

## **EFFECT OF LONG PHOTOPERIOD (L:D 18:6) ON ORGAN GROWTH KINETICS, HISTOLOGY AND SERUM PROFILES OF $T_3$ , $T_4$ , CORTICOSTERONE AND PROGESTERONE DURING POST-HATCHED DEVELOPMENT**

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### **SUMMARY**

The pullets of Indian Rhode Island Red (RIR) breed of domestic fowl were reared under a step-down photoperiod of LD 18:6 (long photoperiod; LP) from day 1 to day 90 post-hatch, and then shifted to LD 12:12 (NLD), to assess its effect on growth kinetics of lymphoid and reproductive organs, and serum hormone profiles. The observations were made at 30, 60 and 90 days and the results were compared with pullets reared under NLD alone. The weights of thyroid and ovary in LP chicks showed significant increment, whereas the weights of liver and lymphoid organs were identical to that of NLD chicks. The weights of adrenal and oviduct decreased significantly. In general, serum levels of  $T_3$ ,  $T_4$ , corticosterone and progesterone tended to show a gradual decrement with age in NLD chicks, but the LP chicks showed a differential change marked by high  $T_3$  and  $T_4$  levels throughout and, increased corticosterone and decreased progesterone levels at 30 and 60 days. Long photoperiod seems to have an initial depressive, but later stimulatory effect on growth of thyroid, while it has an inhibitory effect on adrenal. The histometric data of ovary suggest a stimulatory effect of light, indicated by increased number of follicles and decreased degree of follicular atresia. It is concluded from the present observations that exposure of chicks to LP has differential effects on growth kinetics and, a favourable influence on reproductive hormones suggesting inherent photosensitivity and, no setting in of photorefractoriness during this period.

Key words : Chick ovary ; Corticosterone ; Growth kinetics ; lymphoid organs ; Photoperiod ; Progesterone ; Thyroid hormones.

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## INTRODUCTION

Studies on post-hatched body and organ growth have been carried out in the domestic fowl, to assess the normal growth kinetics as well as to understand the influence of altered thyroid hormones and corticosteroid profiles on the same (1-3). Though more attention had been paid to the histomorphological features of ovary and oviduct in adult laying hens, few studies on this line have also focused in the immature fowl (4-6). Similarly, most studies on serum corticosterone,  $T_3$  and  $T_4$  are in relation to ovulation and oviposition in hens only, except for a few studies in the immature stages (7-8).

Temporal studies on such aspects in the immature stages, from hatch till sexual maturity, would be a useful paradigm to understand the possible relationship if any, between pattern of changes during post-hatched development and attainment of sexual maturity and, features of egg laying performance in adult hens. Moreover, though photoperiodic manipulation has been increasingly used as part of poultry management for increasing egg productivity, the influence of such photoperiodic manipulations on body and organ growth kinetics, hormone profiles and histomorphology of ovary and oviduct, has not been studied. It is presumable that alterations in attainment of sexual maturity and, qualitative and quantitative aspects of egg laying would be induced by photoperiodic manipulations, may be a consequence of the changes induced by photoperiod in the immature stages and hence, could provide valid morpho-physiological correlation. In this context, a previous study had shown some influence of long photoperiod in the rearing stages, amounting to a step-down photic schedule, on attainment of sexual maturity, egg laying performance and egg composition (9). The present study makes an attempt to assess the influence of a long photoperiod on body and organ growth kinetics. Other parameters assessed includes histometry of ovary and serum profiles of  $T_3$ ,  $T_4$  corticosterone and progesterone. These changes have been related with the previously reported effects on sexual maturity and egg laying performance.

## MATERIALS AND METHODS

### *Procurement and maintenance of chicks :*

One day old pullets of the domestic fowl *Gallus gallus domesticus* of Rhode Island Red (RIR) breed were procured from Model Poultry farm, Baroda, Gujarat, India. From day 1 to day 90, the chicks were housed in cages (4x2.5x2 ft.) and placed in light proof enclosures. The chicks were fed with water *ad libitum* and a rationed diet as follows:

Chick mash	days 1-56	30 g/bird/day
Grower mash	day 57 to initiation of egg laying (1L)	90 g/bird/day

The lighting of the cages was done by four florescent tubes fitted atop of the cages. The light intensity was maintained at 250 lux and checked with a lux meter.

*Experimental protocol :*

A total of 24 chicks were used for the present study. They were divided into 2 groups of 12 chicks each.

Group I : These birds were reared under a photoperiod of L:D 12:12 throughout referred to as NLD.

Group II : These birds were reared under a long photoperiod of L:D 18:6 from day 1 to day 90 post hatch.

The lights were switched on for both the groups at 0700 hrs. and switched off at 1900 hrs. for group I and at 0200 hrs for group II by an automatic timer.

*Parameters and methods of evaluation :*

Changes in the weights of body, thyroid, adrenal, ovary, oviduct, liver and lymphoid organs-thymus, bursa and spleen were measured gravimetrically. The birds were quickly decapitated under mild anaesthesia to avoid stress during handling. The viscera was cut open and the organs were quickly excised, blotted free of blood and tissue fluids and gravimetric evaluations were carried out using a digital Mettler balance. The organs were weighed upto 0.01 mg. accuracy. The absolute weights thus obtained were converted into relative weights and expressed in terms of percentage of body weight. Per day growth rate and growth rate kinetics were calculated on the basis of absolute body weight of individual birds.

*Radioimmunoassay :*

The blood samples were collected by puncturing the right jugular vein and centrifuged at 4000 RPM for 40 minutes to obtain the serum. RIA for  $T_3$ ,  $T_4$ , progesterone and corticosterone was carried out by commercial kits purchased from INSTAR corporation, Minnesota, USA and were expressed as ng/dl and, for corticosterone from Diagnostic products corporation (DPC), Los Angeles, CA, USA and expressed as  $\mu\text{g/dl}$ . The ratios of these hormones with respect to each other and also with organ and body weights was calculated.

The ovary of pullets were fixed in Bouin's fluid and processed further, dehydrated and embedded in paraffin. Sections of 5  $\mu$  thickness were cut on a microtome and stained with haematoxylin-eosin and mounted in DPX. The histometrics of the ovarian follicles was done with the help of an oculometer. A specific region in each of the section was selected for the follicular count. Initially, a total count of the follicles was made, counting the pre-ovulatory follicles (POF) and atretic follicles (AF) separately. The POF, on the basis of their size were categorised as small (6-200), big (200-300) and large (>300) follicles and counted separately.

The data were subjected to Students 't' test with 95% confidence level.

**RESULTS**

**Body and organ weights**

The body weight of LP chicks was similar to that of NLD chicks at 30 and 90 days, though at 60 days it was greater than NLD chicks. The growth rate of LP chicks was similar from 0-30 days, significantly more between 30 and 60 days and less between 60-90 days, compared to that of NLD chicks (Fig.1). The weight of thyroid of LP chicks was lesser at 30 days (by 25%) while that at 60 and 90 days was greater (by 15.2% and 25.75%, respectively) than that of NLD chicks (Fig. 2a). The growth rate of thyroid which was lower between 0-30 days, showed a higher rate between 30-60 days and between 60 and 90 days, compared to that of NLD chicks. The adrenal showed a reduced growth rate in LP chicks, with lower weights between 0-30 days and 60-90 days. The ovary of LP chicks was heavier than the NLD chicks at both 60 and 90 days, with a maximal and significantly greater growth rate between 30 and 60 days. The oviduct of LP chicks showed reduced growth rate between 0-30 and 60-90 days and significantly greater growth between 30-60 days, with the result, the oviduct weighed significantly more at 60 days and less at 90 days. The overall growth kinetic ratio showed a significantly greater growth rate for thyroid and ovary and, lesser for adrenal and oviduct of LP chicks. In the case of oviduct, the growth kinetic ratio was higher than NLD throughout, that of thyroid was higher between 30-90 days, with a lower ratio between 0-30 days. Both, the

**Table 1 : Monthly growth rate and growth indices of body and organs of NLD and LP chicks.**

Parameters	Photo period	30 days		60 days		90 days		overall	
		growth rate	growth index	growth rate	growth index	growth rate	growth index	growth rate	growth index
Body weight (kg)	NLD	3.02		651		9.57		6.37	
	LP	3.12		8.88		7.44		6.48	
Thyroid weight (g)	NLD	0.255	0.084	0.255	0.039	0.444	0.046	0.318	0.049
	LP	0.155	0.049	0.455	0.051	0.628	0.084	0.413	0.063
Adrenal weight (g)	NLD	0.219	0.096	0.303	0.046	1.24	0.129	0.613	0.096
	LP	0.263	0.084	0.611	0.068	0.555	0.074	0.433	0.064
Ovary weight (g)	NLD	0.399	0.132	1.01	0.155	1.70	0.177	1.04	0.163
	LP	0.433	0.138	4.27	0.48	0.822	0.110	1.84	0.183
Oviduct weight (g)	NLD	0.588	0.194	1.19	0.182	1.83	0.191	1.20	0.188
	LP	0.408	0.130	1.83	0.206	0.811	0.109	1.01	0.155

Values : Mean

Table 2 : Monthly growth rate and growth indices of liver and lymphoid organs of NLD and LP chicks.

Parameter	Photo period	30 days		60 days		90 days		overall	
		growth rate	growth index	growth rate	growth index	growth rate	growth index	growth rate	growth index
Liver weight (g)	NLD	0.086	0.028	0.098	0.015	0.017	0.116	0.116	0.018
	LP	0.131	0.041	0.146	0.016	0.008	0.111	0.111	0.017
Thymus weight (g)	NLD	0.003	0.001	0.033	0.005	0.002	0.021	0.021	0.003
	L.P	0.021	0.001	0.045	0.005	0.0002	0.016	0.016	0.002
Bursa weight (g)	NLD	0.0021	0.001	0.010	0.001	0.003	0.016	0.016	0.002
	LP	0.0004	0.0001	0.046	0.005	0.001	0.017	0.017	0.0002
Spleen weight (g)	NLD	0.0004	0.0001	0.013	0.006	0.001	0.006	0.006	0.001
	LP	0.002	0.001	0.016	0.005	0.001	0.007	0.007	0.001

Values : Mean

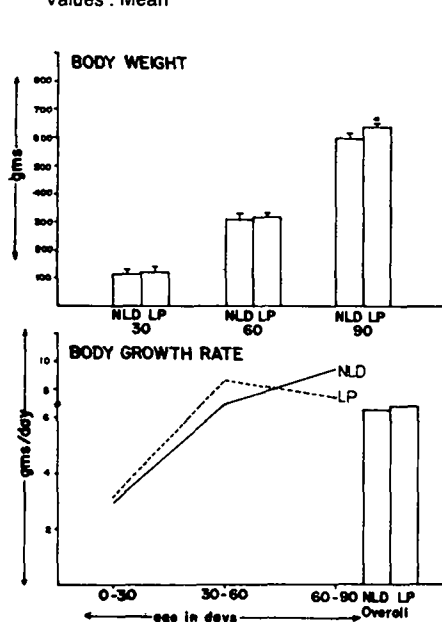


Fig. 1. Changes in body weight and growth rate of NLD and LP pullets at 30, 50 and 90 days post hatch.

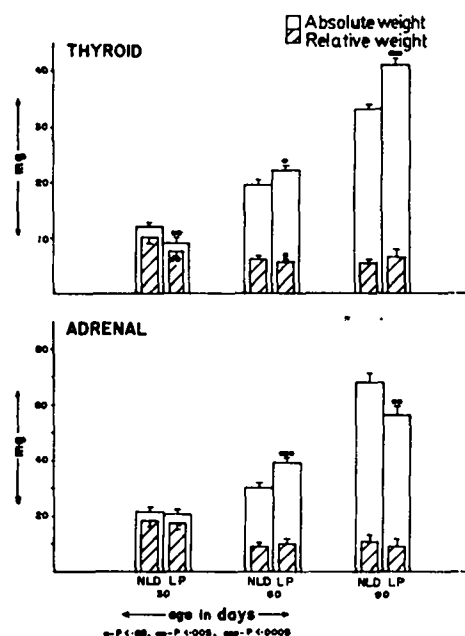
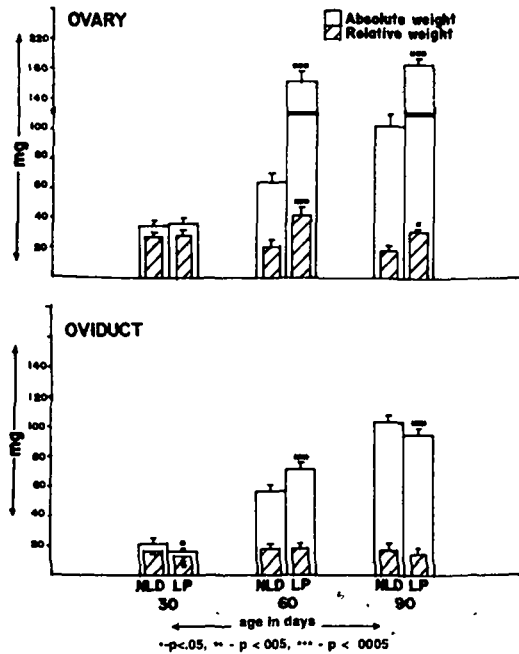
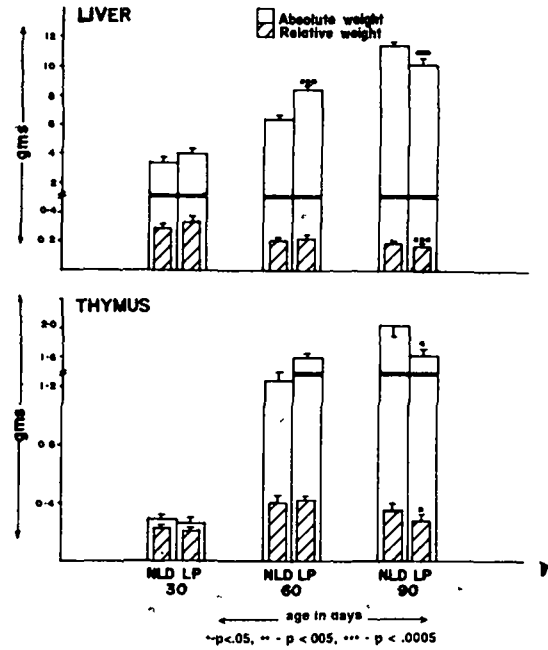


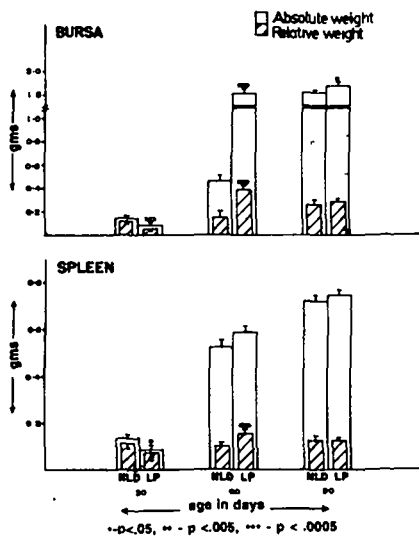
Fig. 2a. Changes in absolute and relative weights of thyroid and adrenal of NLD and LP pullets at 30, 60 and 90 days post hatch.



**Fig. 2b.** Changes in absolute and relative weights of ovary and oviduct of NLD and LP pullets at 30, 60 and 90 days post hatch.



**Fig. 2c.** Changes in absolute and relative weights of liver and thymus of NLD and LP pullets at 30, 60 and 90 days post hatch.



**Fig. 2d.** Changes in absolute and relative weights of bursa and spleen of NLD and LP pullets at 30, 60 and 90 days post hatch.

adrenal and oviduct of LP chicks showed a similar pattern of growth kinetic ratio with higher ratios between 30-60 days and lower between 60-90 days (Table 1). The absolute weights and relative weights of thymus, bursa and spleen showed an increment at 60 days, whereas, liver showed a decrement (Fig. 2c, d). However, at 90 days the relative weights of liver and thymus were significantly decreased. The overall body growth rate of LP chicks was higher than the NLD chicks. The overall growth rates and growth indices of thyroid and ovary were increased and, that of adrenal and oviduct decreased. The liver showed an overall decrement in the growth rate and growth indices, whereas, the lymphoid organs showed marginal changes (Tables 1,2).

#### Hormonal changes

Both NLD and LP chicks showed a similar trend of decreasing CORT and  $T_3$  levels from 30 to 90 days reading the nadir at 60 days. Serum  $T_4$  however, showed almost a constant level in NLD chicks, whereas in LP chicks, it showed continuous decrease, reaching the nadir at 90 days. Though the concentration of CORT was significantly higher in LP chicks at 30 days, it was significantly lower at 60 and 90 days when compared with NLD chicks. The levels of  $T_3$  and  $T_4$  were significantly higher in LP chicks at all ages compared to NLD chicks. The serum

**Table 3 : Changes in serum corticosterone,  $T_3$ ,  $T_4$  and progesterone and their corresponding ratios in NLD and LP pullets.**

Parameters		30 days	60 days	90 days
$T_3$ : $T_4$	NLD	0.288	0.174	0.186
	LP	0.260	0.149	0.205
$T_3$ : CORT	NLD	0.278	0.236	0.286
	L.P	0.337	0.222	0.286
$T_4$ : CORT	NLD	1216	1.350	1.530
	LP	1.295	1.490	2.430
$T_3$ : Body weight	NLD	0.006	0.002	0.0009
	LP	0.010	0.002	0.001
$T_4$ : Body weight	NLD	0.025	0.009	0.005
	LP	0.038	0.010	0.007
$T_3$ : Thyroid weight	NLD	0.057	0.026	0.018
	LP	0.134	0.027	0.020
$T_4$ : Thyroid weight	NLD	0.251	0.154	0.096
	LP	0.516	0.182	0.096
CORT : Body weight	NLD	0.210	0.007	0.003
	LP	0.029	0.007	0.003
CORT : Adrenal weight	NLD	0.115	0.072	0.030
	LP	0.173	0.071	0.029

Values : Mean

progesterone level showed a similar trend of decrease with age, with maximum decrement at 60 days in both NLD and LP chicks. The concentration of progesterone was significantly lower at 30 days and, higher at 60 and 90 days in LP chicks, compared to NLD chicks (Fig. 3).

The histometrics of the ovarian follicles (Table 4) showed a temporal progression from 6-30  $\mu\text{m}$  to 240-440  $\mu\text{m}$  sized follicles from 30-90 days in both NLD and LP chicks. Though the total number of follicles appeared to be more in the ovary of LP chicks, the rate of progression of the follicles into the higher hierarchial classes seemed to be very slow as the number of follicles of large sizes were less. The percentage of atretic follicles was high at 30 and 60 days but significantly decreased at 90 days in the case of LP check ovary. However, the ovary of NLD chicks did not show the presence of 6-30  $\mu\text{m}$  follicles at 90 days, the ovary of LP chicks showed the presence of such follicles in almost the same number as at 30 and 60 days. The number of follicles, of size less than 200  $\mu\text{m}$  was more in LP chick ovary at 30 and 90 days but, at 60 days, it was significantly less.

### DISCUSSION

In general, the growth rate and growth kinetics of various organs were reduced in LP chicks, except for the thyroid and ovary, which showed significant increment and the body

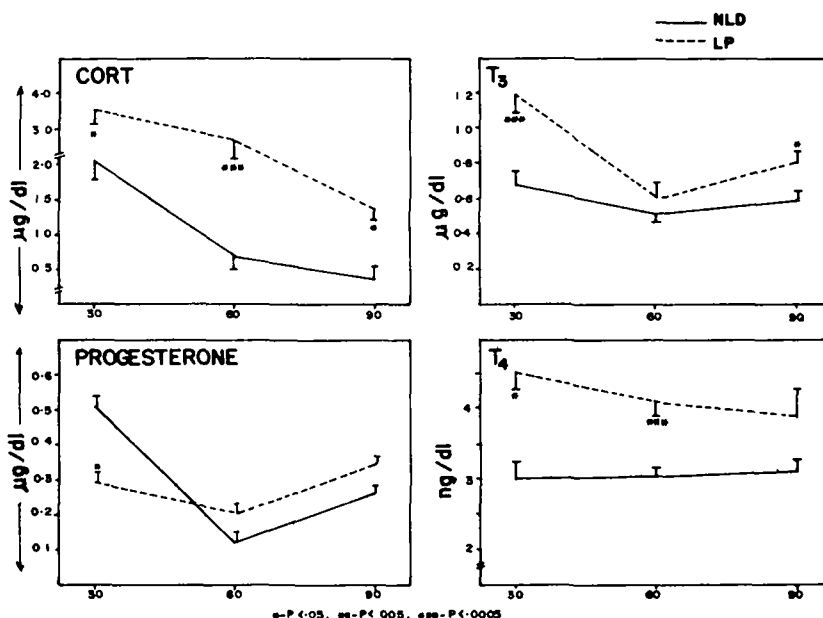


Fig. 3. Changes in levels of serum corticosterone, T<sub>3</sub>, T<sub>4</sub> and progesterone in NLD and LP pullets at 30, 60 and 90 days.



Table 4 : Histometrics of the ovarian follicles of birds under NLD and LP.

Age	Photo period	Follicles	S <sub>1</sub> (<30µm)	S <sub>2</sub> (31-90µm)	S <sub>3</sub> (91-120µm)	B <sub>1</sub> (121-240µm)	B <sub>2</sub> (241-440µm)	S (6-200µm)	B (200-300µm)	L (>300µm)	Total
30 days	NLD	POF	23(38.3%)	28(48.3%)	3 (5%)	5 (8.3%)	-	58	2	-	60
		AF.	2(8.6%)	-	-	-	-	-	-	-	2 (3%)
	LP	POF	10(13.5%)	57(72%)	10(13.5%)	-	-	74	-	-	74
		AF.	4(40%)	12(22.2%)	2(20%)	-	-	-	-	-	18(24%)
60 Days	NLD	POF	21(19.1%)	35(48.6%)	7(9.7%)	6(8.3%)	3(4.1%)	66	6	-	72
		AF.	10(21.2%)	3(8.5%)	1(14.2%)	1(16%)	-	-	-	-	12(16%)
	LP	POF	-	18(38.2%)	4(8.5%)	13(27.6%)	2(4.2%)	32	10	6	47
		AF	-	5(28.7%)	1(25%)	4*30%	-	-	-	-	10(21.2%)
90 days	NLD	POF	29(34.9%)	25(47.1%)	5(9.4%)	17(32%)	6(11.3%)	35	14	4	53
		AF	-	11(44%)	2(40%)	39(17.6%)	-	-	-	-	16(30.8%)
	LP	POF	29(34.9%)	37(44.5%)	4(4.8%)	8(9.6%)	5(6%)	73	65	5	83
		AF	-	-	2(50%)	2(25%)	-	-	-	-	4(4.8%)

Values : Mean

weight, which was marginally higher. Most noticeable effect in this connection is a significantly increased growth rate at 60 days and a perceptibly reduced growth rate at 90 days. Since there are no reports regarding changes in body weight and growth rates of organs during the period of chronic exposure to LP, it is difficult to make any detailed comparative discussion on these aspects. The significant changes noticed at 60 and 90 days in the present study (increased growth rates of organs and body at 60 days and decreased growth rate of the same at 90 days) in LP chicks seem to bear some correlation with the recorded alterations in the serum CORT level which was higher at 60 days and significantly lower at 90 days. The most significant effect of LP seems to be on thyroid and ovary, which showed significantly greater weights at the end of exposure to LP. However, the body weight and the weights of liver and lymphoid organs in general, were almost identical to that of NLD chicks, the weights of adrenal and oviduct were significantly lower. Apparently, exposure to LP has differential effects on growth kinetics of various organs, suggesting the need for different optimal hormonal balances.

In general, serum levels of T<sub>3</sub>, T<sub>4</sub>, CORT and progesterone tended to show a gradual decrement with age in NLD chicks, but in the case of LP chicks, except for serum T<sub>4</sub> level, the other hormones tended to show a differential change, marked by increased CORT level and decreased T<sub>3</sub> and progesterone levels at 60 days. A comparison between NLD and LP chicks reveals relatively higher T<sub>3</sub> and T<sub>4</sub> levels throughout and increased CORT level and decreased progesterone level at 30 and 60 days in LP chicks. The clear correlation between CORT levels and body growth of liver and lymphoid organs suggest a favourable influence of CORT on growth of liver and lymphoid organs in the domestic fowl, as has also been inferred by some authors (10-12). Since both, thyroid hormones and CORT have been implicated in growth kinetics, a cursory glance at the T<sub>3</sub> : CORT and T<sub>4</sub> : CORT ratios indicate lower ratios at 60 days and significantly greater ratios at 90 days in LP chicks, corresponding to the increased growth rates at 60 days and retarded growth rates at 90 days. Obviously, supra optimal ranges

of  $T_3$  : CORT and  $T_4$  : CORT ratios are unfavourable for growth while, optimum ranges of these ratios are favourable (Table 3). Apparently, the present results suggest that the growth of the body and growth rates of various organs are not related to the absolute levels of thyroid and adrenal hormones but to optimal ranges of the same.

The data on thyroid and adrenal indicate a differential effect of LP with peak growth rates for thyroid at 60 and 90 days and for adrenal at 60 days with consequent increased absolute and relative weights of thyroid and decreased absolute and relative weights of adrenal at the end of 90 days. Interestingly, the thyroid showed a lower growth rate and weight at the end of (9-30 days) 30 days, while adrenal showed significantly lower growth rate at 60 and 90 days. Long photoperiod seems to have an initial depressive effect on thyroid growth and stimulatory effect thereafter. Persistent LP seems to have depressive effect on adrenal growth in the later phase, though initially favourable. The hypothalamo-hypophyseal-thyroid (HHT) axis seems to be stimulated by LP, as revealed by the higher absolute  $T_3$  and  $T_4$  levels, as well as the higher ratios of  $T_3/T_4$  : body weight and  $T_3/T_4$  : thyroid weight, implying increased thyroid output. The inhibitory effect of prolonged LP on hypothalamo-hypophyseal - adrenal (HHA), axis is not only indicated by the lower CORT level but also by the lowered CORT : body weight or adrenal weight ratios. The histological features of these two glands also corroborate well with the above inferences.

Previous studies on photoperiodism, sexual maturation and egg laying had highlighted the importance of exposure to a short photoperiod (SP) prior to exposure to LP, for the stimulatory response of photoperiod on the HHG axis (13). A hypothesis put forth by integrating the various available experimental evidences has suggested the hypothalamic GnRH neurons to be sensitive to both short and long photoperiods in the form of inhibitory and stimulatory inputs, respectively (14). However, photosensitivity of juvenile birds has not been studied in greater detail (13). The HHG axis seems to be stimulated under exposure to LP, which is well reflected in the weight and histometrics of the ovary. In this context, the present study evaluates the effect of continuous exposure to LP from the day of hatch till 90 days of age. Though it is not possible to specifically identify the exact phase of photosensitivity from the time of hatch, the present observations definitely suggest stimulatory effects of LP during 1-90 days of age. The histometric studies of the ovary reflect the stimulatory influence of light at 30 days itself, as indicated by the increased number of follicles by 23%. The stimulatory influence of LP is also indicated by the higher rate of transition of follicles from small to big and big to large size at 60 and 90 days.

Another aspect of differential effect of LP, as against NLD, is the quantum of follicular atresia from 1 to 90 days, which decreases from 24% to 4.5% in LP chicks, as against an increase from 3.3% to 30.8% in NLD chicks. There is also an apparent increase in the number of small follicles throughout in LP chicks, which is well reflected in the presence of small follicles of 6-30  $\mu$ m in sizable numbers as contrasted with the total absence of such follicles in the ovary of NLD chicks. The increased number of follicles and, reducing rate of atresia, attest a stimulatory influence of photoperiod during the first 90 days of age. However, LP chicks laid significantly less number of eggs than NLD chicks (9). This was speculated to be due to

the probable higher incidence of follicular atresia consequent to exposure to a shorter photoperiod at the end of 90 days (step-down photo schedule). Androgens have been implicated in follicular atresia in mammals (15-17) and, in this respect, it can be speculated that there is decreased androgen production in the ovary of LP pullets. This is substantiated by the relatively higher levels of serum progesterone observed at 60 and 90 days.

Despite the observed stimulatory effects in the ovary of LP chicks, sexual maturity was delayed and the total number eggs laid decreased subsequent to exposure of the pullets to NLD schedule (9). Continuous exposure to LP seems to induce photo refractoriness between 60 and 90 days of age, as can be gauged by the growth rate and growth index of the ovary, which are both significantly reduced and, also by the percentage decrease of large follicles relative to the second month (13-14). A step-down photoperiod from 90 days apparently strengthens the inhibitory component. Explicably, the HHG axis is set back and, gradually restarted in the new photo schedule which may explain the delayed onset of sexual maturity (9). It is also speculated that the sudden arrest in ovarian development due to switch to relatively short photoperiod, could result in increased follicular loss and ultimately in re-initiation of development. The available pool of follicles is depressed resulting in decreased oviposition as reported earlier (18). Overall, it can be concluded from the present observations that exposure of freshly hatched pullets from 1-90 days has differential affects on growth kinetics and a definite favourable influence on HHG axis, suggesting inherent photosensitivity and, no settling in of photo refractoriness during this period.

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