CHAPTER- 8

Rearing photoperiod and tissue protein and ascorbic contents in RIR pullets

As a structural constituent, protein represents 1/5th to 1/4th of the fat free body of mammals and birds (Mitchell *et al.*, 1931; Griminger and Gamarsh, 1972). In birds, 20-30% of body proteins are found in the feathers, while substantial amounts of structural proteins are also found in bone, muscle and skin. The tissue proteins play a functional role for provision of energy during chronic starvation and tissue degradation. Amniotes in general have similar protein metabolism. Tissue protein turnover is characterized by simultaneous synthesis and degradation of proteins and, the prevailing level of tissue proteins are a reflection of the relative rates of synthesis and degradation. The post-hatch phase of avian growth can be considered to favour faster synthetic rate than degradation, thereby leading to building up of tissue protein and growth as a whole. Protein turn - over studies in birds are however very sparse and limited to some muscles in domestic fowl (Griminger and Scanes, 1986). Hormones can be considered as agents that can exert control over synthesis or break down of proteins. Even endocrine control

of protein metabolism in avian species has received only meagre attention (Griminger and Scanes, 1986). The major vertebrate hormones that can influence protein metabolism are insulin, thyroid hormones, growth hormones, glucocorticoids and sex steroids.

Vitamin C or ascorbic acid (AA) has been functionally related with cellular process such as electron transport, metabolism, collagen synthesis and steroidogenesis (Szent gyorgii, 1957; Bacq and Alexander, 1961; Biswas and Deb, 1970; Chinoy, 1972 a,b).

With such functional involvements, ascorbic acid turnover during the post hatch development, a phase of progressive attainment of functional maturity, could be of great relevance. Once again literature on tissue AA turnover during ex-ovo development of birds is very scant, except for the restricted studies on adrenal AA contents in adult and developing chicks (Chinoy *et al.*, 1974 a,b; Chinoy and Parmar, 1975 a, b).

In the above contexts, the present study is an attempt to evaluate the influence of different rearing photoperiods on tissue protein and ascorbic acid contents during the first three months of post hatch development in RIR pullets.

Results:

Protein content of liver and ovary:

The hepatic protein content of ovary of NP and SP pullets shows a gradual increase from 30-90 days, more pronouncedly in the former. The hepatic protein content of SP birds is significantly lower than that of NP birds at all ages. In contrast, the LP pullets showed a continuous decrease of hepatic protein content from 30-90 days.

The protein content of ovary also shows a similar pattern of gradual increase from 30-90 days in both NP and SP pullets though relatively more pronounced and with a higher content in NP birds. The ovarian protein content of LP birds, like the hepatic protein content showed a decline from 30-90 days with the result that lowest levels were seen in these birds.

Ascorbic acid content of liver, kidney, adrenal and ovary:

Normal photoperiod (NP):

Whereas the hepatic, renal and adrenal AA content showed significant increment at 90days, with a decrement at 60 days, the ovarian AA content remained more or less same at 30 and 90 days, with a decrement at 60 days.

Short photoperiod (SP):

The SP pullets are characterized by almost unchanged steady AA content in liver, marginal increase in kidney and unchanged

but with significantly decreased level at 60 days in adrenals and significantly decreased ovarian AA content at 60 and 90 days.

Long photoperiod (LP):

AA content of all organs (liver, kidney, adrenal and ovary) showed steady decrease from 30-90 days, more significantly that of adrenal and ovary.

Tables 8.1: Total Protein profile in Liver and Ovary of RIR Pullets

Treatment	Liver			Ovary		
	Age in Days			Age in Days		
	30	60	90	30	60	90
(NP) LD (12:12)	20.54 ±0.860	21.94 ±0.95	22.09 ±0.525	8.97 ±0.98	9.11 ±0.830	12.40 ±0.85
(SP) LD (6:18)	16.34^{c} ±0.251	17.54^{b} ±0.620	17.02^{c} ±0.678	7.46 ±0.349	9.31 ±0.757	9.56^{a} ±0.702
(LP) LD (18:6)	16.70^{b} ±0.533	15.60^{c} ±0.33	14.15^{c} ±0.22	11.41^{a} ±0.620	7.22 ±0.315	7.85^{c} ±0.228

NP: Normal photoperiod; SP: Short photoperiod; LP: Long photoperiod

Values expressed as Mean ± S.E, n=6;

a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$

Table 8.2: Ascorbic Acid Level in different tissues of RIR Pullets

Treatment	Liver			Kidney			Adrenal			Ovary		
	Age in Days			Age in Days			Age in Days			Age in Days		
	30	60	90	30	60	90	30	60	90	30	60	90
(NP) LD (12:12)	0.036 ±0.0001	0.030 ±0.0017	0.061 ±0.0013	0.022 ±0.0032	0.016 ±0.0014	0.031 ±0.0037	0.178 ±0.0101	0.135 ±0.0013	0.222 ±0.016	0.083 ±0.0045	0.052 ±0.0034	0.078 ±0.0038
(SP) LD (6:18)	0.041[c] ±0.0011	0.042[c] ±0.0018	0.042[c] ±0.0017	0.021 ±0.0008	0.024[c] ±0.0013	0.025[c] ±0.0003	0.173 ±0.0107	0.134 ±0.0104	0.180[c] ±0.004	0.112[c] ±0.0069	0.036[c] ±0.0031	0.058[c] ±0.0026
(LP) LD (18:6)	0.037 ±0.0031	0.050[c] ±0.0021	0.035[c] ±0.0008	0.033[c] ±0.001	0.031[c] ±0.0027	0.028[b] ±0.0009	0.223[c] ±0.0024	0.158[c] ±0.0086	0.126[c] ±0.001	0.155[c] ±0.0032	0.065[c] ±0.0046	0.038[c] ±0.002

NP: Normal photoperiod; SP: Short photoperiod; LP: Long photoperiod

Values expressed as Mean ± S.E, n=6;

a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$

Fig. 8.1: Total protein in the liver of RIR pullets at different photoperiod

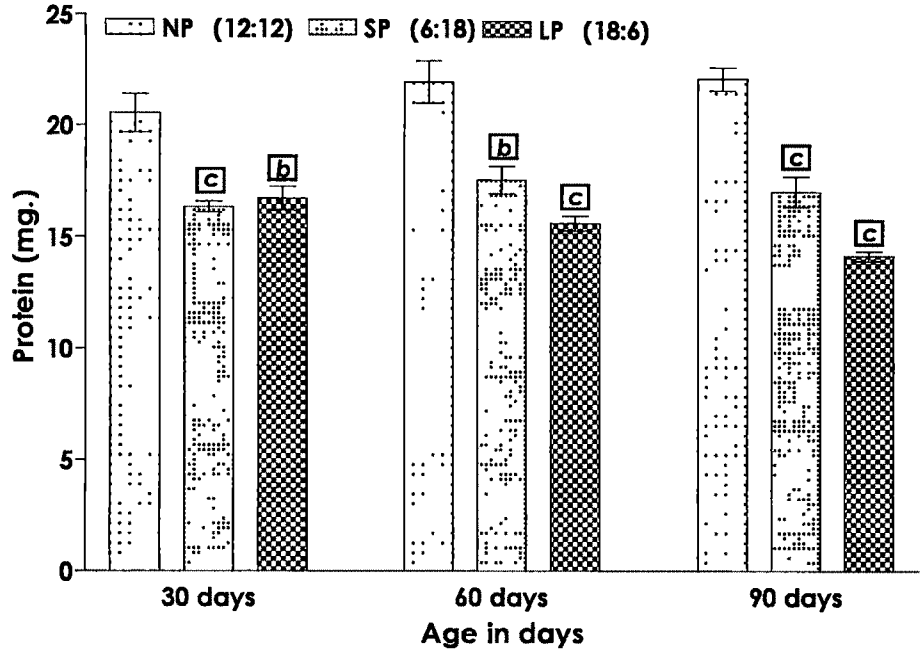
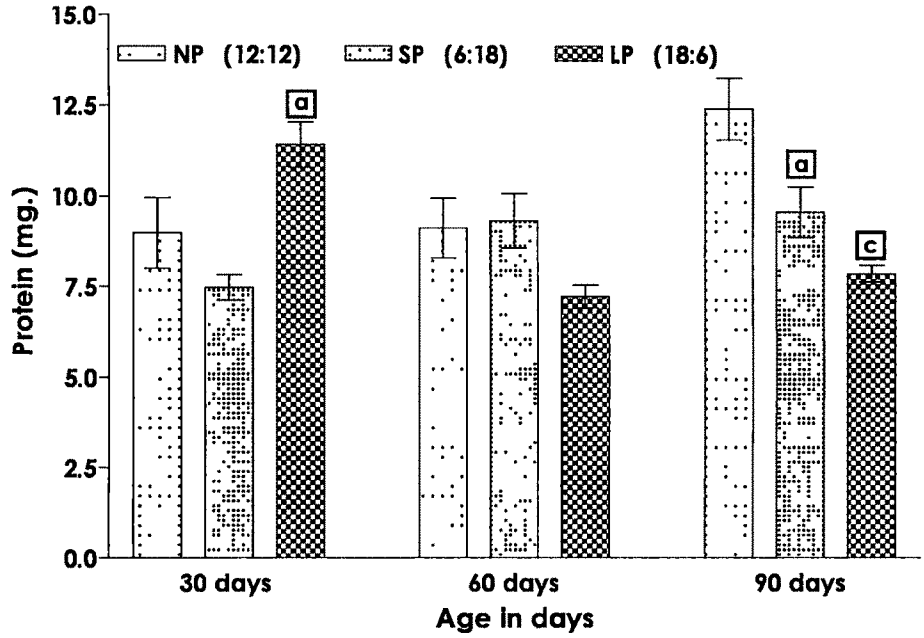


Fig. 8.2: Total protein in ovary of RIR pullets at different photoperiods



Control: Normal photoperiod, SP: Short photoperiod, LP: Long photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 3 animals

Fig. 8.3: Ascorbic acid levels in liver of RIR pullets at different photoperiods

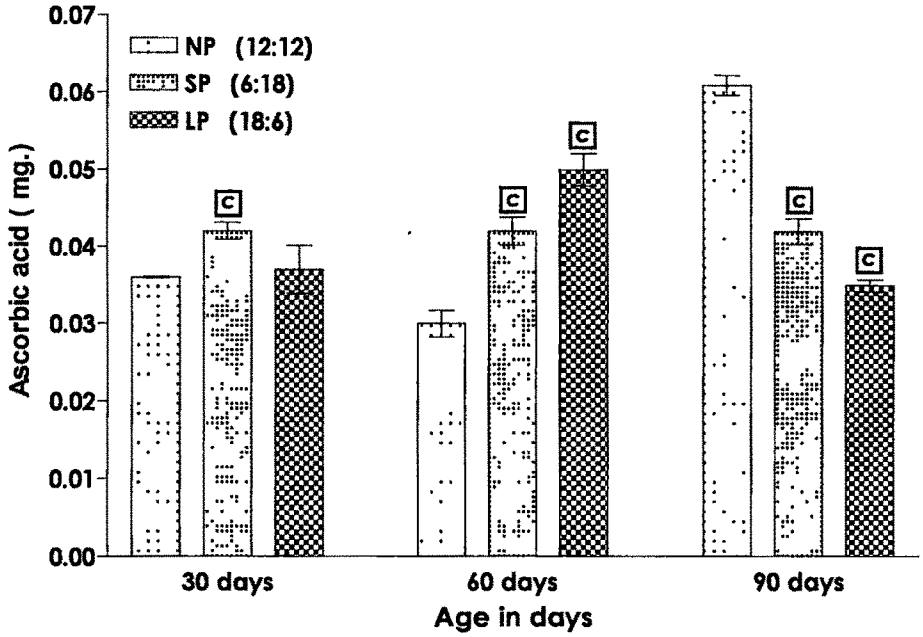
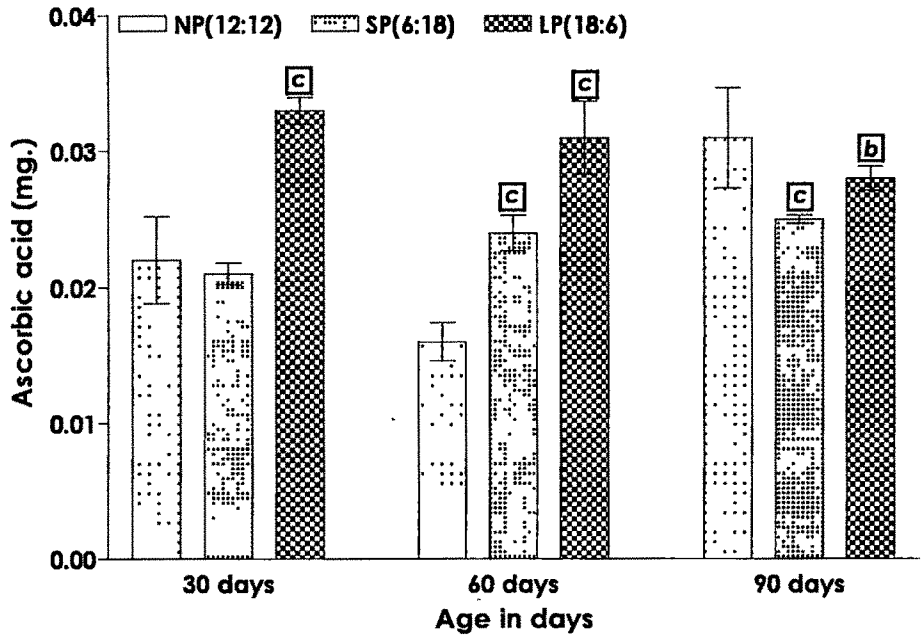


Fig. 8.4: Ascorbic acid levels in kidney of RIR pullets at different photoperiods



Control: Normal photoperiod, SP: Short photoperiod, LP: Long photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 3 animals

Fig. 8.5: Ascorbic acid levels in adrenal of RIR pullets at different photoperiods

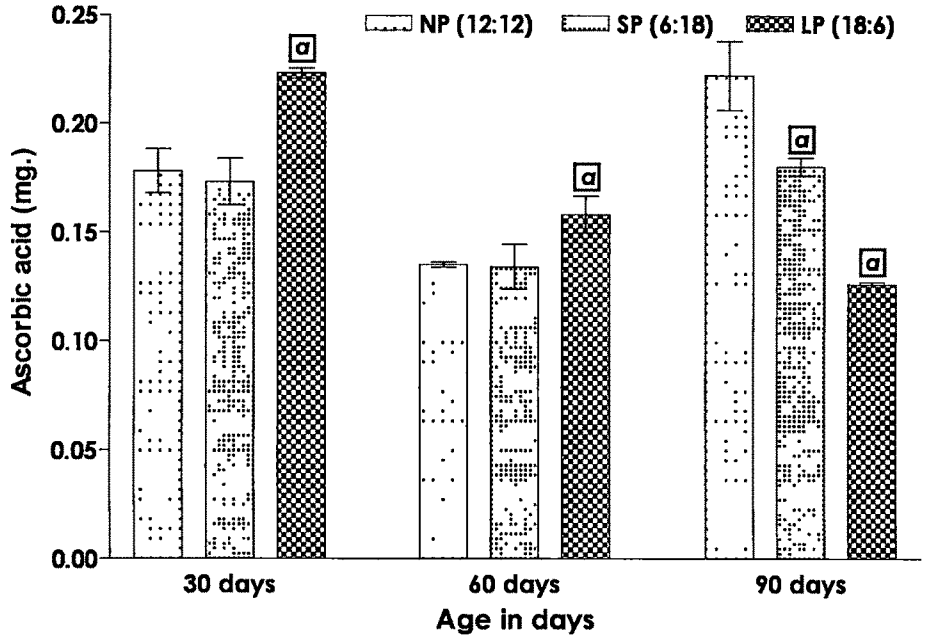
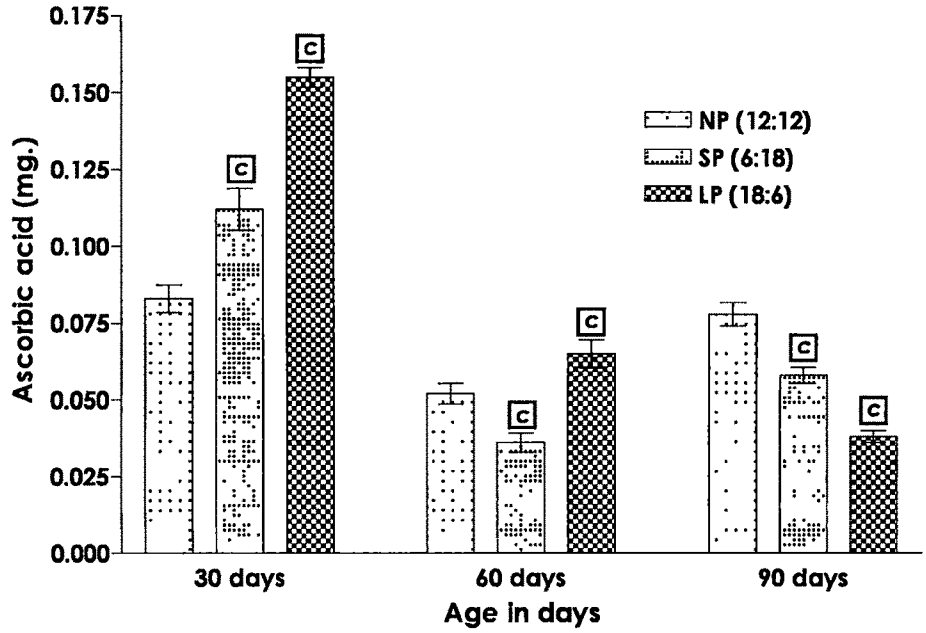


Fig. 8.6: Ascorbic acid levels in ovary of RIR pullets at different photoperiods



Control: Normal photoperiod, SP: Short photoperiod, LP: Long photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 3 animals

Discussion:

Tissue protein and AA turnover can provide an index of overall body protein metabolism and functional homeostasis both of which relate with normal growth. However, both protein metabolism and AA turnover and distribution, can be greatly influenced by many hormones. The present study is essentially an attempt to evaluate the effect of rearing photoperiod on tissue protein and AA contents during the first three months of ex-ovo development.

Clearly, tissue protein content increases gradually during the rearing period under NP and can be related with increased protein synthesis under the anabolic influence of insulin (Chapter- 6, 7), with permissive interactions with thyroid hormones and growth hormones, two essential growth promoting hormones. The most glaring observations are a dampened increase in tissue protein content under SP and decreasing protein content under LP. Obviously a long photoperiod has a negative impact on protein turnover either by decreasing protein synthesis or by increasing protein degradation. It is presumable that the anabolic environment is created in the body during growth by increasing insulin secretion coupled with optimal levels of permissive hormonal out put from the pituitary, thyroid and adrenal. Any imbalances in the permissive hormonal *milieu* either inn the form of oversecretion or undersecretion are

likely to have a negative impact, since higher levels of T_3, T_4 and corticosterone have been reported in pullets reared under a long photoperiod previously (Devkar, 1998). It is likely that this hormonal status has an overall protein catabolic influence. This influence is Supplemented by the reports of decreased DNA, RNA and protein synthesis by cortisol in the tissues of chicks (Bellamy and Leonard, 1965), high rate of amino acid deamination in the liver of chicks by cortisol (Goodlad and Munro, 1959) and decreased nitrogen balance and increased nitrogen excretion and uric acid by glucocorticoid treatment in chickens and quail (Adams, 1968; De la cruz *et al.*, 1981). Further, protein as a source for glucocorticoid induced gluconeogenesis has also been shown in rats and chicks (Bellamy *et al.*, 1968a, b; Harvey *et al.*, 1986). Correlation also comes from the previously reported hyperglycemia in corticosterone treated chicks (Joseph and Ramachandran, 1992) and the hypoglycemic status seen in LP reared pullets (Chapter-6). The observations of protein anabolic influence of insulin in the fowl and the duck (Grimniger and Scanes, 1956; Langslow *et al.*, 1970; Samsel and Ledig, 1976; Laurent and Miahel, 1978) and the corticosterone antagonistic action on insulin responsiveness in one month old leghorn chicks (Joseph *et al.*, 1996). The lesser tissue protein contents even in SP birds, (though more than in LP birds) despite the reported lower corticosterone and thyroid hormone levels (Dandekar *et al.*,

2001) also bespeak of the need for an optimal level of these permissive hormones as well the probable protective action of melatonin against the negative effect on protein synthesis.

Of the various organs evaluated for AA content, kidney serves as the organ of synthesis and liver as the storage organ as is characteristic of galliform birds (Roy and Guha, 1958). Adrenal and ovary represent steroidogenic organs, as is characteristic of all vertebrates. In the light of the reported roles for AA in functions such as enzyme activation, general metabolism, electron transport, collagen synthesis and steroidogenesis, the importance of this vitamin during ex-ovo development and maturation is easily understandable. Two of the hormones reported to extent modulations on AA turnover in the tissues of rats and chicks are sex and adrenal steroids (Stubbs and Mc Kernan, 1967; Dieter, 1969; Dieter and Breitenbach, 1971; Majumdar and Chatterjee, 1974; Overbeek, 1985). Viewed in the above context, the gradually increasing tissue AA content in NP pullets suggests a gradual build up under gradually increasing corticosterone responsiveness. However, a transient decrease noted during the second month cannot be explained and may have to be seen as utilization due to some functional exigencies involving growth. The most noteworthy observation is significantly decreasing tissue AA contents in pullets reared under LP. The increased AA contents at 30 days in LP birds relative to both SP

and NP, can be considered as a consequence of higher corticosterone level (Devkar, 1998; Dandekar *et al.*, 2001), and as such the favorable influence of corticosterone in elevating tissue AA contents is clearly shown by the hypocorticalism induced decrease and hypercorticalism induced increase in tissue AA contents of one month old white leghorn chicks (Joseph and Ramachandran, 1991). In this connection, increased hepatic and blood AA contents in response to ACTH (Stewart *et al.*, 1953; Sinha and Lahiri, 1964) and decreased AA content of liver in adrenalectomised rats (Giovanni *et al.*, 1957; Cuzzocrea *et al.*, 1959) are supporting evidences in favor. Added Support comes from the findings of decreased activity of AA synthesizing enzymes, increased activity of AA catabolizing enzymes and decreased AA content of liver, blood and urine in adrenalactomised rats (Nathani *et al.*, 1971).

This laboratory has also reported decreased tissue AA content under dexamethasone induced adrenal Suppression and increased tissue AA content under corticosterone treatment in pigeons (Ayyer, 1987; Singh *et al.*, 1999) and decreased tissue AA contents under dexamethasone induced adrenal Suppression in lizards (Chacko, 1987).

In contrast, the SP birds show a more or less constant steady hepatic AA content and a sluggish gradual increase in renal AA content. Obviously the increasing AA synthesis by kidney is being

channelised to other tissues for utilization and thereby resulting in no increase in the storage organ (liver). The SP pullets seem to have a differential hormonal *milieu* probably influenced by a higher melatonin level and thereby a differential effect on tissue AA contents. However, on a functional level, the SP pullets mimic the NP pullets as can be deduced from the pattern of changes and, even the SP pullets show the decrease in AA content at 60 days in adrenal and ovary, implying some functional involvement as in NP pullets.

Overall, the present study has revealed corticosterone and melatonin related alterations in the hormonal *milieu* of growing RIR pullets under a long and short photoperiod respectively, contributing to a negative protein and AA balance under LP and, a qualitatively similar but quantitatively less positive protein and AA balance under a short photoperiod compared to a normal photoperiod.

Summary:

The present study is an attempt to evaluate the influence of different rearing photoperiods on tissue protein and ascorbic acid contents during the first three months of post hatch development in RIR pullets. For the long photoperiod day old pullets were reared under LD 18:6 photic schedules till 90 days of

age and for short photoperiod, day old pullets were reared under LD 6:18 photic schedules till 90 days of age. Birds were sacrificed after each treatment termination with their respective control animals. Tissue protein and ascorbic acid contents were measured during the first three months of post-hatch development in the pullets. Results obtained were; the hepatic protein content of SP birds is significantly lower than that of NP birds at all ages, and the protein content of ovary also shows a similar pattern of gradual increase from 30-90 days in both NP and SP pullets. Hepatic, renal and adrenal AA contents showed significant increment at 90days with a decrement at 60 days in NP pullets, significantly decreased level of AA was observed at 60 days in adrenals and significantly decreased ovarian AA content at 60 and 90 days in SP pullets. Overall, it can be concluded that corticosterone and melatonin related alterations in the hormonal *milieu* of growing RIR pullets under a long and short photoperiod respectively contribute to a negative protein and AA balance.