REARING PHOTOPERIOD : EFFECT OF LD 6:18 ON HISTOMORPHOLOGY AND HORMONES OF ADRENAL, THYROID AND OVARY AND GROWTH KINETICS OF LIVER AND LYMPHOID ORGANS IN RIR PULLETS.

General body and organ growth kinetics have been studied during posthatch development, as well as, under thyroid or growth hormone excess or deficiency (Singh *et al.*, 1968; Bermudez *et al.*, 1983; Scanes *et al.*, 1984; Hayashi *et al.*, 1986; Yadav and Arneja, 1993). Many studies have also focused on the histomorphology of ovary and oviduct in the post-hatch phase of development, though most such studies are predominantly on adult laying hens (Williams and Sharp, 1978a; Etches *et al.*, 1983; Sharp *et al.*, 1992). Similarly, except for a few studies on adrenal corticosteroids and thyroid hormones in the immature stages, most such studies on hormonal profile are in the adult birds in relation to ovulation and oviposition (Soliman and Huston, 1974; Beauving and Vonder, 1977; May, 1978; Sharp and Beauving, 1978; Etches, 1979; Wilson and Cunningham, 1980; Gibson *et al.*, 1986). A continuous evaluation of these parameters in the immature stages from the period of hatch till sexual maturity would be meaningful in understanding the temporal changes during post-hatch

development and, may reveal, causal relations with initiation of egg laying as well as various features of egg laying. Moreover, the influence of rearing chicks on different photoperiodic conditions on these aspects has not been studied. It is likely that, the photoperiod induced alterations in attainment of sexual maturity and quantitative and qualitative aspects of egg laying, may be reflected on the changes induced by photoperiod in the immature stages and, might ultimately lead to the development of valid morpho-physiological correlates in relation to laying performance. In this respect, previous study had reported a favourable influence of, short photoperiod in the rearing stages amounting to a step-up photic schedule, on attainment of sexual maturity, egg yield and laying performance (Chapter 1,4). Hence, in the present study an attempt has been made to evaluate the changes in body and organ weights and histological features of the ovary, together with changes in  $T_3$ ,  $T_4$ , progesterone and corticosterone during exposure to a short photoperiod (SP) and possibly correlate these changes with the previously observed effects on sexual maturity and laying performance.

## RESULTS

## Body and organ weights :

The body weight of SP pullets was significantly lower than that of the NLD pullets at 90 days, though at 30 and 60 days it was non-significantly greater. The growth rate showed a similar pattern. The weight of thyroid gland was significantly lower in the SP hens throughout with, significantly lesser growth rates at 30, 60 and 90 days. The weight of adrenal was also lower at 90 days in SP pullets. The adrenal of SP pullets however showed a significantly increased growth rate between 30 and 60 days and higher weight at 60 days. The absolute and relative weights of liver and lymphoid

organs of SP chicks showed increment at 30 days. However, at 90 days, the absolute and relative weights of liver showed a significant decrement at 90 days, whereas, the absolute weights of all the lymphoid organs showed similar weights as that of controls. The overall growth rate was significantly lower in SP pullets. The ovary of SP pullets was significantly heavier at 30, 60 and 90 days with significantly increased growth rate at 30 and 60 days . However, there was a significantly reduced growth rate between 60 and 90 days.

The organ growth index represented as a ratio of organ growth rate to body growth rate shows that both thyroid and adrenal have lower overall ratios in SP pullets. Whereas the reduced ratio for thyroid was due to a constantly lower ratio from day one to 90, that of adrenal was due to a lowered initial ratio between 0 to 30 days, despite the higher ratios between 30 and 60 days and 60 and 90 days. The growth index of ovary was consistently higher while, that of oviduct was significantly lower between 60 and 90 days resulting in lowered overall ratio. The data on body and organ weights and growth kinetics is shown in table1A,1B,2 &3.

## Hormonal changes :

<u>چ</u>

The serum corticosterone (CORT) level showed a similar pattern of decrease at 90 days from 30 days in both NLD and SP pullets. The CORT concentration in SP chicks at 30 days was significantly greater than that of NLD chicks. However, the CORT concentration at 60 and 90 days was significantly lower in SP chicks than the NLD chicks due to a significant fall at 60 days. Between 60 and 90 days both the groups of chicks showed a gradual decline.

The serum  $T_3$  and  $T_4$  levels were significantly lower in SP chicks at all the three age groups. Both  $T_3$  and  $T_4$  seem to show a significant decrease at 90 days in SP chicks with however an increased level at 60 days. But in the

control chicks, whereas, the serum  $T_4$  level tended to remain constant throughout, the  $T_3$  levels seems to show a decrement.

Whereas the serum progesterone titre in SP pullets, showed a continuous significant reduction at 60 and 90 days, in the NLD pullets it showed a significant decrease at 60 days followed by a significant increase at 90 days, though much lower than the level at 30 days (Table-4).

### Histological observations :

### Thyroid :

The thyroid of NLD chicks showed medium sized follicles with cuboidal epithelium and varying contents of colloid at 30 days. At 60 days, the epithelial cell height was reduced and the follicles showed increased colloid content. By 90 days, the follicles were enlarged with rich colloid content and reduced cell height. The thyroid of SP chicks in contrast showed fully colloid filled follicles with flattened epithelium at 30 days. This condition was more or less seen even at 60 days, though some follicles depicted colloid depletion. At 90 days, the follicles were to medium to large sized with full colloid content and very flat epithelium (Plate 1).

#### Adrenal :

The adrenal of 30 day old NLD chicks, showed prominent active cortical cords with relatively less but active medullary cords. At 60 days, the cortical cords appeared very prominent but less active with condensed nucleus. There were signs of medullary activity. By 90 days, the cortical cords were well formed with active looking cells and depicting secretory exhaustion. The adrenal of SP chicks showed regressed less active cortical cords at 30 days and relatively less medullary cords. At 60 days, the medulla appeared well formed though the cortical cords remained reduced in size with less active cells. However, at 90 days the cortical cords were well formed with hypertrophied active cells showing extensive

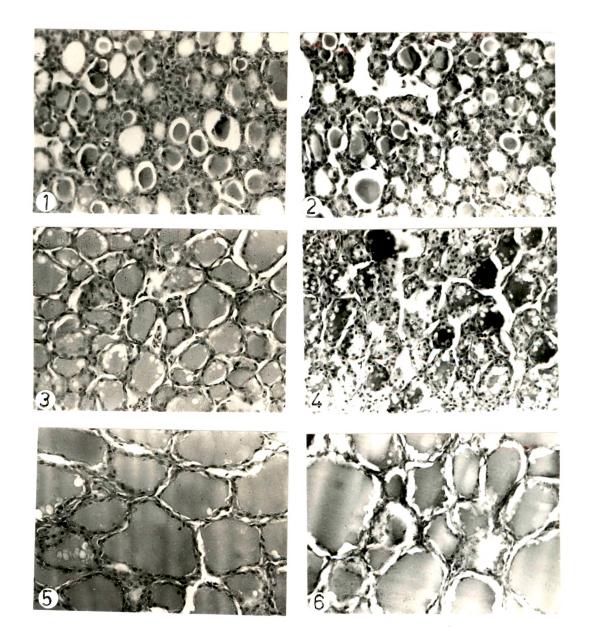
# PLATE I (figures 1-6)

Photomicrographs of sections of thyroid of pullets reared under NLD and SP for first 90 days of post-hatched development (320x).

Fig. 1	Thyroid of 30d NLD chick showing follicles with cuboidal epithelium and varying contents of colloid.
Fig. 2	Thyroid of 30d SP chick showing follicles with reduced cell height and few follicles showing colloid depletion.
Fig. 3	Thyroid of 60d NLD chick showing increased colloid content and reduced epithelial height.
Fig. 4	Thyroid of 60d SP chick showing colloid depletion in follicles.
Fig. 5	Thyroid of 90d NLD chick showing enlarged follicles with rich colloid content.
Fig. 6	Thyroid of 90d SP chick showing colloid filled follicles of medium to large size with flattened epithelium.

.

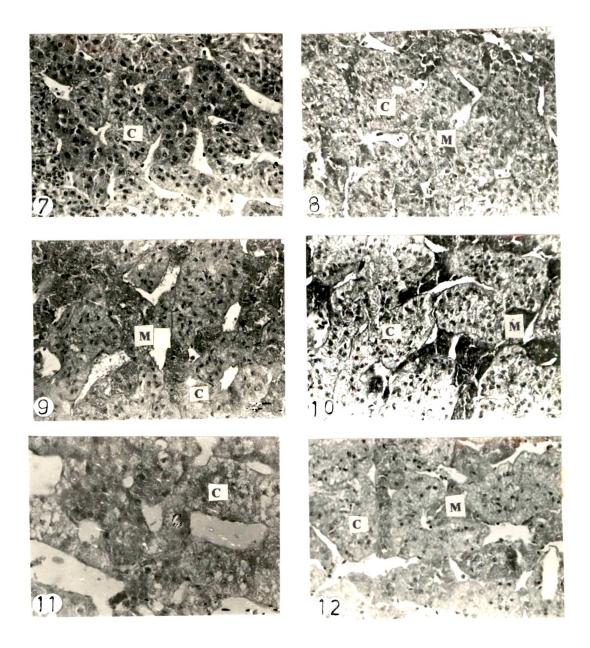
•



## PLATE II (figs. 7-12)

Photomicrographs of sections of adrenal of pullets reared under NLD and SP for first 90 days of post-hatched development (320x).

- Fig. 7 Adrenal of 30d NLD chick showing prominent active cortical cords (C), and less active medullary cords (M).
- Fig. 8 Adrenal of 30d SP chick showing regressed cortical cords and less medullary cords.
- Fig. 9 Adrenal of 60d NLD chick showing prominent but less active cortical cords with condensed nucleus.
- Fig. 10 Adrenal of 60d SP chick showing well formed medullary cords.
- Fig. 11 Adrenal of 90d NLD chick showing well formed cortical cords depicting secretory exhaustion.
- Fig.12 Adrenal of 90d SP chick showing well formed cortical cords with hypertrophied active cells.



## PLATE III (Figs. 13- 16)

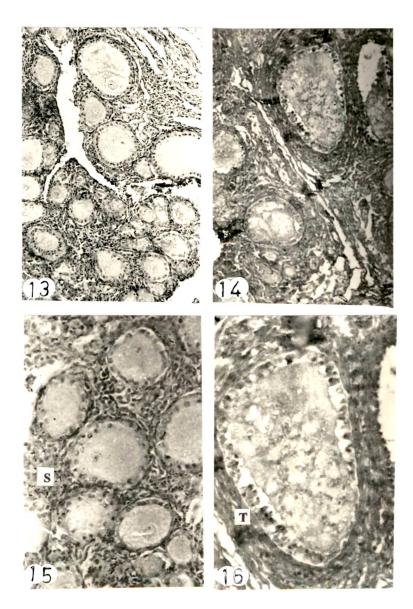
Photomicrographs of sections of ovary of pullets reared under NLD and SP for the first 90 days of post-hatched development.

Fig 13 :	Ovary of 30 d NLD chick showing many primary and primordial
	follicles. (160 x)

Fig 14 : Ovary of 30 d SP chick showing enlarged follicles. (160 x)

•

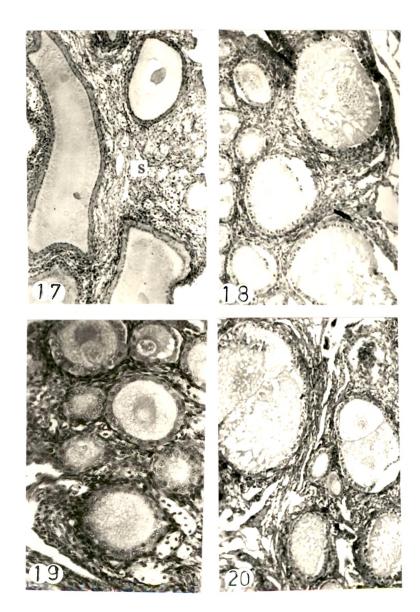
- Fig 15 : Enlarged version of ovary of 30 d NLD chick. Note the stromal CS ) compactness. (320 x)
- Fig 16: Enlarged version of two adjacent follicles showing prominent  $< \pi_{a}$  theca. (320 x)



## **PLATE IV** (Figs. 17- 20)

Photomicrographs of sections of ovary of pullets reared under NLD and SP for the first 90 days of post-hatched development.

- Figs 17 & 18 : Section of ovary of 60 d NLD and SP chicks respectively showing increasing follicular size and prominent Stromal tissue. (160 x)
- Figs 19 & 20 : Enlarged versions of the same. (320 x)



## **PLATE V** (Figs. 21- 24)

Photomicrographs of sections of ovary of pullets reared under NLD and SP for the first 90 days of post-hatched development.

- Fig. 21: Section of ovary of 90 d NLD chick showing a large follicle with well developed granulosa and theca. (160 x)
- Fig. 22: Section of ovary of 90 d SP chick showing the presence of many intermediary sized follicles and an atretic follicle CAD (160x)
- Fig. 23: Section of ovary of 90 d NLD chick at a higher magnification showing large follicles showing well formed granulosa and theca. (320 x)
- Fig. 24: Section of ovary of 90 d SP chicks showing growing and intermediary sized follicles. Note the prominent granulosa (G1) and conspicuous thecal condensation(r)(320 x)

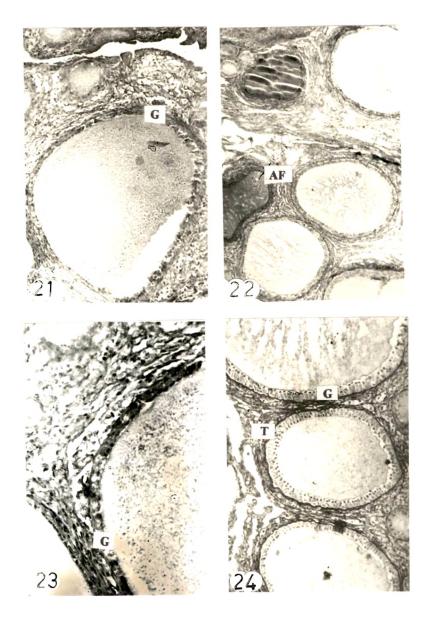
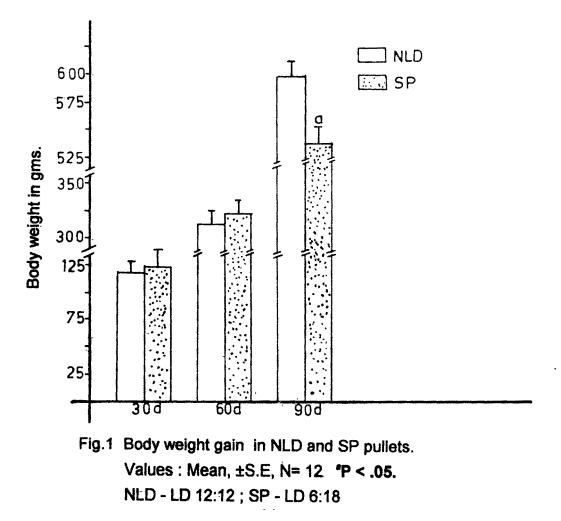


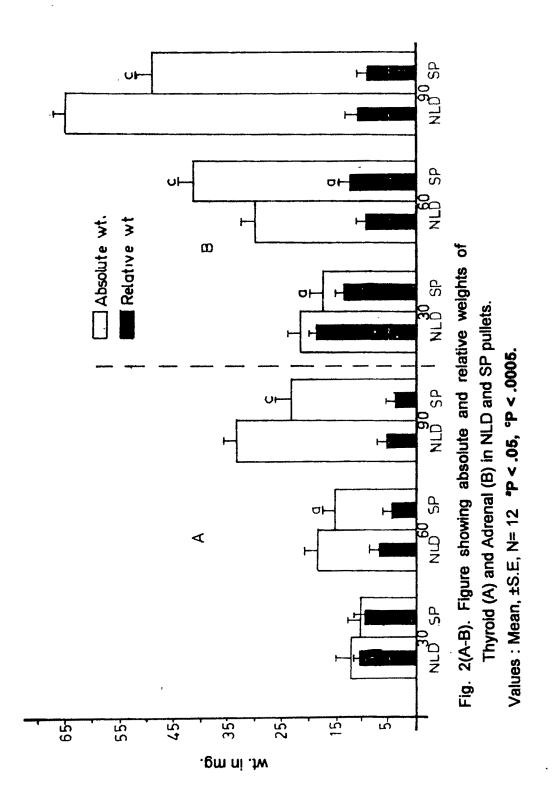
Table 1A: Body weight and organ weight of NLD and SP birds.

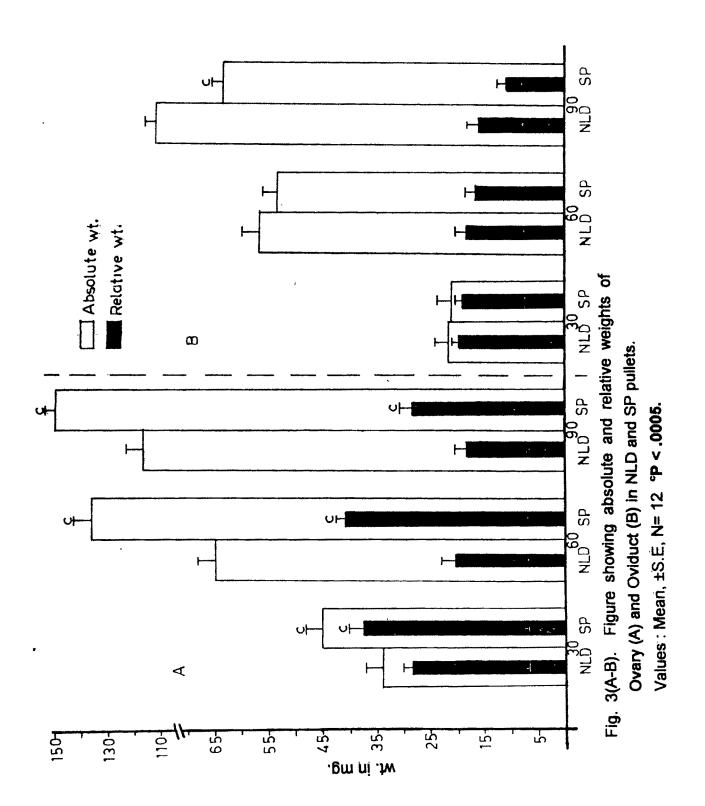
		30 0	30 days	e0 c	60 days	90 days	ays
Body weight	NLD	117.23	17.23 ±16.23	312.85	312.85 ±18.22	600.00 ±16.32	±16.32
E B	SP	122.50	122.50 ±19.25	322.00	322.00 ±13.03	533.33 ±15.87ª	±15.87ª
Organ	Organ weight	Abs. wt	Rel. wt	Abs. wt	Rel. wt	Abs. wt	Rel. wt
Thyroid	NLD	12.00 ±0.81	10.23 ± 1.13	19.66 ±1.20	6.28 ± 1.55	33.00 ±1.80	5.5±0.83
б Ш	SP	10.30 ±0.86	8.57 ±0.607	15.50±1.44ª	<b>4.81 ± 0.75</b>	23.03 ±1.47 <sup>c</sup>	4.31± 0.49
Adrenal	NLD	21.50 ±1.64	18.33 ± 1.13	30.60 ±1.24	9.78 ± 0.80	68.00 ±2.44	11.33±0.87
бш Ш	SP	17.00 ±0.80	13.87 ± 2.32	41.50±2.01 <sup>c</sup>	12.88 ± 0.70 <sup>a</sup>	48.00 ±1.47 <sup>c</sup>	9.00 ± 1.09
Ovary mg	NLD	34.63 ±4.03	28.99 ± 1.58	65.20 ±2.93	20.84 ±1.96	116.33 ±9.31	19.38 ±1.59
	SP	45.33±5.63 <sup>c</sup>	37.00±1.76 <sup>c</sup>	134.66 ±8.61	41.81 ± 1.25 <sup>c</sup>	150.00 ±4.61	28.12±1.09 <sup>c</sup>
Oviduct	NLD	21.40 ±2.52	18.24 ± 1.52	57.33 ±3.19	<b>18.32 ± 1.08</b>	112.30 ±1.88	18.72±0.83
бш	SP	21.33 ±3.79	17.41 ± 1.04	<b>53.00 ±2.93</b>	<b>16.45 ± 0.73</b>	64.00 ±1.54	<b>12.00 ±</b> 0.79
	Abs. w	Values : Mear Abs. wt : Absolute weigt	: Mean, ±S.E, N= 12. t weight; Rel. wt : Rel	Mean, ±S.E, N= 12. <sup>a</sup> P < .05, <sup>b</sup> P < .005, <sup>c</sup> P < .0005 weight; Rel. wt : Relative weight; NLD: LD 12:12; SP: LD 6:18	<b>005, °P &lt; .0005</b> D: LD 12:12; SP:	LD 6:18	

181

• • •





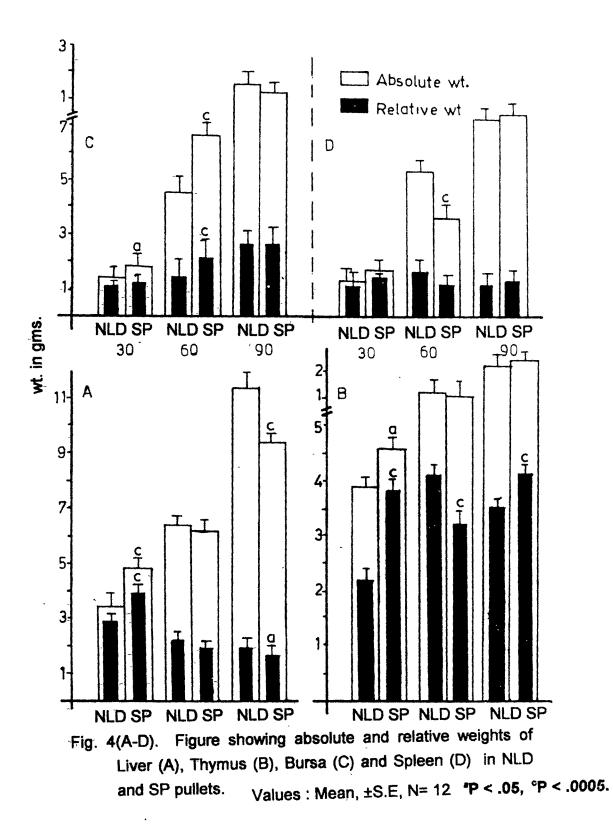


		30 c	30 days	- 60 c	60 days	<i>30</i> (	90 days
Lymphoi	Lymphoid Organs	Abs. wt	Rel. wt	Abs. wt	Rel. wt	Abs. wt	Rel. wt
Liver	NLD	3.48 ±0.15	2.970 ±0.30	6.44 ±0.199	2.06 ±0.43	11.41 ±0.218 1.902 ±0.17	1.902 ±0.17
E B	SP	4.86 ±0.31 <sup>c</sup>	3.973 ±0.24 <sup>c</sup>	6.23 ±0.263	1.93 ±0.23 <sup>b</sup>	9.42 ±0.138 <sup>c</sup>	1.766±0.68ª
Thymus	NLD	0.392 ±0.036	0.248 ±0.02	1.282 ±0.012	0.409 ±0.008	<b>2.12 ±0.218</b>	0.354±0.019
ED	SP	0.46 ±0.02ª	0.381±0.303 <sup>c</sup>	1.100±0.19	0.341±0.108 <sup>c</sup>	2.20 ±0.201	0.412±0.022 <sup>c</sup>
Bursa	NLD	0.148 ±0.007	0.126 ±0.01	<b>0.450 ±0.010</b>	0.143±0.026	1.56 ±0.289	0.261±0.028
ED	SP	0.170 ±0.01ª	0.138±0.01 <sup>c</sup>	0.670±0.014 <sup>c</sup>	0.670±0.014 <sup>c</sup> 0.208±0.014 <sup>c</sup>	1.37 ±0.138	0.257±0.013
Spleen	NLD	0.137 ±0.019	0.116 ±0.01	0.528 ±0.005	<b>0.168 ±0.01</b>	0.720 ±0.021	0.120±0.025
шĝ	SP	0.175 ±0.021	0.142±0.018 <sup>c</sup>	0.142±0.018 <sup>c</sup> 0.359±0.008 <sup>c</sup> 0.11 ±0.013 <sup>c</sup>	0.11 ±0.013 <sup>c</sup>	0.728 ±0.037	0.728 ±0.037 0.136±0.019 <sup>C</sup>
	7	Values : Mear	Values : Mean, ±S.E, N= 12. <sup>a</sup> P < .05, <sup>b</sup> P < .005, <sup>c</sup> P < .0005	<sup>a</sup> P < .05, <sup>b</sup> P < .(	005, °P < .0005		

•

Table 1B: Table showing absolute and relative weights of lymphoid organs.

Abs. wt : Absolute weight; Rel. wt : Relative weight; NLD: LD 12:12; SP: LD 6:18



		0-30	30-60	60-90	Overall
	NLD	3.02	6.51	9.57	6.37
Body weight	SP	3.19	6.65	7.04	5.62
	NLD	0.255	0.255	0.444	0.318
Thyroid	SP	0.205	0.166	0.251	0.207
	NLD	0.291	0.303	1.24	0.613
Adrenal	SP	0.141	0.816	0.216	0.391
	NLD	0.399	1.01	1.70	1.04
Ovary	SP ·	0.755	2.97	0.511	1.41
	NLD	0.588	1.19	1.83	1.20
Oviduct	SP	0.586	1.05	0.366	0.669
	NLD	0.086	0.098	0.165	0.116
Liver	· SP	0.132	0.048	0.106	0.103
	NLD	0.0033	0.033	0.027	0.021
Thymus	SP	0.0091	0.021	0.036	0.022
	NLD	0.0021	0.010	0.037	0.016
Bursa	SP	0.0029	0.016	0.023	0.014
	NLD	-0.00043	0.013	0.0064	0.0063
Spleen	SP	0.0008	0.0061	0.0123	0.0064

Table 2: Per day growth rate in NLD and SP pullets.

Values : Mean

•

ł,

		0-30	30-60	60-90	Overall
	NLD	0.084	0.039	0.046	0.049
Thyroid	SP	0.064	0.024	0.035	0.036
	NLD	0.096	0.046	0.129	0.096
Adrenal	SP	0.044	0.122	0.030	0.069
	NLD	0.132	0.155	0.177	0.163
Ovary	SP	0.236	0.446	0.072	0.250
<u> </u>	NLD	0.194	0.182	0.191	0.188
Oviduct	SP	0.183	0.157	0051	0.199
	NLD	0.028	0.015	0.017	0.018
Liver	· SP	0.041	0.007	0.015	0.018
	NLD	0.001	0.005	0.002	0.003
Thymus	SP	0.002	0.003	0.005	0.004
0	NLD	0.0006	0.001	0.003	0.002
Bursa	SP	0.0009	0.002	0.003	0.0025
	NLD	0.0001	0.001	0.0006	0.0009
Spleen	SP	0.0002	0.0009	0.001	0.001

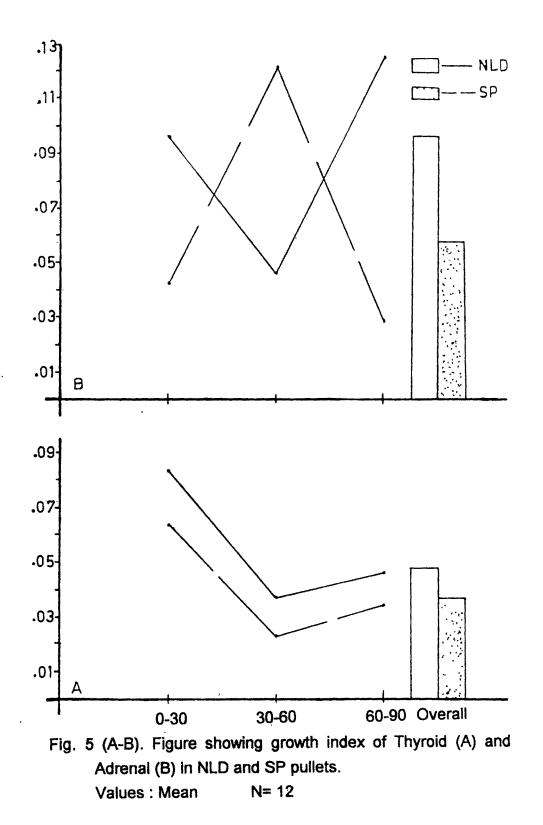
Table :3 Growth Index in NLD and SP pullets

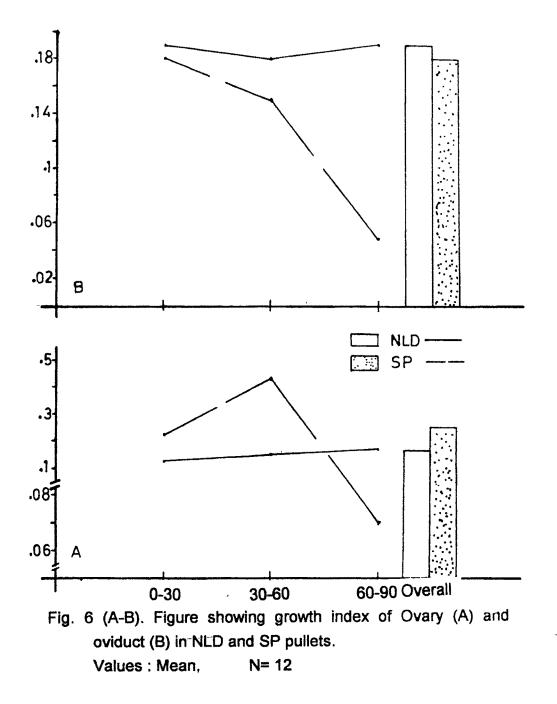
Values : Mean

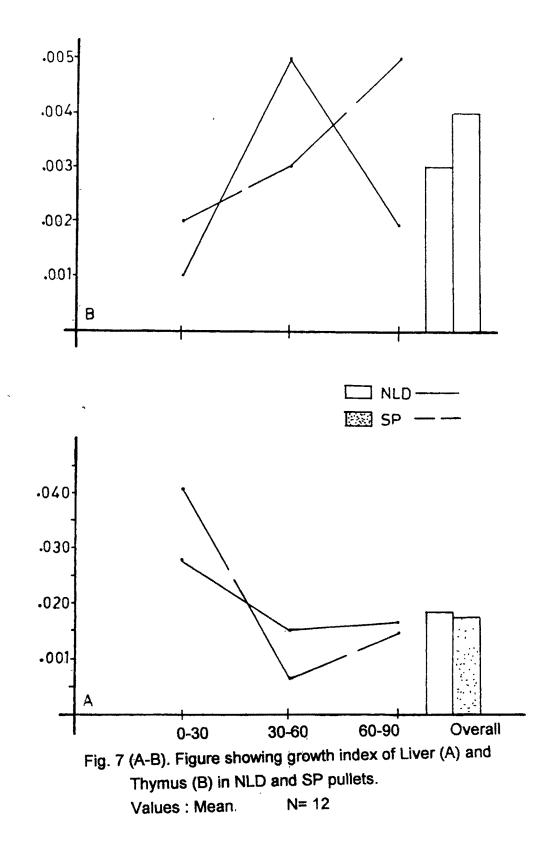
١

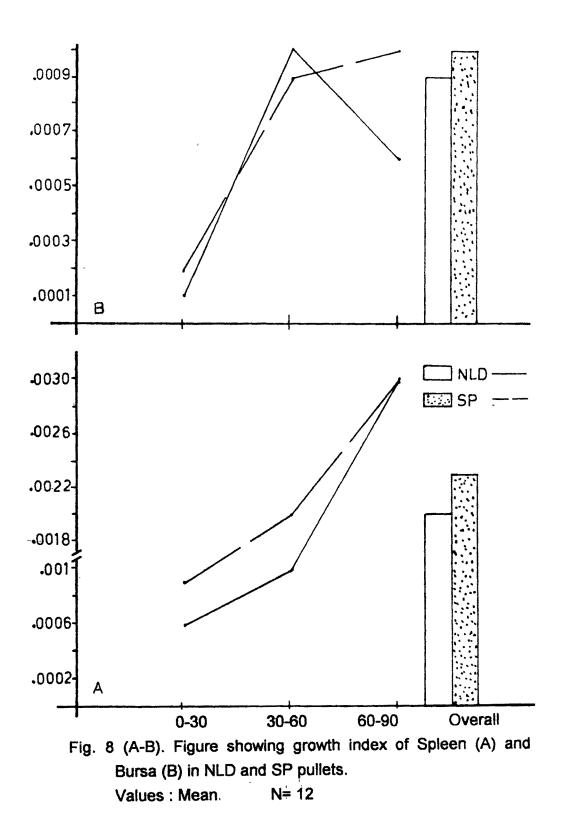
•

•









;

		1		00 4
		30 days	60 days	90 days
Corticosterone	NLD	2.48 ±0.037	2.23±0.20	2.02 ±0.42
(ng/ml)	SP	3.78 ±0.340 <sup>c</sup>	1.57±0.09 <sup>b</sup>	1.36 ±0.94 <sup>ª</sup>
T <sub>3</sub>	NLD	0.690 ±0.073	0.528 ±0.029	0.593±0.089
(ng/ml)	SP	0.389 ±0.062 <sup>b</sup>	0.428±0.022 <sup>b</sup>	0.305±0.017 <sup>b</sup>
T₄	NLD	3.016 ±0.237	3.03 ±0.183	3.18 ±0.154
(µg/dl)	SP	2.59 ±0.39	2.90 ±0.32	1.37 ±0.06 <sup>c</sup>
Progesterone	NLD	0.511 ±0.013	0.120 ±0.013	0.266 ±0.006
(ng/ml)	SP	$0.573 \pm 0.029^{a}$	0.180±0.031 <sup>a</sup>	0.070 ±0.008 <sup>c</sup>

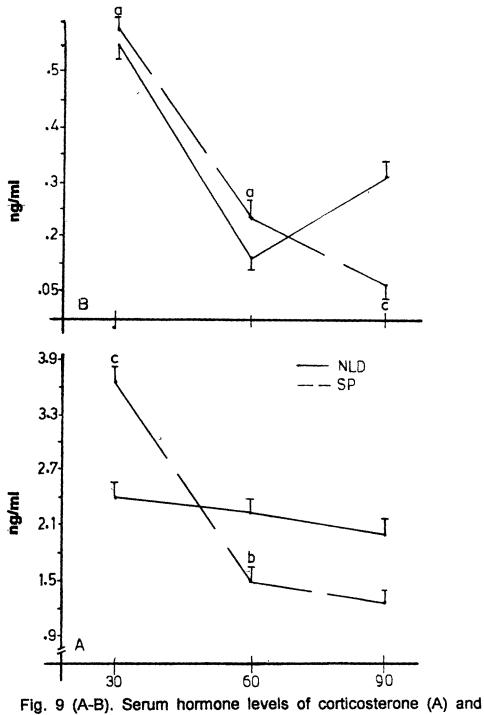
Table : 4 Serum hormone levels of NLD and SP pullets

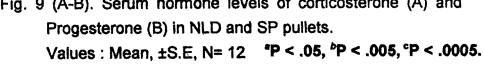
.

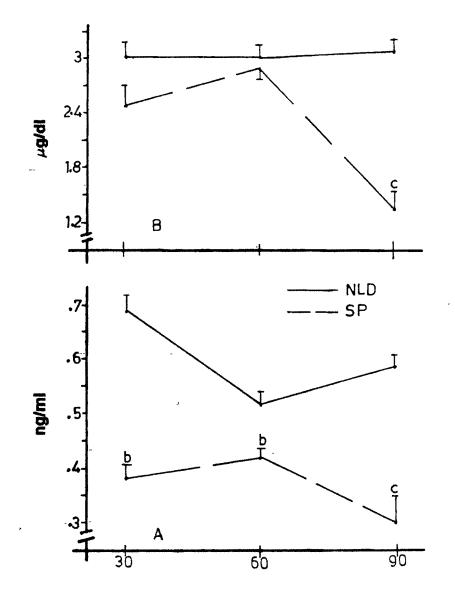
Values : Mean, ± S.E, M- 12. <sup>a</sup>P < .05, <sup>b</sup>P < .005, <sup>c</sup>P < .0005

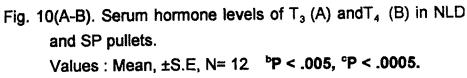
.

-3









		30 days	60 days	90 days
T <sub>3</sub> : T <sub>4</sub>	NLD	0.228	0.174	0.186
	SP	0.150	0.147	0.222
T <sub>3</sub> : Corticosterone	NLD	0.278	0.236	0.286
	SP	0.102	0.272	0.224
T <sub>3</sub> : Body wt.	NLD	0.0058	0.0016	0.0009
	SP	0.003	0.001	0.0005
T <sub>3</sub> :Thyroid wt.	NLD	0.057	0.020	0.0179
	SP	Q.037	0.0276	0.0132
T <sub>4</sub> : Corticosterone	NLD	1.216	1.351	1.530
	SP	0.685	1.84	1.007
T₄ : Body wt.	NLD	0.025	0.0092	0.0053
	SP	0.021	0.009	0.002
T₄ :Thyroid wt.	NLD	0.251	0.154	0.0963
	SP	0.246	0.187	0.059
Corticosterone :Body wt.	NLD	0.021	0.0071	0.0034
	SP	0.030	0.0042	0.0022
Corticosterone:Adrenal wt.	NLD	0.115	0.0720	0.0301
	SP	0.222	0.037	0.028

Table : 5 Table showing ratios of various hormones with respect to other hormones, body weight and respective organ weight.

r

r

Values : Mean

•

Table : 6 Table showing histometric data of ovary of NLD and SP pullets.

•

			Small <sup>1</sup> <30µm	Small <sup>2</sup> 31-90µm	Small <sup>3</sup> 91-120µm	Big <sup>1</sup> 21-240µm	Big <sup>2</sup> 241- 440µm	Small 6-200µm	Big 200- 300µm	Large >300µm	Total
	NLD	Total	23 (38%)	29(48%)	3 (5%)	5 (8%)		58	3	4 2 3	60
30days		A.F	2 (8%)	ł							2(3%)
	SP	Total	22 (27%)	42(52%)	12 (15 %)	4 (5 %)	B	80	8	*	80
		A.F	4 (18%)	17(40%)	2(16 %)	1(25 %)	<b>,</b>				24(30%)
	NLD	Total	21(29 %)	35(48%)	7(8 %)	6(8 %)	3(4 %)	66	9		72
60days		A.F	8(38 %)	3( %)*	1(14 %)	1( %)*					12( %)
	SP	Total	28(35 %)	40(51%)	4(6 %)	5(6 %)	2(2 %)	77	2		62
		A.F	4(14 %)	5(12 %)	2(50 %)		-				11(13 %)
	NLD	Total		25(47%)	5(9 %)	17(32 %)	6(11 %)	35	14	4	55
90days		A.F	1	11(44%)	2(40 %)	3(18 %)					16(31 %)
	SP	Total	18(20 %)	31(35%)	12(13 %)	20(22 %)	7(8 %)	65	18	5	88
		A.F	1(5 %)	4(13 %)	5(41 %)	4(20 %)					14(15 %)
		•		NLD: LD	12:12; SP: L	NLD: LD 12:12; SP: LD 6:18; A.F.: Atretic follicles;	: Atretic fol	llicles;			

30	Dd	60	Dd	90	)d
S⇒ B	B⇒L	S ⇒ B	B⇒L	S⇒B	B⇒L
33%		9.8%	dan dak dar	26.4%	7.5%
0.00		2.5%		20.4%	5.7%
	S⇒ B 33%	33%	S $\Rightarrow$ BB $\Rightarrow$ LS $\Rightarrow$ B33%9.8%	S $\Rightarrow$ BB $\Rightarrow$ LS $\Rightarrow$ BB $\Rightarrow$ L33%9.8%	S $\Rightarrow$ BB $\Rightarrow$ LS $\Rightarrow$ BB $\Rightarrow$ LS $\Rightarrow$ B33%9.8%26.4%

•

.

.

T able 6. Table showing percentage rate of transition from small (0-120 $\mu$ ) to big (121-240 $\mu$ ) and big to large(>300 $\mu$ ) follicular hirearchy in NLD and SP pulléts.

.

•

.

1

.

.

Values: Mean, N=12

signs of secretion exhaustion in the form of vacuoliasation (Plate 2). Ovary :

The 30 day old ovary of both NLD and SP chicks was characterised by the presence of many primordial and primary follicles; however the granulosa of the follicles of SP chicks was quite prominent with visible signs of thecal condensation and prominent germinal vesicle. By 60 days, the follicles of both NLD and SP chicks were enlarged and few follicles depicted atretic changes. The stroma was, in general, well developed with visible signs of interstitial gland differentiation in the SP ovary. At 90 days, follicles were further enlarged with signs of yolk deposition in the oocyte. Thecal differentiation was well evident with active granulosa. Overall, the ovary of SP chicks showed lesser number of atretic follicles (Plate 3,4, and 5).

The approximate follicular count as represented in table-6, reveals a temporal progression from 6-30  $\mu$ m diameter follicles to 240-440  $\mu$ m diameter follicles from 30 days to 90 days in the ovary of both NLD and SP chicks. However, the total number of follicles and the relative number of follicles of various sizes were comparatively more in the ovary of SP chicks. There was also lesser number of atretic follicles. At 90 days, whereas the ovary of NLD birds showed no follicles of 6-30  $\mu$ m range, the SP ovary showed the presence of such follicles in almost the same number as seen in the 30 and 60 days old ovary. The rate of transition in terms of percentage follicles entering into higher size hierarchy (table-7) revealed a slower rate in SP chicks with the presence of a higher percentage of follicles of less than 200  $\mu$ m size at all periods.

## DISCUSSION

The body and organ weights and the growth kinetics indicate an overall reduced weight and growth kinetics ratios in SP pullets. This decrement was essentially due to the significantly reduced weights and kinetics during

the third month, while the same in the first two months were greater than the NLD pullets. Apparently, there is some growth retardatory influence in the third month. Though adrenal, thyroid and oviduct showed significantly reduced weights and growth indices in SP chicks, the ovary showed significantly higher weights and growth indices throughout. Though, rearing of chicks of domestic fowl under different photoperiodic conditions has been attempted by many workers, mainly to evaluate the effect of same on attainment of sexual maturity and egg laying, none of these studies has reported the effect of photoperiod on organ weights and growth kinetics. It is clear from the present study that SP has a favourable influence on a short term basis (60 days) but has a definite retardatory influence on growth and growth kinetics on prolonged long term exposure (beyond 60 days). Favourable influence of SP on organ growth is clearly manifested in the case of ovary (throughout) and also in the case of liver (first two months). However, the growth of adrenal, thyroid and oviduct has shown a negative correlation with SP throughout.

In general, the serum levels of  $T_3$ ,  $T_4$ , CORT and Progesterone show a decrement with age in both the groups of chicks. The relative levels of all the hormones were lower in SP chicks at all the ages, except for CORT level at 30 day and the Progesterone levels at 30 and 60 days, which were higher. The higher level of CORT in the first month in SP chicks is well correlated with the recorded high relative weights of liver, thymus, bursa and spleen (Garren and Barber, 1955; Garren, 1957; Garren *et al.*, 1966; Davison *et al.*, 1985). Apparently, CORT has a favourable influence on the growth of liver and lymphoid organs during the first 30 days. This correlation is validated by the few previous reports showing increased weights of liver and lymphoid organs during the first month (Garren *et al.*).

al., 1961; Davison et al., 1979 Siegel et al., 1979; Davison et al., 1985). The higher serum level of CORT at 30 days is not supported by the weight and histological feature of adrenal. In this context, it may be speculated that, the metabolic clearance rate of CORT is significantly reduced under SP during the first month leading to a higher serum CORT level which results in favourable growth of CORT responsive organs. Despite the increased weights of liver and lymphoid organs in the first month in SP chicks, the weights of these organs levelled off at 90 days indicating a gradual nullification of initial growth burst. This is well corroborated by the significantly reduced CORT level during the second and third months. Though both  $T_3$  and  $T_4$  levels showed age dependent decrease in both the groups of chicks, the relative levels of both T<sub>3</sub> and T<sub>4</sub> were lower in SP chicks, more pronouncedly with respect to  $T_3$  (approximately 40%). However, the serum  $T_4$  level was significantly lower in the third month. There have been no studies regarding the measurement of hormone levels during the period of photoperiodic manipulations in rearing chicks, except for an isolated report of Renden et al. (1994) showing no alteration or slight reduction in adrenal and thyroid hormone levels at different ages during photoperiodic manipulations. The above study had however used shifting combinations of photoperiods which do not conform to an experimental design involving a fixed photoschedule as employed in the present study. The present report is therefore the first one, which projects reduced serum CORT,  $T_3$  and  $T_4$  profile due to exposure to SP. The significantly reduced growth rates of body, liver, ovary and oviduct between the 2<sup>nd</sup> and 3<sup>rd</sup> months and, the consequent effect on the weights of these organs at 90 days in the SP pullets, seem to be mainly due to lower levels of all the three hormones i.e T<sub>3</sub>, T<sub>4</sub> and CORT. The T<sub>3</sub>: CORT ratio and  $T_4$ : CORT ratios were also lower during the 3<sup>rd</sup> month (Table-5).

However, the body weight and the weights of these organs were identical

or even more than those of NLD chicks, with better growth rates. These observations tend to imply that the serum titres of  $T_3$  and  $T_4$  in the SP chicks during the first two months are within the optimum threshold level with no effect on Growth Hormone (GH) secretion/action. However, between  $2^{nd}$  and  $3^{rd}$  month the levels of  $T_3$  and  $T_4$  are below the optimum threshold level which may result in dampened GH secretion, as, correlation between GH secretion and thyroid hormone secretion has been revealed in both birds and mammals (Harvey et al., 1978; Scanes et al., 1981; Harvey et al., 1983. Kameda et al., 1984; Huybrechts et al., 1985; Thedaropoulos, 1985; Kuhn et al., 1986; Lam et al., 1986; Decuypere and Kuhn, 1988). The highly retarded growth of body and organs during the 3<sup>rd</sup> month, could be related to the reduced levels of growth promoting hormones like T<sub>3</sub>, T<sub>4</sub>, GH and Corticosterone. The consistently lower weights and growth rates of adrenal and thyroid in SP chicks seem to indicate SP induced slow maturation/activation of hypothalamohypophysial-thyroid and hypothalamo-hypophysial-gonadal axes. This is confirmed by the significantly lower hormone to body weight ratios with reference to thyroid and adrenal hormones.

The hypothalamo-hypophysial-gonadal axis however seems to be potentiated under exposure to SP from hatch, as marked by the significantly increased weight and histological picture of the ovary. The greater follicular pool and the presence of greater number of follicles in the size range of 31 - 90  $\mu$ m and 91 - 120  $\mu$ m in the ovary of SP chicks attest to the presumed activation of HHG axis. As an alternative to the potentiation of HHG axis, increased responsiveness/sensitivity of the ovarian tissue could also be considered feasible. The total follicular pool was significantly greater during the three months of study in the ovary of

SP pullets. There was a progressive transition from small to big and big to large category of follicles during the 2nd and 3rd months respectively, in the ovaries of both NLD and SP pullets. However, the relative number of such follicles was always lesser in the SP condition. The histometric data clearly reveals that the relative hierarchial progression of follicles in terms of size, is slower in the SP pullets compared to NLD. A comparison of the rate of transition shows that, while 3.3% of the follicles progress from small to big in the 1st month and 9.8% in the 2nd month in NLD chicks, in the SP chicks, it was nil in the 1st month and 2.5% in the 2nd month. Similarly in the 3rd month, whereas 26.4% of the follicles progressed from small to big and 7.5% from big to large in NLD pullets, only 20.4% progressed from small to big and 5.7% from big to large in SP pullets. Obviously, the pace of follicular progression is slowed down due to a exposure to a short photoperiod. Another aspect of clear distinction, is the degree of follicular atresia which decreased from a maximum 30% in the 1st month to a minimum of 5% in the 3rd month in SP chicks while, it increased from a minimum of 3% in the 1st month to a maximum of 31% in the 3rd month in NLD chicks. A related consequence of these changes is the presence of higher number of follicles of less than 200µm size at any time in the ovary of SP chicks and, more clearly, the continued presence of follicles of 0 - 30um size in the ovary of SP chicks while, there was a total absence of follicles of this size range in the ovary of NLD chicks in the 3rd month. The higher follicular pool and the persistent presence of follicles of smaller size range tend to suggest augmented oogonial proliferation coupled with lesser degeneration of germ cells, so that more germ cells are available for recruitment into follicles. The reduced rate of atresia seems to extend even to the follicles during folliculogenesis, as noted by the decreasing follicular atresia under SP condition. From extensive studies on mammals, androgens have been implicated as one of the factors

involved in follicular atresia (see Knobil, 1990) and in this respect it can be speculated that there is reduced androgen production in the ovary of SP pullets which finds corroboration in the herein observed relatively higher levels of serum progesterone in the SP chicks in the 1st and 2nd months. The significantly reduced degree of follicular atresia and serum progesterone level in the 3rd month in SP chicks, suggest increased oestrogen production. The slower rate of follicular growth transition in SP chicks might provide them with more time for maturational changes and consequently an augmented stimulatory response to the increased hypothalamo-hypophysial output in response to a step-up photoperiod after 90 days (Sharp, 1993; see Etches, 1996), resulting in faster progression through white and yellow yolky follicular hierarchy, as characteristic of hens closer to sexual maturity and oviposition (see Etches, 1996). In this respect, our previous study had reported early attainment of sexual maturity and commencement of egg laying (by 53 days) in pullets exposed to SP to a step-up photoperiod (Chapter 1).

Overall, our present observations extend, the already established potential of a stepup photic schedule during rearing period to increase the stimulatory component of the HHG axis at the time of switch from short to long photoperiod (Sharp, 1993; see Etches, 1996), by providing evidence for favourable intraovarian changes during the period of exposure to SP and, consequent competence of the ovary in a quantitative and qualitative sense to respond to the increased hypothalamo-hypophysial output. The early initiation of egg laying and, relatively higher egg yield from these pullets as reported previously (Chapter 1), could be related to the present observations during the period of exposure to SP.