Age | | | | |
|---|---|---|---|---|---|
| (nmol/L) | 1 month | 6-8 weeks | 20 weeks | 30 weeks | 40 weeks |
| Triiodothyronine (T3) | 3.37±0.2 | 3.23±0.30 | 4.18±0.26 | 3.11±0.42 | 2.92±0.28 |
| Thyroxine (T4) | 15.06±3.9 | 12.01±3.88 | 3.72±1.05 | 9.94±4.19 | 6.12±1.49 |
| Testosterone | $0.04±0.01^{C}$ | $0.06±0.01^{C}$ | $0.15 ±0.03^{BC}$ | $0.3±0.11^{AB}$ | $0.42±0.08^{A}$ |
| Cortisol | $9.37±4.33^{B}$ | $7.99±2.05^{B}$ | $4.71±1.4^{B}$ | $12.84±6.28^{B}$ | $30.78±4.39^{A}$ |

Note; Means with different superscript are significantly different.

**Table 2:** Average hematological parameters of Pati duck during post-natal development.

| Haematology | Age | | | | |
|---|---|---|---|---|---|
| | 1 month | 6-8 week | 20 week | 30 week | 40 week |
| RBC ($10^3$/$mm^3$) | 2.51±0.12 | 2.38±0.06 | 2.83±0.11 | 2.85±0.18 | 2.72±0.19 |
| WBC (M/$mm^3$) | $58.20±1.55^{AB}$ | $52.81±4.30^{AB}$ | $60.37±1.26^{A}$ | $47.00±7.15^{B}$ | $30.82±3.88^{C}$ |
| Lymphocyte (%) | 58.59±2.55 | 53.48±1.29 | 54.82±4.24 | 54.39±3.71 | 55.53±2.88 |
| Monocyte (%) | $4.80±0.07^{B}$ | $5.17±0.12^{B}$ | $5.00±0.16^{B}$ | $5.80±0.45^{B}$ | $12.31±1.24^{A}$ |
| Heterophil (%) | $34.63±2.91^{B}$ | $30.77±1.75^{BC}$ | $37.78±4.20^{AB}$ | $43.52±2.25^{A}$ | $25.44±2.76^{C}$ |
| Eosinophil (%) | 3.30±1.80 | 5.82±1.33 | 2.27±0.52 | 3.65±0.21 | 5.48±0.83 |
| Basophil (%) | 0.33±0.12 | 0.47±0.11 | 0.27±0.07 | 0.27±0.03 | 0.45±0.04 |
| Hb (g/dl) | 13.92±0.64 | 12.92±0.99 | 16.08±0.55 | 14.92±1.04 | 14.13±1.03 |
| PCV (%) | $36.15±1.92^{B}$ | $35.68±2.02^{B}$ | $42.15±1.13^{AB}$ | $42.87±2.76^{A}$ | $45.12±2.43^{A}$ |

Note; Means with different superscript are significantly different.

erythrocyte values in the male (Fried *et al*., 1964). Olayemi *et al*. (2006) suspected that testosterone plays an insignificant role in the erythropoiesis of the Nigerian laughing dove.

Among the different age groups WBC count in 20 weeks was significantly higher (P<0.05) while it was significantly lower (P<0.05) in 40 weeks (Table 2). The finding was much higher than the recorded count by Menon *et al*. (2013) in Emu; Mostaghni *et al*. (2005) in Flamingo and Black-headed gull. Olayemi *et al*. (2006) also recorded the WBC count in male Nigerian laughing dove (0.75±0.28 × $10^9$/L) and Nigerian duck (16.96±2.23 × $10^9$/L). Okeudo *et al.* (2003) recorded that in Southern Nigerian Duck WBC count was (23.81±0.88 × $10^3$/$mm^3$). The difference in finding may be attributed to species and breed difference.

The average lymphocyte percentage observed showed no significant difference among the different age groups of male Pati duck (Table 2). Lymphocyte was observed to be highest in 1 month old whereas it was lowest in 6-8 weeks old. The finding was in conflict with the observations by Kecici and Col (2011) in pheasants where it was lowest in chick and highest in adult which were comparatively higher that any age groups of Pati duck. The observed values in the present study were also higher than the findings by El-Katcha *et al*. (2017) in Pekin ducking, Menon *et al.* (2013) in Emu; by Mostagni *et al.* (2005) in Flamingo and Black headed gull. However the finding in Pati duck was much lower than the observation by Okeudo *et al.* (2003) in Southeastern Nigerian Duck and Sturkie (1986) in male Indian native duck. The difference in findings may be ascribed to differences in avian species, the management procedure and the physical and environmental conditions (Niedojadlo *et al*., 2018).

The monocyte percentage was significantly higher (P<0.05) in 40 weeks age groups of Pati duck. The finding at 40 weeks was similar to the earlier findings by El-Katcha *et al*. (2017) in Pekin duckling while the finding in other age group was much lower. The observed value at 1 months old was in accordance with the findings by Menon *et al*. (2013) in male Emu. The observation in Pati duck of Assam was in conflict with Kecici and Col (2011) in pheasants as a decreasing trend was observed from chick to adult. The difference in findings may be attributed to differences in avian species, the management procedure and the physical and environmental conditions.

The percentage of heterophils in 30 weeks age group was significantly higher (P<0.05) than the other age groups, while 40 weeks was significantly lower (P<0.05). The mean percentage neutrophil was recorded by El-Katcha *et al*. (2017) in Pekin duck which was in the same range as Pati duck of Assam. The findings by Mostaghni *et al*. (2005) in Flamingo and Black-headed gull; Menon *et al*. (2013) in male Emu were much higher than the findings in Pati duck of Assam. Sturkie (1986) recorded that the heterophils in Pekin duck was 52%. However, a mean heterophils of 15.33±4.16% was recorded by Okeudo *et al*. (2003) in male Southeastern Nigerian duck which was lower than the findings in Pati duck. Kecici and Col (2011) observed a decreasing trend from chick to adult pheasant which was in conflict with the findings in Pati duck of Assam. The difference in findings may be attributed to differences in avian species, breed, the management procedure and the physical and environmental conditions of the birds studied.

The eosinophil percentages of male Pati have no significant difference among the different age groups. Eosinophil was highest in 6-8 weeks age while it was lowest in 20 weeks old. The finding was in the same range with the findings by Okeudo *et al*. (2003) in Southeastern Nigerian male duck. However, the observation was in conflict with the findings by Kecici and Col (2011) in pheasants where eosinophil concentrations decreased from chick to adult. The mean percentage eosinophil was recorded by El-katcha *et al*. (2017) in Pekin duck was much higher while the findings by Menon *et al*. (2013) in male Emu was much lower than the findings in Pati duck of Assam. The differences in findings may relate to season, species and/or technique.

The average basophil concentrations in the blood of male Pati duck showed no significant difference between the different age groups. It was observed that basophil was highest in 6-8 weeks and lowest in 30 weeks of age. The finding was in accordance with Menon *et al*. (2013) record in male Emu. The finding was in conflict with the observations by Kecici and Col (2011) in pheasants where basophil percentage decreased from chick to adult. The mean percentage basophil was recorded by El-Katcha *et al*. (2017) in Pekin duck was much higher than the observed value in Pati duck of Assam. The differences in findings may relate to season, species and/or technique.

There was no significant difference in the Hb value among the different age group with highest Hb observed in 20 weeks age group while lowest was observed in 6-8 weeks age group. The Hb value observed in the present study was in a similar range with the observation by Okeudo *et al*. (2003) in Southeastern Nigerian male duck and Oladele *et al*. (2007) in Mallard duck. Mostaghni *et al*. (2005) recorded the Hb value in Flamingo (117.8±59 g/l) and Black headed gull (123±13.3 g/l). Olayemi *et al*. (2006) also recorded the Hb value in male Nigerian laughing dove (148.60±22.10 g/L) and Nigerian duck (136.10±20.40 g/L). Birds that don't fly have higher Hb than bird which frequently flies (Olayemi *et al*., 2006) which may be the reason for lower Hemoglobin observed in Pati duck. The change in Hb may be attributed to change demands of oxygen for activity.

The average PCV count for 1 month and 6-8 weeks was significantly lower than the other age groups. The findings in Pati duck of Assam were in the similar range with the observation by Mulley (1979) in Black duck, Oyewale and Ajibade (1990) in Pekin duck, Okeudo *et al*. (2003) in Southeastern Nigerian male duck and Oladele *et al*. (2007) in Mallard duck. Mostaghni *et al*. (2005) recorded the PCV percentage in Flamingo (35.21±1.6%) and Black-headed gull (39±2.52%). Olayemi *et al.* (2006) recorded the PCV in male Nigerian laughing dove (43.58±7.33%) and Nigerian

duck (42.58±5.67%). A much higher percentage of PCV was recorded by Menon *et al*. (2013) in Emu. Higher PCV and hemoglobin concentrations in tropical poultry breeds over exotic breed might be due to inherent physiological traits in these local breeds involving their hemopoetic system Nwosu (1979); Oluyemi and Ologhobo (1998) which probably enhances the dissipation of useless energy Okeudo *et al*. (2003). The difference in the PCV concentrations observed may also be attributed to breed and species difference as Orji *et al.* (1986), reported strong species and sex effects on avian hematological parameters.

## CONCLUSION

Age related change observed with testosterone hormone. T3 and T4 hormone were higher in younger age while Cortisol was higher in older groups. Among the hematological parameters significant changes was found in packed cell volume, white blood cells, monocyte and heterophils. Heterophils was the predominant leucocytes in Pati duck. The study provides physiological groundwork of blood and hormone at different stages of development specifically in male which can further help in diagnostic baseline.

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### Ethics statement

The animal used for the experiment was ethically approved by the institutional Animal Ethics Committee, Faculty of Veterinary Science, AAU, Khanapara, Guwahati-22 vide Approval No. 770/GORe/S/03/CPCSEA/FVSc/AAU/IAEC/18-19/60 dated 28.12.2018.

### Conflict of interest

There is no conflict of interest.

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