# INTRODUCTION

The production of eggs has been the factor of greatest economic importance in poultry raising as far as the chicken industry is concerned.

For the most part, therefore, the problem of the poultry breeder has been how to develop efficient egg-laying strains, at the same time giving due consideration to the economic importance of meat production.

Of the 6000 or more species of birds, only a few have been domesticated, but those that have provided food for mankind particularly are distinguished by their high rate of reproduction. India ranks fourth largest producer in eggs and eighth largest producer in poultry broiler in the world.

In spite of India being in the first top ten countries, there has always been a need felt to raise egg production by another 8-10 folds to be at par with the developed countries.

The poultry population in India consists of indigenous (desi) and exotic (imported) strains of hens. The desi breeds are sturdy and well adapted to withstand diversified agro climatic conditions prevailing in Indian villages. These breeds have better resistance towards most of the diseases. The genetically Superior individuals from the desi as well as exotic breeds are identified and bred on

**RIR pullets**

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**(Fig. A)**

Eggs of RIR hen

**(Fig. B)**

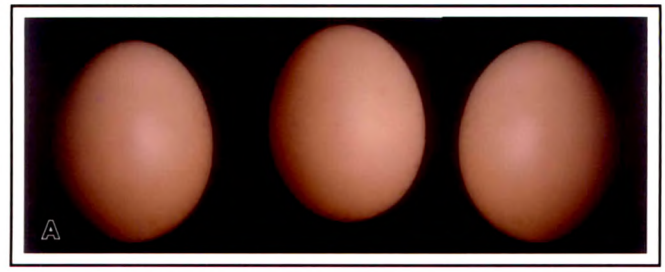
Day old chicks

**(Fig. C)**

Mature hen

**(Fig. D)**

Grower hen


A


B


C


D

pure lines to develop a parent stock. The nucleus population of selected stock in the great grandparent generation is expanded by mating with pure line to produce grandparent generation. In the succeeding generations, parent males are mated with the parent females to produce commercial egg laying hens or broiler chickens.

It is well known that India has gifted the world the species, Red Jungle and Silver Jungle fowls, out of whose progenies, domesticated and cross breed, have emerged the pure lines of today.

The Indian RIR (Rhode Island Red) breed is obtained by crossing the English RIR breed with the Kalinga Brown of Bhuvaneshwar. The subsequent generations have better resistance against disease and environmental factors and can survive well in the hilly areas. The Indian RIR is one of the common breeds in the Indian poultry industry used for dual purpose (egg production and meat). The laying hens are reared in well-ventilated hen houses (deep litter system) or in laying cages (tire system). The lighting regimen comprises of continuous light phase for the first eight weeks of post hatch development. The pullets at 17-20 weeks of age are transferred to the laying cages and are provided a lighting regimen of 16 h of light per day, which includes 12 h of natural daylight and 4 h of artificial light. This

**(Fig. A)**

Chicks in Deep litter system

**(Fig. B)**

Growers in deep litter system


A


B

**(Fig. A & B)**

Layers in two tier cages

A

B

rearing schedule is followed in India for the poultry birds in most of the government and private poultry firms.

During the last four decades, poultry science has made an all round progress on varied aspects like genetic, nutrition, disease control, management technology and marketing.

The field of physiology of reproduction has witnessed the emergence of newer concepts involving the role of non-classical hormones emanating from thyroid and adrenal in modulating reproductive functions in mammals and, is gaining increasing validity. In the avian species, these glands have been reported to show seasonal variations in structure and activity in relation to breeding activities which have lead to tentative suggestions of, parallel or inverse thyroid gonadal axis in some birds (Thapliyal, 1969; Thapliyal and Carg, 1969; Jallageus and Assenmacher, 1974; Thapliyal, 1980; Knowlton *et al.*, 1999) and parallel or inverse adrenal gonadal axis in other birds (Riddle *et al.*, 1924; Ramachandran and Patel, 1986; Petite and Etches, 1991). However, role of thyroid gland and its hormones on the reproductive cycle of seasonally breeding birds is well recognized (Oishi and Konishi, 1978; Thapliyal, 1982; Wilson and Reinert, 1996; Wilson and Reinert, 1999; Wilson, 2001).

Panda and Thapliyal (1964) and Thapliyal and Pandha (1965) demonstrated for the first time the importance of thyroid in the regulation of gonadal activity in Spotted Munia, (*Uroloncha*

*punctulata*). This was followed by a series of works showing role of thyroid in annual breeding cycles of both sexes of adult and juvenile Lal and spotted Munia, black headed Munia, Chest nut-bellied Munia, Weaver bird, red vented bulbul, migratory red headed bunting, common myna, rain quail and male house sparrow (Thapliyal, 1969;1978;1980;1981; Thapliyal and Chandola 1972; Chandola *et al.*, 1973; Chandola and Thapliyal, 1978; Lal, 1982; Thapliyal and Gupta, 1984; Lal and Thapliyal, 1985 a; b).

In some species like Munia and Finches, thyroid activity is sufficiently high in non-breeding season to inhibit gonadal development. Thus, if the thyroid glands are removed, the birds come into breeding condition but the gonads can be made to regress by giving thyroxine (Thapliyal and Chandola, 1972; Wilson and Reinert, 1996). Even the studies conducted in our laboratory to assess the role of pineal adrenal axis in reproduction of feral blue rock pigeon (*Columba livia*) have shown the involvement of thyroid hormones in the reproductive cycle of this bird (Singh *et al.*, 1993).

Recently Cook *et al.* (2004), has proposed that thyroid hormone is a critical regulator of somatic growth, metabolism, brain development and other vital process in developing adult animals. However, thyroid hormone was not historically viewed as a major regulator of the gonads. This paradigm has undergone a major reappraisal over the past fifteen years, and it

is now clear that thyroid hormone has critical actions on the ovary and testes, especially during development in the male. Despite extensive clinical data showing that both hypo and hyperthyroidism can improve reproduction in the female. Mechanistic data in this area are not conclusive and a clear consensus of the role of thyroid hormone in the ovary has not been developed.

The role of thyroid hormones in reproduction of poultry bird has also been documented (Singh and Parshad 1978; Peebles *et al.*, 1994; Knowlton *et al.*, 1999) and appropriately timed Propyl 2-Thiouracil (PTU) treatment brings about precocious puberty and abnormal spermatogenesis resulting in increased testes size and sperm production in domestic fowl (Kirby *et al.*, 1996) while dietary PTU treatment in commercial layers affected egg production and egg shell quality (Peeble *et al.*, 1994). Further, Knowlton *et al.* (1999) have also reported early adult gonadal function and sexual maturity in Turkey hens. Lien (1989) has proposed that thyroid is essential for initiation and maintenance of egg production in Turkey. Even series of experiments on Turkey hens showed reinitiation of egg laying after thyroidectomy (Siopes, 1997; Lien, 1989; Siopes, 2002).

The reproduction in birds involves processes like gametogensis, nest building, fertilization and oviposition which are intricately interrelated and controlled by environmental stimuli like light,

temperature, food etc. which trigger a cascade of changes in the general physiology and hormonal *milieu* of birds. A range of environmental stimuli is transmitted through the CNS to affect the liberation of neurohormones or releasing hormones (RH) in the median eminence of hypothalamus. These neurohormones are transported via portal blood vessels to the anterior lobe of pituitary to release pituitary hormones, mostly gonadotropins which in turn regulate gametogensis and synthesis and secretion of steroid hormones (Sturkie, 1986).

A definite role of pineal in reproduction is however highlighted by many studies, which have shown the influence of photoperiodism in regulating the annual gonadal cyclicity of temperate species of birds (Wingfield and Farner, 1980; Follett *et al.*, 1974). The influence of light on reproduction was first demonstrated by Rowan (1926) in migratory Juncos, it became a starting point for a series of physiological studies aimed at understanding how photoperiodic signals initiate the events that culminate in egg and sperm production. In birds, light is perceived through photoreceptors in the hypothalamus which acts as biological transducer that converts the photon energy into neural impulse, which in turn is amplified by the endocrine system to control ovarian and testicular function and consequently, the multitude of reproductive activities, behavior and secondary sexual characters (Etches, 1996). Photoperiodism

is the process whereby endogenous circadian and circannual rhythms of body functions are synchronized with external daily and seasonal rhythms.

Birds exhibit a wide spectrum of adaptive photo responsiveness, which phase the hypothalamic neurosecretion, which eventually regulate migratory fattening, moult, gonadal development and other photoperiodic functions. Since studies on tropical species of birds have indicated that annual gonadal cyclicity may not be directly dependent on photoperiodism, though many of them are shown to be responsive/ sensitive to photoperiodic manipulations (Marshall and Disney, 1956; Lofts, 1962; Epple *et al.*, 1972; Thapliyal, 1981; Patel, 1993). The house finch for example is non stimulatory to short days (LD 6:18), but long days (LD 18:6) leads to rapid testis growth and spermatogenesis (Hamner, 1964). Further studies on European starlings shows that the long photoperiod stimulated LH secretion but also induced photorefractorines to a degree that was exactly countered by the following 13 short photoperiods (Dawson *et al.*, 2001). Further (Kumar, 1997; 1988; Kumar and Tiwari, 1989; Kumar and Kumar, 1991; 1995) have rigoursly demonstrated that a circadian photoperiodic clock is involved in the process of induction and termination of seasonal reproduction.

As in seasonally breeding birds, photoperiodism is an important factor in poultry birds, and manipulation of the same is one of the

most powerful management tools in poultry industry. The onset of lay can be advanced or retarded, the rate of lay can be influenced, the timing of lay can be altered, shell quality can be improved, egg size can be optimized, and feed efficiency can be maximized by providing the appropriate lighting regime. Hence, it is important to know how many hours of light must be given before domestic birds will recognize that they have been exposed to long day and to know how day length is related to subsequent rate of gonadotropin secretion and gonadal growth. Poultry birds like chicken, Turkey, and Quail are known to utilize internal circadian rhythms to differentiate between short day and long day. The long days are known to stimulate gonadotropin secretion, since they are known to illuminate the photosensitive phase of the 24 hrs cycle (Follet, 1974). Similar effects are observed by intermittent lighting regimens, which are equally effective in controlling the reproductive process in domestic birds (See Etches, 1996). Further, ahemeral lighting increased egg weight and poult weight in turkey hens photo-stimulated early (Siopes and Neely 1997), and long ahemeral day's results in early initiation of lay in broiler breeder's hens (Spies *et al.*, 2000).

Short exposure to long days provides a large stimulatory response resulting in increased plasma LH and increases egg production in layers. Long exposure to long days sets in photo refractoriness

and dissipates the stimulatory effect of long days. These responses are assumed to be the sum of inhibitory and stimulatory inputs to the GnRH secreting neurons depending upon previous exposure to light (Sharp, 1993).

Increasing (step-Up) photoperiod advances sexual development in pullets (Lewis, 1996) and tends to stimulate the rate of lay after maturity, whereas decreasing (Step-Down) photoperiod has an opposite effect. By contrast, it is possible to obtain a difference of 6 weeks or more in age at 50% lay and there are important consequential effects on yield.

Most of the studies are restricted to the temperate species of laying hens, like ISA Brown, Shaver 288, Babcock 300, etc. (Lewis *et al.*, 1996, a, b and c; Gous, 2000) but reports are scant on tropical species of domestic fowl with practically no reports on Indian RIR on its laying performance, except for studies conducted in our laboratory (Dandekar, 1998; Devkar, 1998).

Hence it was thought pertinent to study the effect of step –up photic schedule on the reproductive performance in RIR hens.

The avian egg is a marvel in nature's architecture. A highly complex reproductive cell, it is essentially a very small center of life, a world of its own.

The egg is the single most complete food known to man, versatile and nutritious. It is used every day in the preparation of most common or the most fanciful meals. Although, human

nutritional requirements are not the same as those of the chick, they are so similar in many respects that the egg has become a convenient, economical source of many essential proteins, minerals and vitamins necessary to our good health.

The composition of the egg in the domestic fowl is of important economic interest, as many factors can affect their size, composition and viability. A variety of factors or combinations of same are known to have a definitive effect on the egg size and composition. However most of the studies are involving influence of dietary and genetic manipulations on egg composition of domestic fowl (Christie and Moore, 1970; Christie and Moore, 1972; Cunningham and Lee, 1978).

Egg size is another important parameter, which has been worked upon by scientists to increase the egg size, as smaller eggs have low economic value. One of the ways of achieving desired egg size is by restricted diet during the pullet stage and /or a non stimulatory photo schedule which delays sexual maturity. This enables the pullets to attain ideal body weight prior to lay. Further, Methionine and Linolic acid fed with diet are known to improve the egg size in laying hens (Sandoval and Gernat, 1996).

A chicken egg is made up of 11% shell, 31% yolk and 58% albumen. Egg on the whole is known to have 11.5% fat, 12.9% protein, 73% water and 0.9% carbohydrates. The major component of albumen is water 88% besides having protein and

fat 12.5% and 11.8% respectively. The yolk is known to have 48% water, 16.6% protein and 32.6% fat. Taylor (1960) has reported that out of all those components of egg studied, yolk is resistant to drastic change in composition and hence is called as the conservative component of a Hen's egg. In recent years, much attention has been focused on the fat, fatty acid and cholesterol contents of egg.

Although, cholesterol content of egg was found to be increased / altered by factors like genetic constitution, higher rate of lay and seasonal variations, factors like oral hypo-cholestremic agents are also known to reduce the same (See Panda, 1995).

As there is no report from anywhere depicting effect of photoperiod or endocrine manipulations on egg composition and physical properties of egg, except studies conducted in our laboratory (Dandekar, 1998; Devkar, 1998), it was thought pertinent to carry out studies on the effect of step-up photo schedule and hypothyroidism and a combination of both, on the egg composition of RIR hen.

Thyroid and Adrenocortical hormones are physiological indicators of various forms of stress in the fowl (Edens and Siegel, 1975; Etches, 1976; Freeman, 1978; Beuving and Vonder, 1978; Siegel, 1980). Changes in the circulating levels of these hormones in the growing period would affect the growth and metabolism of various developing tissues. The study of effect of thyroid

hormones on various facets of metabolism during post hatch development has shown that though the inadequacy of growth hormones and thyroid hormones have several retarding effects, administration of either of the two above mentioned hormones to the normal animal was unable to stimulate growth appreciably. Apparently growth is regulated by complex and subtle endocrine *milieu* and not under the purview of a single hormone *per se*. Role of thyroid hormones in avian post hatch development is well known (Leung *et al.*, 1984, 1985; Scane, 1986).

In the back ground of the literature reviewed above and based on our own experimental findings, it was thought pertinent to attempt experimental manipulations involving thyroid hormones and photoperiodic manipulations in chronological systematic sequence during post hatched developmental period; and the objectives behind the present study were to see whether the manipulation of external factor (photoperiod) or internal factor (Thyroid hormone deficiency) or a combination of the two factors may bring about early egg laying. Even the presence of a critical period for PTU treatment has been reported previously for both domestic fowl (Kirby *et al.*, 1996) and rodents (Cooke *et al.*, 1992; Joyce *et al.*, 1993; Jannini *et al.*, 1995) and in this context we also wanted to scrutinize the critical time period for

the induction of HPOT so as to get favourable results in female domestic fowl.

It becomes evident from above review of literature that both photoperiodic manipulations (representing an exogenous change) and manipulations of thyroidal status (representing an endogenous change) in birds have potential effects on attainment of sexual maturity but there is no or very scant studies showing the effect of combination of both photoperiod and hypothyroidism (Freg and Bryan, 1998). However, such studies were performed in our laboratory on RIR pullets, involving effect of hyper or hypo corticalism under the influence of long or short photoperiodism (Dandekar *et al., 2000*; Devkar *et al.*, 1998, 1999). The results of above studies were early initiation of egg laying, effects on egg composition and related favourable changes in histomorphology of ovary and serum hormone profile. These studies involving photo-endocrine manipulations are a novel approach in enhancing the reproductive potential of birds.

# MATERIALS AND METHODS

**Procurement and maintenance of animals:**

Day-old pullets of domestic fowl (*Gallus gallus domesticus*) of Indian RIR (Rhode Island Red) breed were used for the study. The animals were procured from the Model Poultry Farm, Vadodara, Gujarat. The Indian RIR breed of domestic fowl is obtained by cross breeding the American Rhode Island Red and the Kalinga Brown of Bhuvaneshwar, for better survival in the tropical climatic conditions. The Indian RIR is considered to be a dual-purpose breed, as it is used for both egg laying and table purpose.

Day-old pullets were housed in cages (5×10×8 ft) under deep litter system for 90 days. Thereafter, they were shifted to a 3 tier laying cages. Both cages were placed in a dark room and photoschedule was controlled by fluorescent tube-light, regulated by automated timer.

The control and each of the experimental groups had six animals each. The pullets were vaccinated for Ranikhet disease on the 4$^{th}$ day of hatch and after 2 months. The vaccines against fowl pox and fowl cholera were also given.

**Feed and Feeding Regimens:**

The feed and water were provided to all the chicks *ad libitum* in a plastic feeder. From day 1 till day 56, chicks were fed with Chick-Mash twice a day at an average of 30gm/chick and from 57th day onwards till lay, they were fed on Grower-Mash twice a day at an average of 90gm/bird. After the initiation of egg laying, the birds were fed on the Layer-mash twice a day at an average of 110gm/bird. The feed was obtained from Model Poultry Farm, Vadodara. The composition of chick, grower and layer mash is given in (Table 1). Calcium Supplement in the form of shell grit was provided whenever required.

**Table: 1. Composition (Kg/ ton) of chick, grower and layer mash**

| CONSTITUENTS | | CHICKS | GROWER | LAYER |
|---|---|---|---|---|
| 1. | Corn | 481 | 420 | 385 |
| 2. | Groundnut cake | 180 | 110 | 120 |
| 3. | Rice bran | 150 | 200 | 200 |
| 4. | Wheat bran | 76 | 100 | - |
| 5. | Rice polish | - | 90 | 130 |
| 6. | Fish meal | 50 | 30 | 40 |
| 7. | Proto Liv. | 30 | 15 | 40 |
| 8. | Mineral mixture | 25 | 25 | 26 |
| 9. | Dicalcium phosphate | 4 | 5 | 15 |
| 10. | Coxidot | 0.5 | - | - |
| 11. | Ventrimix ( A, B, 2D, 3K) | 0.1 | 0.1 | 0.1 |
| 12. | Salt | 4 | 5 | 4 |
| 13. | Calside/ Shell Grit | - | - | 40 |
| 14. | Neftine - 200 | - | 0.25 | - |

**Lighting regimens:**

Fluorescent tubes fitted on top of the cages did the lighting and the light intensity was maintained at 250 lx and checked regularly by lux-meter.

The present investigation is divided into four phases each of which is elaborated separately.

**Phase I**

Few RIR pullets were subjected to photoperiodic (Step-Up photoperiod), some are subjected to thyroid manipulations and others are subjected to both Step-Up photo schedule and Hypothyroidism (HPOT). Controls of respective treatments are maintained under normal light dark conditions (LD 12:12), to assess age at first egg, number of eggs laid in the first month after initiation of lay.

**Phase II**

The eggs of the hens (collected from Phase I) were analysed to study the changes in their physical features and biochemical composition.

**Phase III**

RIR pullets were subjected to the experimental schedule (same as in Phase I) and were sacrificed at 45, 60, 75 and 90 days of

age. The changes in organ growth, serum hormone profiles and histomorphometry of ovary were studied to have a better understanding of the photo-endocrine interactions and ovarian development.

**Phase IV**

Day old RIR pullets were subjected to three different photo schedules

1. Long Photoperiod (LP): LD 18:6
2. Short Photoperiod (SP): LD 6:18
3. Normal Photoperiod(NP): LD 12:12

and maintained till 90 days of age and were sacrificed at 30, 60 and 90 days of age. The changes in carbohydrate metabolism of muscle and liver, lipid metabolism of liver and ascorbic acid level in liver and ovary were assessed.

**Photoperiodic manipulations:**

Photoperiodic manipulations were studied by employing three experiments.

## Experiment – 1

Day old chicks were maintained under a short photoperiod (LD 8:16) for 90 days of age. After 90 days, they were transferred to a long photoperiod (LD 16:8) for a period of 30 days till 120 days of

age and lastly, they were transferred to normal light/dark conditions (LD 12:12) and maintained thereafter. This shifting involved an increase of 8 hrs of light, from 8 hrs of light to 16 hrs. Hence, this photoperiodic manipulation has been referred to as a **step-up photic schedule**. Another set of birds maintained under normal light/dark conditions (LD 12:12) (NLD) since the day of hatch, served as the control.

The lights were on at 06.00 hrs in all the cages and switched off at 18.00 hrs for control birds. Whereas for step-up photoschedule, lights were on at 06.00 hrs and switched off at 14.00 hrs till 90 days and switched on at 06.00 hrs and switched off at 22.00 hrs from 90 to 120 days.

## Experiment – 2

In this experiment, birds were maintained under long photoperiod (LD 18:6) for a period of 90 days; the lights were switched on at 08.00 hrs and switched off at 20.00hrs.

## Experiment – 3

In this experiment, birds were maintained under short photoperiod (LD 6:18) for a period of 90 days, the lights were switched on at 08.00 hrs and switched off at 14.00hrs.

In both second and third experiments control birds were maintained under NLD (12:12) till 90 days.

**Thyroid manipulation:**

The birds were subjected to hypothyroidism (HPOT) by mixing anti-thyroid substance Methimazole (MMI) (Sigma Chemical Co., USA) in the diet.

Mechanism of action of Methimazole (MMI)

Methimazole belongs to the group of thyouraylenes. MMI interferes with the incorporation of iodine into tyrosyl residues in thyroglobulin and inhibits the coupling of iodotyrosyl residues to form iodotyronines (see Haynes, 1991). Taurog (1976) proposed that the drug inhibits the thyroperoxidase enzyme, thereby preventing oxidation of iodine or iodotyrosyl groups to the required active state. The anti-thyroid drug binds and activates the thyroperoxidase only when the heme moiety of the enzyme is in an oxidized state (Davidson *et al.*, 1978; Engler *et al.*, 1982). Over a period of time the inhibition of hormone synthesis results in hypothyroidism due to depletion of iodinated thyroglobulin stores. The half-life of MMI in plasma is 6 to 13 hours.

Birds were initially fed with MMI (10mg/kg bd. Wt.) mixed in feed (10-25 gm) and after completing that feed, animals were allowed to feed on normal diet throughout the day. HPOT was induced in four different groups of study for 30 days

**Group I:** Birds were fed with MMI from 15th day till 45th day of age

**Group II:** Birds were fed with MMI from 30th day till 60th day of age

**Group III:** Birds were fed with MMI from 45th day till 75th day of age

**Group IV:** Birds were fed with MMI from 75th day till 90th day of age

**Experimental groups:**

**Group 1 (NLD):**

Day old pullets reared were under LD 12:12 photic schedules.

**Group 2 (Step-up Photo schedule):**

Day old pullets were reared under LD 8:16 (short photoperiod) till 90 days and then transferred to long photoperiod LD 16:8 till 120 days, and maintained under 12:12LD thereafter.

**Group 3 (Long photoperiod):**

Day old pullets were reared under LD 18:6 photic schedule till 90 days of age.

**Group 4 (Short photoperiod):**

Day old pullets were reared under LD 6:18 photic schedule till 90 days of age.

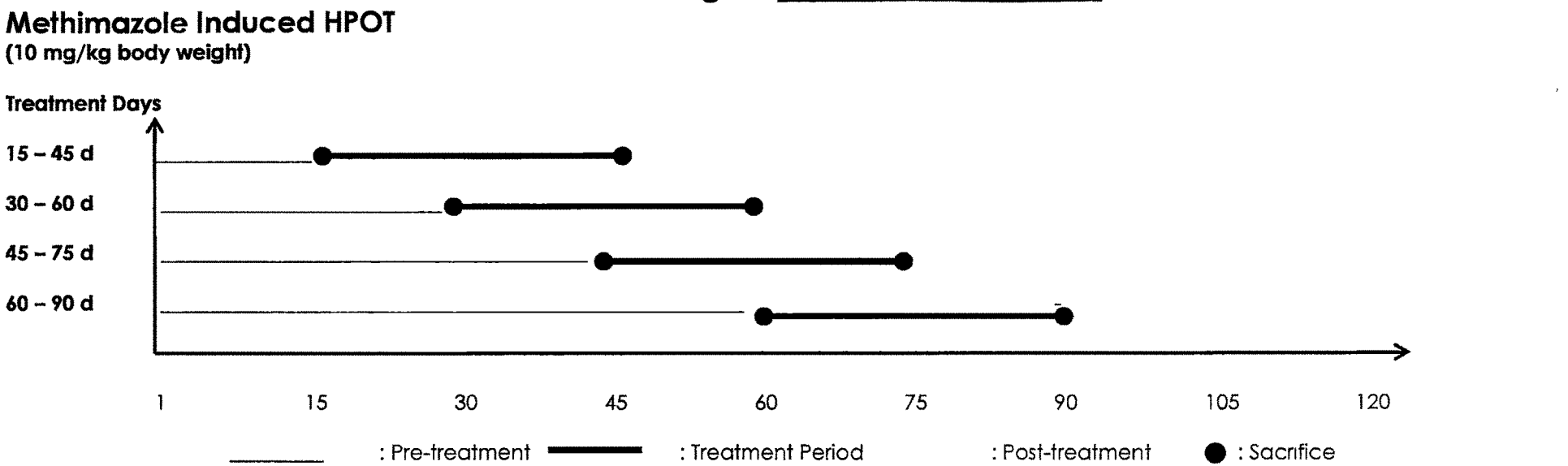
**Group 5 (NLD + HPOT):**

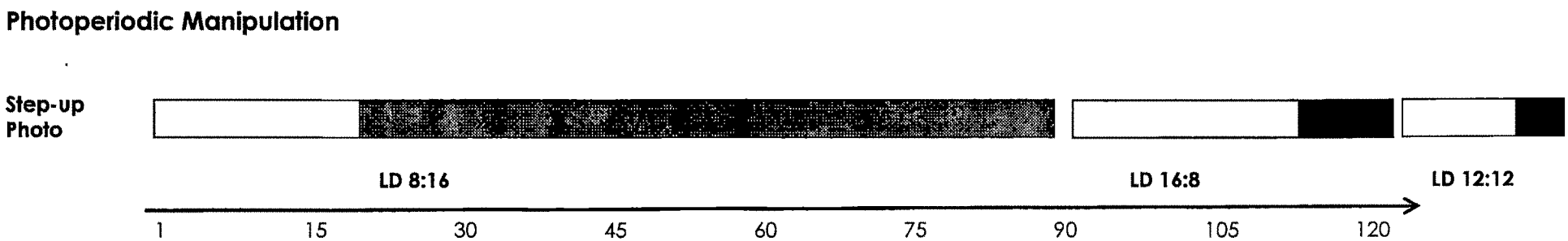
Pullets fed with MMI at different age groups as mentioned earlier and maintained under NLD.

**Group 6 (Step-up + HPOT):**

Pullets fed with MMI at different age groups as mentioned earlier and maintained under step-up photic schedule.

## Fig: 1 Experimental Schedule



Birds were sacrificed after each treatment termination with their respective control animals.

**Phase I:**

The age at first egg or initiation of lay and egg weight was noted for each bird, and the no. of eggs laid during 30 days after initiation was recorded, for each group. Mean values of the same under different experimental schedules are represented in the respective chapters.

**Phase II:**

A study of changes in physical features and biochemical composition of eggs subjected to photoperiodic and endocrine manipulations was carried out at initiation of lay and 30 days after initiation of lay.

Three eggs were selected by random sampling for each group and their analysis was carried out. The physical features of the eggs of control and experimental groups of birds analyzed were the egg weight, yolk, albumen and shell weight measured on a pan balance. The width and height of eggs were measured using a Vernier caliper and the shell thickness was measured using a micrometer screw gauge. The egg volume was measured by water displacement method.

The percentage water and solid contents, total protein, total lipid, and total cholesterol were estimated in both yolk and albumen according to the following methods:

**Percentage water content and solids:**

Yolk or albumen was taken in pre-weighed lipid tubes and then weighed again. These tubes were oven dried at 50-60°C for 24 hrs. These tubes containing dry yolk or albumen were weighed again. The difference of the two readings gave the water content and the total dry matter left was the total solid content. Both parameters were expressed in terms of percentage.

**Total Protein (Yolk and Albumen) Lowry et al. (1951):**

The known amount (50 mg) of yolk/albumen is mixed in 5ml chilled distilled water and 0.1 ml of sample is taken and diluted further by adding 0.9 ml distilled water, so as to make the final volume up to 1 ml. Blank as well as standard tubes are also run simultaneously. To each of the test tube, 5 ml of reagent C (freshly prepared alkaline copper solution) is added. After 10 min, 0.5 ml of color reagent is added in each tube. The optical density (OD) is read at 660 nm wavelength against blank tube after 30 min and the protein content was expressed in terms of mg percent of wet yolk/albumen.

**Total Lipid (Yolk and Albumen) Folch et al. (1957):**

Total lipid content was measured using a chloroform-methanol mixture (2:1; v/v) as an extractant. The known quantity of fresh yolk (80-100 mg) and albumen (3-5 gm) is taken in test tubes and chloroform methanol mixture (2 ml) and 1% $CaCl_2$ (1 ml) are added to each of the tubes. The aqueous layer is separated after 24 hrs with a syringe and a 2 ml aliquot was taken in pre-weighed lipid tubes and oven dried at 50°C. The tubes were weighed again and the lipid is thus measured gravimetrically and expressed as mg lipid in 100 mg yolk/albumen.

**Total Cholesterol (Yolk and Albumen) Crawford, 1950:**

The lipid, after extraction, is dried (as mentioned above) and then dissolved in 2 ml of chloroform-methanol mixture (2:1 v/v) known quantity of aliquote (0.2 ml for yolk and 2 ml for albumen) was taken in test tubes and oven dried at 50°C. Ferric chloride (2 ml) is added to each of the tubes and is boiled for 5 min. in water bath and then cooled in ice bath. Sulphuric acid (2.5 ml) is added along the side of the test tube and mixed by swirling the tubes and the readings are taken after 30 min. on a colorimeter at 540 nm green filter.

The water and lipid indices, non-lipid dry and calorific value were also calculated.

**Calorific value:**

Energy/gm fresh egg was calculated utilizing energy equivalents of 9 cal / gm lipid and 4 cal / gm of protein (see Rama Rao, 1986).

**Phase III:**

The day old chicks were subjected to photoperiodic and/or endocrine manipulations (as mentioned earlier) for a period of 90 days. The pullets were sacrificed under mild anesthesia at 45, 60, 75 and 90 days of age and following parameters were assessed.

**Gravimetry:**

Changes in the weights of body, thyroid, ovary, oviduct were recorded in control and experimental groups of animals.

The birds were quickly decapitated under mild anesthesia 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 digital Mettler balance. The organs were weighed up to 0.01 mg accuracy. The absolute weights thus obtained were converted into relative weights and expressed in terms of percentage of body weight. The per day growth rate, and growth kinetics were calculated on the basis of absolute body weight of individual birds.

**Histology and Histometry:**

The ovary of pullets were fixed in Bouin's fixative and processed further, dehydrated and embedded in paraffin wax. Sections of 5 µm thickness were cut on a microtome and stained with Haematoxylin-Eosin stain and mounted in DPX. These sections were photographed on a photomicroscope.

The histometrics of the ovarian follicles was done with the help of an occulometer. A specific region in each of the sections was selected for the follicular count. Initially a total count of the follicles was made, counting the pre-ovulatory follicle and atretic follicles (AF) separately. The pre-ovulatory follicles on the basis of their size were catergorised as small (60-120 mm), big (121-300 mm) and large (>300 mm) follicles and counted separately.

**Serum Hormone Assay:**

The blood samples were collected by puncturing the right jugular vein and later centrifuged at 3000 rpm for 30 min to get the serum. The hormone assay was carried out by using ELISA kits. The ELISA kits for T3 and T4 were obtained from Medix Biochemica Oy Ab, Finland and kits for progesterone were purchased from General Biological Corporation, Taiwan.

**Phase IV**

Day old pullets were subjected to three different photoschedules as mentioned earlier and was sacrificed on 30,

60 and 90 day of age and following biochemical parameters were assayed.

**Glucose 6-phosphatase (G-6Pase):**

Homogenate for estimation of this enzyme activity was prepared in cold citrate buffer a 6.5 pH. Enzyme activity was assayed by the method of Harper (1960). Glucose 6-phosphate (disodium salt, Sigma Chemicals, USA) was used as the substrate. Inorganic phosphate released was measured as per the method described by Fiske and Subbaraw (1925) and the color intensity was read at 660 nm (red filter) on a Klett-Summerson colorimeter. Enzyme activity was expressed as µg phosphate released / mg protein / 10 minutes.

**Succinate dehydrogenase (SDH):**

Activity level of SDH was assayed as per the method of Kun and Abood (1949) using denitro-triphenyl-tetrazolium salt (INT) as the hydrogen acceptor. The formazan formed was extracted in 7 ml of acetone and the colour intensity was read with a blue filter on a colorimeter. Enzyme activity was expressed as µmoles formazan formed / mg protein / 30 minutes.

**Phosphorylase:**

Total phosphorylase activity was assayed by the method of Cahill *et al.* (1957) using glucose-1-phosphate (Sigma Chemicals, USA) as the substrate. The inorganic phosphate released was measured as per the method of Fiske and Subbaraw (1925).

Enzyme activity was expressed as µg phosphate released / mg protein / 30 minutes.

**Ascorbic Acid:**

Total ascorbic acid content in the tissues was estimated by the method of Roe (1954). The ascorbic acid was extracted with 6% TCA and oxidized to dehydro-ascorbic acid by shaking with norit (activated animal charcoal) for 15 min, 4 ml of the filtrate was incubated with 2, 4-dinitrophenyl hydrazine for 3 hrs at 37°C to yield ozazone. This was treated with 85% H2SO4 to form a reddish brown colour, which was read colorimetrically at 540 nm (green filter). Ascorbic acid content was expressed in terms of mg/100 mg tissue weight.

**Total Lipid:**

Total lipid content was measured employing the method of Folch *et al.* (1957) using a chloroform-methanol mixture (2:1; v/v) as an extractant and measured gravimetrically. The total lipid content was expressed as mg/100 mg fresh tissue weight.

**Cholesterol fractions:**

Total, free and esterified cholesterol contents were estimated by the method of Crawford (1950) using alcohol-ether mixture (3:1, v/v). Free cholesterol was precipitated with digitonin and estimated as the digitonide. $FeCl_3$ was used as the colour reagent; colour intensity was read at 540 nm on a colorimeter.

Total, free and esterified cholesterol were expressed as mg/100 mg tissue.

**Blood Glucose:**

Blood glucose level was estimated by GOD-POD method. The glucose concentration was expressed as mg/100 ml blood.

**Glycogen:**

The glycogen content was estimated employing the method of Seifter *et al.* (1950). Small pieces of tissue were dropped in pre-weighed test tubes containing 2 ml of 30% KOH. Glycogen was precipitated with 95% alcohol. The diluted precipitated were treated with anthrone reagent and the colour intensity was read calorimetrically at 620 nm. Glycogen content was expressed as mg/100mg wet tissue weight.

**Protein:**

Tissues of known weight was homogenized in a pre-chilled mortar and diluted to a required concentration with chilled glass distilled water. The protein content was estimated by the method of Lowry *et al.* (1951) using folin-ciocaltean as the colour reagent. The colour produced was read at 720 nm (Red filter) on a colorimeter and protein content was expressed in terms of mg percent of fresh tissue weight.

**STATISTICAL ANALYSIS:**

All the results were statistically analyzed by student's test with 95% confidence level and are depicted as Mean ± SE.

# CHAPTER: 1

## Effect of step-up photoperiod on egg lay and egg composition in RIR breed of hens

Many environmental factors like nutrition, management techniques, humidity, light and temperature can all impact on growth and sexual maturation as well as on egg productivity in poultry birds. The growth rate of pullets, the age at which egg laying is initiated, the attainment of sexual maturity, the size of eggs at initiation of lay, weight and total number of eggs laid and the total duration of laying period are all dependent on environmental conditions. Feed management and nutritional manipulations have been shown to influence egg production in poultry birds (Dunn *et al.*, 1990; Sandoval and Gernat, 1996). Virtually all temperate zone wild bird species are photoperiodic in that, seasonally changing duration of daylight hours influences gonadal development and gonadal regression (Nicholis *et al.*, 1988; Dawson *et al.*, 2001). Although photoperiod itself influences the timing of sexual maturation in *ad libitum* fed domestic pullets (Lewis *et al.*, 1996a), a change in photoperiod is more potent, and is the most influential proximate environmental factor affecting the rate of sexual development (Whetham,

1933; Morris and Fox, 1958; Sharp, 1993; Lewis *et al.*, 1996b). Absolute photoperiod is not consequential in the domestic hen as, regardless of the duration of photoperiod, they undergo ovarian development and become sexually mature at about 5-6 months under widely different lighting regimen (Lewis *et al.*, 1994). A reduction in photoperiod has been shown to reduce the rate of egg production in laying hens (Sykes, 1956; Hutchinson *et al.*, 1957; Morris *et al.*, 1964). Birds maintained under 6 hrs or 18 hrs of constant photoperiod from day one, become sexually mature later than birds kept on a 10 hrs constant photoperiod (Morris, 1968) and, a 12 hrs increase in photoperiod from 8 hrs to 20 hrs did not effect ovarian growth (Dunn, 1990). The sexual response of domestic hen to changes in photoperiod is age dependent and, Morris (1968) showed that they become more responsive to photic changes closer to sexual maturity. Delayed sexual maturity is characteristic of pullets reared under normal or long days versus short (8 hrs) days (Payne, 1975; Proudfoot, 1980; Renden and Oates, 1989). Exposure of growing pullets to an increased photoperiod advanced the age at first egg and the exposure to decreased photoperiod delayed the same (Morris, 1968). The degree of advancement or delay was shown to be dependent on the size and timing of change in photoperiod (Morris, 1963; Lewis *et al.*, 1992). The above studies have also suggested that the sensitivity

of hens to changes in photoperiod is not uniform in terms of photoperiod or age as, changes made outside the range from 8-16 hrs prove to be less potent than the changes made within that range. Further, increase in photoperiod made closer to sexual maturity was less effective than when given at younger ages.

Extensive studies on photoperiodic manipulations on poultry birds of temperate breeds have been carried out while, such studies have not found application on breeds of tropical countries. The present study has tried to evaluate the effect of rearing of pullets of RIR breeds on a short photoperiod of 8 hrs from day one till 90 days of age and then shifting them to a long photoperiod of 16 hrs till 120 days and then maintaining them under constant photoperiod of 12 hrs, on age at first egg, total lay during the first month, physical parameters of eggs and biochemical composition compared to pullets reared under a constant photoperiod of 12 hours.

## RESULTS:

### Age at first egg and total number of eggs/month:

Age at first egg was 116.48 day in Step-up photoperiod birds and 158 days in NLD birds, which showed significant early initiation of egg lay of SUP birds (42 days) than the NLD birds. Further, there was no much difference in the total number of eggs laid/month in SUP and NLD hens (Table 1.1).

**Egg weight:**

The weight of eggs laid by SUP hens on the day of initiation of lay was less compared to those laid by NLD hens (23.04 gm in SUP hens and 28.34 gm in NLD hens). Though there was an increment in egg weight from first laid eggs to 30$^{th}$ day eggs in both the groups, the increment in NLD hens was slightly greater than that of SUP hens (Table 1.2).

**% Water and Dry content of Egg:**

There was a significant increment in the water content and concomitant decrement in the total solid content in the yolk of SuP eggs, both during initiation of lay and after 30 day. However, the water and the dry content remained invariantly identical in the albumen of the eggs of both NLD and SuP hens (Table 1.3).

**Physical Parameters of Egg:**

The egg weight, volume and shell weight of SUP eggs were significantly lesser at initiation of egg lay as well after 30 days compared to those of NLD eggs. There was no much difference in shell thickness, egg width, height, yolk weight and albumen weight in either 1$^{st}$ day eggs or 30$^{th}$ day eggs of SUP hens compared to NLD hens. The yolk weight of SUP eggs was less and that of albumen weight was higher than NLD eggs (Table 1.4)

**Biochemical composition of Egg:**

The protein content of yolk and albumen of first day eggs were significantly lower in the SUP eggs but the yolk protein content of 30$^{th}$ day eggs were almost similar in both SUP and NLD hens, while the albumen protein content was significantly higher in SUP eggs.

There was no much difference in yolk total lipid content of 1$^{st}$ day eggs and 30$^{th}$ day eggs of NLD and SUP hens. However, there was significant increase in albumen total lipid content in 1$^{st}$ day eggs and significant decrease at 30$^{th}$ day eggs of SUP hens compared to NLD hens.

The SUP eggs at initiation of lay and 30$^{th}$ day after initiation showed decreased total cholesterol level when compared to NLD eggs both in yolk and albumen (Table 1.5; 1.6).

**Water and Lipid Index:**

SUP eggs did not show any increase in yolk lipid index and albumen water index, though there was an increase in yolk water index and decrease in albumen lipid index (Table 1.7).

**Table 1.1: Age at first egg and total no. of eggs in a month after initiation of lay**

| Treatment | Age at first egg | Total no. of eggs/month |
|---|---|---|
| **NLD** | 158<br>±2.502 | 25.33<br>±2.027 |
| **SuP** | 116.481[c]<br>±4.842 | 24.66<br>±2.403 |

**Table 1.2: Average egg weight under the effect of step-up photoperiod**

| Days | NLD | SuP |
|---|---|---|
| 1 | 28.34<br>±2.822 | 23.04<br>±1.142 |
| 5 | 29.18<br>±1.896 | 24.83<br>±1.620 |
| 10 | 32.51<br>±1.646 | 28.43<br>±1.98 |
| 15 | 34.96<br>±1.367 | 30.16<br>±0.138 |
| 20 | 35.81<br>±1.367 | 33.51<br>±0.138 |
| 25 | 40.18<br>±1.535 | 33.89[a]<br>±1.16 |
| 30 | 43.74<br>±1.943 | 34.83[b]<br>±1.28 |
| **Over all Egg wt.** | **34.96<br>±2.117** | **29.81<br>±1.75** |

NLD: Control; SuP: Step-up 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 1.3: Effect of Step-up photoperiod on % water content and % dry content /100gm of egg's yolk and albumen**

| Treatment | Day of Initiation of Egg lay | | | | 30th day after initiation of egg lay | | | |
|---|---|---|---|---|---|---|---|---|
| | Yolk | | Albumen | | Yolk | | Albumen | |
| | % water content | % dry content | % water content | % dry content | % water content | % dry content | % water content | % dry content |
| NLD | 46.968 ±4.58 | 53.029 ±4.581 | 85.843 ±0.539 | 14.155 ±0.539 | 44.87 ±1.429 | 55.128 ±1.429 | 82.404 ±3.399 | 17.595 ±3.99 |
| SuP | 52.04 ±2.368 | 48.48 ±2.389 | 85.54 ±0.054 | 14.45 ±0.053 | 51 668[a] ±1.477 | 48.330[a] ±1.477 | 81.404 ±0.549 | 18.595 ±0.549 |

NLD: Control; SuP: Step-up 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 1.4: Physical parameters of Egg under the effect of Step-up Photoperiod**

| Treatment | Day of Initiation | | 30th Day after Initiation | |
|---|---|---|---|---|
| | NLD | SuP | NLD | SuP |
| Weight (gm) | 27.30 ±0.8158 | 22.41 ±3.246 | 42.62 ±3.975 | 33.65[a] ±0.124 |
| Volume (cc) | 23.93 ±2.245 | 19.53 ±5.783 | 39 03 ±3.790 | 29.61[a] ±0.348 |
| Shell Wt. (gm) | 2.39 ±0.1194 | 1.92 ±0.404 | 3.38 ±0.278 | 2.94 ±1.050 |
| Shell Thickness (cm) | 0.033 ±0 0003 | 0.035 ±0.002 | 0.026 ±0.002 | 0.028 ±0.001 |
| Width (cm) | 3.44 ±0.0223 | 3.18 ±0.117 | 3.86 ±0 1624 | 3.76 ±0.450 |
| Height (cm) | 4.36 ±0.0683 | 4.21 ±0.391 | 5.43 ±0.2376 | 5.42 ±0.386 |
| Yolk Weight (gm) | 7.31 ±0.247 | 7.01 ±2.877 | 14.34 ±1.678 | 12.13 ±0.461 |
| Albumen Weight (gm) | 18.39 ±0.87 | 19.35 ±2.800 | 19.72 ±1.965 | 27.89 ±3.024 |
| Yolk: Albumen ratio | 0.39 | 0.36 | 0.72 | 0.43 |

NLD: Control; SuP: Step-up 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 1.5: Egg composition in mg/100mg yolk or albumen under the effect of step-up photoperiod**

| Treatment | On the day of initiation of lay | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total protein | | Total lipid | | Total cholesterol | | Calorific value |
| | Yolk | Albumen | Yolk | Albumen | Yolk | Albumen | |
| NLD | 23.026 ±1.90 | 22.183 ±2.383 | 20.56 ±1.440 | 0.020 ±0.0013 | 1.733 ±0.266 | 0 0095 ±0.0081 | 155.26 |
| SuP | 19.675 ±2.181 | 15.996 ±2.219 | 18.437 ±1.984 | 0.037 ±0.005 | 1.0839 ±0.259 | 0.004 ±0 0009 | 120.85 |

NLD: Control; SuP: Step-up 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 1.6: Egg composition in mg/100mg yolk or albumen under the effect of step-up photoperiod**

| Treatment | On 30th day after initiation of lay | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total protein | | Total lipid | | Total cholesterol | | Calorific value |
| | Yolk | Albumen | Yolk | Albumen | Yolk | Albumen | |
| NLD | 23.895 ±3.733 | 17.343 ±2.203 | 32.162 ±2.721 | 0.028 ±0.0035 | 2.461 ±0.293 | 0.0005 ±0 0001 | 167.98 |
| SuP | 23.562 ±2.322 | 24.329 ±3.640 | 30.052 ±2.835 | 0.0142 ±0.0003 | 1.138 ±0.226 | 0.0006 ±0.0001 | 204.42 |

NLD: Control; SuP: Step-up Photoperiod

Values expressed as Mean ± S.E, n=6;

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

**Table1.7: Effect of Step-up photoperiod on water and lipid indices of egg's yolk and albumen**

| Treatment | Day of Initiation of Egg lay | | | | 30th day after initiation of egg lay | | | |
|---|---|---|---|---|---|---|---|---|
| | Water Index | | Lipid Index | | Water Index | | Lipid Index | |
| | Yolk | Albumen | Yolk | Albumen | Yolk | Albumen | Yolk | Albumen |
| **NLD** | 0.88 | 6.06 | 0.38 | 0.0014 | 0.81 | 4.68 | 0.58 | 0.0015 |
| **SuP** | 1.07 | 5.91 | 0.38 | 0.002 | 1.06 | 4.37 | 0.62 | 0.0075 |

NLD: Control; SuP: Step-up 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.1.1: Age at First Egg

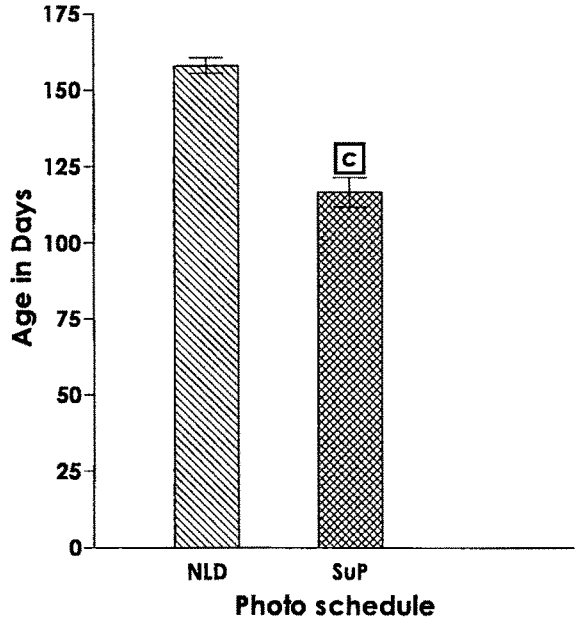
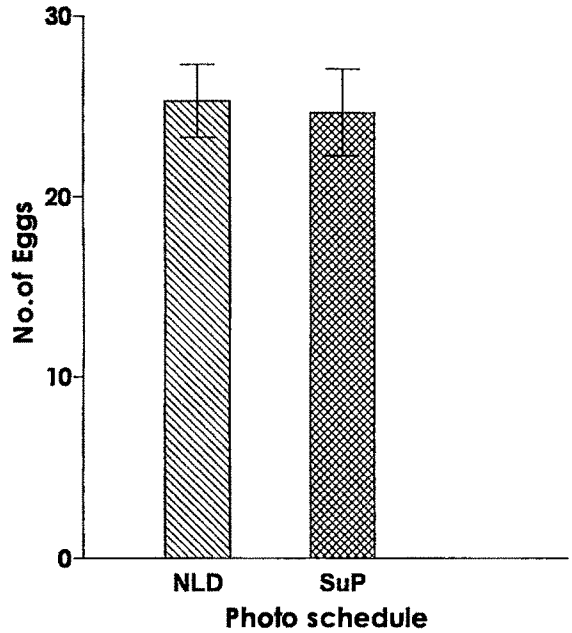



Fig. 1.2: Total No. of Eggs laid




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.3: Average Egg Weight

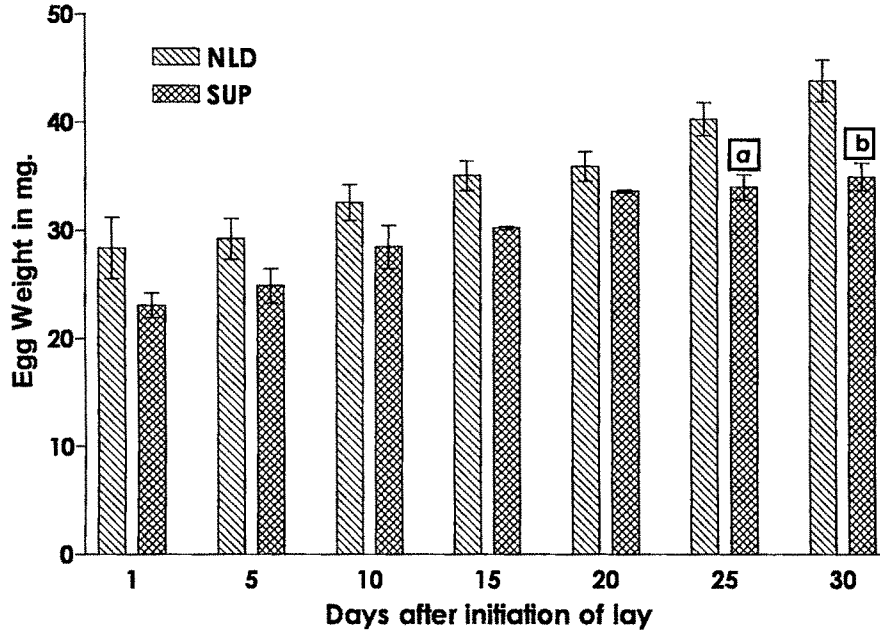
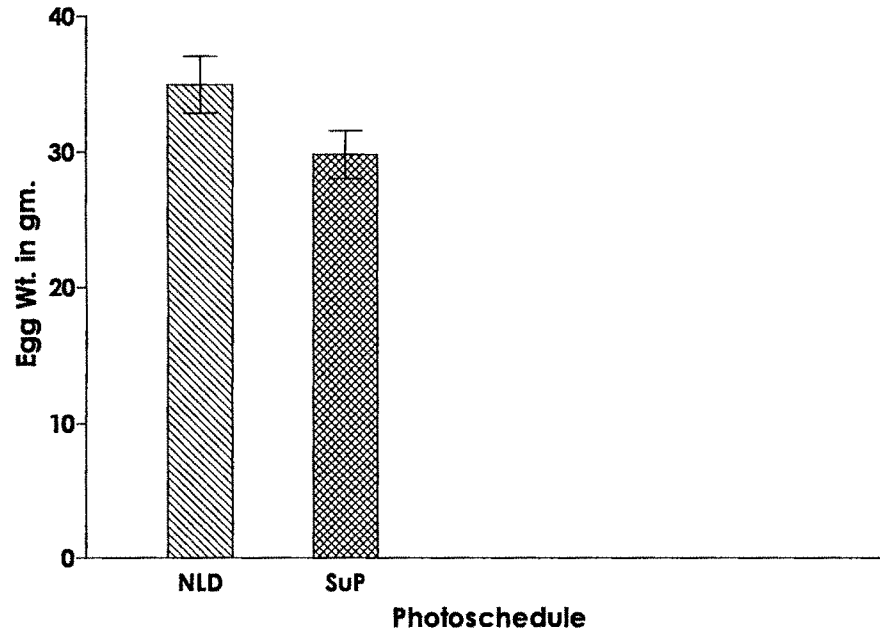



Fig. 1.4: Overall Egg Weight, 30days after initiation.




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.5: % Water and Dry content in the yolk and albumen on the day of initiation of lay

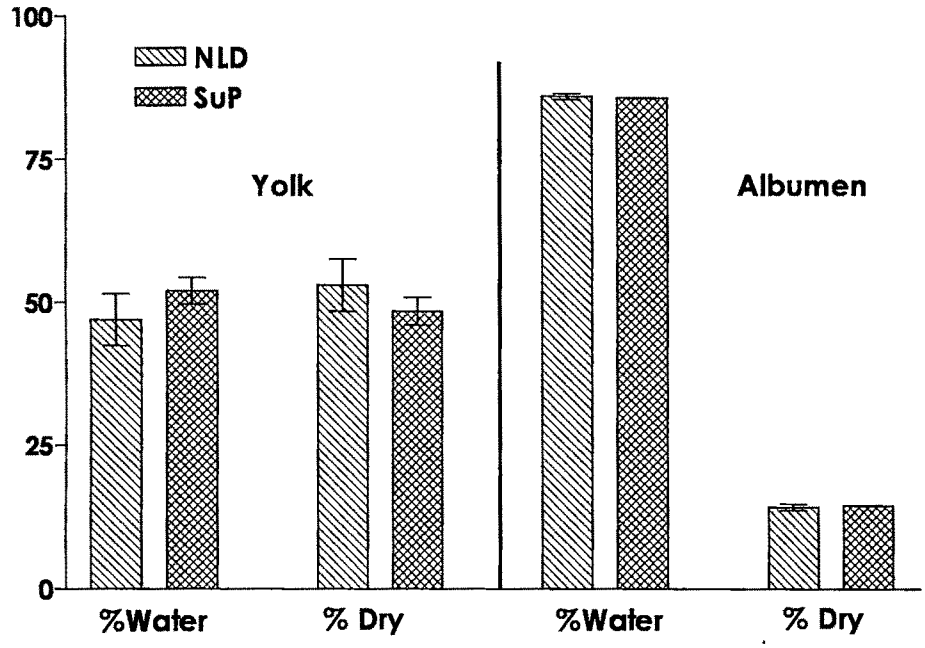
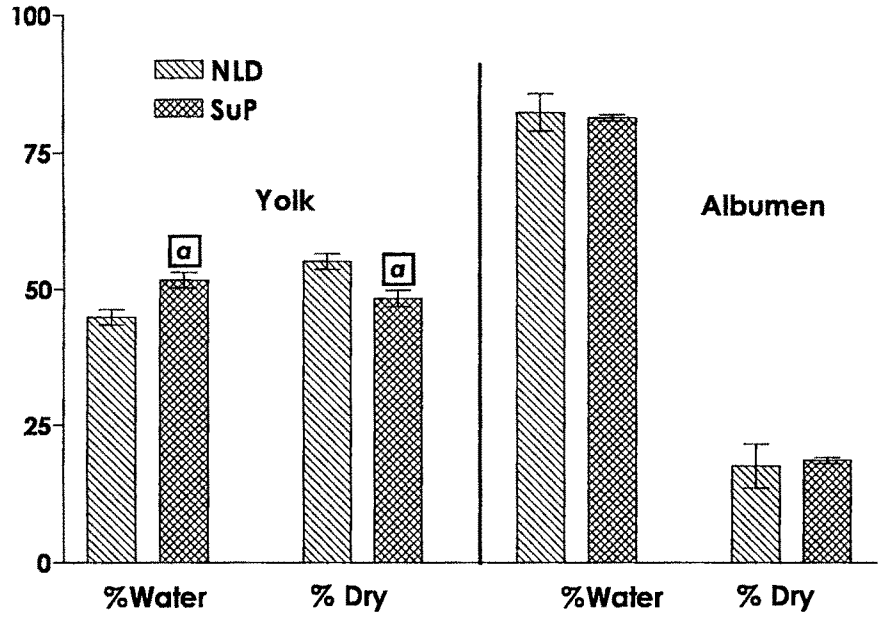



Fig. 1.6: % Water and Dry content in the yolk and albumen on the 30th day after initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.7: Total protein in yolk and albumen on the day of initiation of lay

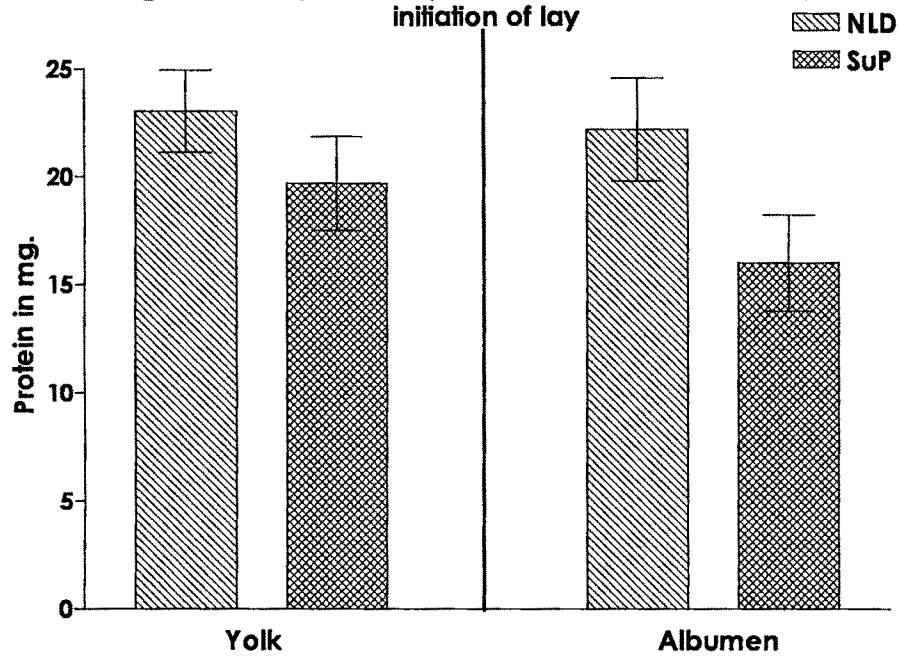
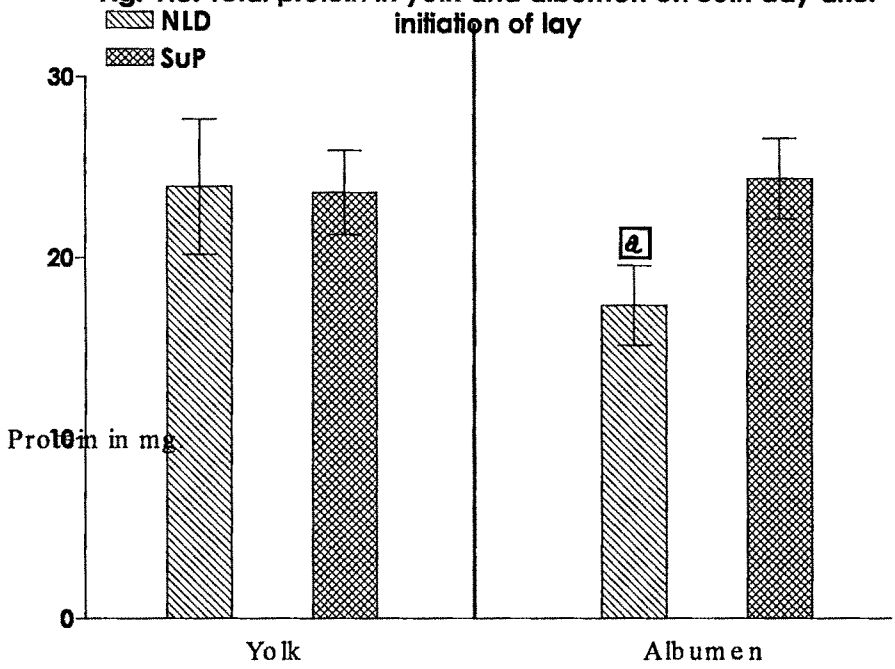



Fig. 1.8: Total protein in yolk and albumen on 30th day after initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.9: Total Lipid in Yolk on the Day of initiation of lay

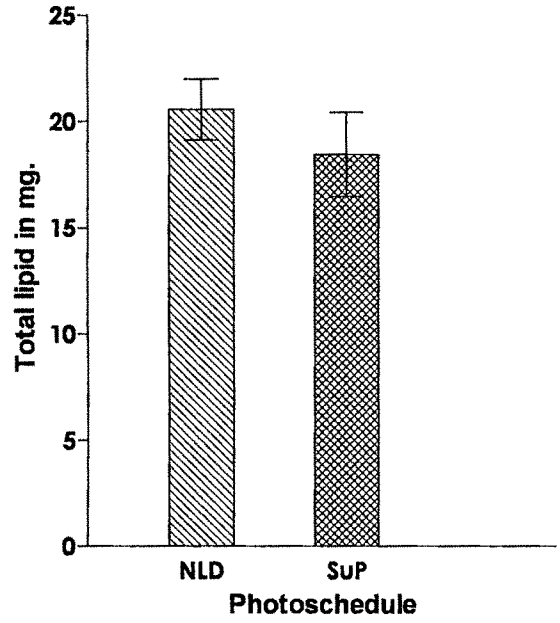
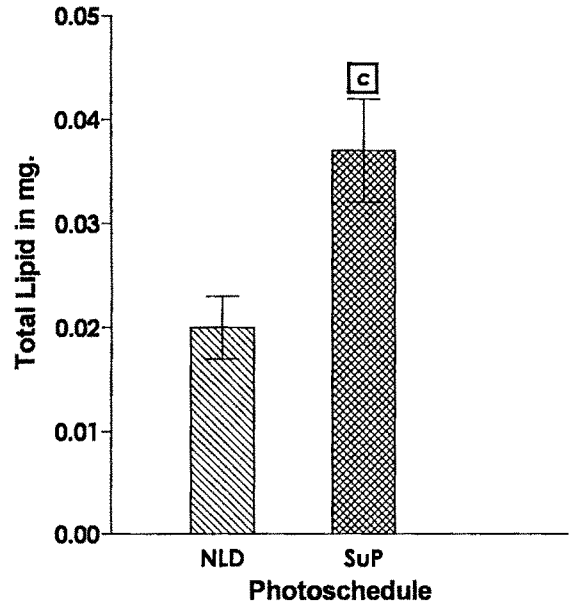



Fig.1.10: Total Lipid in albumen on the day of initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.11: Total Cholesterol inYolk on the day of initiation of lay

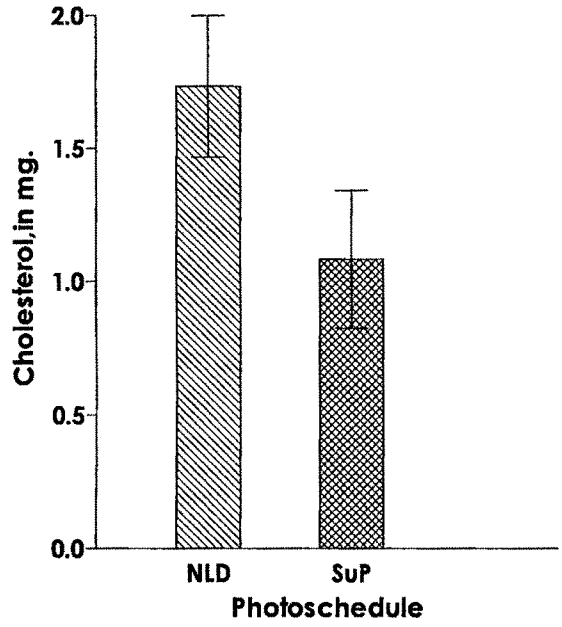
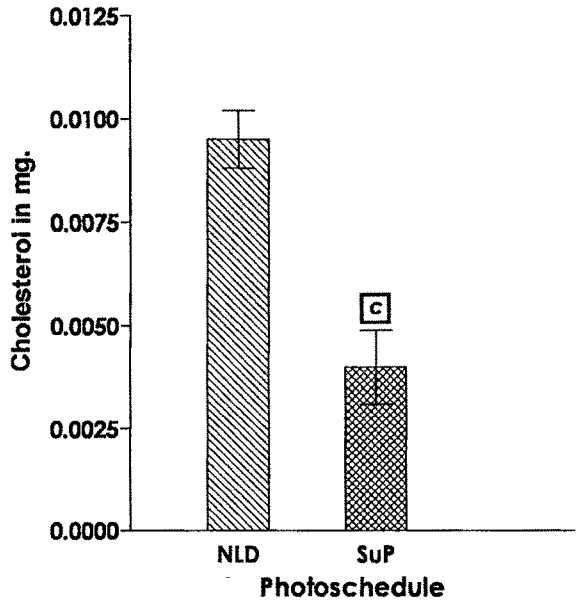



Fig. 1.12: Total Cholesterol in albumen on the day of initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.13: Calorific Values of eggs

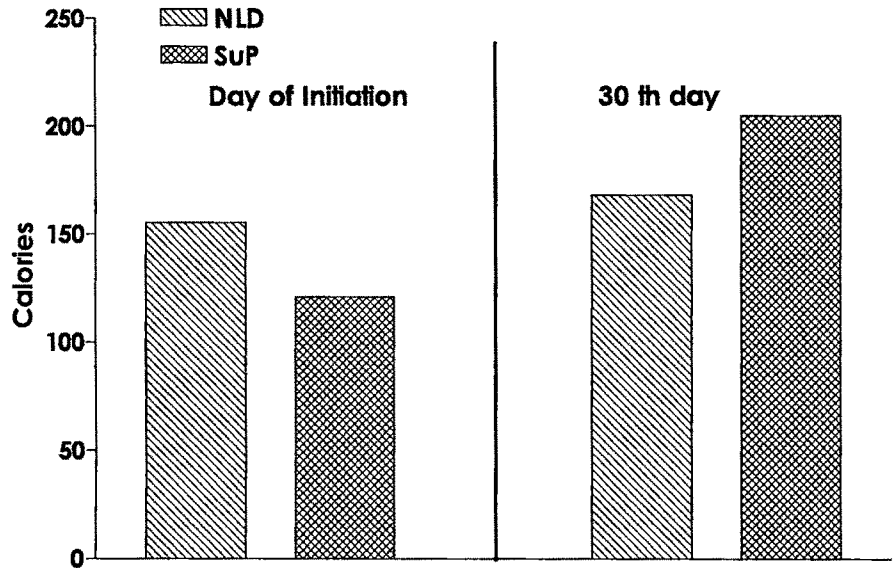
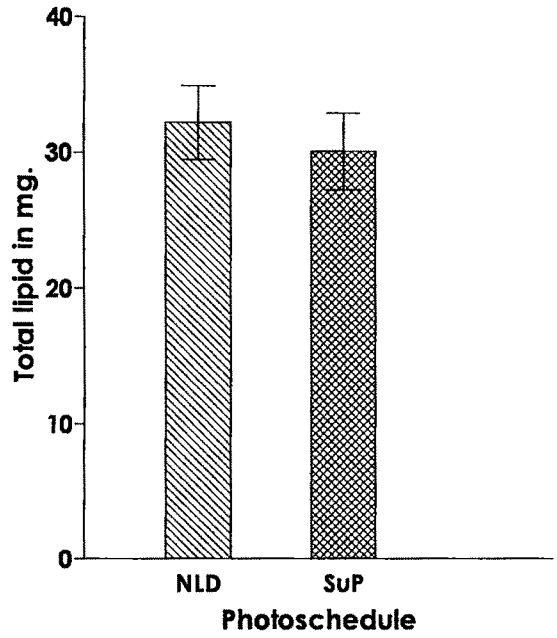



Fig. 1.14: Total Lipid in Yolk on 30th day after initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.15: Total lipid in albumen on 30th day after initiation of lay

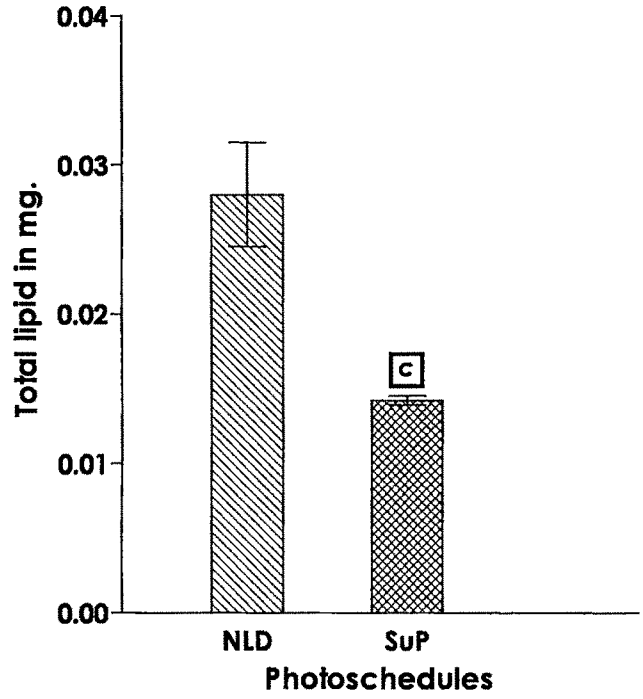
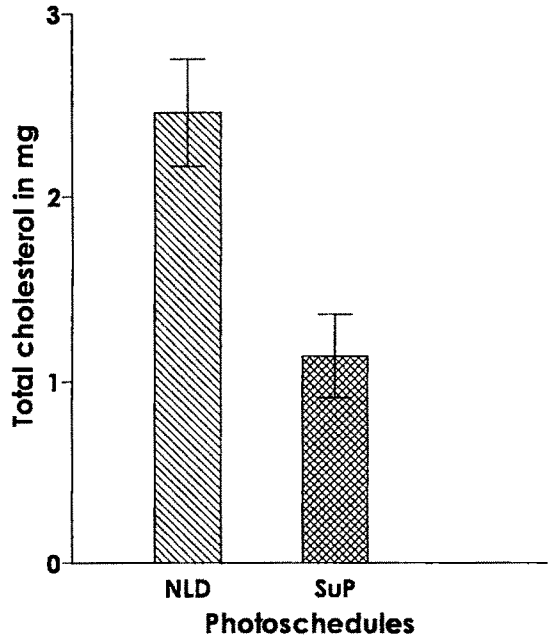
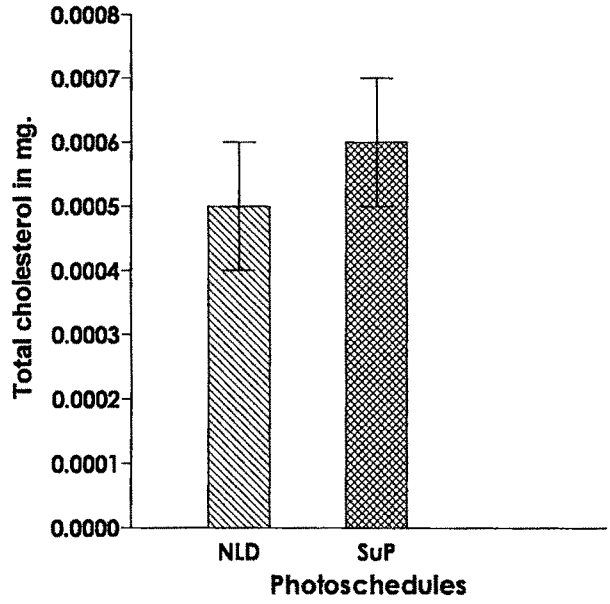



Fig. 1.16: Total cholesterol in yolk on 30th day after initiation of lay




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

Fig. 1.17: Total cholesterol in albumen on 30th day after initiation of lay.




NLD: Normal light dark, SuP: Step Up photoperiod
a: $p \leq 0.05$, b: $p \leq 0.02$, c: $p \leq 0.001$ of 6 animals.

## DISCUSSION:

The results of the present study involving exposure of rearing chicks to a step-up photoperiod (SuP) of 8 hrs (from LD 8:16 to LD 16:8) at 90 days significantly advances age at first egg with, gradually increasing egg weight and calorific value towards the end of the first month of lay. Chicks reared under a constant photoperiod of LD 12:12(NLD) throughout, initiated egg laying on 158$^{th}$ day while chicks subjected to a SUP initiated egg laying on 116$^{th}$ day. The advancement in initiation of lay by 42 days obtained in the present study is a greater response compared to an 18 day advancement seen in ISA brown and Shaver 288 breed of hens when the photoperiod was changed from LD 8:16 to 13:11 (Lewis *et al.*, 1996a, 1997). In the above breeds, maximum advancement in initiation of lay was only by 33 days, when the photoperiod was changed from 8 to 13 hrs at 63 days and, a minimum advancement by 6 days when the shift was done at 119 days (Lewis *et al.*, 1996a). Based on their studies, they have concluded that a SUP from 8-13 hrs made at 63 or 84 days is most optimal and that the ISA birds are generally more responsive than the Shaver birds (Lewis *et al.*, 1997). A previous study from our laboratory on RIR hens had shown an advancement in initiation of lay by 58 days by a SuP from 6 hrs to 12 hrs given at 90 days (Dandekar, 1998). Clearly, SuP of 6 hrs duration is more optimal than 8 hrs duration in advancing

initiation of egg laying in RIR breed. Though tropical animals are considered to be less photosensitive, our observations suggest that the RIR pullets are definitely photosensitive and can be manipulated for reproductive efficiency and egg laying performance. It is interesting to note that the RIR breed developed under tropical conditions have shown a greater response to photoperiodic changes compared to temperate adapted breeds like ISA brown and Shaver 288. Since the studies in RIR breeds involved a light intensity of 250 lux as compared to only 10 lux in the temperate breeds, the observed difference could either be related to a potential genetic difference or even to the intensity of light used. Since no studies have been carried out with different light intensities on RIR breed, it is difficult to ascertain the optimal intensity of light for reproductive maturation in this breed. However differential responses towards different intensities of light have been recorded in the temperate breeds as ISA brown pullets has been shown to respond maximally at 2 lux intensity with no further change at 25 lux intensity and at the same time Shaver white pullets has been shown to respond maximally at 25 lux than at 3 lux (Lewis *et al.*, 2004). The total number of eggs laid during the first month shows no significant difference between NLD and SUP birds. Since the previous study showed a 15% higher egg yield during the first month (Dandekar, 1998), the difference may be accredited to

more favourable SuP of 6 hrs durations than of 8 hrs duration. There is no significant difference either in laying performance in first month nor in increase in egg weight between NLD and SUP birds. The eggs laid by SuP hens during the first month were at an average 19% lighter than those laid by NLD hens. The eggs of both groups of birds showed 51% (SuP) to 54 % (NLD) increment in egg weight from first laid eggs to 30$^{th}$ day eggs.

In terms of physical parameters, the egg weight, volume and shell weight of first laid SuP eggs were significantly lesser by 17.9%, 18% and 19.6% and of the 30$^{th}$ day eggs by 21%, 24% and 13% respectively compared to those of NLD eggs. Though there was not much difference with reference to shell thickness, egg width, egg weight, yolk weight and albumen weight in either 1$^{st}$ day eggs or 30$^{th}$ day eggs, the yolk weight and albumin weight of 30$^{th}$ day eggs were different. Whereas the yolk weight of SuP eggs was lesser by 15.4%, the albumen weight was higher by 27.8%. Such a generalized reduction of various physical parameters even in overall lay was also reported by Dandekar *et al.* (1999) in their experiments involving 6-hour increment in rearing photoperiod. It is clear that the observed albumen and yolk contents are much lesser than the range reported for poultry eggs (31-35% for yolk and 52-60% for albumen) (see Panda, 1995; Etches, 1996). Since Dandekar *et al.* (1999) had registered yolk and albumen contents in this range in their study involving overall

lay, it is surmisable that the first month eggs usually have much lesser amounts which would gradually attain the average higher level during the later lay period. Relatively higher albumen content is the feature of SuP eggs as denoted by the lesser yolk:albumen ratio of these eggs compared to NLD eggs (0.35 v/s 0.39 for first eggs and 0.48 v/s 0.72 for 30$^{th}$ day eggs). Such an observation was made by Dandekar *et al.* (1999) as well, for the initial phase eggs in their study involving complete laying cycle. A comparison of the percentage content of water and solids in the yolk and albumen shows that, while they remain invariantly identical in the albumen of the eggs of both NLD and SuP hens, there is significant increment in the water content with a concomitant decrement in the total solid content in the yolk of SuP eggs. Importance of water content for avian embryonic development and the relatively lesser content of water in the yolk due to increase in lipid load have been well recognized (Roca *et al.*, 1984).

The protein content of yolk and albumen of first day eggs was significantly lower in the SuP eggs but the yolk protein content of 30$^{th}$ day eggs was identical in both SuP and NLD while, the albumen protein content was significantly higher in the SuP eggs. The herein recorded protein content seems to be higher than the range documented by Etches (1996) in their reviews. However, Sainz *et al.* (1983) and Roca *et al.* (1984) have reported similar

protein content in the yolk of RIR hens. But the protein content in the albumen of Indian RIR breed is slightly more than that reported by the above workers. Whereas there was no difference in yolk protein content and a decrease in albumen protein content of NLD eggs from first day to 30$^{th}$ day both the yolk and albumen protein contents increased significantly in the SUP eggs from first day to 30$^{th}$ day. In keeping with the observed increase in protein content from 1$^{st}$ day to 30$^{th}$ day, Superchi *et al.* (2002) have observed increasing protein content of albumen in Ostrich eggs during the laying period, towards the 60$^{th}$ day. Since one of the functions of albumen and particularly the protein fraction consisting of ovalbumen, ovatransferrin, ovamineoid, lysozyme etc. is protection of the embryo against bacterial attack, the increased albumen protein content recorded in the SUP eggs would suggest a favourable influence of step-up rearing photoperiod in affording protection against bacterial infections. This bacterio-protective effect is due both to a higher albumen viscosity, which restricts bacterial movements and, to the direct action of lysozyme that catalyzes hydrolysis of β-glycosidic bonds of polysaccharides in the cell wall (Mac Donnel *et al.*, 1954).

Whereas both the yolk and albumen total lipid contents increased from 20.50% to 32.16% and 0.02% to 0.028% respectively in NLD eggs from day 1 to day 30 only the yolk lipid

content increased from 18.4% to 30.0% while the albumen lipid content decreased from 0.037% to 0.014% in the SuP eggs. The presently recorded lipid contents are in range with the values of 34% and trace (Brody, 1945; Romanoff and Romanoff, 1949), 35.2% and 0.42% (Roca, 1984), 32.2% in yolk (Hall and McKay, 1993) and 29-34% and trace to 0.05% (Panda, 1995; Etches, 1996). The lipid contents of SuP eggs are lesser than those of NLD eggs. This is in contrast to the higher lipid content in the SuP eggs reported previously (Dandekar *et al.*, 1999).

Since the above study took into consideration all the eggs laid in the complete lay period, it is presumable that the lipid content of SuP eggs can increase as a long-term consequence of photoperiodic manipulation. A temporal increase in yolk lipid content from 1$^{st}$ to 60$^{th}$ day of lay has also been reported in the Ostrich (Superchi *et al.*, 2002). Lipids usually constitute about 30% of yolk and they are the primary nutrient source to the developing embryo (Speake *et al.*, 1998). Lipids also provide a range of essential components for tissue development and functionality (Noble *et al.*, 1996a) and also Supply over 90% of energetic needs. The β-Oxidation of fatty acids is the predominant pathway of energy provision in this system (Freeman and Vince, 1974) and approximately 50% of the initial fatty acid content of the yolk is recovered in the tissue lipids of the chick (Noble and Coechi, 1990; Lin *et al.*, 1991) while, the

remaining part is used for energy production. Dandekar (1998) had reported higher lipid and water indices in SuP eggs. But in the present study, SuP eggs did not show any increase in yolk lipid index or albumen water index, though there is an increase in yolk water index and a decrease in albumen lipid index.

Yolk cholesterol content is relatively higher than the albumen cholesterol content and in both the 1st day as well as 30th day SuP eggs the cholesterol content in general was significantly lower than in the NLD eggs. Interestingly, Hall and McKay (1993) had inferred the occurrence of higher yolk cholesterol content in the early eggs of Hisex-Brown breed of hens commencing lay at an earlier age to be due to a high cholesterol laden plasma lipoprotein in the immature birds as against low cholesterol laden plasma lipoprotein in the mature birds. In conformation with the above, previous study SuP in RIR hens showed higher yolk cholesterol content in the initial phase of lay (Dandekar, 1998). However in the present study the same could not be duplicated and may be speculated to be related with the difference in the SuP schedule of six hours increment in the former and of eight-hour increment in the present. In terms of nutritional value, the eggs of SUP hens at 30 days is Superior as the energy content of 100 gms of edible egg was higher by 22% compared to that of NLD eggs, despite the fact that the energy content of 1st day SuP eggs was lower by 22%. Apparently, the reduction in egg size is

adequately compensated by higher energy content in the SuP eggs.

Overall, the present results provide evidences for an alteration in egg composition and energy content of eggs due to exposure of immature chicks to SuP during the rearing period.

## Summary:

The present study has tried to evaluate the effect of rearing of pullets of RIR breeds on a short photoperiod of 8 hrs from day one till 90 days of age and then shifting them to a long photoperiod of 16 hrs till 120 days and then maintaining them under constant photoperiod of 12 hrs, on age at first egg, total lay during the first month, physical parameters of eggs and biochemical composition compared to pullets reared under a constant photoperiod of 12 hours. Results were, significant early initiation of egg lay of SuP birds (42 days) than the NLD birds. The weight of eggs laid by SuP hens on the day of initiation of lay was less compare to those laid by NLD hens. The protein content of yolk and albumen of first day eggs were significantly lower in the SuP eggs. Overall, it can be concluded that exposure of rearing chicks to a step-up photoperiod (SuP) has a significant effect on the age at first egg, egg weight and calorific value.