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# IMPACT OF NEOMERCAZOLE AND TESTOSTERONE ON GONAD WEIGHT, GONAD VOLUME AND FEATHER REGENERATION IN MALE RAIN QUAIL, *Coturnix-coromandelica*

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## Abstract

The present study aims to investigate the effect of neomercazole and testosterone on gonad and feather regeneration in male Rain Quails, *Coturnix-coromandelica*. Birds were divided in to four groups, Birds of group III and IV were castrated and were treated with salve and testosterone respectively. Birds of group I and II were normal & treated with 0.1 ml normal Saline and neomerecazole respectively. In both groups I and II testis volume tended to decrease till February. In group II this significant decrease occurred till February followed by a sharp rise during March. Group II showed highly significant decrease when compared to control group. Feather regeneration was valuable in different groups.

**Key words -** Neomercazole, testosterone, saline, regeneration.

## Introduction

This study aims to find the effect of neomercazole and testosterone on gonad and feather regeneration in male Rain Quail, *Coturnix-coromandelica.* A comparative study of testosterone and melanin-based black plumage correlation has been done by Bokony *et al.* (2008). Stoehr and Hill (2001) found that elevated testosterone have various effects on plumage hue in male house finches.

Administration of testosterone after castration in amounts that restore plasma concentrations to physiological or supra-physiological levels can reverse all the effects of castration (Gross, 1980; Kalra and Kalra, 1980) and reduce the responsiveness of the pituitary gland toward GnRH in vivo. The role of thyroid gland in the regulation of the gonad development cycles has been demonstrated in a number of avian species (Meier and Ferrell, 1978; Thapliyal, 1981). Thyroid is also involved in the regulation of breeding cycles of temperate zone birds (Assenmacher and Jallageas 1978, Jallageas and Assenmacher, 1974; Jallageas *et al.*, 1978; Sharp and Klandorf, 1985). Hypothyroidism in Indian finches, whose gonadal cycles are not regulated by light, leads to gonadal recrudescence. Effects of surgical thyroidectomy have been extensively studied in wild birds (Thapliyal, 1969; Thapliyal and Chandola, 1972; Wieselthier and Van Tienhoven, 1972). Willow *et al.* (2011), observed that in a Tropical Passerine Bird the Red-Backed Fairy-Wren (*Malurus melanocephalus*), sexually selected male plumage color is testosterone dependent. The feather is a complex ectodermal organ and it provides functions in endothermy, communication, and flight. Studies of feather growth, cycling, and health are of fundamental importance to avian biology and poultry science. Many reviews have covered feather bud development (Lin, CM *et al.* 2006) and feather follicle generation, repository (Lin, SJ *et al*. 2013). A specialized region with in the father sheath, the collar bulge, houses the epithelial stem cells (Lucas, AM and Stettenheim PR 1972, Lillie FR and Wang H. 1941 and Yuez *et*

*al.* 2005). The feather follicle provides a proceed region in which to store these epethetial stem cells. Feathers can regenerate through out a bird's life (Lucas and Stettenheim, 1972). Significant changes in feather morphology take place during feather cycle (Lin, SJ *et al.* 2013). Birds undergo a periodic process to shed worn feathers and replace them with new feather types at different physiological stages. Old feathers are expelled from the follicle as young new feathers grow in the following cycle. Surgical thyroidectomy is a very crucial stage and it includes complete removal of thyroid gland which leads to a severe hypothyroid state. In the present work attempt has been made for gradual regression of thyroid by chemical thyroidectomy (by administration of an antithyroid drug neomercazole diluted in 0.9% vehicle). The study aims to find out the effect of neomercazole solution and testosterone on gonad and plumage regeneration of male Rain quails, *Coturnix-coromandelica.*

**Material and Methods**

Twenty birds were separated from the stock. Ten birds were caponized in October, 2016. In November remaining ten birds were laparotomized to measure the size of the left testis in situ. The plumage of all the birds were plucked from the chest region to study the plumage pigmentation. All birds were divided into four groups comprising of equal number and housed in separate wire net cages of same size (18" x 12" x .9"). The duration of the experiment, the groups, status and number of birds and the dosages used are listed in the Table-1. Group I and III birds received 1.5 ml. vehicle per month. In group II each bird received a total dose of one tablet of Neomercazole (5 mg.) in one month and in group IV the bird received a total dose of 7.5 mg testosterone (Aquaviron; 1 ampoule = 25 mg. in 1 ml.) during one month.

The birds were injected intramuscularly on alternate day (15 days in a month) in the morning between 9:00 - 10:00 hours. The treatment was started from November 2016 and experiment was terminated on April, 2017.

The feathers of half side of the abdominal region were removed completely to observe feather regeneration in all the birds. The feather regeneration was examined in each month on day $9^{th}$, $16^{th}$, $23^{rd}$ and $30^{th}$ starting from the day of injection treatment till the termination of experiment. Feather regeneration was recorded in terms of size of feather papillae emerged from the ectoderm. After the 24 hours of last injection of the month the birds of group I and II were laparatomized and the size of the left testis was measured in situ. On day of termination of the experiment, i.e. April, 2017, the birds were sacrificed and testis of birds of group I and II were removed to measure the gonad size. Testis weight was also taken to the nearest mg. Food and water was freely available to all birds through out the experiment. The data was analysed statistically by student's 't' test. Gonad volume was compared (November Vs other months).

**Testis Volume**

In both the groups I and II testis volume tended to decrease till February. In group I the decrease was not significant during December, significant ($P>0.01$) during April while highly significant ($P>0.005$) during other months, when compared to November. In group II significant decrease occurred upto February followed by a sharp rise during March (Table-1). During April testis

volume in group I increased significantly when compared to March whereas in group II it decreased significantly when compared both with March and November (Table 1).

**Testis Weight**

Group II showed highly significant decrease (P>0.005) in testis weight when compared to control group (Table-1).

**Plumage Regeneration**

Table 2 shows the rate of feather regeneration (in cm) of different groups during different months on 9th, 16th, 23rd and 30th day, respectively. On all the four days comparison of treated groups was made with control group.

**Table 1: Showing the effect of Normal Saline and Neomercazole solution on the gonad (volume and weight) of male Rain quails, (*Coturnix-coromandelica*).**

| Groups | I | II | III | IV |
|---|---|---|---|---|
| Status of Bird | Intact | Normal | Castrated | Castrated |
| Dose per Bird/day | 0.1 ml. Saline | 0.1 ml. Neomecrazole solution | 0.1 ml. Normal saline | 0.5 mg Testosterone |
| November 2016 | 40.29 ±5.48 (5) | 47.27 ±4.11 (5) | - | - |
| December 2016 | 38.95 ±0.93 NS (5) | 35.77 ±7.82** (5) | - | - |
| January 2017 | 17.50 ±6.11*** (5) | 15.56 ±2.00*** (5) | - | - |
| February 2017 | 11.72 ±1.83*** (5) | 7.86 ±0.37*** (4) | - | - |
| March 2017 | 8.71 ±1.23*** (5) | 30.38 ±8.15*** (4) | - | - |
| April 2017 | 29.43 ±2.30*** (3) | 12.28 ±0.58*** (4) | - | - |
| Testis wt. in Mgs/Gm Body Wt. M±SE | 119.54 ±4.24 (3) | 58.37 ±3.52*** (4) | - | - |

Values expressed as Mean ± SE

* P>0.025, ** P>0.01, *** P>0.005, NS - Not significant

Figures in parentheses indicate number of birds

Significance test - Testis volume - November 2016 Vs other months

Testis volume - Control Vs. treated group

**November**

On 9th day of injection treatment maximum growth of feather (papillae) occurred in group III and minimum in control group. When other treated groups were compared to control group, highly significant (P>0.005) increase in growth of feather papillae occurred in group III and IV whereas group II showed increase of lesser significance (P>0.01).

On 16th day group II and III showed marginal increase (P>0.025) and group IV exhibited non-significant decrease when compared to control group (Table-2). Observations of 23rd day showed significant increase (P>0.01) in group II and III whereas slight decrease (P>0.025) was found in group IV. Insignificant and fast significant (P>0.005) rise occurred on 30th day of treatment in group III and II respectively. Marginal decrease (P>0.025) was found in group IV (Table 2). During November maximum growth of feather was found in group III (castrated-treated with 0.1 ml. of 0.9% saline) but last observation (on 30th day) showed maximum growth in group II (Normal-treated with 0.1 ml. neomercazole solution).

**Table 2: Effect of Normal saline (in normal and castrated birds), Neoaecrazole ((in normal) and testosterone (in castrated birds) on feather regeneration (in cm) in Male Rain quails *Coturnix-Coromandelica* on 9th, 19th, 23rd and 30th day, respectively, starting from the day of injection treatment.**

| Months | Group | 9th day | 16th day | 23rd day | 30th day |
|---|---|---|---|---|---|
| November 2016 | I | 0.43 (5) ±0.05 | 1.63 ±0.06 | 2.37 ±0.10 | 3.05 ±0.28 |
| | II | 0.83 (5) ±0.28 | 1.83 ±0.18* | 2.73 ±0.23** | 3.52 ±0.13*** |
| | III | 1.17 (5) ±0.04*** | 1.87 ±0.20* | 2.77 ±0.25** | 3.13 ±0.38 NS |
| | IV | 0.88 (5) ±0.05*** | 1.55 ±0.06 NS | 2.17 ±0.12* | 2.73 ±0.10* |
| December 2016 | I | 0.43 (5) ±0.03 | 1.23 ±0.06 | 1.73 ±0.07 | 1.87 ±0.06 |
| | II | 0.43 (5) ±0.03 NS | 1.13 ±0.08 NS | 1.67 ±0.05 NS | 1.83 0.03 NS |
| | III | 0.40 (5) ±0.05 NS | 1.17 ±0.12 NS | 1.51 ±0.14* | 1.78 ±0.13 NS |
| | IV | 0.25 (5) ±0.15* | 1.27 ±0.17 NS | 1.80 ±0.14 NS | 2.02 ±0.11* |
| January 2017 | I | 0.17 (5) ±0.10 | 1.17 ±0.23 | 1.73 ±0.14 | 2.12 ±0.13 |
| | II | 0.13 (5) ±0.03 NS | 1.30 ±0.02 NS | 2.10 ±0.22** | 2.35 ±0.14* (4) |
| | III | 0.20 (5) ±0.08 NS | 1.53 ±0.0** | 2.43 ±0.31*** | 2.83 ±0.40*** |
| | IV | 0.13 (5) ±0.07 NS | 1.42 ±0.05* | 1.93 ±0.07* | 2.17 ±0.04 NS |

| | | | | | |
|---|---|---|---|---|---|
| February 2017 | I | 0.23 (5) ±0.12 | 0.8 ±0.16 | 1.23 ±0.22 | 1.53 ±0.15 |
| | II | 0.45 (4) ±0.11* | 1.15 (4) ±0.20 | 1.85 (4) ±0.18*** | 2.15 (4) ±0.14*** |
| | III | 0.37 (5) ±0.05* | 1.22 ±0.05*** | 1.67 ±0.05** | 1.90 ±0.20** |
| | IV | 0.20 (5) ±0.05 NS | 1.08 ±0.18* | 1.70 ±0.08*** | 1.93 ±0.22** |
| March 2017 | I | 0.30 (5) ±0.07 | 1.70 (3) ±0.17 | 2.05 (3) ±0.04 | 2.40 (3) ±0.07 |
| | II | 0.28 (4) ±0.05 (NS) | 1.30 (4) ±0.11** | 1.98 (4) ±0.09 (NS) | 2.30 (4) ±0.14 (NS) |
| | III | 0.20 (5) ±0.08 (NS) | 0.88 (5) ±0.05*** | 1.45 (5) ±0.11*** | 1.60 (5) ±0.11*** |
| | IV | 0.33 (5) ±0.10 (NS) | 1.68 (4) ±0.16 | 2.30 (3) ±0.14* | 2.70 (3) ±0.17* |

Values expressed as Mean ± SE

* P>0.025, ** P>0.01, *** P>0.005, NS - Not significant

Figures in parentheses indicate number of birds

Significance test - Testis volume - November 2016 Vs other months

Testis volume - Control Vs. treated group

The minimum growth occurred in group IV (castrated-treated with testosterene) except on 9th day of injection treatment on which the minimum growth was exhibited by the control group (Intact-treated with saline). It was concluded that maximum growth of feather occurred during $9^{th}$-l$6^{th}$ day of treatment in al the groups except group III where maximal growth was shown during $16^{th}$ - $23^{rd}$ day. The minimum growth occurred between $23^{rd}$-$30^{th}$ day of treatment in all the other groups except the control in which it occurred during $1^{st}$ - $9^{th}$ day of treatment.

**December**

$9^{th}$ day observations showed that rate of feather regeneration in group I and II was same. When compared to control group non-significant and marginal (P>0.025) decrease was found in group III and IV respectively (Table 2) . After the 16 days of treatment no significant alteration occurred in any treated group. On day 23rd non-significant decrease and increase occurred in groups II and IV respectively. In group III marginal reduction (P>0.025) occurred in feather growth when compared to group I (Table-2). Non-significant decrease was found in group II and III on 30th day of treatment whereas in group IV marginal increase (P>0.025) was found.

When maximum and minimum growth was compared, group IV showed maximum growth on 16th, 23rd and 30th day but. It exhibited minimum growth up to day 9th of treatment. The maximum growth of feather papillae on day 9th was shown by group I and II. There was quite variation about the minimum growth. Through out the month, it was not exhibited by any one group. On day 9th it was

found in group IV, on day 16th it was noticed in group II, whereas on 23rd and 30th day minimal growth of feather was recorded in group III (Table-2).

In all the groups the maximum growth of feather occurred during 9th-l6th day (approximately in 2nd week of treatment) whereas minimal growth occurred during 23rd-30th (last week of treatment).

**January**

When treated groups were compared with control group no significant change was recorded in any of the group on day 9th. On day 16th non-significant, significant (P>0.01) and marginal (P>0.025) increase was found in groups II, III and IV respectively (Table 2).

Insignificant, marginal (P>0.025) and fast P>05 rise in groups IV, II and III respectively was found on 30th day. Thus, in January on 16th, 23rd and 30th day of treatment, increase in feather growth in all the treated groups occurred when compared to control group but of varying significance.

The maximum growth of feather was shown by group III throughout the month and the minimum by the control group except on day 9th on which the minimum growth was shown by the group II and IV (treated with Neomercaole and castrated-testosterone treated). The maximum growth of feathers in all the groups occurred during 9th - 16th day and the minimum during 1st - 9th day (Table 2). The ascending order of rate of growth of feathers was as follows - lst-9th day < 23rd-30th day l6th-23rd day < 9th-l6th day.

**February**

When compared to control group, first observation (of 9th day) showed. slight increase (P > 0.025) in group II and III and non significant decrease in feather development in group IV. On 16th day increase was noticed in all the groups but of different significance. The increase was marginal (P>0.025), significant (P>0.005) and highly significant (P>0.005) in groups IV, II and Ill respectively. On 23rd day fast (P> 0.005) rise occurred in all treated groups. On 30th day increase was noticed in Group II (P>0.005) and Group III and IV (P>0. 001).

Out of all groups maximum growth occurred in group II (treated with 0.1 ml. neomercazole solution) except on day 16th on which maximum growth occurred in group III. The minimum growth, except day 9th (in group IV) was found in control group I (Table-2). The rate of feather growth was maximum during 9th-l6th day in all the groups. In group II same rate of growth was shown during l6th-23rd day (third week). The minimum growth of feather in all the groups was not recorded using a particular period as that of maximum growth. The minimum growth during $1^{st}$-$9^{th}$ day occurred in group I and TV and during 23rd-30th day in group II and III.

**March**

On comparing the treated groups with control group no significant alteration in rate of feather growth occurred in any of the group on 9th day (Table-2). On 16th day non-significant, significant (P>0.01) and highly significant (P>0.005) reduction occurred in groups IV, II and III respectively. Observations of 23rd day showed non-significant and highly significant (P>0.005) decrease in groups II and III Marginal (P>0.025) increase was observed in group IV. The last observation (30th day of

March) of the experiment regarding feather growth showed exactly similar results as on 23rd day. The maximum growth occurred in group IV except on day 16th on which maximum growth was recorded in control group. The minimum growth occurred in group III which started from 9th day and lasted upto 30th day. The rate of feather regeneration was maximum during 9th-l6th day and minimum during 1st-9th day except in group III in which the minimal growth occurred during 23rd-30th day of treatment.

**Discussion**

Chemical thyroidectomy in Indian finches failed to stimulate the regressing gonads and plumage regeneration (Thapliyal and Pandha, 1967). The failure of gonads to recrudescence may be due to the inability of the antithyroid drug to impair thyroid function completely.

In wild birds, made hypothyroidic, increase in gonadal activity has been recorded Pandha and Thapliyal, 1964; Thapliyal and Pandha, 1967; Thapliyal, 1969; Wieselthier and Van Tienhoven, 1972). Lindsay W.R. *et al.* (2009), investigated that plumage color acquisition and behavior are associated with androgens in a phenotypically plastic tropical bird. Kimball, R.T. (2006) studied on hormonal control of coloration.

The effects of testosterone and neomercazole (anti-thyroid drug) on feather regeneration is different in different months. During November and January minimum feather regeneration occurred in castrates treated with testosterone but the reverse was true during December and March. It may be due to the fact that the same amount of hormone may have different effects during different tine of year (Maitra and Ghosh, 1989). The anti-thyroid drug also exerts similar effect.

Cell proliferation in the feather bud epithdium is another major factor contributing to the elongation process. During the short bud stage, epithelial cell proliferation localizes mainly to the distal compartment, and as growth progresses, the proliferation zone shifts proximally. The dermal papilla is induced to grow by exposure to the thyroid substance, as well as progesterone (Juhn M., and Harris PC 1955). Studies revealed that molting is associated with changes in retabolism and neuroendocrime regulation (Kuenzel WJ 2003, Vezina *et al.* 2009). A unique property of feathers is the ability to molt, regenerate and renew. Through the process of evolution, the stem cells and dermal papilla become clustered at the proximal follick poised to generate a new feather (Yue Z *et al.* 2005). Regenerative cycling is based on cyclic activation of Wnt/DKK (Chu Q *et al.* 2014). Maximum feather regeneration occurs during November and February and in rest of the months the growth was not significant. It appears that the same dose facilitate plumage regeneration in some months whilst in others exert a negative affect or/and non-significant effect. These data indicate that testosterone and neomercazole both interferes with the feather regeneration process in Rain quail. Perhaps the effects of testosterone on the plumage regeneration in Rain quail is mediated by thyroid, as suggested in other species (Vinod and Sanjay, 1990). A detailed study of Patik and Pathak (1986) on the role of the thyroid hormones and male hormones in feather regeneration also concludes that the effect of castration on feather regeneration is brought about through change in thyroid functions. Nevertheless our data clearly demonstrate that castrates treated with testosterone showed maximum plumage regeneration during December and March. Although sex steroids have been reported to inhibit molt in a number of species (Meier and Ferrell, 1978) could it be that exogenous administration of

testosterone in castrated Rain quail causes considerable change in the normal circulating thyroxine testosterone ratio responsible for feather regeneration. However, more investigations are required before such a proposition is accepted.

Nevertheless, the results of the present study clearly indicate that antithyroid drug and testosterone will have a differential effect on the body weigh, testes and plumage regeneration and that the same amount of hormone may have different effects during different time of a year.

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