Endocrine based molecular investigation on induced moulting and interventions to improve post-moult production in layers

Thesis

Submitted in partial fulfillment of the requirement for the degree

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In

POULTRY SCIENCE

Submitted by

ANISH. D.

Roll No: 970

To

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Dedicated to

MY PROFESSION

Division of Physiology and Reproduction
Central Avian Research Institute
Izatnagar, Bareilly, UP-243122

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Principal Scientist and Head

Dated: th September 2008

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Certified that the research work embodied in this thesis entitled **"Endocrine based molecular investigation on induced moulting and interventions to improve post-moult production in layers"** *submitted by* **Dr. Anish. D. (Roll No. 970)**, *for the award of* **PhD** *degree of Deemed University, Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

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We have carefully gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented by him for award of ***PhD Degree*** *of this institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of ***PhD Degree*** *of Indian Veterinary Research Institute, Izatnagar.*

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-Anish D-

Contents

Abbreviations

$	:	Dollars
%	:	Per cent
α	:	alpha
β	:	Beta
µl	:	Microliter
µM	:	Micromolar
µg	:	micrograms
ACTH	:	Adrenocorticotropic hormone
ANOVA	:	Analysis of variance
AVMA	:	American Veterinary Medical Association
bp	:	Base pairs
BW	:	Body weight
Ca	:	Calcium
cDNA	:	Complementary deoxyribonucleic acid
cm	:	Centimeter
CP	:	Crude protein
d	:	days
DEPC	:	Diethyl pyrocarbonate
DNA	:	Deoxyribonucleic acid
dNTP	:	Deoxynucleotide triphosphate
dUTP	:	Deoxyuridine triphosphate
EDTA	:	Ethylenediamine tetraacetate
ER	:	Estrogen receptor
FSH	:	Follicle stimulating hormone
FW	:	Feed withdrawal
g	:	Gram(s)
GH	:	Growth hormone
GHRH	:	Growth hormone releasing hormone
GHSR	:	Growth hormone secretagogue receptor
GLP	:	Glucagon-like peptide
GnRH	:	Gonadotropin releasing hormone
H:L	:	Heterophil:Lymphocyte
I	:	Iodine
ie	:	that is

IGF	:	Insulin-like growth factor
kg	:	Kilogram(s)
LH	:	Lutenizing hormone
M	:	Molar
ME	:	Metabolizable energy
$MgCl_2$	:	Magnesium chloride
ml	:	Milliliter
mm	:	Millimeter
mM	:	Millimolar
mRNA	:	Messenger ribonucleic acid
Na	:	Sodium
nm	:	Nanometer
NTC	:	No-template control
^oC	:	Degrees centigrade
PCR	:	Polymerase chain reaction
PCV	:	Packed cell volume
pg	:	Picogram
PMD	:	Post-moult diet
pMol	:	Picomol
ppm	:	Parts per million
PR	:	Progesterone receptor
RBC	:	Red blood corpuscles
Re	:	Rupee
RNA	:	Ribonucleic acid
Rs	:	Rupees
s	:	seconds
T_3	:	Triiodothyronine
T_4	:	Thyroxine
Taq	:	*Thermus aquaticus*
TBE	:	Tris boricacid EDTA
UEP	:	United Egg Producers
UK	:	United Kingdom
USA	:	United States of America
UV	:	Ultra violet
wk	:	weeks
Zn	:	Zinc

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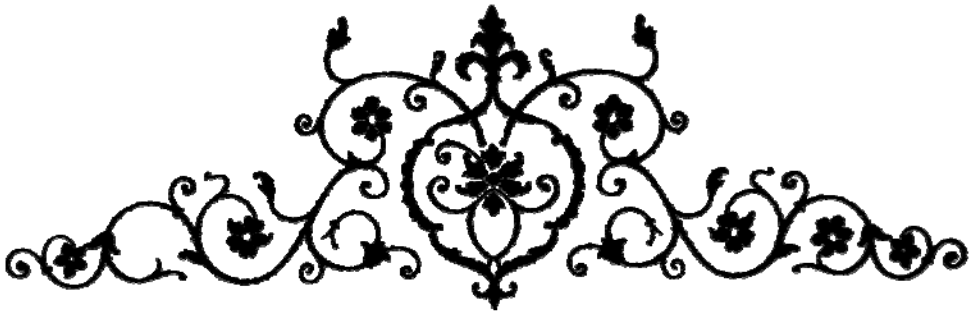
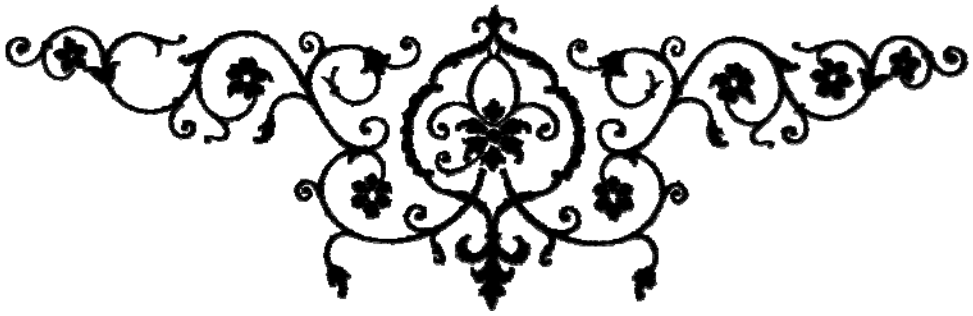


INTRODUCTION



Introduction

India, the world's second largest developing country, is contributing to the development through the tremendous growth of its poultry sector. In India, poultry sector growth is being driven by rising incomes, together with the emergence of vertically integrated poultry producers that have reduced consumer prices by lowering production and marketing costs (Landes *et al.*, 2004). In recent years, global ranking position of India with respect to egg and meat production is improving.

With the fast growth, however, egg industry has been facing several challenges due to more or less stabilized or reduced egg prices in contrary to alarmingly increasing trend in feed costs, lower returns from spent hens and higher cost of replacement pullets and chicks. Collectively, all these factors result in a shrunken profit margin to the egg farmer sometimes even leading to losses.

Subject to the use of layer for a second cycle of egg production, the chick cost and feed cost for raising layer pullets can be saved and this is likely to reduce the expenditure to a great extent. Moulting of birds is a method of recycling of old layer birds. An egg farmer resorts to the practice of moulting when;

1. Funds to replace new pullets are scarce or not available.
2. The seasonal egg rates are low.
3. The replacement flock (chicks) and the facilities to raise are costly and not available.
4. There is a market for extra large eggs at premium rates.
5. There are low returns from spent hens.

The conventional all-pullet programme (one cycle programme) requires about 8.4 flocks of layers per house for a period of 10 years. In case the flocks are moulted after a year of lay for a second cycle of production, it would require only 5.4 flocks per house for 10 years and moreover, moulting results in at least 30% higher profit margins compared to all-pullet programmes (Bell, 2003).

Moulting in avian species is a natural phenomenon resulting in periodic shedding and replacement of feathers. In wild species of birds, moulting also involves reproductive quiescence. The domestic hen likewise experiences a period of moult about the end of the laying season. However, during a naturally occurring moult in domestic hens, the reproductive quiescence is incomplete and it continues to lay at a lower rate for a prolonged period (Berry, 2003). This is an unprofitable time for the farmer, who, to avoid the escalating losses, discard the stock and purchases a new batch of chicks / pullets. But, an artificial induction of moulting stimulates the birds to shed the feathers at a faster rate and grow expeditiously a new set of feathers. It also provides a rest period (pause) to the layers after a long period of egg production in which the bird gets sufficient time for regression and rejuvenation of reproductive organs. Thereafter the bird restarts egg production at a profitable rate.

Many methods are reported to cause initiation of moulting in chicken. Moulting by feed withdrawal is the most prevalent. Alternative methods of moulting include manipulation of some dietary minerals such as zinc, calcium, iodine and sodium and administration of some drugs or hormones. Besides spotlighted as an inhumane method, the starvation moult also results in a reduction in humoral and cell mediated immune status in birds causing an increased risk of salmonella infection (Holt, 1993; 2003). The colonization of salmonellae in reproductive tract results in its presence in eggs causing a public health hazard. As the resistance against moulting by feed withdrawal is gaining momentum, the search for a more humane, alternative method of induced moulting has intensified all over the world. However, any of the existing alternative methods are not efficient compared to classical feed withdrawal method. A thorough knowledge about the physiology of moulting will be helpful in designing an equally animal-friendly and profitable method of induced moulting.

One of the most spectacular physiological changes during moulting relates to the variations in levels of different hormones in blood during an induced moulting in chicken. A main mechanism suggested on moulting explains that the withdrawal of feed and reduction in the light period causes a reduction in the

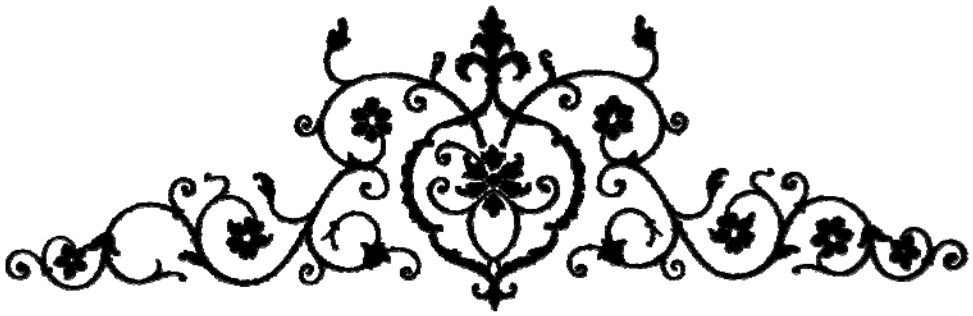
GnRH production from the hypothalamus resulting in a decline in gonadotropin production from the pituitary gland. Subsequently there is a massive follicular atresia in the ovary, resulting in a lower steroid hormone support to oviduct causing atrophy of the latter (Etches *et al.,* 1984). To understand the exact role of steroid hormones, the mRNA expression profile estradiol and progesterone receptor during moulting at different parts of oviduct is still obscure.

Even though the primary target of the moulting methods is a regression of the reproductive tract and its subsequent remodeling, there are sufficient evidences to suggest that the cue for this might be originated from elsewhere in the body. The role of digestive tract in moulting has not received a deserving attention of research workers. Ghrelin hormone inhibits food intake (Saito *et al.,* 2005) and mRNA of ghrelin receptor has shown an increase during fasting in chicken (Chen *et al.,* 2007). Another polypeptide hormone, leptin, is produced by both adipose tissue and the liver and has been shown to induce satiety in chickens (Ashwell *et al.,* 2001). The essential role of leptin predominantly in hypothalamus levels has been well evidenced in literature in regulating the feed intake by markedly inhibiting the feed intake (Brunner *et al.,* 1997; Taouis *et al.,* 2001; Shi *et al.,* 2006). These studies suggested an interactive association between the digestive and reproductive tracts. Further understanding on the mRNA profiles of these hormonal factors/receptors in different parts of digestive tract during induced moulting needs to be sorted out.

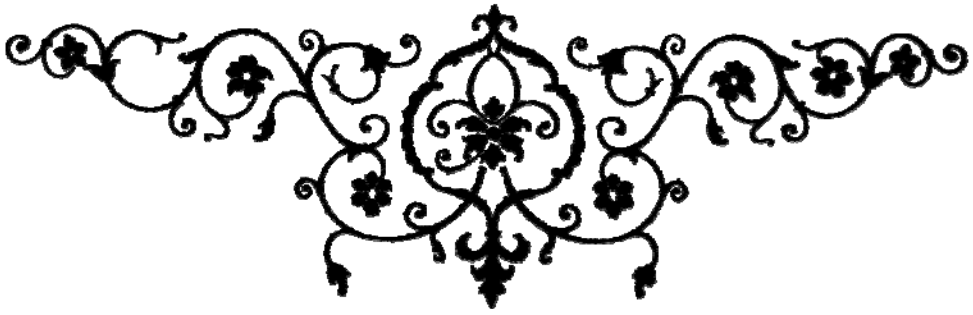
Another hormonal event associated with moulting is the change in serum thyroxine levels. Serum thyroxine increases during a moult in wild and captive birds, and thyroidectomy prevents induction of moulting. Oral thyroxine, in purified form or iodinated casein induces a molt and may enhance animal well-being by reducing the need for feed withdrawal (Bass *et al.,* 2007). Administration of thyroxine orally may cause a moult faster and thus may be advantageous on economic terms. The present study has also considered the use of thyroxine along with estrogen and progesterone hormone analogs to manipulate the pre- and post-moult periods in hens.

In view of the foregoing rationality, the stated objectives of the thesis work were:

1. To investigate the mRNA expression patterns of certain endocrine constituents and/or their receptors in association with induced moulting in chicken.
2. Endocrine interventions in California method of induced moulting to replace / shorten the induction period and to improve post treatment reproductive performance.



REVIEW OF LITERATURE



Induced or forced moulting is a management practice where in the birds are forced to shed their feathers and stop temporarily their egg production temporarily. This practice leads to regression and rejuvenation of reproductive organs resulting in a better performance after the rest period. Induced moulting has wide spread acceptance during 1930s and, the egg producers in California, USA adopted the practice almost commonly by 1950s. In 1970s, this practice has spread to most major egg producing countries (Bell, 2003).

2.1 Justification for Moulting

Moulting, in nature or induced by the farmer has the same effect-rejuvenation of the flock with higher egg production, renewal of feathering, and improvements in egg quality. This not only lengthens the productive life of flocks but also provides a means of optimizing the use of resources with enhanced returns on investments (Bell, 2003).

2.2 Performance of Moulted Birds

2.2.1 Egg production: Moulting improves egg production about 14% on comparison to the start of treatment (Narahari, 2001). An increased egg production rate would need to extend beyond six months post-moult to give induced moulting an advantage in total number of eggs produced (Wilson *et al.,* 1967).

2.2.2 Egg quality: Post-moult eggs are larger than the pre-moult eggs (Bell, 2003; Narahari, 2001). Albumen height significantly improves due to moulting (Lee, 1982). The Haugh unit scores improves by induced moulting to a level of pullets at 5 months of lay but this increase is temporary (Len *et al.,* 1964). Shell thickness significantly improves due to moulting (Lee, 1982; Len *et al.,* 1964). The shell quality restoration is to a level of pullets of 5 months of lay (Len *et al.,* 1964). Significant improvement in shell thickness is observed 4 weeks after the induced-moulting period (Lee, 1984). Feed withdrawal for 4 days results in a significant increase in eggshell specific gravities when compared with the control (Christmas *et al.,* 1985).

2.2.3 Feed consumption: During the early postmoult period, birds consume significantly more feed (Lee, 1982). Induce moulted hens eat less feed per dozen eggs produced than control hens (Len *et al.,* 1964). Normally, feed consumption is slightly more in the second cycle, but if weight loss was great, the birds have a tendency to remain light in the second cycle, and therefore consume less feed (North, 1984). However, Bell (2003) observed that practically there are no differences in feed consumption except during the moult period.

2.2.4 Mortality: There is an increase in mortality during the first three weeks of moult, but decreases over the next seven or more weeks to levels below corresponding pre-moult levels (Bell, 2002). Mortality of healthy hens subjected to feed deprivation typical of induced moult programmes is usually not greater than hens left on full feed (Berry and Brake, 1987). Induced moult programmes probably hasten the death of moribund hens, compressing a certain total amount of mortality into a shorter time (Webster, 2003). Individual flocks demonstrate large ranges in moult mortality during this period due to different moulting programmes and to strain and management conditions. Thus it is recommended that daily mortality must be monitored during this critical period (Bell, 2003).

2.2.5 Age at moulting

The average age followed at first moult is 69 wk and second moult is 104 wk. Most producers, however, will vary their selection of moulting age relative to existing egg quality and the price of the eggs. If quality is acceptable and the egg price is high, most producers will delay the regular moult date. If, on the other hand, shell quality is poor and prices are low, an earlier moult may be justified. The moulting age for a flock replacement policy is usually scheduled years in advance in order to optimize the use of buildings and equipment (Bell, 2003). When flocks are moulted young, the second-cycle egg production peaks and rate of lay are higher (Bell and Adams, 1992).

2.3 Economic Analysis of Induced Moulting

It costs less to moult a flock and bring it back to egg production than it does to grow a new flock. This is the major factor in making the decision of whether to moult or not to moult. The most profitable two-cycle programme is moulting the birds at 65 weeks of age and sell them 40 weeks later at 105 weeks (Bell, 2002). In opposition, Shirasaki and Kashiwagi (1987) judge that the most profitable programme was conducting an induced moulting at 80 weeks of age. Moulting causes a 10.7% reduction in egg production costs (Narahari, 2001). Induced moulting, in general, results in better monetary returns when compared to the non-moulted control group (Ravindran *et al.*, 1987). It is estimated that replacement programmes that include moulting results in at least 30% higher profit margins for the egg producer compared to all-pullet programmes. This is based upon a return of $0.65 per year per hen housed for a two-cycle programme compared to $0.50 for a one-cycle programme (Bell, 2003). Narahari (2001) reports that the total cost of egg production was Rs. 1.12 during the pre-moult 20-75 weeks period, whereas the post-moult egg production cost was Re. 1.00, with a saving of 3.57%.

2.4 Public Health Significance of Induced Moulting

Induced moulting through feed removal depresses cellular immunity in hens (Holt, 1992). Delayed type of hypersensitivity was significantly depressed on day 3 of moulting. Dietary restriction has been shown to alter humoral and cellular responses and to increase the susceptibility to disease, including salmonella infection (Holt and Porter, 1992). Moulting increases susceptibility of hens to *Salmonella enterica* serovar Enteritidis infection and moulting birds sheds significantly more *S*. Enteritidis than unmoulted hens (Holt, 1993). The intestinal tract of infected moulted hens shows increased inflammation compared to unmoulted experimentally infected hens (Holt and Porter, 1992).

The primary site of *S*. Enteritidis infection is the intestines, where the organism replicates and then disseminates to various organs including the ovaries, where subsequently replication may occur. Birds that are fasted to

induce a moult and then infected with *S.* Enteritidis release a large number of organism to the intestine, which could be of special concern to the egg producers. The organism is readily transmitted to uninfected birds from infected hens in adjacent cages during moulting (Holt and Porter, 1992). Alternative moulting strategies must not cause the increased severity of intestinal infection found with fasting and yet still allow producers to extend the egg-laying life of their hens.

2.5 Need for Alternative Techniques for Inducing Moulting

In recent years, the induction of moult by feed withdrawal has become more of a concern to the poultry industry in the UK and Europe due to the hen welfare and human food safety reasons. Almonst all egg producers in USA still moult their flocks by feed removal method (UEP, 2006). Feed deprivation results in hunger, which lowers immunity and increases mortality losses of the birds. Additionally, induced moulting by feed removal resulted in a decreased mineralization of the medullary tibia and the humerus although a gradual recovery during the post-moult period was observed (Mazzuco and Hester, 2005).

While the feed withdrawal method has been most effective in inducing moulting, it is deemed unacceptable on hen welfare grounds (Yousaf and Chaudhry, 2008). Therefore poultry scientists have the responsibility to develop and implement suitable alternatives if induced moulting of laying hens is considered essential by the poultry industry (AVMA, 2002). In the UK and Europe, induced moulting by feed withdrawal is strictly banned (DEFRA, 1994). Therefore, the need for an alternative moulting method has dramatically increased with the recent change in United Egg Producers guidelines where feed withdrawal can no longer be used to induce moult in laying hens (Yousaf and Chaudhry, 2008).

2.6 Methods of Induced Moulting

An effective induced moulting procedure causes a complete cessation of egg production for a period of 4 to 8 weeks (Wolford, 1984). The best program

must be able to get a flock out of production rapidly and uniformly, with relatively low mortality, be simple to follow, less expensive and postmoult egg production and egg quality must be only slightly poorer than first-cycle results (Bell, 2002).

2.6.1 Moulting by feed and water withdrawal

During the feed removal, about 25-30% body weight reduction is achieved (Webster, 2003). Several types of feed restriction procedures are used in the egg industry. Some versions include light and/or water restriction while others use grain and/or oyster shell. In addition, within these techniques, there are variations in the restriction period and differences in the amounts of feed used in the recovery period (Hussein, 1996). California method, North Carolina method and Washington method are of prominence among feed withdrawal methods. Conventional fasting techniques for induced moulting cause complete cessation of egg production within 10 d from initiation of fasting (Swanson and Bell, 1971). Considerable variation in the details of moulting procedure was evident with feed removed for as little as 5 d to as much as 14 d. A second source of variation is whether or not to include a resting period following feed removal (Bell, 2003). United Egg Producers (UEP) suggest that the body weight loss must be limited to 30% of the starting weight and that feed should be returned when mortality reaches no more than 1.2% of the starting count (cited by Bell, 2003). Withholding of water increased egg size, which is undesirable in most cases. Withholding of water is not recommended due to increased egg size concerns and increased mortality (Brake and Carey, 1983).

2.6.2 Low nutrient diet programmes

These are used for moulting especially in countries where regulations exist against feed withdrawal. In general, these methods have not proven satisfactory as they account for lesser weight loss (Bell, 2002).

2.6.3 Moulting by changing the level of dietary minerals

Manipulation of different minerals of the diet is also used as a method for inducing the moulting. These include high zinc, high aluminium, high iodine, low calcium and low sodium diets.

High zinc diet has attracted much attention because addition of a minor element is much easier to perform in practice than to get a well-balanced low calcium or sodium diet (Decuypere and Verheyen, 1986). Feeding of high levels (10,000-20,000 ppm) of Zn resulted in cessation of egg production within 5-7 days and upon removal of high Zn diet, hens return to egg production by 3-4 weeks (Wolford, 1984). Among different salts, zinc oxide, zinc carbonate and zinc sulphate are generally used. Except to high zinc diets, other moulting techniques have not proven to be as consistent in halting egg production (Berry, 2003). Combining a low calcium diet with a relatively low level of Zn (2,800 ppm) induces reproductive involution and moulting similar to that of 20,000 ppm dietary zinc (Breeding *et al.,* 1992).

A diet devoid of or with relatively low levels of calcium can reduce egg production to less than 5% by 10-14 days or can lead to complete cessation of egg production by 21 days (Wolford, 1984). Salt free rations have also been used for the induction of moulting, but the success was limited (Berry, 2003). The efficacy of low Ca diets to halt production varies widely. A dietary Ca level of 0.3% caused a complete cessation of lay within 14 d in 69-wk-old Leghorn hens (Martin *et al.,* 1973*)*. However, after 3 d on the low Ca diet, many hens exhibited paralysis (Berry, 2003).

Low Na rations for moulting laying hens have received considerable attention. Diets deficient in Na inhibit egg production when administered to pullets prior to egg laying. However, use of low Na for induced moulting has met with limited success.

High dietary level of iodine is found to induce a pause in egg production with our without the loss of feathers. Iodine can be added (5,000 ppm) to ration

for inducing moulting. It is added as potassium iodide and this result in cessation of egg production within 5-7 days, and will be resumed 7-10 days after the withdrawal of iodine from the ration (Wolford, 1984).

Dietary aluminium has also been tried as a moulting agent (Hussein *et al.*, 1989). The procedure takes more than 2 wk to cause egg production to cease, but production performance during 14 wk second cycle comparison was similar to that of hens moulted by feed withdrawal. Dietary aluminium also causes reduced feed intake in hens. However, the food safety implications of aluminium treatment has to be ascertained before the industrial use.

2.6.4 Other methods of inducing moulting

Hens infused with corticosterone showed hyperphagia, a two-fold increase in liver weight and regression of ovary (Etches *et al.*, 1983). Corticosterone causes rapid ovarian and oviductal regression apparently by reducing secretion of lutenizing hormone (Etches *et al.*, 1984; Williams *et al.*, 1985).

Progesterone, both parenterally or orally cause termination of egg production in 2-4 days and cause moulting in 7-12 days after administration and egg production resume about 3-4 weeks after the end of administration (Kuenzel, 2003).

Enheptin if given through diet can reduce feed consumption and cause dose related body weight reduction in laying hens (Pino, 1955).

Administration of prolactin cause moult in fowls and turkeys, but increase of it is slight during fasting and egg laying stop (Juhn and Harris, 1956).

Kuenzel *et al.* (2003) cited that feeding thyroid glands or their extracts to birds resulted in a robust loss of body feathers. Nicarbazin treatment for induction of moult has been reported (Bar *et al.*, 2003).

Tamoxifen, and anti-estrogen compound, has been shown to cause cessation of ovulation, induced moult, and is shown to be comparable in results

with that of high zinc or feed/water restriction methods of induced rest methods (Stake *et al.,* 1979).

Clomiphene citrate fed at high levels for one week produced a severe reduction in egg production and a heavy moult in hens. GnRH agonist infusion induced an ovarian regression (Dickerman and Bahr, 1987).

It has been proposed that GnRH agonists cause ovarian regression via a down regulation of pituitary GnRH receptors followed by reduced gonadotropin release (cited by Dickerman and Bahr, 1989).

2.7 Physiological and Biochemical Changes During Moulting

2.7.1 Changes in blood picture

The total circulating leukocytes were significantly lower in molted hens than in control hens. Differential leukocyte counts were affected by all induced molting programs and the heterophil to lymphocyte ratio was significantly increased, than controls (Alodan and Mashaly, 1999). These changes in H:L ratio can be due to changes in the blood corticosterone levels (Webster, 2003). Glucocorticoids such as corticosterone can cause changes in circulating populations of blood leucocytes by suppressing the proliferation and activity of lymphocytes (Munck *et al.,* 1984). An Increased packed cell volume (PCV), eosinophilia and increased monocyte numbers are associated with an induced moult (Brake *et al.,* 1982). Reticulocytosis is observed during moult recovery (Berry *et al.,* 1987). A psychogenic influence of H:L ratios can occur in feed-deprivation situations (Zulkifli *et al.,* 1995). Medvedev *et al., (*2002) found that hens moulted with alfalfa diets had H:L ratios intermediate to those of full-fed controls and hens moulted by feed withdrawal. Heterophils from feed-deprived hens had lesser ability to phagocytize *S.* Enteritidis and to generate an oxidative burst in response to inflammatory agoinsts (Kogut *et al.,* 1999).

PCV (haematocrit) increases during the period of reproductive involution and decreases again as the reproductive tract regenerates. This is due to removal of estrogen inhibition of red blood cells formation, reduced vascular volume due

to involution of the ovary and oviduct, and presumably due to decrease of fluid intake leading to haemoconcentration (Berry *et al.,* 1987). Numbers of RBC decline as ovarian function returns during moult recovery. Reticulocytosis is observed during moult recovery.

2.7.2 Changes in visceral organs

Moulting affects the liver size in chicken significantly (Cleaver *et al.,* 1986). Zinc feeding causes seven-, six- and threefold increase in zinc concentration in pancreas, liver and kidney respectively (Verheyen *et al.,* 1990). The significant decrease in relative liver weight of moulting turkey hens during starvation may be attributed to the depletion of glycogen, protein and lipids in liver. The lower relative liver weights prior to egg production and during the forced moult reflected the absence of both estrogen, as indicated by reduced relative ovary and oviduct weights, and the estrogen-dependent synthesis of phospholipoproteins and other lipids by the liver. Once feed was returned to the moulting birds, relative liver weight began to increase, reflecting the probable replenishing of nutrient reserves (Cleaver *et al.,* 1986). An increased spleen weight during the induced moult was coincident with an increase in haematocrit and haemoglobin (Brake *et al.,* 1981). This may have reflected either a loss of inhibition of erythropoiesis due to lowered estrogen levels, a decreased blood vascular space due to tissue regression which forced blood cells into the peripheral blood, or haemoconcentration.

2.7.3 Changes in body weight

Feed withdrawal causes a reduction in body weight proportional to the days of fasting. Baker *et al.* (1983) found out that a body weight loss of approximately 27-31% produces optimum postmoult performance with respect to egg production, egg weight, specific gravity and shell weight. Bell (2002) recommended that the body weight loss should not exceed 30% of the original weight in White Leghorn flocks.

2.7.4 Reproductive organ weights and involution

The weight lost by ovary and oviduct constitutes 25% of the body weight loss by hen moulted by feed restriction (Brake and Thaxton, 1979). According to McCormick and Cunningham (1984), Zn treatment results in a decrease in ovary weight up to 75% by 4 days and up to 80% by 10 days of feeding. Relative oviduct weight of moulting turkey hens appeares to decline more slowly than relative ovary weight (Cleaver *et al.,* 1986).

Lack of gonadotrophic support during fasting causes ovarian involution. The follicles in the maturational hierarchy become atretic and yolk material is resorbed. Ovary weight declines as follicles become atretic. Beyond 25% body weight loss, the ovary is fully regressed (Berry, 2003). There is a reduction in the length and weight of the oviduct parts and the hen day average reaches 0% by the day 5 of the starting of the feed withdrawal, the ovarian weight loss reaches more than 90% (Harimurti *et al.,* 2000). Oviduct involution is a consequence of ovarian regression due to a reduced steroidogenesis and a lack of steroidal support. The cells of oviduct become shrunken and glandular tissue lost by a process of apoptosis (Heryanto *et al.,* 1997). Eroschenko and Wilson (1974) reported that during moulting period, a reduction in cell size of mucosal epithelium and involution of tubular glands occurres in the oviduct. The regression of chick oviduct during estrogen withdrawal involves a process of apoptosis (Monroe *et al.*, 2000) and involves caspase gene expression (Monroe *et al.*, 2002). Caspases-1 and -2 and inducible nitric oxide synthase mRNA are found to be differentially expressed in ovary and magnum in hens moulted by feed withdrawal and zinc supplementation (Anish *et al.,* 2007). Furthermore, cytokines play a major role in regression of the ovary and oviduct during induced molting in chickens (Sundaresan *et al.,* 2007).

2.8 Digestive Tract and Moulting

Duodenal weight decreases during fasting and returns to original size upon refeeding. Following the moult, intestinal Ca binding protein concentration increases (Berry and Brake, 1991) compared to unmoulted hens. Intestinal uptake

of Ca also increases following moulting. In most domestic animals, reproductive function is considerably affected by nutrition.

2.9 Role of Different Hormones in Moulting

Various hormones, including growth hormone, insulin-like growth factors (IGFs) and insulin, have been proposed as potential mediators affecting reproductive function (Cassy *et al.,* 2004) in addition to the established role of the reproductive steroids and thyroid hormones. Hormones like ghrelin and leptin have potential effects on the food intake, fasting and appetite regulation in animals and human beings.

2.9.1 Ghrelin

Ghrelin is a 28-amino-acid peptide with an n-octanoyl modification at the third position (Ser-3) indispensable for its activity, was originally discovered in human and rat stomach as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHSR) (Kojima *et al.,* 1999). Ghrelin is found to be five times potent than GHRH in releasing GH (Ahmed and Harvey, 2002).

Ghrelin is attributed to have overwhelmingly diverse functions. Discovered in stomach and other tissues, it is involved in control of food intake and adiposity acting mainly at hypothalamic levels. Ghrelin and its receptor is believed to be the interlink between the diet and growth (Vallejo-Cremades *et al.,* 2004). Ghrelin administration is found to have suppressing feed intake in neonatal chicks (Furuse *et al.,* 2001). It also regulates pituitary secretion of growth hormone and to a lesser extent, FSH, LH, prolactin, ACTH and vasopressin. Growth hormone secretagogue receptor type 1a (GHSR) has potential action in central as well as peripheral locations. Ghrelin is reported to promote proliferation of a variety of cell types including adipocytes (Kim *et al.,* 2004) whereas suppresses proliferation of some variety of cells. Direct effect of ghrelin in promoting and suppressing apoptosis is described. It is also reported to inhibit insulin secretion and to stimulate glucagon secretion (Sirotkin *et al.,* 2006).

Direct gonadal action of ghrelin is stressed by the expression of it and its receptor in ovary in humans and mouse. In chicken also, ghrelin and GHSR (cGHSR) mRNA is found in ovary. Ghrelin modulates the cell proliferation action in chicken ovarian cells. It also modulates apoptosis and the expression of several apoptotic markers like caspase-3, bax, bcl-2 and p53 genes (Sirotkin *et al.,* 2006). However, many of the proliferating, anti-apotptotic and steroidogenic activity of ghrelin might be due to its stimulatory effects on the release of IGF-I.

Chen *et al.* (2007) reported that chicken ghrelin mRNA and its receptor mRNA gets upregulated in the chicken liver after a short fasting period. The same has been true for proventriculus also. They reported that in proventriculus of young broiler birds, the level of ghrelin mRNA has significantly upregulated in response to 12-36 h fasting. However, cGHSR mRNA increased with 12 hr fasting, not 36 h fasting. mRNA levels of both cGhrelin and cGHSR in liver was upregulated in response to fasting in a time dependent manner and returned to control levels subsequent to refeeding.

Low protein diets increased plasma ghrelin levels in rats (Beck *et al.,* 2002). In humans, high carbohydrate diet dropped stomach ghrelin levels whereas in rats, high fat diet increased stomach ghrelin expression (cited by Chen *et al.*, 2007). These reports indicate that the composition of diet can cause variation in the level of ghrelin expression in stomach.

In chicken, ghrelin has a dose dependant effect in the release of GH (Baudet and Harvey, 2003). Chicken ghrelin is a 26-aminoacid peptide which is predominantly synthesized in the proventriculus (Kaiya *et al.,* 2002) where as it is absent in gizzard (Kojima and Kangawa, 2005). *In situ* hybridization and immunohistochemical analyses indicate that ghrelin-containing cells are a distinct endocrine cell type found in the mucosal layer of the stomach in rats (Date *et al.*, 2000). Ghrelin-immunoreactive cells are also found in the duodenum, jejunum, ileum, and colon (cited by Kojima and Kangawa, 2005). In the intestine, ghrelin concentration gradually decreases from the duodenum to the colon. The chicken ghrelin mRNA level in the proventriculus increases after a short fasting

period. Moreover, the chicken ghrelin mRNA and its receptor mRNA gets upregulated in the chicken liver, after fasting, in a time-dependant manner (Chen *et al.,* 2007). Unlike in mammals, where ghrelin stimulates the feed intake, chicken ghrelin is found to have an opposite effect on feed intake. This seemingly contradictory effect may actually reflect its potent GH-releasing capability (Ahmed and Harvey, 2002), because GH administration or signaling through the GH secretagogue receptor inhibits feeding in chickens (Rosebrough *et al.,* 1991; Saito *et al.,* 2002). Furthermore, ghrelin increases plasma corticosterone levels (Richards *et al.,* 2006).

2.9.2 Leptin

The polypeptide hormone leptin is produced by both adipose tissue and liver and has been shown to induce satiety in chickens (Ashwell *et al.,* 2001). Leptin is the product of the obese (*ob*) gene and functions to regulate energy homeostasis (Gura, 1997). As an adipose derived hormone, leptin may play a role in metabolism to increased lipid storage by regulating fatty acid homoeostasis in chicken (Unger *et al.,* 1999). Liver leptin expression is associated with fat depot size in lines of birds divergently selected for abdominal fat pad size (Dridi *et al.,* 2000). Leptin expression in liver is increased by insulin and dexamethasone and decreased by glucagon and estrogen. However, only estrogen could change (decrease) the expression of leptin in adipose tissues (Ashwell *et al.,* 1999). In rats and humans, estrogen has been reported to increase leptin production *in vivo* (Shimizu *et al.,* 1997).

The avian liver is responsible for as much as 95% of the *de novo* fatty acid synthesis in birds and is also the site of considerable lipid storage in obese chickens (Ashwell *et al.,* 1999). In general, the regulation of leptin expression appears to be similar to that of mammals. It is possible that the significance of avian leptin expression in liver is directly related to its role in lipogeneis. In the mammalian liver, leptin has direct effects on the regulation of carbohydrate and lipid metabolism (Cohen *et al.,* 1998). The role of leptin in the liver has not yet been determined in avian species, but the central role played by the liver in lipid

metabolism in this species suggests that leptin may be associated with a role in controlling lipogenesis. Moreover, in chicken, it has been found that the leptin receptor mRNA is downregulated by leptin and insulin in liver cells (Cassy *et al.*, 2003).

According to Dridi *et al.* (2000), leptin plasma levels are higher in fed chickens than in fasted ones, showing that the expression of this signaling molecule is regulated by the nutritional status. Moreover, the intracerebroventricular injection of human recombinant leptin in Single Comb White Leghorn chickens decreased feed intake, a feature that demonstrates the existence of a functional link between leptin and satiety in chicken (Denbow *et al.*, 2000). In the pancreas, it has been demonstrated that leptin has a profound inhibitory influence upon insulin secretion (Benomar *et al.*, 2003).

In chickens, leptin attenuates the negative effects of fasting on ovarian function. Injections of leptin during fasting delays cessation of egg laying, attenuates regression of yellow hierarchical follicles, alters ovarian steroidogenesis and limits apoptosis (Paczoska-Eliasiewicz *et al.*, 2003). Expression of leptin receptor in laying hens has been determined by RT-PCR. The highest expression of leptin receptor is found in hypothalamus. Lower amounts were found in hypophysis, and lowest expression found in the ovary. In chickens, leptin might be involved in the adaptation to starvation due to attenuation of follicular apoptosis (Paczoska-Eliasiewicz *et al.*, 2003).

2.9.3 Cholecystokinin

Cholecystokinin, a potent inhibitor of feeding, has been well studied in birds. CCK stimulate gastric emptying and the release of pancreatic enzymes to aid in the digestion of feed, but it also functions as a satiety signal to the brainstem and is capable of depressing appetite (Richards, 2003).

2.9.4 Insulin-like growth factor

A polypeptide hormone, IGF-I is structurally related to insulin (McMurtry *et al.*, 1997). The synthesis and secretion of IGF-I is under both growth

hormone-dependent and independent control (McMurtry *et al.,* 1997). It is synthesized by various tissues, where it acts via autocrine and paracrine mechanisms to effect an array of anabolic pathways (McMurtry *et al.,* 1997). The metabolic effects of avian IGF include an increased amino acid and glucose uptake and the upregulation of DNA and protein synthesis (McMurtry, 1998).

Insulin-like growth factor system includes IGF-I, IGF-II, IGF receptor and IGF binding proteins. Chicken IGF-I and –II exert their function by binding to it specific type 1 receptors (Zhou *et al.,* 1995). The primary, secondary and tertiary structures if IGFs are homologous to proinsulin (Onagbesan *et al.,* 1999). Even though there is a 60% homology between the amino acid sequences of these two IGFs, many physiologically significant differences were found among them.

IGF system plays major roles in reproductive functions in mammalian species. IGF-I and –II act in autocrine and paracrine fashions for ovarian cellular growth and differentiation. The granulosa, theca and luteal cells contain receptors that mediate the action of IGFs. The regulatory role of IGFs in the ovary is mediated through the IGF binding proteins. In chicken, both granulosa and theca shows secretion of IGF-I. FSH, LH and GH combinely stimulate follicles to secrete IGFs. Larger follicles tend to secrete more IGFs than medium sized follicles (Onagbesan *et al.,* 1999).

Avians lack functional IGF-II receptors. It is suggested that the functions of IGF-I and –II both are mediated by IGF type I receptors (Onagbesan *et al.,* 1999). Higher levels of IGF-I was found in the systemic blood of feed restricted broiler breeder hens than *ad libitum* fed birds (Hocking *et al.,* 1994). IGF-I and –II enhance progesterone production by granulosa cells *in vitro*. Some reports also indicated that estrogen production by theca cells is inhibited by IGF-I (Onagbesan *et al.,* 1999).

Plasma levels of IGF-I and –II decrease with fasting and increase with age (Beccavin *et al.,* 2001). Moreover, IGF might influence growth rate, body composition and lipid metabolism in poultry. Mazzuco *et al.* (2005) reported that circulating IGF-I was markedly sensitive to nutritional status. Moulting had a

profound effect on circulating concentration of IGF-I and its mRNA in the liver of hens. They reported that IGF-I plasma concentrations decrease by 2 days of moulting. The levels overshooted after 3 days of refeeding (moulting regimen included only 10 d fasting). This elevated level was maintained for another 20 days. The authors noticed similar pattern of IGF-I expression in liver. However, they collected liver samples for expression studies only 3 times, ie, during beginning of moulting, on 25th day and 64th day.

Induced molt by fasting as well as non-fasting methods affects circulating IGF-I concentrations in hens. IGF-I concentrations were decreased 54 hours postmolt, thereafter increase from 13 to 43 d postmolt, all molted birds have elevated IGF-I as compared with controls (Mazzuco *et al.,* 2005).

2.9.5 Insulin and glucagon

There are fundamental differences between the carbohydrate and lipid metabolism of chicken and mammals. Chicken maintain a "chronic hyperglycaemic" blood glucose concentration and have to meet the high demands of lipid synthesis and transport due to egg formation. It is well established that insulin and glucagon play a key role in the control of carbohydrate and lipid metabolism in avian species (Simon, 1989; Cogburn, 1991). Single administration of glucagon increases plasma glucose concentration in laying hens (Mitchell and Raza, 1986). Glucagon inhibits hepatic lipogenesis and stimulates lipolysis in adipose tissue (Pal *et al.,* 2002). Moreover, birds are relatively resistant to high doses of insulin. Insulin stimulates hepatic fatty acid production. In mammals, insulin inhibits lipid mobilization from adipose tissues whereas in birds, it is not antilipolytic (Hazelwood, 2000). Exogenous glucagon administration increases plasma glucose whereas insulin has an opposite effect. Exogenous administration of insulin triggers pancreatic glucagon release in order to counter the effect of insulin (Pal *et al.,* 2002). However, central administration of leptin and glucagon as well as insulin suppresses food intake in chicken and mammals (Honda *et al.,* 2007). In rats, glucagon is found to increase the ghrelin

secretion from stomach via gastric glucagon receptors thus controlling the appetite (Katayama *et al.,* 2007).

In contrast to mammals, glucagon is the dominant pancreatic hormone in order to meet the requirements of the constant high-carbohydrate supply for metabolic processes (Hazelwood, 2000). Birds are relatively resistant to high doses of insulin (Hazelwood and Lorenz, 1959; Simon, 1989).

Changes in the plasma glucose levels are observed in fasted birds. In chicken, plasmas glucose concentrations generally decline somewhat at the beginning of feed restriction. Within 24 h of starvation, glycogenolytic activity in the chicken liver greatly increases, promoting net glucose efflux from the liver, associated with a 90% reduction in the liver glycogen (Davison and Langslow, 1975). Brake and Thaxton (1979) found that plasma glucose concentrations actually increases in hens during periods of feed and water restriction.

The glucagon and glucagon receptor expression is regaining considerable attention in recent times due to the discovery of a group of glucagon-like peptides and receptors in the gut and their role in control of diabetes. Glucagon-like peptide-1 is derived from the transcription product of the proglucagon gene. Carbohydrates, proteins and lipids induce the secretion of this peptide from the L cells of small and large intestines. Glucagon-like peptide-1 (GLP-1) is found to be the most insulinogenic of the glucagon-like peptides (Valverde *et al.,* 2004). GLP (1) increases the insulin secretion from the pancreas in a glucose-dependent manner, (2) decreases glucagon secretion from the pancreas, (3) increases beta cells mass and insulin gene expression, (4) inhibits acid secretion and gastric emptying in the stomach and (5) decreases food intake by increasing satiety. The glucagon-like peptide-1 receptor (GLP1R) binds specifically the glucagon-like peptide-1 (GLP1) and has lower affinity for related peptides such as the gastric inhibitory polypeptide and glucagon (Fehmann *et al.,* 1994). GLP1R expresses in pancreatic beta cells and activated GLP1R stimulates increased insulin synthesis and release of insulin (Drucker *et al.,* 1987). GLP1R is also expressed in the brain where it is involved in the control of appetite (Kinzig *et al.,* 2002). GLP gene has

been characterized in chicken and GLP1R and GLP2R are found to be expressed in gastrointestinal tract, brain, pancreas and abdominal fat (Richards and McMurtry, 2008).

2.9.6 Estrogen and progesterone

Fasting reduced serum progesterone and estradiol levels, and estradiol levels decreases after only one day of fasting (Etches *et al.,* 1984). Plasma progesterone concentration decreases more gradually than that of estradiol (Braw-Tal *et al.,* 2004). Progesterone, either injected or fed, typically terminates egg production in 2 to 4 d, whereas molt commences from 7 to 12 d after P4 administration (Kuenzel, 2003).

ER-α is the classical estrogen receptor (Walter *et al.,* 1985) whereas ER-β also has been demonstrated (Sakimura *et al.,* 2002). ER-β shows a different pattern of expression from ER-α in the brain of quails (Bernard *et al.,* 1999). Estrogen binds with both these receptors with similar affinity (Kuiper *et al.,* 1998). Estrogen bound ER-α and ER-β mediates gene transcription through estrogen response element (ERE), but has opposite effect when activated via Activator Protein pathway. Thus gene expression pattern of ER-α and ER-β determines the tissue specific response of estrogen (Paech *et al.,* 1997).

There is a dramatic decrease of estrogen in chicken during old-age (70 wk) compared to those at peak production while ER-α also decrease with age in hens (Hansen *et al.,* 2003). Estrogen and its receptor also has important role in the Ca absorption, metabolism and medullary bone formation. Duodenal weight decreased during fasting and returns to original size upon refeeding (Berry and Brake, 1991). Upon refeeding, the ovarian production of steroids resumes and oviduct regenerates (Berry, 2003). ER-α has been discovered in avian duodenum which is an important site of Ca abosorption. Intestinal Ca binding protein concentration increases and Ca uptake improves following a moult (Berry and Brake, 1991; Al-Batshan *et al.,* 1994).

Progesterone is involved in differentiation and protein synthesis of oviduct cells in the glands and luminal epithelium. Progesterone receptor is increased after treatment with estrogen in chicken (Gasc *et al.,* 1984). Syvälä *et al.* (1997) reported two isoforms of progesterone receptor in chicken (isoforms A and B). They also reported that the expression of PR-A is induced by estrogen during young stages. González-Morán *et al.* (2003) reported that PR-A is the predominant isoform found in the magnum of newly hatched chicks.

2.9.7 Corticosterone

Etches *et al.* (1983) reported that corticosterone is elevated in hens during molting induced by fasting; however, the measured amount of corticosterone is lower than the amount of exogenously administered corticosterone required for inducing ovarian regression. The corticosterone increase is transitory and decreases toward prefast levels as fasting continues. Upon refeeding, corticosterone levels again increase. The reproductive functions of hens are depressed by increased corticosterone (Moudgal *et al.,* 1991). Corticosterone also affects the food intake, food passage time and nutrient uptake in chickens (Nasir *et al.,* 1999).

Glucocorticoids stimulate hepatic gluconeogenesis, helping to maintain levels of plasma glucose (Munck *et al.,* 1984). In the chicken, the temporary increase of plasma corticosterone during the initial adjustment to feed deprivation probably stimulates glucose output from the liver, helping to ward off hypoglycemia until glucose production from fat catabolism is established.

2.9.8 Adiponectin

Adiponectin is an adipokine abundantly expressed in adipose tissues in mammals (Kadowaki and Yamauchi, 2005). It plays a dominant role in lipid and carbohydrate metabolism. Moreover, adiponectin stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing insulin sensitivity (Chabrolle *et al.,* 2007). It is also implicated in regulation of

energy balance and body weight. The synthesis of Adiponectin is regulated by several factors including insulin and insulin-like growth factor (IGF).

Two specific receptors are identified in humans (AdipoR1 and AdipoR2), Adipo-R1 which is expressed abundantly in skeletal muscles and Adipo-R2 which is expressed in liver. Adiponectin and its receptors mRNAs are expressed in various chicken tissues including ovary. Adipo-R1 and Adipo-R2 genes have been cloned and sequenced in chicken. Chicken adiponectin mRNA is expressed in various tissues including ovary. Expression of Adipo-R1 mRNA is about 2 folds higher in granulosa cell than in theca cells, where as Adipo-R2 mRNA expression is similar in these cells. Furthermore, expression of Adiponectin receptor in some tissues such as adipose tissue, liver and pituitary is reduced by food deprivation for 48 h (Chabrolle *et al.,* 2007).

The level of adiponectin mRNA is about 10- to 30-fold higher in theca cells than in granulosa cells in hierarchal follicles. In chicken, adiponectin could exert paracrine or autocrine effect on the ovarian steroidogenesis (Chabrolle *et al.,* 2007).

A 48h feed deprivation significantly decreased AdipoR1 mRNA quantity in the chicken pituitary gland, while AdipoR2 mRNA quantity was significantly increased in adipose tissue. AdipoR1 and AdipoR2 genes are ubiquitously expressed in chicken tissues and that their expression is altered by feed deprivation in the anterior pituitary gland and adipose tissue (Ramachandran *et al.,* 2007). An interaction between the ovarian steroids and pituitary gonadotropic hormones along with changes in Adipo-R1 and Adipo-R2 in the anterior pituitary (Ramachandran *et al.,* 2007) has been reported. The adiponectin produced in ovary might exert an effect in ovarian steroidogenesis. In mammals, adiponectin is known to increase the progesterone secretion in the presence of IGF-1 in chicken granulosa cells (Chabrolle *et al.,* 2007).

2.9.9 Thyroxine and triiodothyronine

Feeding thyroid glands or their extracts to birds resulted in a robust loss of feathers (Torrey and Horning, 1922). It was not clear which thyroid hormone was responsible for initiating a moult.

Plasma thyroxine (T_4) levels decrease initially after feed withdrawal and increases above control levels after 6th day of feed withdrawal. Triiodothyronine (T_3) level is consistent throughout the fasting period, and on resumption of feeding, there is a decrease of T_4 and a concomitant increase in T_3 (Brake *et al.*, 1979). Verheyen *et al.* (1986) proposed a separate function for T_3 and T_4. T_3 injected birds displayed significant reduction in egg production rate, and some moulting. T_4 injection caused moulting which matched the force-moulted control birds that underwent feed withdrawal with respect to intensity of moult.

Iodine can be added (5,000 ppm) to ration for inducing moulting, this result in cessation of egg production within 5-7 days, and will be resumed 7-10 days after the withdrawal of iodine from the ration (Wolford, 1984). Praharaj *et al.*, (1994) observed that a salt-free diet containing either 7500 ppm Zn or a combination of 3750 ppm I + 5000 ppm Zn could be used efficiently for moulting of White Leghorn layers.

Oral thyroxine, in purified or non-purified form, induces a molt and may enhance animal well-being by reducing the need for feed withdrawal (Bass *et al.*, 2007).

2.10 Rejuvenation of Tissues Following Moulting

Upon resumption of sex steroid production by the ovary during recovery from the moult, the oviduct recrudesces. Relatively little effort has been expanded to study the physiological basis for the apparent rejuvenation of egg production and eggshell quality that occurs following moulting. This rejuvenation might be mediated by an increasing tissue sensitivity or efficiency (Berry, 2003). Reorganization of metabolic processes, removal of inhibiting

substances at the cellular level, or reversal of the normal, but as yet undefined, ageing processes of the laying hen could be involved (Brake and Thaxton, 1979).

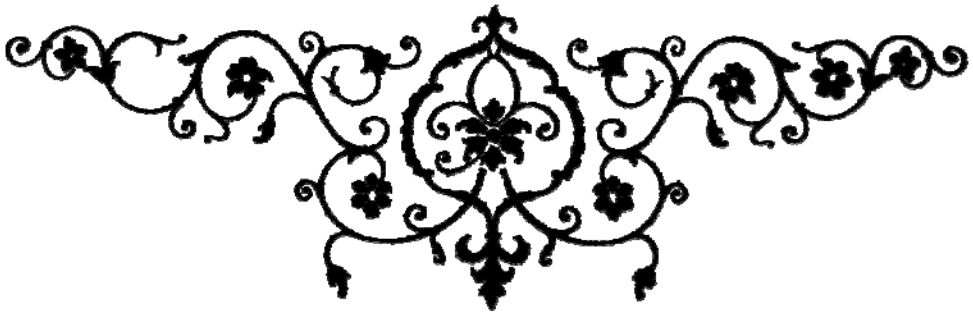
Oviduct tissues are remodeled by replacing the old glandular cells with new ones that are derived from mucosal epithelium and uninvoluted glandular cells (Heryanto *et al.,* 1997). It is hypothesized that during induced moulting, apoptosis of oviductal tubular gland is induced in the earlier stages and autolysis involving lysosomal hydrolases occurs at later stages leading to regression of tubular glands. Improved shell gland function following induced moulting may be due to remodeling at the cellular level. Cellular proliferation in the oviduct replaces cells lost during the regression.

Remodeling of shell gland tissue may also be responsible for removing substances that interfere with shell gland function. The lipid content of the shell gland increases as hens age. Induced moulting reduces shell gland lipid and alters the balance of lipid types (Baker *et al.,* 1980). Baker (1981) observed that the uterine glandular epithelium, which is the site of eggshell Ca transport and deposition, contain quantities of intracellular lipid visibly detectable by histological staining. Roland *et al.* (1977) reported that hens laying shell-less eggs have significantly higher uterine lipid content compared to hens producing normal eggs. Uterine lipid is not lost until the body weight decreases to that point, which coincides with maximum oviduct regression.

2.11 Future of Induced Moulting

If moulting were eliminated as a replacement option, there will be an increased flock size and resultant higher house utilization. Higher costs of production would occur as replacement costs increased. An all-pullet industry would produce a higher percentage of less popular egg sizes-medium to small, which are already burdensome to industry. Moreover, egg producers will have less flexibility to adjust production to meet market demand. In the future, moulting as a part of an optimum replacement strategy is to be continued, but better alternative methods would be used for initiation of moulting. These alternative methods must be profitable to the farmer, as being animal-friendly while satisfying the food-safety concerns.

MATERIALS & METHODS



3.1 EXPERIMENT I

Objective: To investigate the mRNA expression patterns of certain endocrine constituents and/or their receptors in association with induced moulting in chicken.

3.1.1 Materials and Reagents

For the molecular biology work, only molecular biology grade chemicals were used for preparation of the buffers and solutions. Autoclaved deionized distilled water was used for preparing buffers and solutions for molecular biology. The buffers and reagents used for RNA handling were prepared in DEPC-treated and autoclaved Milli-Q water.

3.1.1.1 Chemicals

Diethyl pyrocarbonate (DEPC), RNA stabilization solution (RNA*later*®, Ambion Inc., USA), 10x *Taq* buffer, 25mM $MgCl_2$, 10mM dNTP mix, *Taq* DNA polymerase (6U/mL, Bangalore Genei), Agarose (Merck), molecular size quantitative marker (Genei), 6X loading dye, 5X tris boric acid EDTA buffer.

3.1.1.2 Kits

RNA isolation kit (RNAgents® Total RNA Isolation System, Promega Corporation, USA), cDNA synthesis kit (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas Life Sciences), Real-time SYBR Green kit (MESA GREEN qPCR MasterMix Plus for SYBR Assay with flurescein, Eurogentec, Belgium).

3.1.1.3 Equipments and Software

Cooling microcentrifuge (3500 Table-top micro refrigerated centrifuge, Kubota Corporation, Tokyo, Japan), Tissue homogenizer (Polytron, Kinematica AG, Switzerland), Micropipettes (Eppendorf AG, Germany), plasticwares (Axygen Scientific, Inc. CA, USA), UV/Vis Spectrophotometer (NanoDrop 1000, Thermo Scientific, Singapore), Thermalcyclers (IQ5 Multicolor Real-time PCR Detection System; iCycler; MJ Mini Gradient Thermal Cycler, Bio-rad

laboratories, Inc. Hercules, USA), horizontal submarine electrophoresis apparatus (Scie-Plas Ltd., Warwickshire, England), Laminar air-flow apparatus (Tanco, India), Real-time PCR detection software (IQ5 Optical System Software, Bio-rad Laboratories, Inc. Hercules, USA), Primer design software (Beacon designer 7.02, Premier Biosoft International, CA, USA).

3.1.1.4 Feeds and Feed Preparation

The feed and shell grit were procured from the Feed Unit, CARI. Different feeds used in the current experiment were (1) Standard layer diet and (2) Cracked maize.

3.1.2 Experimental Birds and Housing

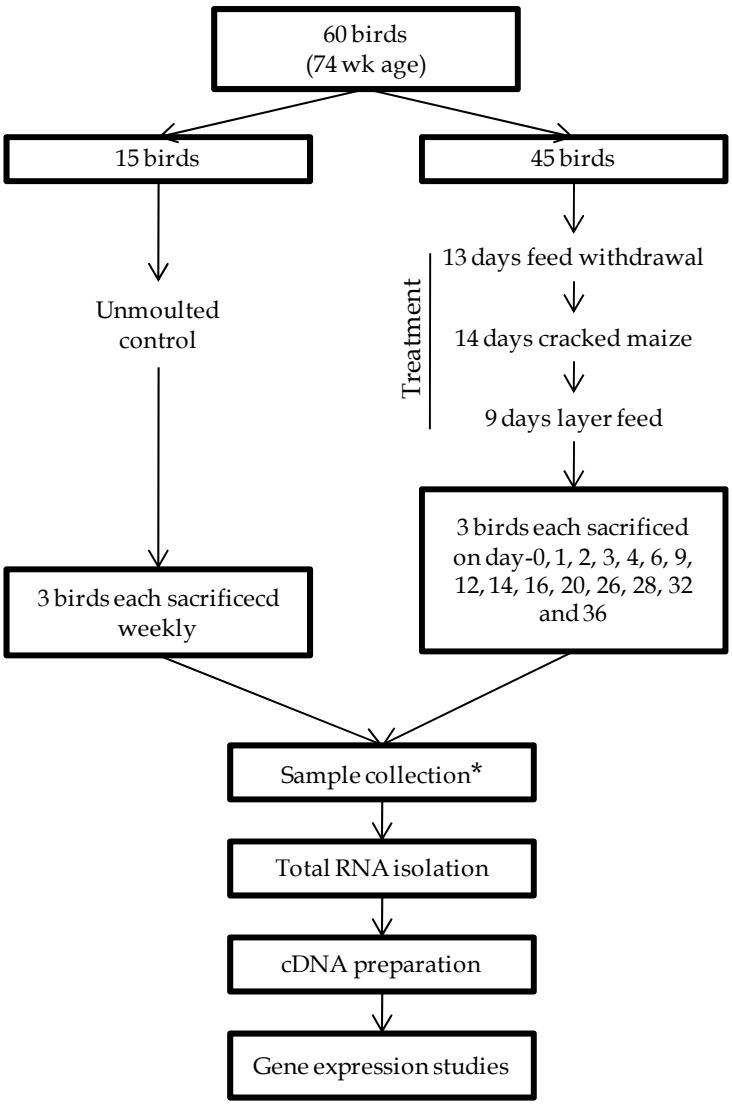
Sixty Rhode Island Red hens from the same hatch at the end of the production cycle (about 74 wk age) were used in this study. The birds were housed in individual cages and were maintained under uniform management conditions.

3.1.3 Experimental Design and Methodology

The experimental design is briefly presented in Fig 3.1. Sixty hens were divided to two groups comprising of a control group of 15 birds and a treatment group of 45 birds. The control group was fed a standard layer ration throughout the study period. The treatment group received a moulting treatment (California method of moulting; Bell, 2002).

3.1.3.1 Moulting

In the California moulting programme, feed was withdrawn for a period of 14 days (the birds lost about 25% of body weight on a group basis on 14th day). *Ad libitum* drinking water was provided during the same period. After 14 days of feed withdrawal, the birds were given a rest period of two weeks (14 days) days, during which they were provided with a post-moult diet (PMD) of cracked maize (Bell, 2002). Thereafter a standard layer ration was provided.



Tissues Collected	Hormone mRNA Studied	Receptor mRNA Studied
Digestive Tract (Liver Jejunum Proventriculus)	Ghrelin IGF-I Leptin	Adiponectin Receptor-1 Ghrelin Receptor (cGHSR) Glucagon Receptor Insulin Receptor IGF-Receptor
Reproductive tract (Magnum Shell gland)		Adiponectin Receptor-1 Estrogen Receptor-α Progesterone Receptor

Fig 3.1 Design of Experiment I

3.1.3.2 Collection of Tissues

From the treatment group, three birds each were sacrificed on days zero, 1, 2, 3, 4, 6, 9, 12, 14, 16, 20, 26, 28, 32 and 36 days of starting the treatment for collecting tissues. Three unmoulted birds were sacrificed on a weekly basis to serve as a control similar to day zero birds.

All samples were collected aseptically in DEPC-treated and autoclaved microcentrifuge tubes. The scissors and forceps were treated with DEPC and autoclaved before use. Digestive and reproductive tracts were dissected out after sacrificing the birds. Small pieces of magnum and uterus were collected from the reproductive tract. From the digestive tract, liver, proventriculus and jejunum were collected. About 50 mg of each tissue were collected for the total RNA isolation.

For contingency use, about 150 mg of tissue were immersed in 5 volumes of RNA stabilizing solution in 1.5 ml microcentrifuge tubes. These samples were kept at 4^{o}C overnight and transferred to -20^{o}C freezer till used.

3.1.3.3 Total RNA Isolation

The total RNA isolation was performed using an RNA isolation kit based on thiocyanate guanidium method. The procedure followed for each sample was briefly as follows:

1. Fifty mg of tissue was weighed and put in 600 µl of chilled denaturing solution taken in DEPC-treated and autoclaved 2 ml microcentrifuge tubes.
2. The tissue was homogenized using a tissue homogenizer for about 15-30 seconds.
3. Sixty microlitres of chilled 2M Sodium Acetate (pH 4.0) was added in to the tube and were thoroughly inverted 4-5 times.
4. Six hundred microlitres of Phenol:Chloroform:Isoamyl Alcohol was added to the tube, taking care to remove only from the lower organic phase. The microcentrifuge tube was capped and was carefully mixed by inversion 3-

4 times. Thereafter the tube was shaken vigourously for 10 seconds. The tube was then chilled in ice for 15 minutes.

5. The mixture was transferred in to new DEPC-treated and autoclaved 2 ml microcentrifuge tubes and was centrifuged at 10,000 x g for 20 minutes at 4^{o}C in a microcentrifuge.
6. The top aqueous phase that contains RNA was carefully removed and transferred to a fresh 1.5 ml DEPC-treated and autoclaved microcentrifuge tube.
7. Equal volume of chilled isopropanol was added to the aqueous phase and incubated at -20^{o}C for 20 minutes to precipitate the RNA.
8. The RNA was pelletted by centrifugation at 10,000 x g for 10 minutes at 4^{o}C.
9. The isopropanol supernatant was decanted and the pellet was washed with 1 ml of ice-cold ethanol (75%). The pellet was dispersed and the tube was then centrifuged at 10,000 x g for 10 minutes at 4^{o}C.
10. The ethanol was decanted and the RNA pellet was air dried in an RNAse-free environment (laminar air flow apparatus).
11. The dried RNA pellet was resuspended in about 30 µl of Nuclease-free water.

3.1.3.4 RNA Quantification

The purity and concentration of the total RNA was checked using UV spectrophotometry at 260 and 280 nm.

3.1.3.5 First-strand cDNA Synthesis

The first-strand cDNA synthesis was performed using 200 µl PCR microtubes in a thermal cycler using first strand cDNA synthesis kit. The steps followed for each total RNA sample were described as follows:

1. Eleven µl of diluted total RNA (containing 5.0 µg of RNA) was taken in a PCR tube.

2. One µl of Oligo(DT)$_{18}$ primer (0.5 µg /µl) was added to the tube. The sample was mixed gently and spinned down for 3-5 sec. in a spinner.
3. The mixture was incubated at 70^{o}C for 5 minutes and were chilled on ice.
4. The following components were added in the following order: 5x reaction buffer (4 µl); Ribonuclease inhibitor (20 u/l; 1 µl); 10 mM dNTP mix (2 µl).
5. The mixture was incubated at 37^{o}C for 5 min.
6. Thereafter, 1 µl of M-MuLV reverse transcriptase (200 u/µl) was added to the mixture.
7. The mixture was incubated at 42^{o}C for 60 min. Thereafter, the reaction was stopped by heating at 70^{o}C.
8. The resultant first-strand cDNA was stored at -20^{o}C till used.

3.1.3.6 Primer Standardization for PCR

Oligonucleotide primers (Table 3.1) were synthesized and used for polymerase chain reaction (PCR) for studying the mRNA expression profiles. The primers were standardized for the PCR reaction conditions. The amplification was carried out in 25 µl volume, containing 10 pM of each primer, 0.1mM dNTP mix, 1 unit of *Taq* DNA polymerase (BioTools, B&M Labs, S.A.) and 2 µl cDNA (about 16.6 ng) in 1x *Taq* polymerase buffer. PCR cycling conditions were: initial denaturation at 94^{o}C (5 min); 30 cycles of denaturation at 94^{o}C (45 s), annealing (Table 3.1) (1 min) and extension at 72^{o}C (1 min); and final extension at 72^{o}C (10 min). The sizes and quantity of PCR products were verified by comparison with a DNA ladder. The samples yielded products of the expected sizes following PCR with gene-specific primers.

3.1.3.7 Gene expression study

The relative expression of specific gene mRNA was quantified by real-time PCR detection system. The gene-specific primer pairs used are mentioned in Table 3.1. All PCRs were performed in nuclease-free 8-tube-strips with optically clear flat caps.

Table 3.1 Oligonucleotide primer pairs used for gene expression studies.

Sl No.	Gene	Primer	Annealing Temperature	Length (bp)	Reference / Acc. Number	Manufacturer
1	Estrogen receptor-alpha (ER-α)	F- TGC GAG CTC CAA CCC TTT GGA CA	57°C	338	Imamura *et. al.,* 2006.	MWG Biotech, Germany
2		R- GGA GCG CCA GAC TAA GCC GAT CA				
3	Progesterone receptor	F- GGG CTG CTC TAC CCC AGA AG	55°C	76	NM_205262.1	Eurogentec, Belgium
4		R- GTT CTT CCT CCT CCT CCT CCT C				
5	Adiponectin receptor-1 (Adipo-R1)	F- CCA GGA GAA GGT TGT GTT TG	57°C	149	Chabrolle *et. al.,* 2007.	MWG Biotech, Germany
6		R- TGA TCA GCA GTG CAA TTC CT				
7	Insulin-like growth factor-I (IGF-I)	F- TGT ACT GTG CTC CAA TAA AGC	58°C	127	Guernec *et al.,* 2003.	Invitrogen Corporation
8		R- CTG TTT CCT GTG TTC CCT CTA CTT G				
9	Insulin-like growth factor receptor (IGF-R)	F- TAG ATC CCT CCT CTA CCC TCC AA	58°C	291	Heck *et al.,* 2003	Invitrogen Corporation
10		R- TCT GAA GAT CCA CTG AGG TAC AG				
11	cGhrelin	F- AAA GGA TAC AAG AAA ACC AAC AGC	58°C	91	NM_001001131.1	Eurogentec, Belgium
12		R- ATT GTC ATC TTC TCC CTC TGT TTC				
13	Ghrelin receptor (cGHSR) Type A	F- GTT ACC GGA GAG CCA TCA CG	56°C	80	AB095996.1	Eurogentec, Belgium
14		R- CCA GGT GAC GAC ACA AGT AGA G				
15	Insulin receptor	F- CTT AGC AGA GCA GCC ATA TC	55°C	182	AF111857	Integrated DNA Technologies, Inc. USA
16		R- CCT TCA GCA TCT CAA TGA CC				
17	Glucagon receptor	F- TCT CCC AGC CAC ATC TCC TC	56°C	99	NM_001101035.1	Eurogentec Belgium
18		R- TGT AAG CCT TCC AGC TCT CAA AG				
19	Leptin	F- CGT CGG TAT CCG CCA AGC AGA GGG	56°C	261	Ashwell *et. al.,* 1999	Operon, Germany
20		R- CCA GGA CGC CAT CCA GGC TCT CTG GC				
21	28s rRNA	F- GGC GAA GCC AGA GGA AAC T	55°C	62	Rothwell *et. al.,* 2004	Operon, Germany
22		R- GAC GAC CGA TTT GCA CGT C				

The amplification was carried out in 20 µl volume with final concentration of 1x SYBR Green PCR master mix which contains SYBR Green I dye, fluorescein additive, Meteor *Taq* hot-start DNA polymerase, dNTPs including dUTP and $MgCl_2$ with 4mM final concentration in optimized buffer components, a 0.25 pM concentration of each gene-specific primer, and 2.5 µl of cDNA template.

Real-time PCR cycling conditions were: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s; annealing (variable temperatures for different primer pairs; Table 3.1) for 20 s and extension 72°C; 30 s. For each sample a dissociation curve (melt curve) was generated after completion of amplification. For this, the temperature was increased from 55°C to 95°C, each degrees centigrade at 30 s intervals. A negative control containing all the ingredients except cDNA template (non-template control; NTC) was setup invariably for each master-mix made for conducting the reactions. 28S rRNA was used as house-keeping gene in the experiments.

Results are expressed in terms of the threshold cycle value (Ct), the cycle at which the change in the SYBR Green dye passes the significance threshold. To convey the inverse relationship between starting template concentration and Ct value, results were expressed and analyzed as a 40 – Ct value. The corrected specific mRNA per sample was calculated using the following formula:

$$\textbf{Adjusted Ct value} = (\textbf{mean 40-Ct}_{\textbf{target}}) / \textbf{28S df}$$

Where,

(mean 40-Ct $_{target}$) = the mean of 40-Ct value of target gene;

28S df = the mean of 28S /overall mean for all 28S values within the experiment;

3.1.3.8 Statistical analysis

The data generated from the Experiment I were recorded. The expression of mRNA was calculated as explained in Section 3.1.3.7. Sample data of different days were analyzed using one-way ANOVA (Snedecor and Cochran, 1989) and compared using Duncan's multiple range test (Duncan, 1955).

3.2 EXPERIMENT II

Objective: Endocrine interventions in California method of induced moulting to replace / shorten the induction period and to improve post treatment reproductive performance.

This experiment was designed to understand the effect of supplementing a progesterone and estrogen analog, thyroxine, vitamin E, and shell grit during the post-moult period compared to the classical post-moult diet of cracked maize. It was assumed that providing a post-moult diet containing progesterone and estrogen analog would enhance the recovery of the reproductive system of moulted birds. It was also assumed that providing thyroxine in feed might improve the feed intake causing a fast recovery of reproductive system. Providing vitamin E may improve the antioxidant status of birds whereas shell grit would improve the shell quality during post-moult production period.

3.2.1 Experimental Birds and Housing

A total of sixty Rhode Island Red layer hens (64 wk age) were used for the study. The birds were reared in cage system, under standard uniform management conditions.

3.2.2 Drugs and Hormones

Progesterone and estrogen synthetic analogue (PE analog) (each tablet containing Levonorgestrel 0.15 mg and Ethinyloestradiol 0.03 mg; Indian Drugs and Pharmaceuticals Ltd., Gurgaon, India), Thyroxine (Eltroxin tablets, each tablet containing 100 µg thyroxine sodium; GlaxoSmithKline Ltd., Nashik, India), Vitamin E (Tocopherol acetate, Merck).

3.2.3 Feeds and Feed preparation

Three types of post-moult diets were prepared for this experiment.

3.2.3.1 Group A (Control) PMD: Twenty-eight kg of cracked maize was thoroughly mixed with 20 ml soybean oil.

3.2.3.2 Group B PMD: Twenty-eight kg of cracked maize was thoroughly mixed with (1) 280 thyroxine tablets (powdered), (2) 5.6 g of tocopherol acetate in 20 ml of soybean oil and (3) 1 kg of shell grit. (Final concentration of ingredients: thyroxine 1 mg/kg feed, tocopherol acetate 200mg/kg feed).

3.2.3.3 Group C PMD: All 3 ingredients of Group B ration along with PE analog (560 PE analog powdered tablets; 0.3 mg progesterone analogue + 0.06 mg estrogen analogue per day per bird assuming 100g feed intake per day) added to 28 kg cracked maize.

3.2.3.4 Standard layer diet: as mentioned in annexure.

3.2.4 Experimental Design and Treatment

The experimental design is briefly presented in Fig 3.2. The egg weight and shell thickness were measured before starting the treatment. Sixty birds were moulted by California method (without water restriction) till a body weight loss of 25% (on group basis). On attaining of 25% body weight loss on day-14, the birds were divided in to three groups as follows (1) Group A (control), (2) Group B and (3) Group C (n=20). These groups were provided with the specific PMD mentioned in section 4.1.3 for a period of 14 days.

After the 14 days period of PMD, eight birds from each group were sacrificed to record the ovary and oviduct weights. Thereafter, the remaining 12 birds from each group were provided with a standard layer feed. The egg production parameters were studied until 50% production attainment. The post-moult egg weight and shell thickness were measured when the birds reached about 50% production.

3.2.5 Analysis of Data

The pre- and post-moult egg weight and shell thickness were analyzed using paired *t*-test. The internal organ weights were analyzed using one-way ANOVA and compared using Duncan's Multiple Range test (Duncan, 1955).

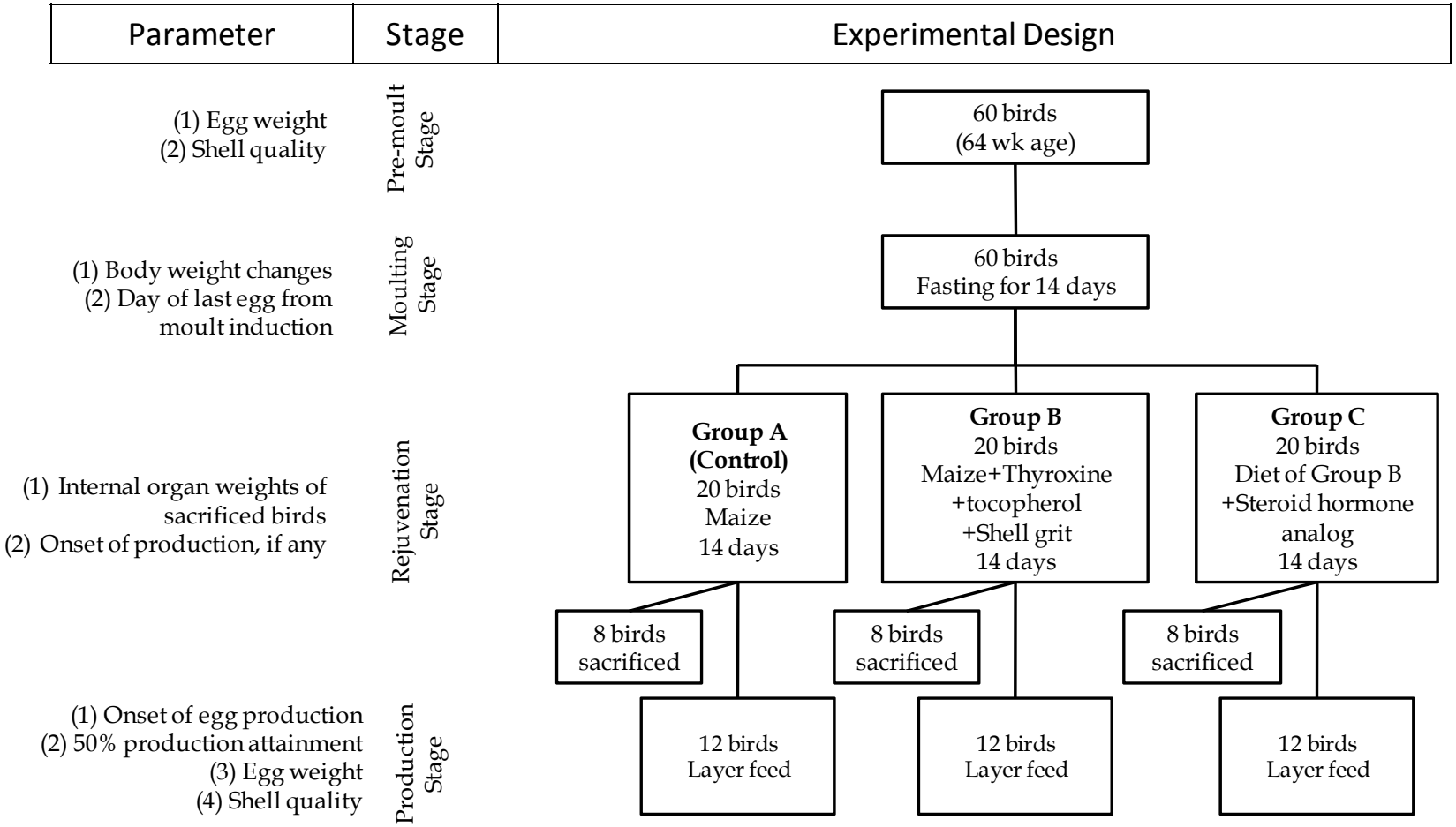


Fig 3.2 Design of Experiment II

3.3 EXPERIMENT III

Objective: Endocrine interventions in California method of induced moulting to replace / shorten the induction period and to improve post treatment reproductive performance.

The experiment was designed to evaluate the ability of a high dose of thyroxine to induce a moult compared to a fasting group provided with a small dose of thyroxine. After moult induction, the birds were provided with a conventional or enriched post-moult diet to understand the production status and egg parameters of moulted birds.

3.3.1 Experimental Birds and Housing

A total of seventy-two Rhode Island Red layer hens (70 wk age) were used for the study. The birds were reared in cage system, under standard uniform management conditions.

3.3.2 Drugs and Hormones

Progesterone and estrogen synthetic analogue (PE analog) (each tablet containing Levonorgestrel 0.15 mg and Ethinyloestradiol 0.03 mg; Indian Drugs and Pharmaceuticals Ltd., Gurgaon, India), Thyroxine (Eltroxin tablets, each tablet containing 100 µg thyroxine sodium; GlaxoSmithKline Ltd., Nashik, India), Vitamin E (Tocopherol acetate, Merck).

3.3.3 Feeds and Feed preparation

Three types of post-moult diets were prepared for this experiment.

3.3.3.1 Moulting diet with thyroxine-40mg/kg: 20 kg of grower feed was mixed with 8000 powdered thyroxine tablets (Final concentration: 40mg/kg thyroxine).

3.3.3.2 Layer ration: Standard layer diet (see annexure).

5.1.3.3 Grower ration: as mentioned in annexure.

3.3.3.4 Conventional PMD: Twenty-eight kg of cracked maize was thoroughly mixed with 20 ml soybean oil.

3.3.3.5 Enriched PMD: Twenty-eight kg of cracked maize was thoroughly mixed with (1) 280 thyroxine tablets (powdered), (2) 5.6 g of tocopherol acetate in 20 ml of soybean oil, (3) PE analog (560 PE analog powdered tablets; 0.3 mg progesterone analogue + 0.06 mg estrogen analogue per day per bird assuming 100g feed intake per day) (4) 1 kg of shell grit. (Final concentration of ingredients: thyroxine 1 mg/kg feed, tocopherol acetate 200mg/kg feed).

3.3.4 Experimental design and treatment

The experimental design is briefly presented in Fig 3.3. Seventy-two hens were randomly assigned into 4 groups. Group 1 and 2 contained 24 birds each. Two control groups of 12 birds each was also maintained (One unmoulted control and one conventional moulting treatment).

The two treatment groups were treated with (Group 1) Fasting along with thyroxine 5mg/lit water and (Group 2) Thyroxine 40 mg/kg of grower feed. The treatment was continued until the birds of fasting group lost about 25% of their body weight. In the thyroxine group, treatment continued till full feather moulting was achieved as no significant reductions in body weight was noticed.

After the treatment period, the birds of treatment groups were divided into two sub-groups each. One sub-group from each treatment group was provided with cracked maize and the other sub-group with enriched PMD. After 14 days of PMD feeding, the birds were provided with a regular layer ration. The conventional moulting group was provided with cracked maize for 14 days.

The time period needed for reaching zero egg-production in each group, presence of shell-less or soft-shelled eggs, days for first egg in each group after moulting, days of reaching 50% production and egg weight and Shell thickness of the eggs before and after moulting were studied.

3.3.5 Analysis of Data

The pre- and post-moult egg weight, body weight changes and shell thickness were analyzed using paired *t*-test.

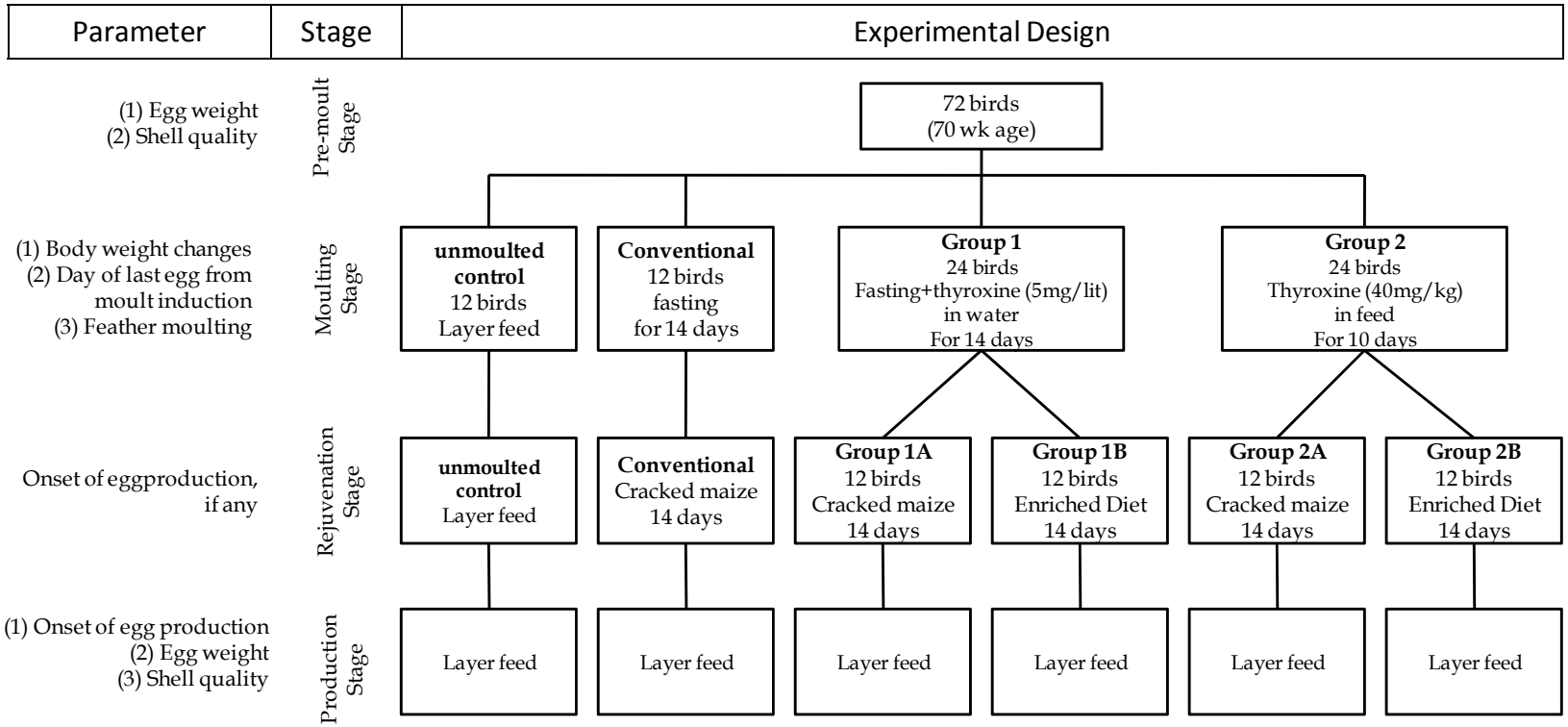
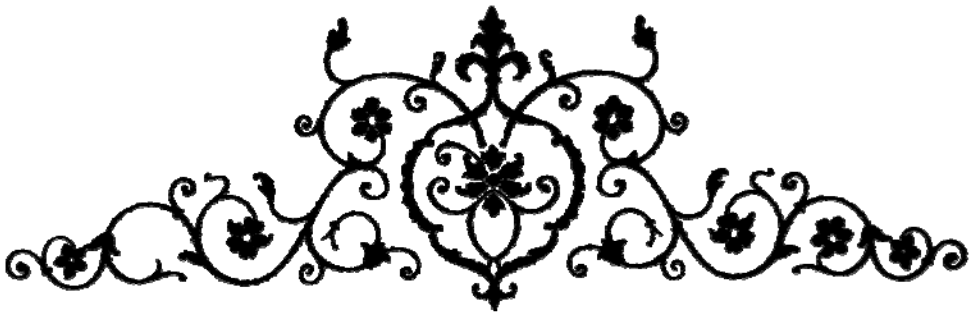


Fig 3.3 Design of Experiment III



RESULTS

4.1 EXPERIMENT I

At the beginning of the experiment, the body weights of all the birds were recorded. On the day-5 of moulting, egg production stopped completely and by day-13, the birds lost about 25% of body weight. A post-moult diet of cracked maize was started on day-14 onwards and layer feed was provided to the birds on day-27 till day-36. The brief results of this experiment are presented in Table 4.1

4.1.1 Expression pattern of various genes (mRNA) in magnum during moulting

The expression profile of estrogen receptor-α (ER-α), progesterone receptor and adiponectin receptor 1 (Adipo-R1) were studied in magnum and uterus (shell gland) of the chicken oviduct.

4.1.1.1 Estrogen Receptor-α

On day-1 of moulting, a significant (P≤0.05) upregulation in the expression was noticed in the magnum. Thereafter a decrease in expression to initial level was observed on day-4 of moulting. The levels increased again on day-6, which was maintained during the rest of moulting and post-moult periods (Fig 4.1).

4.1.1.2 Progesterone Receptor

The expression levels of progesterone receptor mRNA did not vary throughout the experimental period (Fig 4.2). Two separate products with varying melting points were observed in the real-time PCR melting curve cycle. These products correspond to the type A and B receptor variants.

4.1.1.3 Adiponectin Receptor-1

No significant differences in expression level of Adipo-R1 were found in the magnum throughout the experimental period (Fig 4.3).

Table 4.1 Overall results of Experiment I

	Reproductive Tissue		
	Magnum	**Uterus**	
Estrogen Receptor-α	Changes found	Changes found	
Progesterone Receptor	No significant changes	Changes found	
Adiponectin Receptor-1	No significant changes	Changes found	
	Digestive tissue		
	Liver	**Jejunum**	**Proventriculus**
Adiponectin Receptor-1	Changes found	Not expressed	Not expressed
Glucagon Receptor	Changes found	Changes found	Changes found
Insulin Receptor	Changes found	Changes found	Not expressed
Ghrelin	No significant changes	Not expressed	Changes found
Growth Hormone secretagogue Receptor	Changes found	Changes found	Changes found
Insulin-like Growth Factor-I	Changes found	Changes found	Changes found
Insulin-like Growth Factor Receptor	Changes found	Changes found	Changes found
Leptin	Changes found	Not expressed	Not expressed

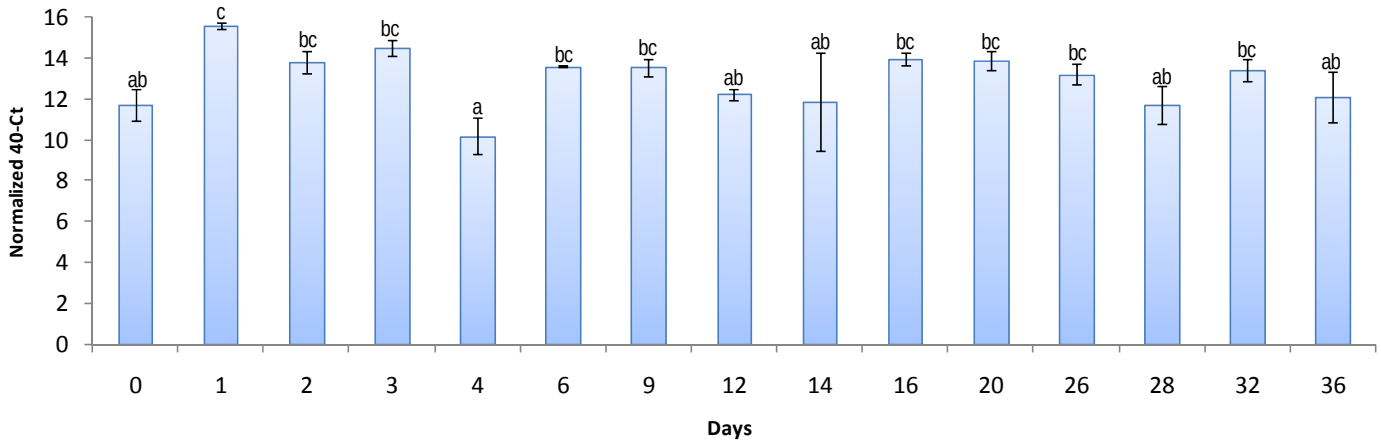


Fig 4.1 Expression profile of Estrogen Receptor-α mRNA in magnum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

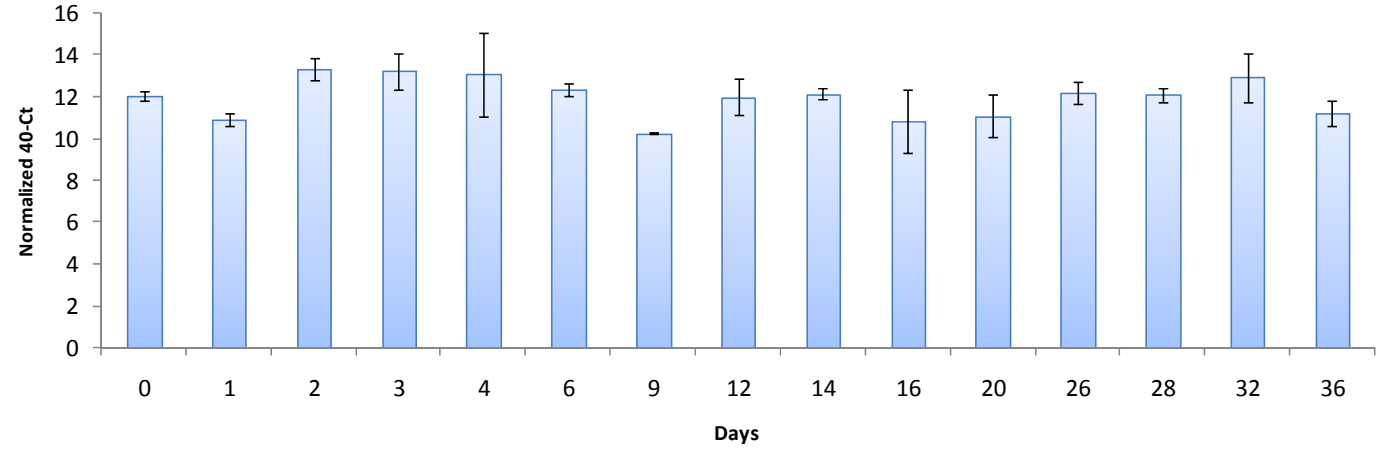


Fig 4.2 Expression profile of Progesterone Receptor mRNA in magnum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

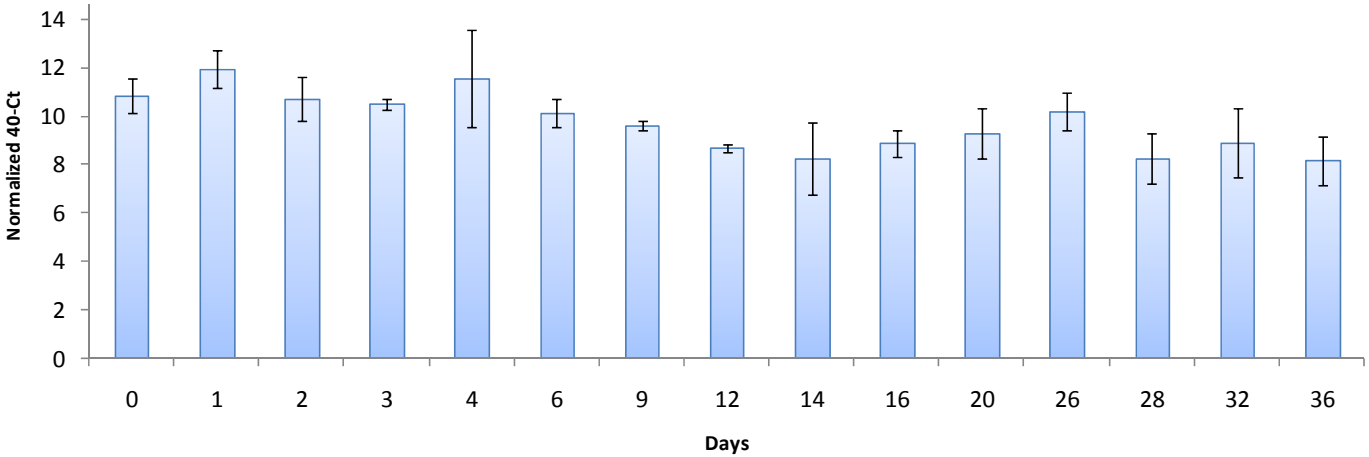


Fig 4.3. Expression profile of Adipo-R1 mRNA in magnum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

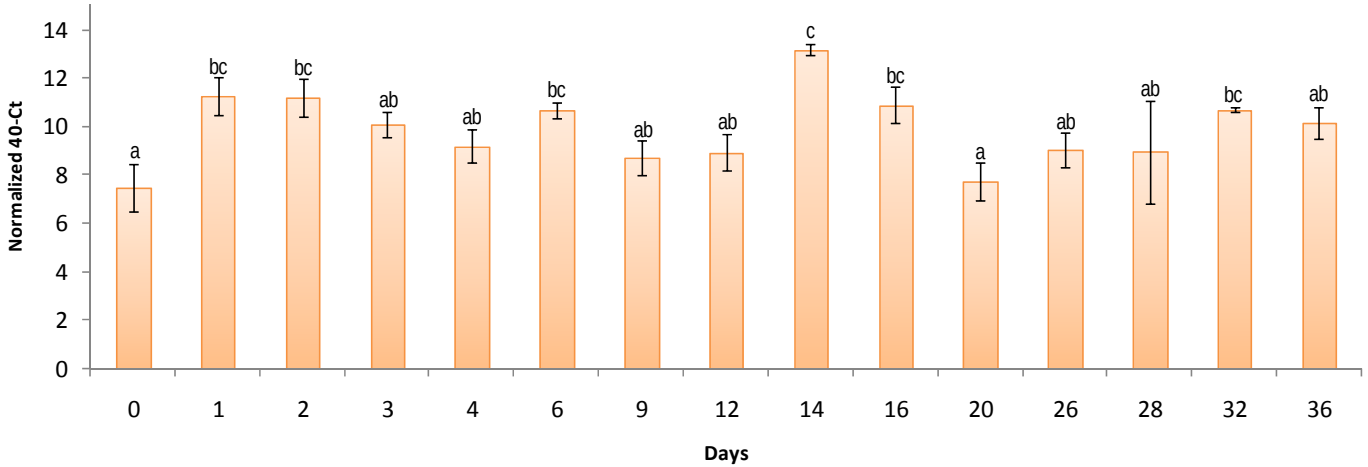


Fig 4.4 Expression profile of Estrogen Receptor-α mRNA in shell gland of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

4.1.2 Expression pattern of various genes (mRNA) in shell-gland (uterus) during moulting

4.1.2.1 Estrogen Receptor-α

Similar to the magnum, a significant (P≤0.05) upregulation of ER-α mRNA was noticed in uterus on day-1 compared to unmoulted (day-0) birds. Thereafter, the levels were maintained without any significant change through the moulting period. However, a further increase in the mRNA expression was noticed on day-14 and thereafter, the levels returned to normal levels (Fig 4.4).

4.1.2.2 Progesterone Receptor

The receptor genes were upregulated from day-2 of moulting except on day-9 with the lowest expression noticed on day-9 (Fig 4.5).

4.1.2.3 Adiponectin Receptor-1

Considerable variation was observed in the expression pattern of adiponectin receptor-1 mRNA in uterine tissue. Upregulation of expression was observed on day-1 to -3, day-6, -14 and -26 (Fig 4.6).

4.1.3 Expression pattern of various genes (mRNA) in liver during moulting

The expression profile of adiponectin receptor 1 (Adipo-R1), ghrelin, chicken growth hormone secretagogue receptor (cGHSR), glucagon receptor, insulin receptor, insulin-like growth factor-I (IGF-I), IGF receptor (IGFR) and leptin were studied in liver.

4.1.3.1 Adiponectin Receptor-1

No significant differences in Adipo-R1 expression was observed during the experimental period (Fig 4.7).

4.1.3.2 Ghrelin

No significant changes in the expression level of ghrelin mRNA was noticed in the liver of chicken during experiment. However, a higher fluctuation in the C*t* values among birds was noticed on all days after day-14 (Fig 4.8).

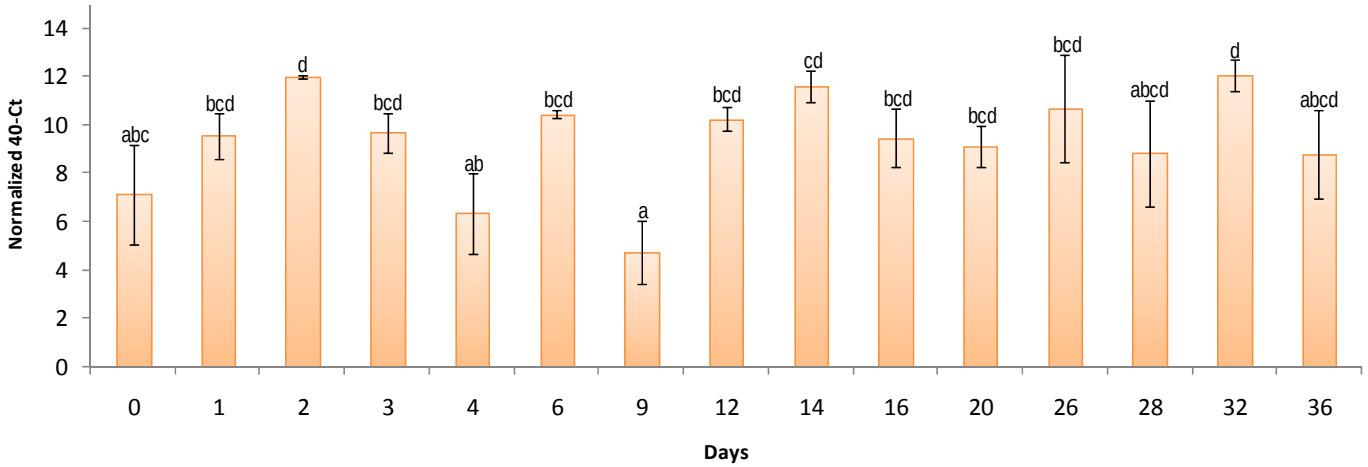


Fig 4.5 Expression profile of Progesterone Receptor mRNA in shell gland of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

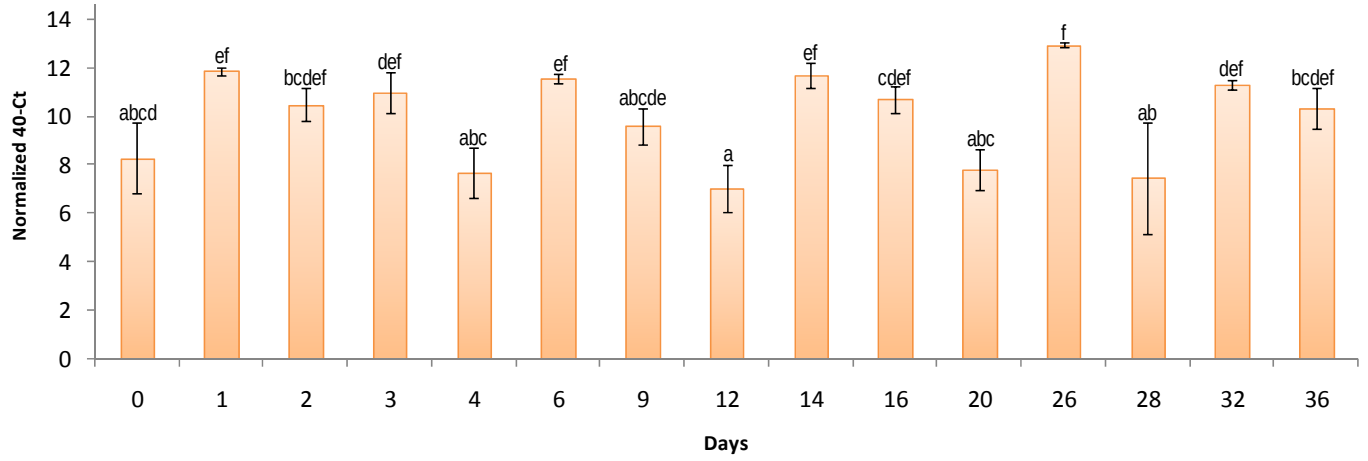


Fig 4.6 Expression profile of Adipo-R1 mRNA in shell gland of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

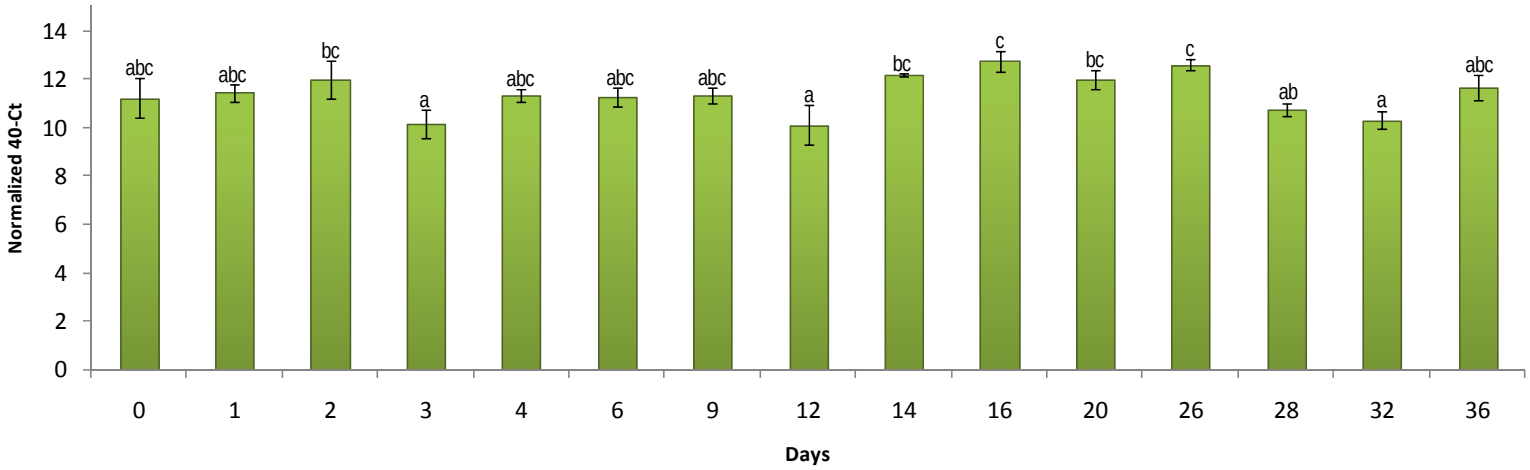


Fig 4.7 Expression profile of Adipo-R1 mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

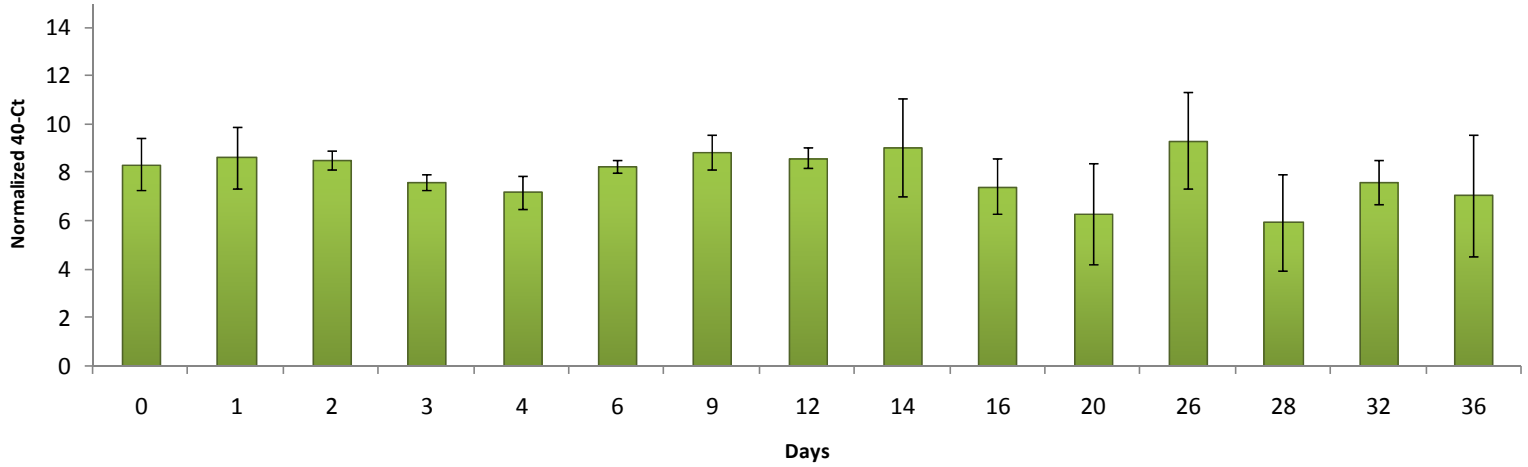


Fig 4.8 Expression profile of Ghrelin mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

4.1.3.3 Growth Hormone Secretagogue Receptor

No significant difference in the expression levels of cGHSR mRNA was noticed in liver throughout the experimental period except on day-2 and day-26 (Fig 4.9).

4.1.3.4 Glucagon Receptor

A significant (P≤0.05) downregulation of glucagon receptor mRNA was noticed on days-1, -3 and -9 compared to day-0. A significant downregulation was found on day-3. No variation in expression levels were noticed from day-14 till the end of experimental period (Fig 4.10).

4.1.3.5 Insulin Receptor

A significant (P≤0.05) upregulation of expression compared to initial level was noticed on day-2. The levels reduced to initial levels on day-3 and from day-4, upregulated again that was continued till the end of the experiment (Fig 4.11).

4.1.3.6 Insulin-like Growth Factor-I

A significant (P≤0.05) downregulation of IGF-I was noticed on day-3 of moulting. Thereafter, the levels again increased and reached the normal level of expression (Fig 4.12).

4.1.3.7 Insulin-like Growth Factor Receptor

The pattern of expression of IGF-receptor conformed the expression of IGF-I in liver (Fig 4.13).

4.1.3.8 Leptin

Considerable variation of leptin mRNA expressions was noticed on different days from day-6 to day-26 (Fig 4.14). Even though clear C*t* values were obtained for the leptin mRNA, the melt curve analysis revealed blunt peaks.

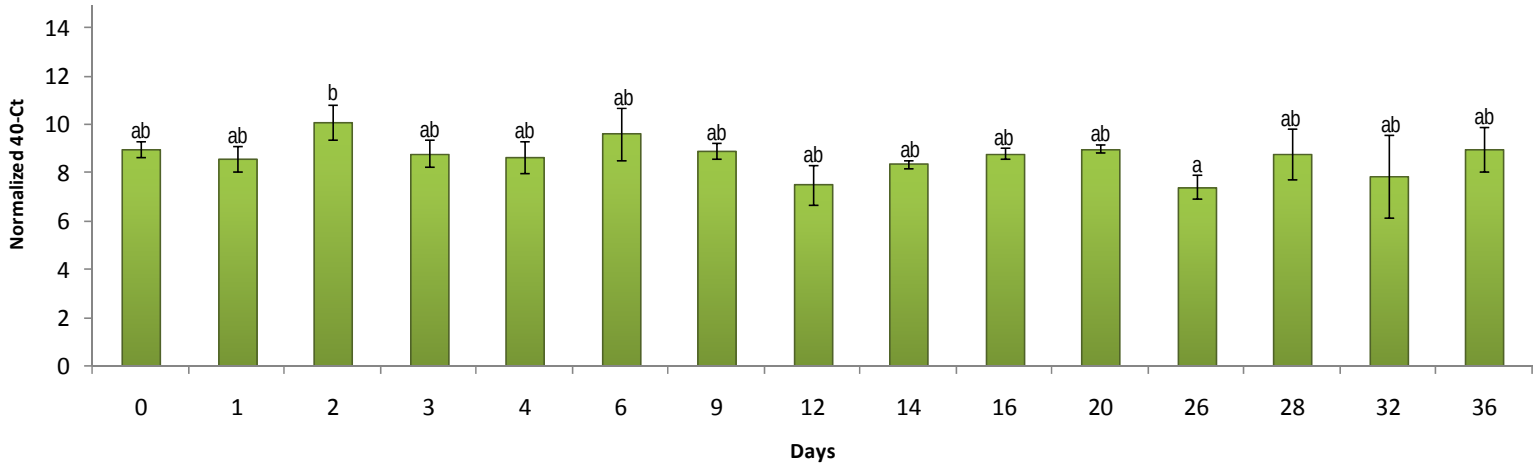


Fig 4.9 Expression profile of Growth Hormone Secretagogue Receptor mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

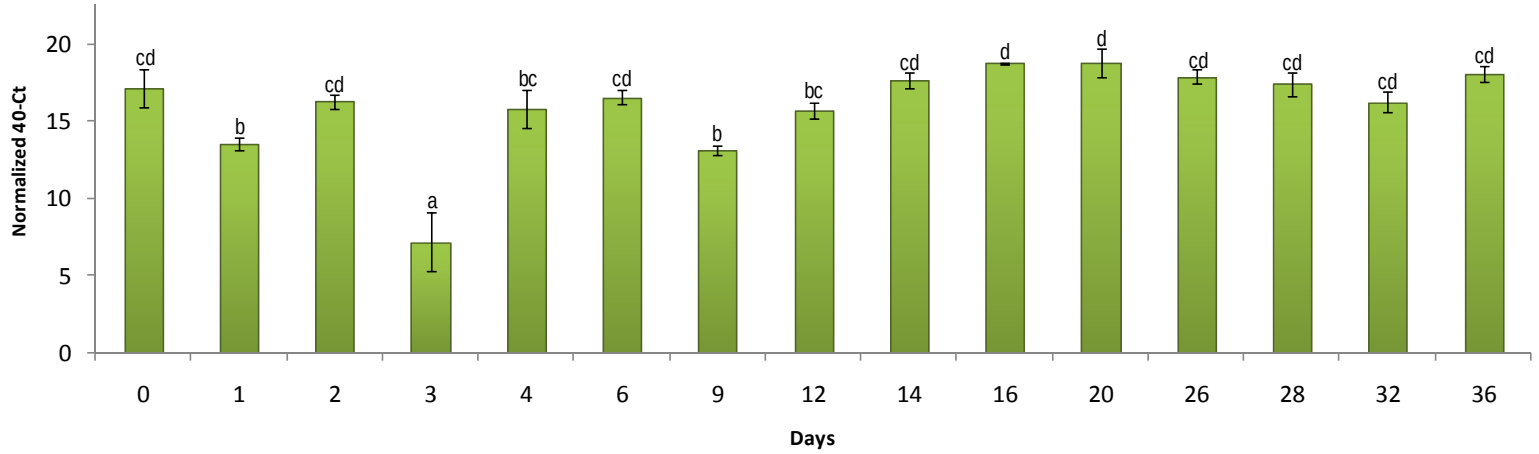


Fig 4.10 Expression profile of Glucagon Receptor mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

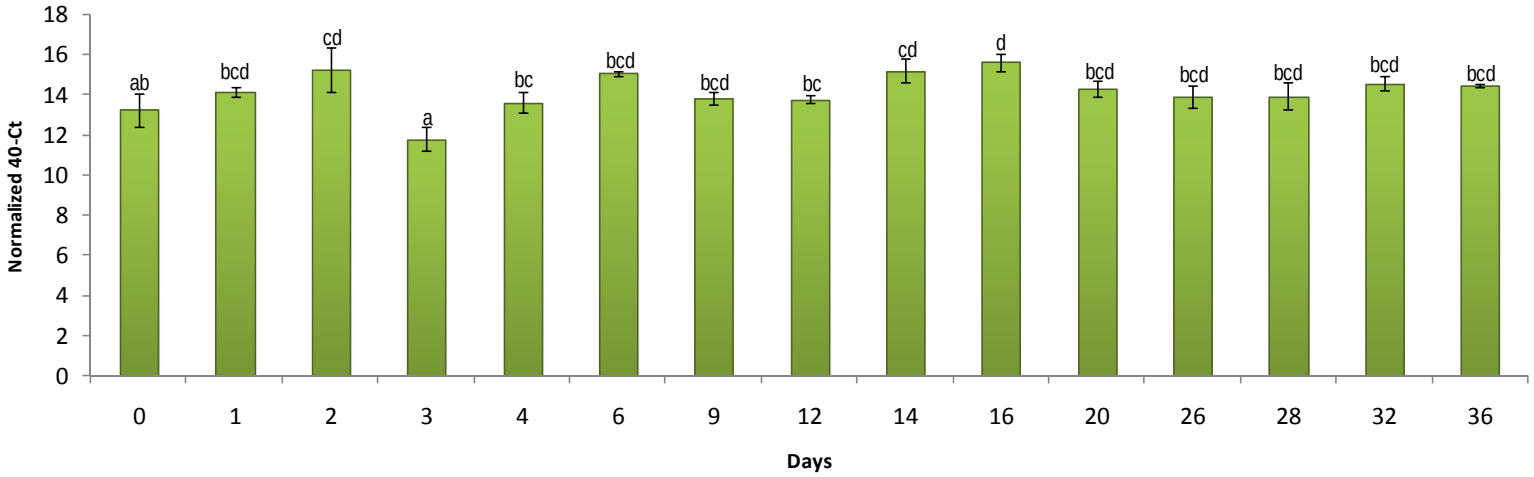


Fig 4.11 Expression profile of Insulin Receptor mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

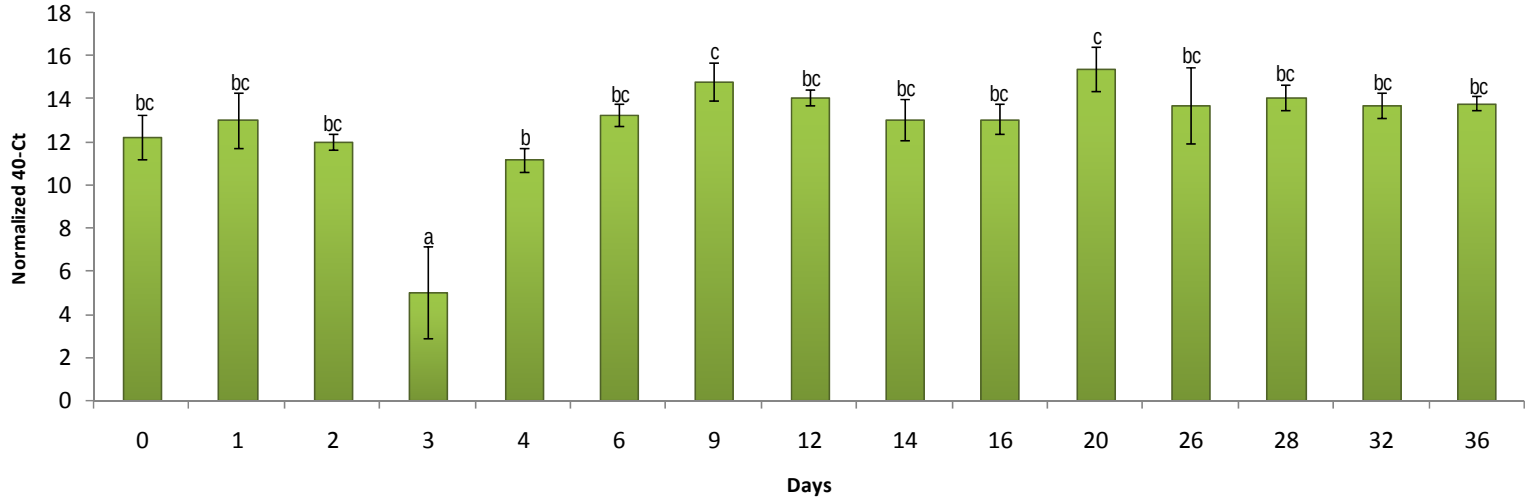


Fig 4.12 Expression profile of Insulin-like Growth Factor-I mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

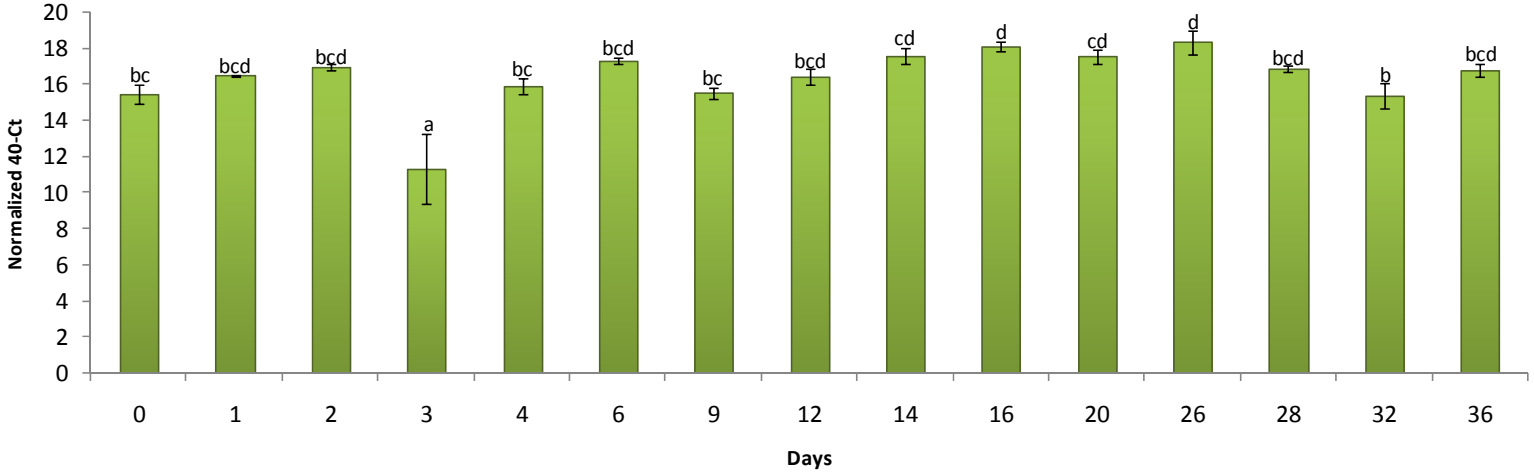


Fig 4.13 Expression profile of Insulin-like Growth Factor Receptor mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

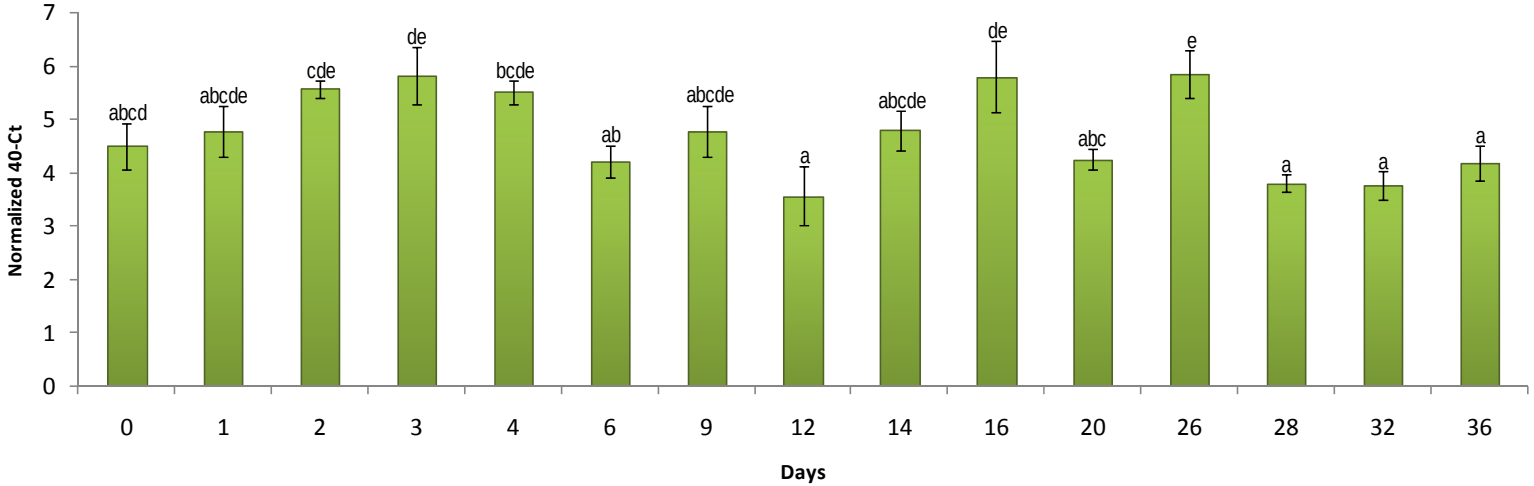


Fig 4.14 Expression profile of Leptin mRNA in liver of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

4.1.4 Expression of various genes (mRNA) in jejunum during moulting

The mRNA expression of ghrelin, cGHSR, glucagon receptor, insulin receptor, IGF-I, IGF receptor, Adipo-R1 and leptin were studied in jejunum part of small intestine. However, Adipo-R1, ghrelin and leptin mRNA expression in jejunum were not detected and therefore the same has not been included in the results.

4.1.4.1 Growth Hormone Secretagogue Receptor

Significant (P≤0.05) downregulation in cGHSR expression was noticed in jejunum on day-12 compared to the initial levels. Thereafter, the expression levels increased to normal levels for the rest of the experimental period (Fig 4.15).

4.1.4.2 Glucagon Receptor

The initial expression levels were maintained up to day-3 of the treatment. A significant (P≤0.05) downregulation was noticed on day-4 of the treatment, which continued till day-12 of treatment. The levels again increased to normal levels on day-14 and were maintained till day-28 of experiment. A significant (P≤0.05) decrease in the levels of glucagon receptor expression was again noticed on day-32 and -36 (Fig 4.16).

4.1.4.3 Insulin Receptor

The normal expression levels were maintained till day-3. On day-4 a decline in expression was noticed which reached the least on day-9. On day-14, the expression levels again increased to initial levels. However, expression was not detected on day-32 of experiment (Fig 4.17).

4.1.4.4 Insulin-like Growth Factor-I

A significant (P≤0.05) upregulation of expression was noticed on day-1, which was followed by reduction in expression levels till day-4 when no expression was noticed. This low level was maintained till day-12. Thereafter the levels were increased on day-14. No expression was noticed again on days-16, -32 and -36 with higher levels found from day-20 to -28 (Fig 4.18).

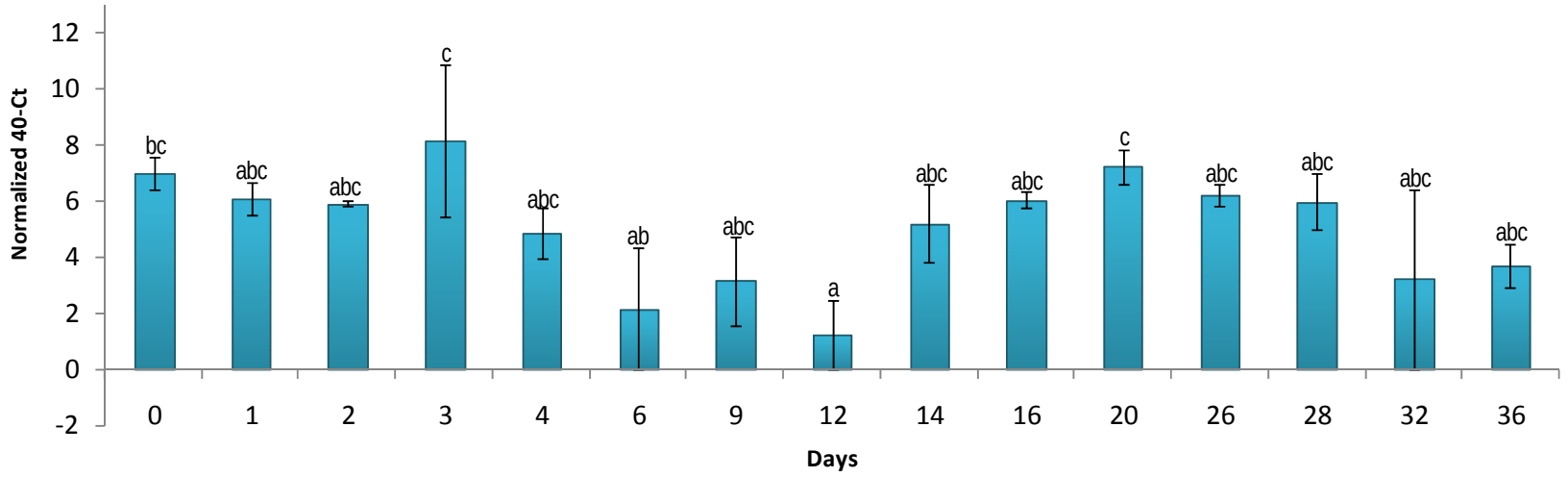


Fig 4.15 Expression profile of Growth Hormone Secretagogue Receptor mRNA in jejunum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

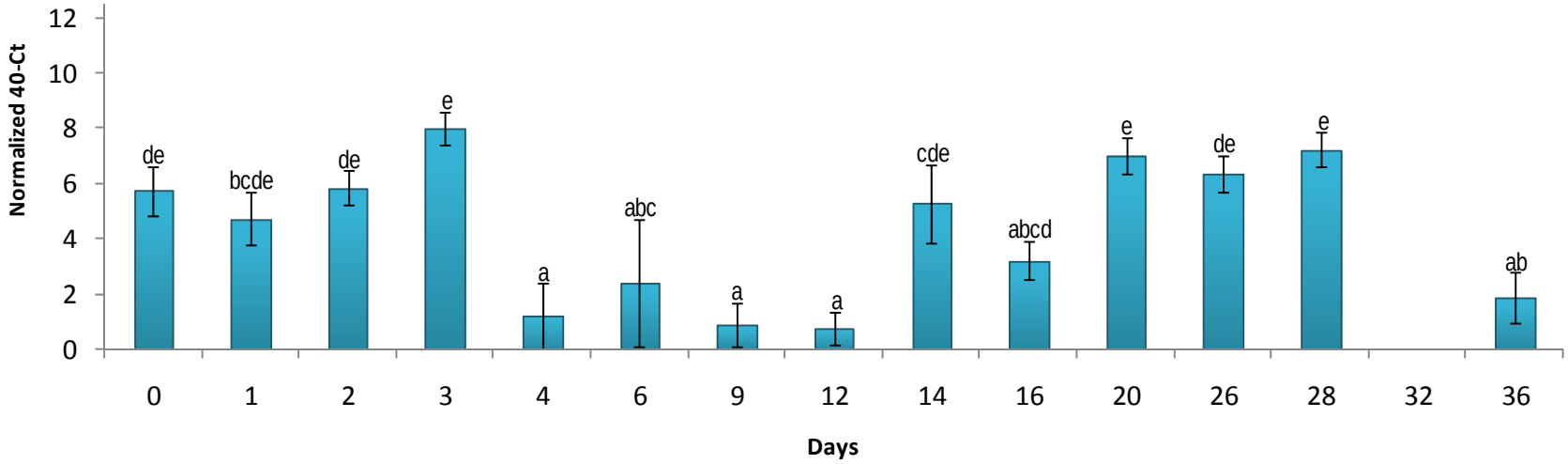


Fig 4.16 Expression profile of Glucagon Receptor mRNA in jejunum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

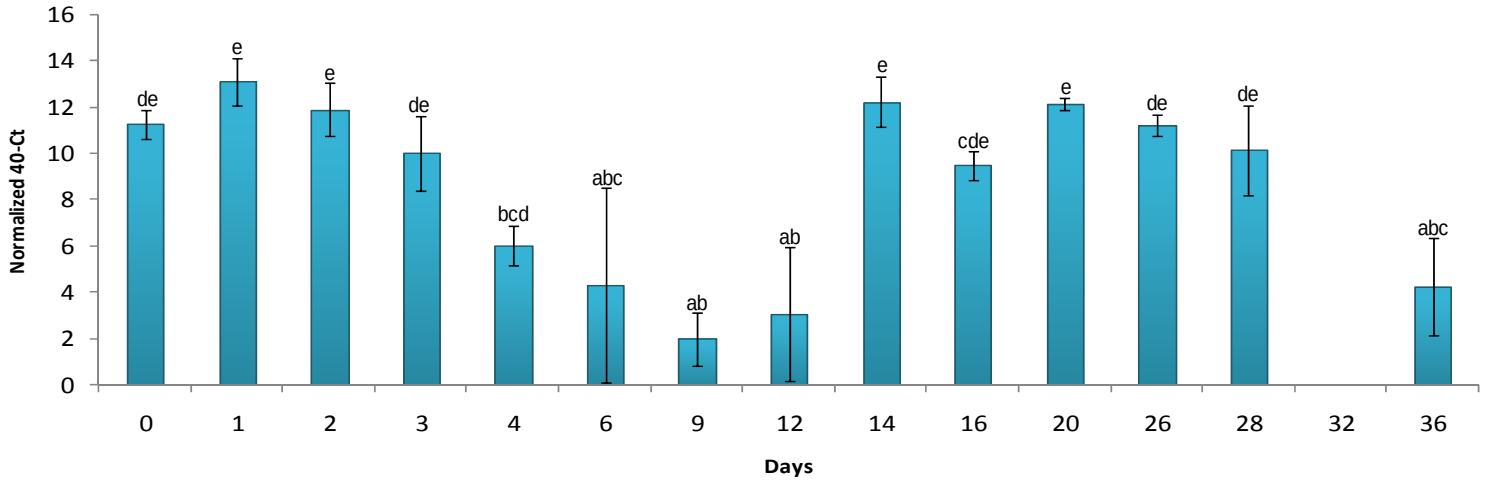


Fig 4.17 Expression profile of Insulin Receptor mRNA in jejunum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

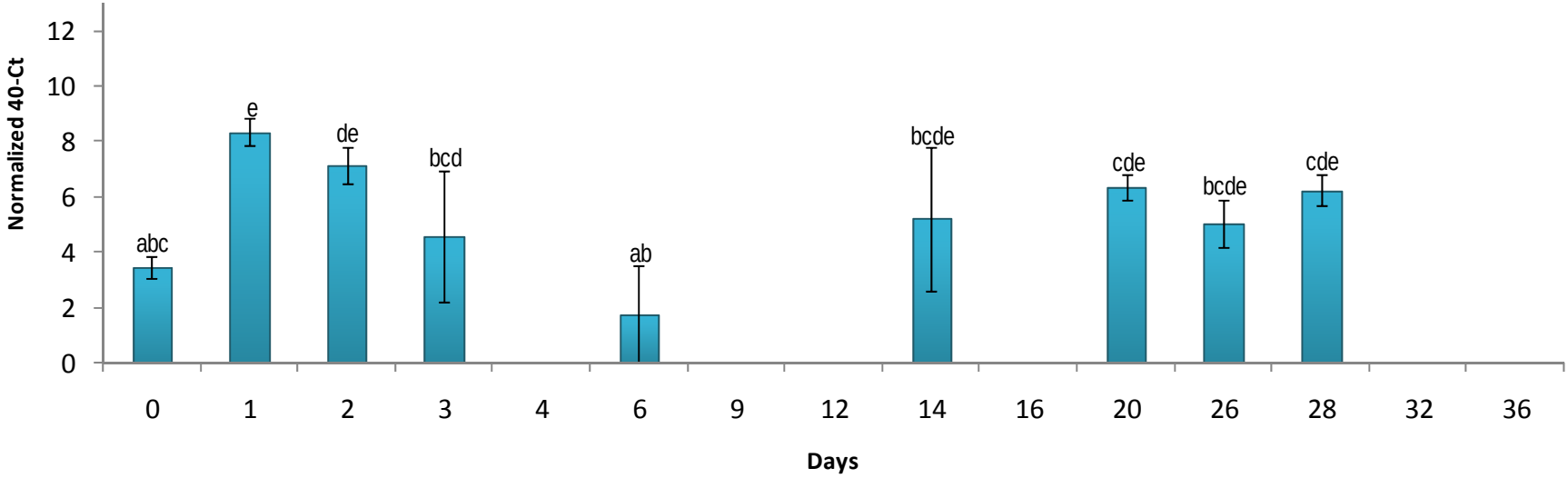


Fig 4.18 Expression profile of Insulin-like Growth Factor-I mRNA in jejunum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

4.1.4.5 Insulin-like Growth Factor Receptor

The pattern of IGF-R expression was similar to that of IGF-I in the jejunum (Fig 4.19).

4.1.5 Expression of various genes (mRNA) in proventriculus during moulting

mRNA expression of ghrelin, cGHSR, glucagon receptor, insulin receptor, IGF-I, IGF receptor, Adipo-R1 and leptin were studied in the glandular stomach (proventriculus) of chicken. Of these, Adipo-R1, insulin receptor and leptin mRNA expressions were not detected and thus not included in the results.

4.1.5.1 Ghrelin

Ghrelin mRNA levels were reduced marginally on day-1 and upregulated again from day-2 till day-6. No expression was noticed on day-9 and -12. Thereafter the levels increased and reached normal levels on day-20. After day-20, no expression was found on day-26 and low levels were maintained subsequently till it reached initial levels on day-36 (Fig 4.20).

4.1.5.2 Growth Hormone Secretagogue Receptor

The expression level of cGHSR was maintained at lower levels throughout the experimental period. However, variation in expression levels was found from day-12 to -26 (Fig 4.21).

4.1.5.3 Glucagon Receptor

The glucagon receptor mRNA levels were almost consistent throughout the experimental period. However, no expression was noticed on day-12 (Fig 4.22).

4.1.5.4 Insulin-like Growth Factor-I

The IGF-I mRNA levels were lower on most of the days and were steady throughout the experimental period except for day-9 and -12 on which no expression was noticed (Fig 4.23).

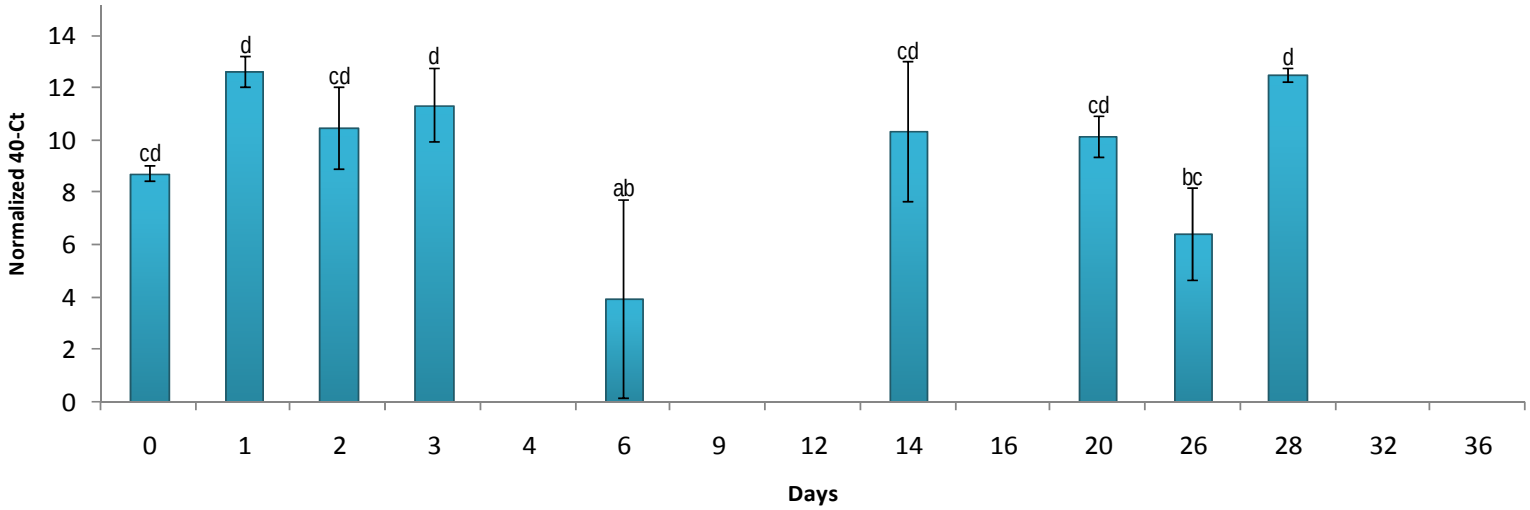


Fig 4.19 Expression profile of Insulin-like Growth Factor Receptor mRNA in jejunum of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

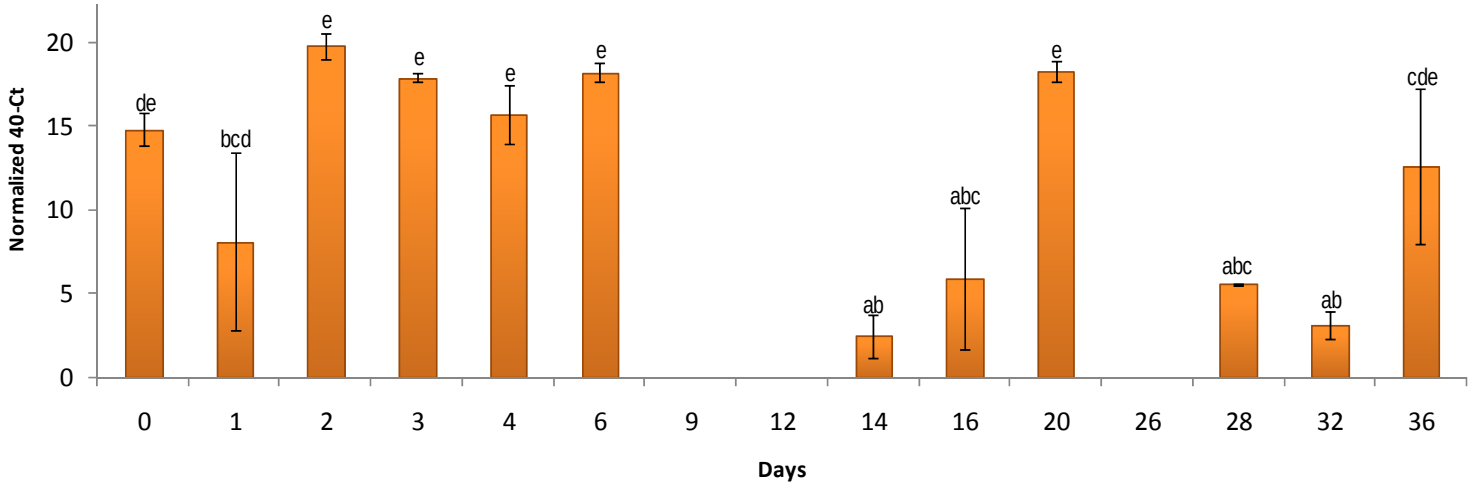


Fig 4.20 Expression profile of Ghrelin mRNA in proventriculus of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

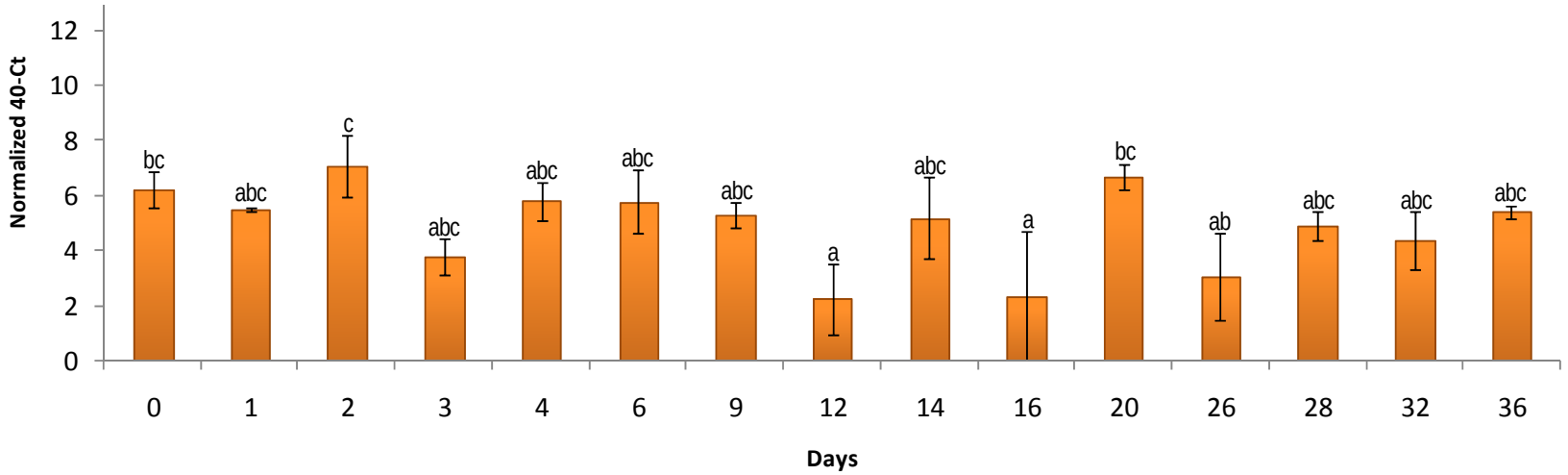


Fig 4.21 Expression profile of Growth Hormone Secretagogue Receptor mRNA in proventriculus of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

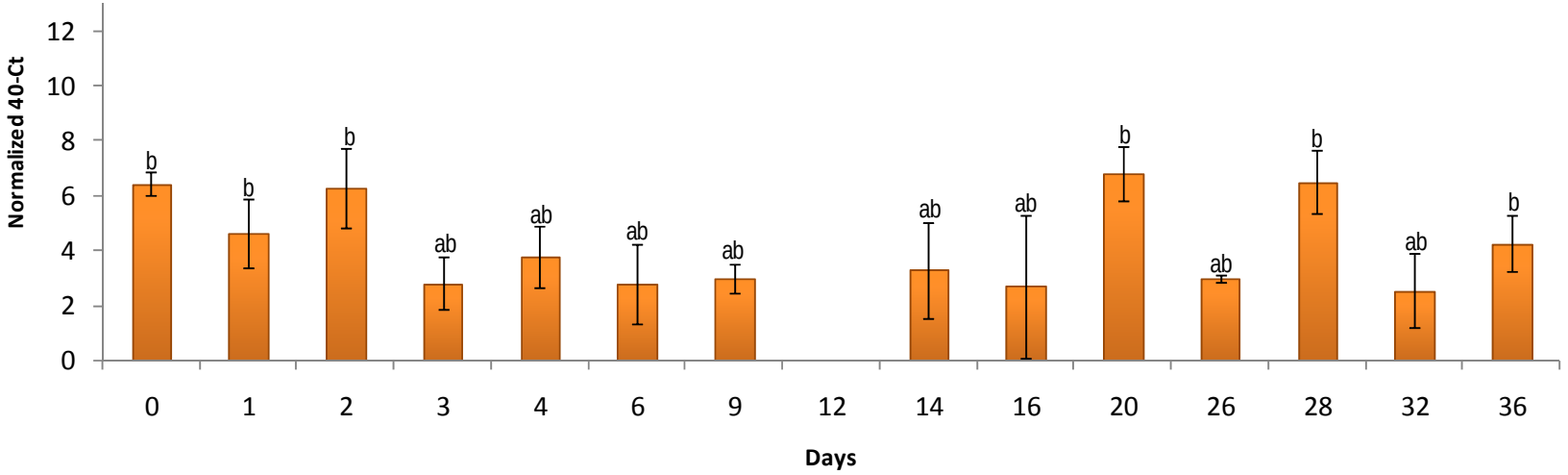


Fig 4.22 Expression profile of Glucagon Receptor mRNA in proventriculus of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

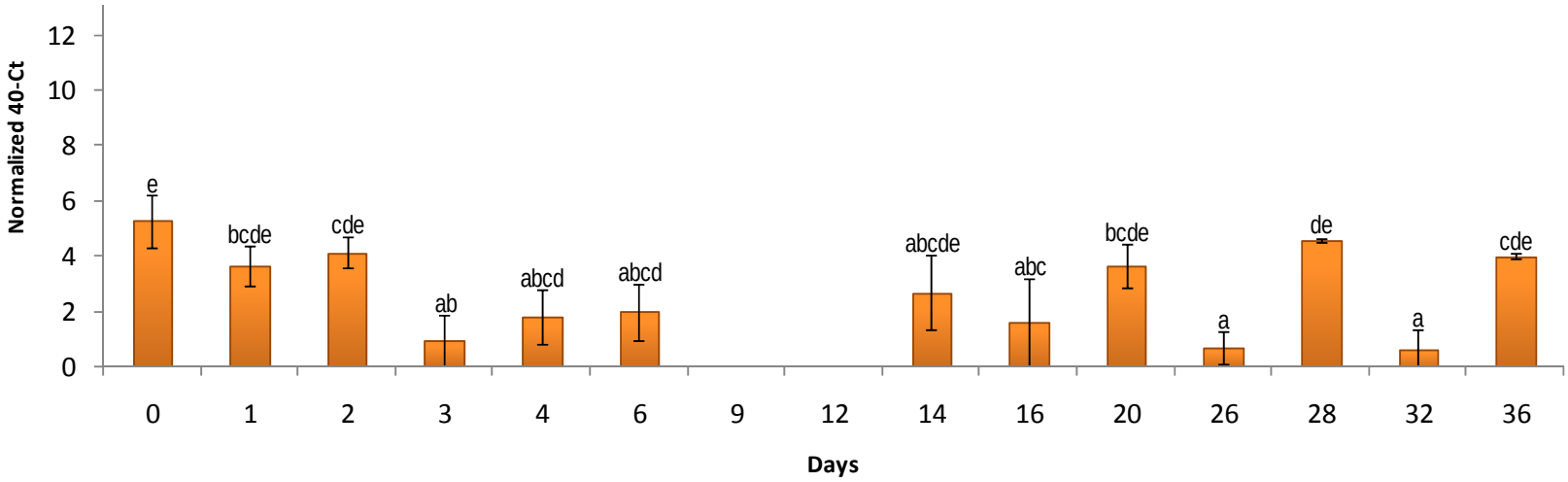


Fig 4.23 Expression profile of Insulin-like Growth Factor-I mRNA in proventriculus of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

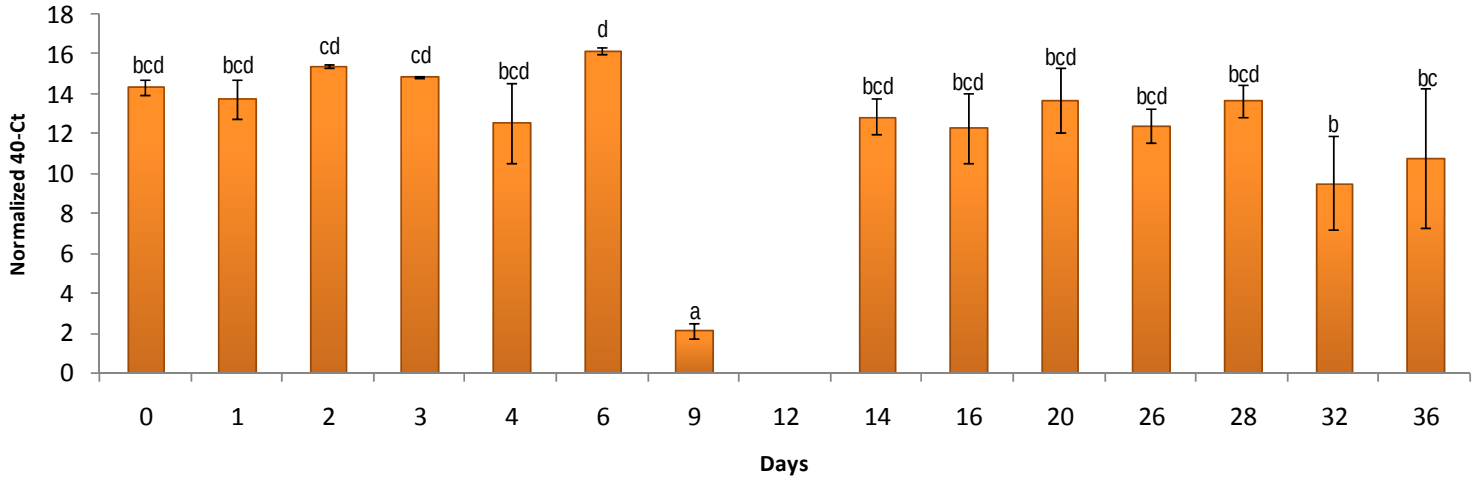


Fig 4.24 Expression profile of Insulin-like Growth Factor Receptor mRNA in proventriculus of birds on different days of treatment as obtained by real-time PCR method (Mean±SE; N=3; means bearing different superscripts differ significantly at P≤0.05).

4.1.5.5 Insulin-like Growth Factor Receptor

The mRNA expression pattern of IGF-R in proventriculus almost conformed that of IGF-I. However, the normal expression levels were very high compared to IGF-1 throughout the experiment. Significant ($P \leq 0.05$) downregulation in expression levels were found on day-9 while no expression was noticed on day-12 (Fig 4.24).

4.2 EXPERIMENT II

In this experiment, the effect of certain hormonal factors (progesterone and estrogen analog along with low levels of thyroxine) in the post-moult diet of birds moulted by conventional method was studied. Sixty birds were moulted by feed withdrawal for a period of 14 days. Thereafter, birds were divided to three groups of 20 birds each and were provided with post-moult diet for 14 days described as follows. Group A served as control and was fed with a conventional post-moult diet of cracked maize. Group B was fed with a post-moult diet enriched with thyroxine (1 mg/kg), vitamin E (200 mg/kg) and shell grit (3 kg/100 kg feed). Group C was fed with a similar diet with the addition of progesterone and estrogen analog (Fig 3.2).

The birds stopped the egg production completely on day-7 of starting the feed withdrawal. A body weight reduction of 25% was attained on day-14 of treatment. Feeding of cracked maize and experimental diets was started on day-15 and ended on day-28. Thereafter, a regular layer feed was provided to the birds.

On day-29 of starting the experiment, 8 birds were sacrificed from each group and the weight of ovary, oviduct, separated magnum, liver and proventriculus were measured. Moreover, the egg weight and shell thickness were also studied before and after treatment.

4.2.1 Weight and shell thickness of eggs before and after moulting and recovery period.

The results are presented in Table 4.2. No significant differences in the egg weight and shell thickness were observed before and after treatment (Fig 4.25 and 4.26 respectively). It was also observed that the incidence of shell-less or soft-shelled eggs were not found in any of the groups up to a period of 50% egg production after moulting.

4.2.2 Visceral organ weights of birds after feeding of post-moult diets

The result of the experiment is presented in table 4.1. Significant ($P \leq 0.05$) differences were found between the mean ovary weights of Group B (13.23±2.79g) and Group A (23.87±3.57). The mean ovary weight of Group B was lesser than that of Group A. However, the mean ovary weights of Group C (15.7±3.27g) were not significantly different from that of Group A or Group B (Fig 4.27 Plate A).

In the oviduct, no significant differences were noticed either in terms of the whole mean oviduct weight or mean magnum weight (Fig 4.27 Plate B and 4.27 Plate C respectively).

Mean liver weight of the Group A (48.96±4.08g) was significantly ($P \leq 0.05$) higher than that of Group B (33.65±1.09g). However, no significant differences were noticed between the mean liver weights of Group C (41.16±4.93g) and Group B (Fig 4.27 Plate D).

No significant differences were found between the mean proventriculus weights of the three groups (Fig 4.27 Plate E).

4.2.3 Egg production parameters after moulting

About 50% of the production loss was noticed within 4 days of starting the feed withdrawal. The production was totally stopped on 7th day of the feed withdrawal.

Table 4.2 Overall results of Experiment II

Parameter		Post moult **Group A** (Cracked maize only)	Post moult **Group B** (Cracked maize+ Thyroxine+Vit E+Shell grit)	Post moult **Group C** (Group B diet + Progesterone and estrogen analog)
Ovary weight (g)		23.87±3.58^{b}	13.23±2.8^{a}	15.69±3.27^{ab}
Liver weight (g)		48.97±4.08^{b}	33.65±1.09^{a}	41.16±4.93^{ab}
Oviduct weight (g)		45.50±3.52	38.83±3.77	42.71±6.07
Magnum weight (g)		29.30±2.73	23.95±2.53	26.57±3.82
Proventriculus weight (g)		5.38±0.32	4.72±0.22	4.67±0.21
Appearance of first egg		12 days of cracked maize	2 days of layer feed	2 days of layer feed
50% production attainment		10 day of layer feed (39 eggs)	10 day of layer feed (26 eggs)	11 day of layer feed (29 eggs)
Egg weight (g)	**Pre-moult**	57.7±0.88	56.18±2.07	56.45±1.79
	Post-moult	57.45±1.47	55.04±1.5	54.68±1.09
Shell thickness (mm)	**Pre-moult**	0.33±0.01	0.33±0.01	0.30±0.02
	Post-moult	0.32±0.01	0.32±0.01	0.31±0.01

Means bearing different superscripts in a row differ significantly (P≤0.05).

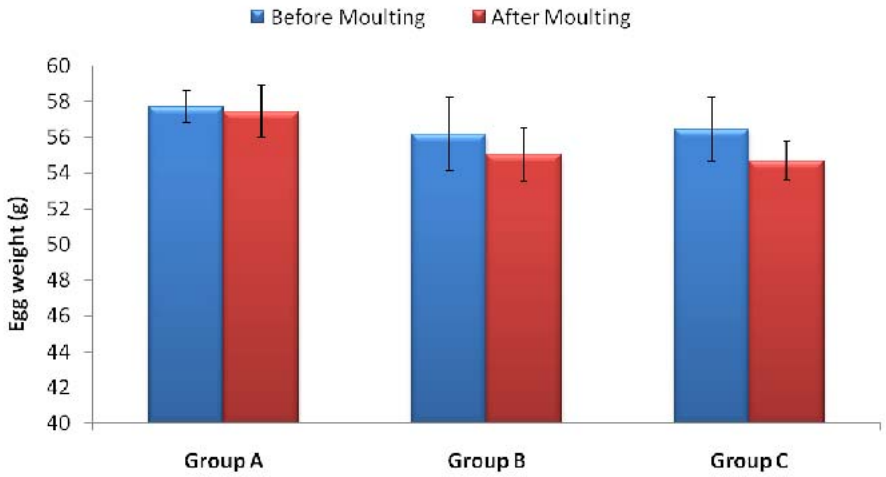


Fig 4.25 Changes in egg weight before and after moulting (Mean±SE).
Group A: Post-moult diet of cracked maize; ***Group B:*** Post-moult diet enriched with thyroxine, tocopherol and shell grit; ***Group C:*** Post-moult diet similar to Group B along with progesterone and estrogen analog.

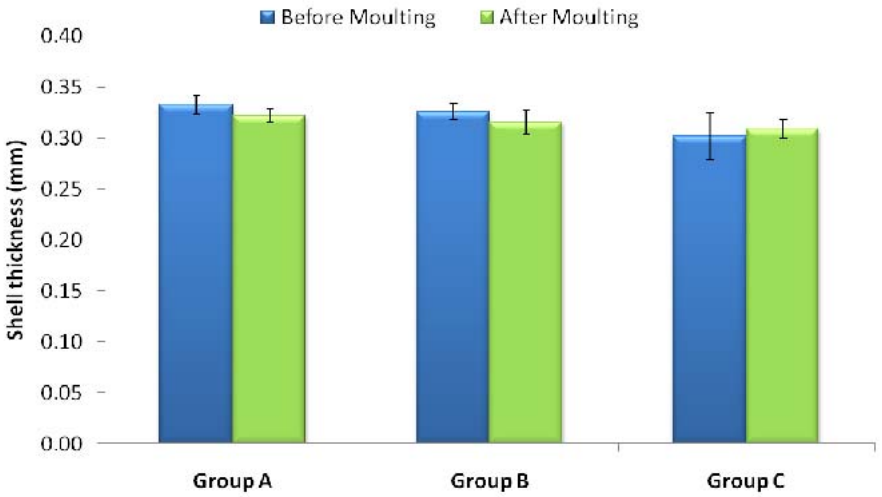


Fig 4.26 Changes in shell thickness before and after moulting (Mean±SE).
Group A: Post-moult diet of cracked maize; ***Group B:*** Post-moult diet enriched with thyroxine, tocopherol and shell grit; ***Group C:*** Post-moult diet similar to Group B along with progesterone and estrogen analog.

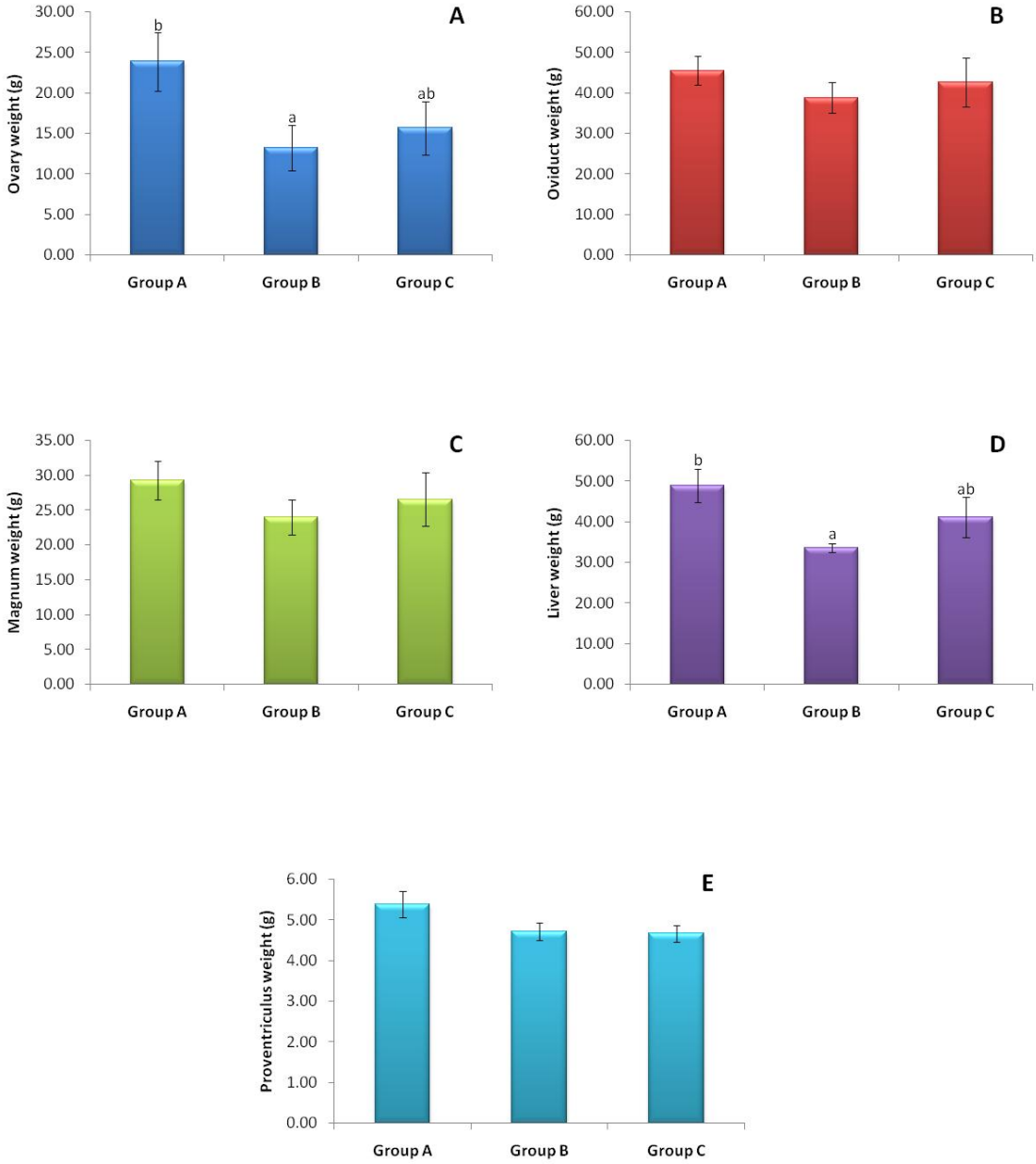


Fig: 4.27 Changes in visceral organ weights after rejuvenation period (Mean±SE; n=8)
Group A: Post-moult diet of cracked maize; ***Group B***: Post-moult diet enriched with thyroxine, tocopherol and shell grit; ***Group C***: Post-moult diet similar to Group B along with progesterone and estrogen analog. Different superscripts above the mean indicate significant difference (P≤0.05).

In the Group A, the first egg was obtained 2 days before starting the layer feed. They reached 50% production on 10th day of starting the layer feed.

In the birds of group B, first egg was obtained on 2nd day of starting the layer diet. 50% production was accomplished on 10th day of starting the layer feed.

In the Group C birds, first egg was obtained on 2nd day of starting the layer diet. This group reached 50% egg production on 11th day of starting the layer feed.

4.3 EXPERIMENT III

The design of this experiment is depicted in Fig 3.3. Group 1 (24 birds) was treated with fasting along with a low amount of thyroxine in water (5mg/lit). The fasting was continued till the birds lost 25% of body weight on 14th day of treatment. Group 2 (24 birds) was treated with 40mg/kg of thyroxine in feed. In this group, the treatment was continued for 10 days as feather loss was obvious by 9 days of treatment. An unmoulted control group (12 birds) and Conventional Moult control group (12 birds) were also kept.

After the treatment period, the treatment groups were divided into two subgroups each, and treated with cracked maize as post-moult diet or an enriched post-moult diet for a period of 14 days. Thereafter, a regular layer feed was provided. The conventional moulting group was given cracked maize as post-moult diet for 14 days. Thereafter, egg weight, shell quality and egg production parameters were studied in all groups.

4.3.1 Body weight of birds during moulting

The changes in body weight are presented in Table 4.3 and Fig 4.28. There was no significant change in the body weights of the unmoulted group (initial BW 2.18±0.07kg and final BW 2.24±0.06) after 14 days.

The conventional moulting group lost about 25% of body weight by 14 days (initial and final body weight 2.15±0.08kg and 1.58±0.13kg respectively).

Table 4.3 Overall results of Experiment III

Parameter		Unmoulted	Conventional Moult	Fasting + Thyroxine in water (Group 1)		Thyroxine (40mg/kg) in feed (Group 2)	
				Cracked maize (Group 1A)	Enriched diet (Group 1B)	Cracked maize (Group 2A)	Enriched diet (Group 2B)
Body weight (kg)	**Pre-moult**	2.19±0.08	2.15±0.18	2.23±0.13		1.97±0.16	
	Post-moult	2.24±0.06	1.58±0.13*	1.71±0.19*		1.89±0.14	
Day of last egg		-	Day-7	Day-5		Day-6	
Full Feather moulting		-	Day-11	Day-11		Day-9	
Appearance of first egg after moulting		-	12th day of cracked maize feeding	12th day of cracked maize feeding	14th day of enriched diet	4th day of layer feed	5th day of layer feed
50% production attainment		-	10th day of layer feed (46 eggs)	11th day of layer feed (48 eggs)	10th day of layer feed (36 eggs)	11th day of layer feed (29 eggs)	12th day of layer feed (24 eggs)
Shell thickness (mm)	**Pre-moult**	0.29±0.02	0.33±0.01	0.29±0.01	0.30±0.01	0.31±0.01	0.31±0.01
	Post-moult	0.22±0.01*	0.32±0.01	0.30±0.01	0.32±0.01	0.29±0.01	0.30±0.01
Egg weight (g)	**Pre-moult**	54.56±1.28	57.7±0.88	55.00±1.05	55.49±1.01	52.66±1.37	55.73±1.32
	Post-moult	51.82±1.45	57.45±1.47	55.08±2.41	53.94±1.62	51.18±3.48	55.54±2.14

Asterisk (*) in a column with respect to a parameter indicates significant (P≤0.05) difference.

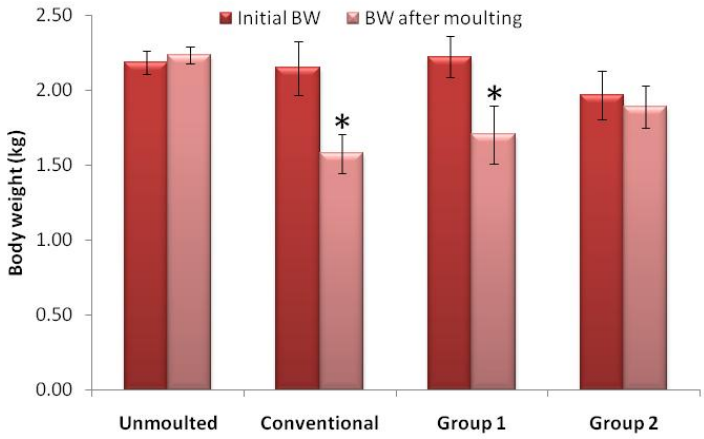


Fig 4.28 Changes in body weights after moulting.
Asterisk (*) indicates significant difference (P≤0.05).

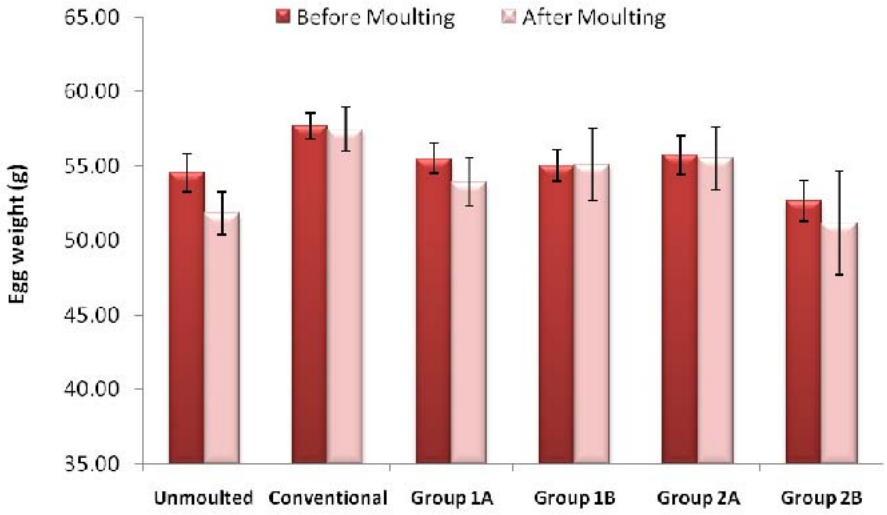


Fig **4.29** Changes in egg weights after moulting

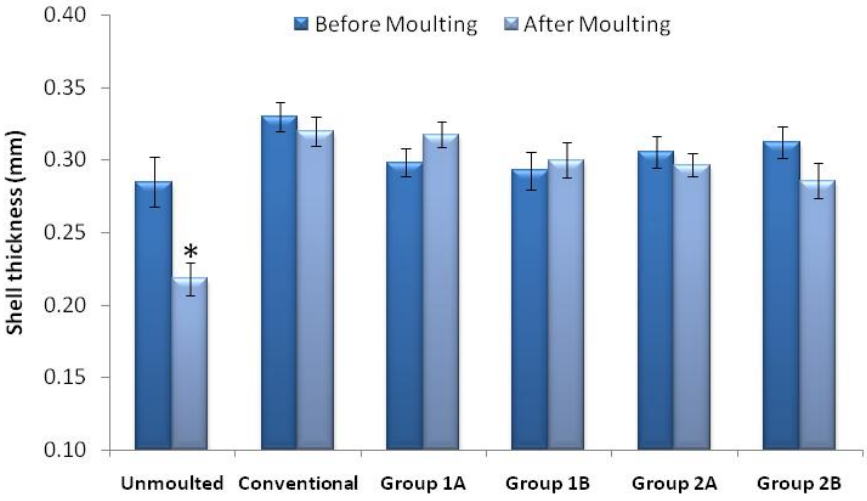


Fig **4.30** Changes in shell thickness after moulting
Asterisk (*) indicates significant difference (P≤0.05).

The birds of Group 1 (feed withdrawal along with thyroxine in water) lost about 25% of their body weight in 14 days (on a group basis). This reduction was statistically significant (P≤0.05). The initial and final mean body weights of this group were 2.23±0.13kg and 1.71±0.19kg respectively.

The thyroxine fed birds (Group 2) lost very less of their body weight even after 10 days of treatment. An overall body weight reduction of 4.5% was observed in this group during this period. The initial and final mean body weights of this group were 1.97±0.16kg and 1.89±0.14kg respectively.

4.3.2 Feather loss in the moulting birds

In the Group 1 birds which received feed withdrawal along with thyroxine in water began feather shedding on 9th day of treatment. The feather loss was obvious from about 11th day of treatment. The same was true for the conventional moulting group also.

Feather loss was initiated in Group 2 birds on 7th day of treatment and total feather loss was evident from 9th day onwards. Due to this reason, the drug was discontinued after 10 days of treatment and PMD was started thereafter.

4.3.3 Egg production changes in moulting birds

In the conventional moulting group, total stoppage of egg production was observed on day-7 of moulting. In the birds of Group 1, total stoppage of egg production was observed on 5th day. In the Group 2 birds, eggs were totally stopped on 6th day of treatment.

In the birds of Group 1A, the first egg was obtained on 12th day of feeding the post-moult diet. 50% production was obtained from 11th day of after providing the layer feed. In the birds of Group 1B, the first egg was obtained on 14th day of starting the post-moult diet. The birds reached 50% production on 10th day of starting the layer feed.

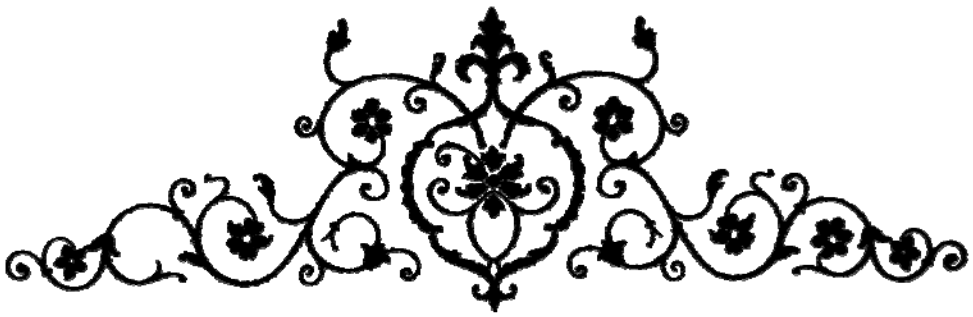
In Group 2, no egg was obtained before starting of layer feed. In Group 2A, the egg production was started on 4th day of starting the layer feed. 50%

production was reached on 11th day of starting the layer feed. The Group 2B, first egg was obtained on 5th day after starting the layer feed. This group reached 50% production on 12th day of starting the layer feed.

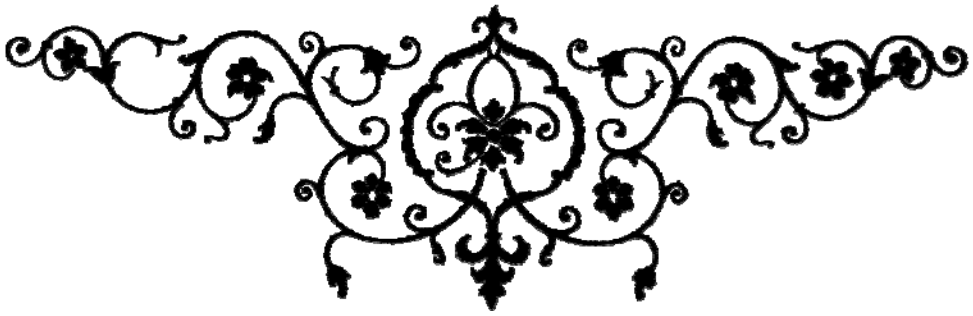
4.3.4 Egg weight and shell thickness before and after moulting

The results are presented in Table 4.3. No significant difference in egg weight was found in the unmoulted birds before and after the experiment (Fig 4.29).

The results are presented in table 4.3. No significant differences were found in shell thickness of the conventional moulting group or the treatment groups before and after moulting. However, it was observed that the shell thickness after moulting was numerically higher in Groups 1A and 1B and lower in Groups 2A and 2B. Moreover, shell-less or soft-shelled eggs were not found after treatment in any of the treatment groups after moulting, while these eggs were found in the unmoulted group. A significant ($P \leq 0.05$) reduction in shell thickness was found in the unmoulted birds during this period (Fig 4.30).



DISCUSSION



Discussion

Forced moulting is a common management practice in commercial poultry production. Farmers resort to this practice for stabilizing the profit margins that are under peril because of escalated production costs. During forced moulting, the reproductive tract of the birds gets regressed and rejuvenated in preparation for a second cycle of lay along with a superficial loss of body feathers. This helps to avoid the purchase of young chicks as well as to postpone the sale of spent hens. Moulting also provides greater flexibility to the farmer for anticipated fluctuations in feed cost and egg prices.

Although the forced moulting is in practice for last 50 years, of late, it attained criticism because of the feed withdrawal practices resulting in animal suffering and public health problems.

Therefore, efforts have been made to explore the alternative methods to feed withdrawal. However, such alternative methods developed so far could nto produce results comparable to the traditional method. The design of a viable, animal friendly and hazard-free method of moulting necessitates a thorough understanding of the molecular and physiological processes in the chicken. The present work is an attempt towards understanding the role of the various hormones/receptors implicated in the digestive and reproductive system of birds during moulting. Besides, attempts were also made to manipulate the purchase moulting and regeneration using progesterone and estrogen analogs and thyroxine.

5.1 EXPERIMENT I

In this study, expression of Estrogen receptor-α (ER-α), progesterone receptor (PR), adiponectin receptor (Adipo-R1), leptin, insulin and glucagon receptors, ghrelin and growth hormone secretagogue receptor (GHSR) as well as insulin-like growth factor (IGF-I) and its receptor (IGFR) were studied in chicken tissues during a moulting regime which included 13 days moulting by feed withdrawal, subsequent 14 days refeeding by cracked maize, and then provision of layer diet for rest of the days till 36th day of experiment. ER-α, PR and Adipo-R1 were studied in the reproductive system (magnum and uterus parts of

chicken oviduct). Adipo-R1, Leptin, insulin and glucagon receptors, ghrelin, GHSR, IGF-I and IGFR were studied in digestive system (liver, jejunum and proventriculus).

3.3.1 Expression of Adiponectin receptor gene (mRNA) in various tissues

Adiponectin is an adipokine abundantly expressed in adipose tissues in mammals (Kadowaki and Yamauchi, 2005). It plays a dominant role in lipid and carbohydrate metabolism. Adiponectin stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing insulin sensitivity (Chabrolle *et al.,* 2007). It is also implicated in regulation of energy balance and body weight. Adipo-R1 and Adipo-R2 genes are ubiquitously expressed in chicken tissues and that their expression is altered by feed deprivation in the anterior pituitary gland and adipose tissue (Ramachandran *et al.,* 2007)

In the present study, no change in the gene expression of Adipo-R1 was detected in magnum throughout all days of moulting and post-moult period. However, a rhythmicity in upregulation and downregulation was noticed in the uterus.

The uterine glandular epithelium, which is the site of calcium (Ca) transport and deposition to egg-shell, contains a higher quantitiy of intracellular lipids that are visible by histological staining (Baker, 1981). Hens accumulate higher amount of lipids in shell gland during old age (Baker *et al.,* 1980; Baker, 1981). It is also common that there is a higher incidence of shell-less or soft-shelled eggs during the end of the production cycle. Shell-less eggs are especially found in those birds with high uterine lipid (Roland *et al.,* 1977). Induced moulting reduces shell-gland lipid and alters the composition of lipids (Baker, 1981). The body weight reduction during moulting is assumed to have a role in the oviduct weight reduction and lipid mobilization. Brake and McDaniel (1981) have reported that optimum post-moult performance was achieved when BW loss was greater than 25% where complete loss of additional uterine lipid was noticed which also coincided with maximum oviduct regression. Thus, the

adiponectin receptor expressed in the uterus might have a role in lipid mobilization from the uterus that might be responsible for a better shell quality in the subsequent production cycle. However, more studies are required to establish the effect of moulting with regard to Adipo-R1 in the shell-gland.

In the liver, Adipo-R1 mRNA expression of moulting birds showed marginal variation. In general, lowest expression level was found on day-3, day-12 and day-32. On day-3, liver weight reduces significantly on fasting during moulting by feed withdrawal compared to other days (Anish, 2005). After day-12, the increase noticed may be as an effect of refeeding. On feeding with layer feed on day-27, the levels again reduced from day-28. It appears that the expression profile of the Adipo-R1 is in tune with fasting and refeeding regime.

3.3.2 Expression of estrogen and progesterone receptor genes (mRNA) in reproductive tissues

A significant upregulation of ER-α gene expression was noticed on day-1 in magnum and uterus compared to initial levels. A similar upregulation in PR expression was noticed in the uterus on day-2.

ER-α levels are increased during estrogen deprivation in estrogen dependent xenografts in athymic mice (Detre *et al.,* 1999). Fasting reduces serum estradiol and progesterone levels after only one day of fasting (Etches *et al.,* 1984), however, progesterone levels decreases more gradually compared to estrogen (Braw-Tal *et al.,* 2004). ER-α receptor population decrease in chicken shell gland at later stage of egg production compared to pre-lay pullets (Hansen *et al.,* 2003). The concentration of calcium binding protein in shell gland increases after moulting (Berry and Brake, 1991) and estrogen and progesterone may play important role in modulation of calcium binding protein in chicken shell gland (Navickis *et al.,* 1979). The results of the present study reiterated that the estrogen and progesterone receptor population is affected by fasting in chickens.

In the present study, no significant change in progesterone receptor gene expression was found in magnum whereas changes in expression were found in

uterus. Further studies are required on PR synthesis and regulation in oviduct in relation to plasma steroid hormone levels in moulting birds. These changes in expression of estrogen and progesterone receptors in the reproductive tissues suggest that there is a differential expression of steroid hormone receptor mRNA in different parts of oviduct of chicken during moulting.

3.3.3 Expression of ghrelin and cGHSR genes (mRNA) in digestive tissues

Discovered in stomach and other tissues, ghrelin is involved in control of food intake and adiposity. Ghrelin and its receptor are believed to be an inter-link between the diet and growth (Vallejo-Cremades *et al.,* 2004). Ghrelin administration suppress feed intake in neonatal chicks (Furuse *et al.,* 2001). Chen *et al.* (2007) reported that ghrelin mRNA is upregulated in liver and proventriculus after 12-36 h of fasting whereas cGHSR mRNA is upregulated after only 12 h fasting and come back to normal levels on refeeding. On the contrary, in the present study, we could not observe significant changes in ghrelin expression in liver on any of the experimental days. With respect to cGHSR, no changes in expression were found except for a marginal upregulation on day-2 and a reduction on day-26.

A notable change in ghrelin mRNA expression was found in proventriculus. These changes may be more or less related with diet modifications that included fasting for 13 days, low quality feed of cracked maize (high carbohydrate, low protein and low fat) for 14 days followed by layer feed (high calcium and high protein) for 9 days. It was already reported that the composition of diet can cause variation in the level of ghrelin expression in stomach (Chen *et al.,* 2007) and the results of the current study corroborate the same. It appears that fasting also influenced the ghrelin gene expression besides diet changes. A significant downregulation of cGHSR gene expression was noticed on day-12. This suggested that fasting regulates ghrelin receptor expression more than the dietary variations.

In jejunum, high variability among samples was detected from day-3 to day-14 as well as day-28 and -32 with respect to cGHSR expression. Lowest levels

were found on last week of fasting. These results indicate that on progression of fasting, the cGHSR mRNA levels come down, and then resumes to initial levels on refeeding. However, ghrelin expression in jejunum was found to be very low and inconsistent and is not included in results.

3.3.4 Expression of leptin gene (mRNA) in liver

Produced by both adipose tissue and liver, leptin is shown to induce satiety in chickens (Ashwell *et al.,* 2001). Leptin increases lipid storage by regulating fatty acid homoeostasis (Unger *et al.,* 1999). Leptin plasma levels are higher in fed chickens than in fasted ones (Dridi *et al.*, 2000) whereas injection of leptin decreases feed intake (Denbow *et al.,* 2000).

In the current study, expression of leptin mRNA was not obtained in jejunum and proventriculus. In liver, no significant differences in leptin mRNA expression were noticed on most of the experimental days with respect to initial days. During fasting, mean expression levels were found to be lowest on day-12. Thereafter the levels increased significantly on day-16 and decreased again on day-20.

The C*t* values obtained for chicken leptin mRNA were reasonable, however, the melt curve analysis revealed that the peaks were rather blunt/rounded. Various problems in amplification of leptin gene were reported by many workers (Amills *et al.,* 2003; Pitel *et al.,* 2000; Friedman-Einat *et al.,* 1999). Amills *et al.* (2003) reported that the chicken leptin primers amplified murine and human leptin targets. It was even suggested that the Genebank chicken leptin sequences may be artifactual and are phylogenically highly similar to rodent leptin sequences (Friedman-Einat *et al.,* 1999; Dunn *et al.,* 2001).

3.3.5 Expression of insulin and glucagon receptor genes (mRNA) in digestive tissues

In birds, glucagon is the dominant pancreatic hormone in order to meet the requirements of the constant high-carbohydrate supply for metabolic processes (Hazelwood, 2000). On fasting, within 24 h, glycogenolytic activity in

the chicken liver greatly increases, promoting net glucose efflux from the liver, associated with a 90% reduction in the liver glycogen (Davison and Langslow, 1975). In neonatal mice, fasting decreases glucagon receptor (GR) mRNA expression in liver whereas in adult mice, GR mRNA increases. Increase in glucose levels is found to increase GR mRNA in hepatocytes. Moreover, liver GR mRNA expression increases during fasting gluconeogenesis. (Burcelin *et al.*, 1998).

In the current study, a significant downregulation in expression of glucagon receptor mRNA was noticed on day-3 of moulting in liver. Thereafter, normal levels were maintained throughout the experimental period except for a slight reduction on day-9. A similar trend was exhibited by insulin receptor gene expression in liver even though the reduction on day-3 was less marked.

Anish (2005) reported that the liver weight decrease to about half on day-3 of fasting in chicken during moulting. Reduction in liver weight is attributed to the depletion of glycogen, proteins and lipids (Cleaver *et al.,* 1986). The continued egg production during these days might be a triggering factor for the fast liver weight loss. The liver weight stabilized after day-3 of fasting (Anish, 2005). The changes in liver metabolism and blood glucose concentrations on these days might have some role in the reduction in glucagon receptor mRNA expression on day-3. Further studies are required in moulting layers on the daily blood glucose variation and GR mRNA levels in liver to find out possible associations.

In jejunum, glucagon receptor mRNA expression levels were downregulated on day-4 and were low for the rest of fasting period. The levels again upregulated on refeeding. The expression pattern of insulin receptor gene in jejunum was almost similar to glucagon receptor.

In the proventriculus, a progressive decline in glucagon receptor mRNA levels was noticed on fasting and no expression was found on day-12. Thereafter, the levels increased again. The insulin receptor expression in proventriculus was highly very low and insignificant therefore was not included in results.

3.3.6 Expression of insulin-like growth factor I and it receptor genes (mRNA) in digestive tissues

IGF is synthesized by various tissues and via autocrine and paracrine mechanisms to effect an array of anabolic pathways (McMurtry *et al.,* 1997). Plasma levels of IGF-I and –II decrease with fasting and increase with age (Beccavin *et al.,* 2001). Moulting had a profound effect on circulating concentration of IGF-I and its mRNA in the liver of hens. IGF-I concentrations were decreased 54 hours of fasting and increase from 3 days of refeeding (Mazzuco *et al.,* 2005).

In the present study, IGF-I and IGFR expression in liver were consistent throughout the study period, except for a significant downregulation on day-3 of moulting. The expression pattern of liver IGF-I and IGFR is highly similar to hepatic glucagon and insulin receptor expression. The reduction in expression of IGF-I and IGFR mRNA is in agreement with the IGF-I blood levels reported by Mazzuco *et al.* (2005) on second day of moulting. They have found a higher level of plasma IGF on 13th day, however, as they have sampled the blood only on 13th day after day-2, direct comparisons are not possible.

In the proventriculus, mean IGF-I expression was noticeably less compared to IGFR. No expression of IGF-I mRNA was noticed for last 7 days of moulting. For IGFR, the least expression was noticed on day-9 and no expression was found on day-12. However, on other days, there was no change in expression levels. It was noticeable that higher variations among the birds were obtained on the days after refeeding. IGFR expression in stomach of growing chicken was reported by Matsumura *et al.* (1996). Nguyen *et al.* (2007) reported that the upregulation of IGF-I in stomach induces re-epithelization during gastric ulcer in mouse. The variations in the IGF and its receptor expression may probably be associated with the re-epithelization during refeeding after feed withdrawal.

The results of experiment-I indicated that elucidation of complete association between expression of genes and the remodeling process during moulting necessitates further studies.

5.2 EXPERIMENT II

In the traditional feed withdrawal method of induced moulting, a body weight reduction of 25-30% is generally aimed at (Webster, 2003). The period during fasting and the following refeeding when egg production is essentially at zero is referred to as the rest period (Berry, 2003). During the rest period, various rations are provided as part of some moulting programmes. With the onset of renewed egg production, a layer ration is returned to the flock.

Various rest diets have been tried and of these the most commonly practiced one is the cracked corn/maize. The length of the rest period in which no eggs are produced is associated with post-moult performance (Hansen, 1960). The rest diet typically contains lower protein and calcium so that the egg production is postponed and the rest period is long. In the current experiments, a rest period of two weeks (14 days) was practiced. However, recently workers have advocated the use of shell grit in rest diet to improve the shell quality in later production period.

It is an early observation that during moulting, there is a reduction in the plasma thyroxine levels. Moreover, feeding of thyroid gland extracts is found to initiate a moult (Torrey and Horning, 1922; Brake *et al.,* 1979). However, feeding of small amount of thyroxine to modify the post-moult diet has not been tried. Intend behind using a small quantity of thyroxine (1mg/kg feed) in the current experiment was to improve the feed intake and to hasten recovery of the reproductive tract.

During fasting, there is a decrease in the steroid hormone concentration in the plasma (Etches *et al.,* 1984). In order to improve the steroid status for faster reproductive recovery, progesterone and estrogen analog (PE analog) was supplemented in one of the diets along with thyroxine. Tocopherol (vitamin E)

supplementation is very effective to reduce the effect of stress (Tengerdy, 1989) and have antioxidant properties (Halliwell and Gutteridge, 1989). Along with tocopherol acetate, shell-grit was also supplemented in the enriched post-moult diet in order to improve the shell-quality.

After the period of moulting and rest, a few birds from each group were sacrificed to compare the visceral organ weights. Significant differences in the ovary and liver weights were obtained in the experiment. The control group, which received cracked maize only, was found to have the highest recovery rate. The mean liver and ovary weights in the groups were in the following order. Group A (Control; cracked maize only) > Group C (PE analog + Thyroxine + Vitamin E + Shell-grit) >Group B (Thyroxine + Vitamin E + Shell-grit).

These results indicate that the thyroxine treatment was ineffective at improving the reproductive organ weight. Rather, it may be interpreted that thyroxine feeding at 1mg/kg rate caused delay in reproductive recovery. However, though the mean ovary and liver weights of the Group I was lesser than Control, they were not significantly different. This may be indicate that the supplementation of the PE analog could effectively counter the negative effect of thyroxine to an extent with respect to regain in reproductive organ weights. The correlation and similarity among the weight of ovary and liver are not surprising. Liver is the primary site of lipid synthesis in poultry. These lipids are later transported across blood stream to be deposited in the ovarian follicles in the form of yolk. As the ovary weight of the Group I was not significantly different from Control, the PMD of this group was selected for usage in the Experiment III.

No significant differences were found among different groups with respect to the oviduct weight and proventriculus weights. However, even though not statistically significant, the mean oviduct weights were conforming to the ovary weights numerically.

No significant changes in the egg weight and shell quality were found among the groups. Bell (2003) stated that post-moult eggs were larger than pre-moult eggs. Swanson *et al.* (1978) reported that the higher egg weights are as a

result of water restriction. However, Christmas *et al.* (1985) could not find differences in egg weight either with 4 days or 10 days of feed withdrawal. Len *et al.* (1964) reported that egg-shell quality is restored to a level of pullets of 5 months lay; however the shell quality is temporary.

With regard to egg production, it was noticed that the egg production initiation in the Control birds were faster than other groups. This is natural when correlating with the higher ovary weights found in this group. The thyroxine-fed groups started their production only after providing the layer feed. However, in these groups, the follicles might have apparently recruited to the hierarchy well before providing the layer feed, so that eggs appeared only 2 days after feeding it. However, it was also observed that all the groups reached 50% production almost simultaneously.

It may be concluded from this experiment that the supplementation of thyroxine, progesterone and estrogen analog and other components did not help in improving the performance significantly compared to the traditional post-moult diet. Thus, it may be assumed that further interventions with supplemented steroid hormones and thyroxine are not required to improve the moulting procedure as far as post-moult diet is concerned. The present post-moult diet of cracked maize is sufficient for good growth of reproductive organs and egg production/quality.

5.3 EXPERIMENT III

Early research indicated that thyroid gland controlled moulting of feathers. Zavadovsky (1925) reported that feeding mammalian thyroid gland to birds caused feather loss. Himeno and Tanabe (1957) reported that even antithyroid compounds like thiouracil caused moulting. T_4 may be an important hormone for moulting (Brake *et al.,* 1979) in fast induced moulting. Injection of T_4 caused moulting (Verheyen *et al.,* 1986).

Bass *et al.* (2007) reported that feeding of thyroxine either in purified form or as iodinated casein causes moulting. Hooge *et al.* (2006) filed patent

application for many methods of moulting encompassing the use of thryroxine. They advocated the use of some form of L-thyroxine administered at about 40mg/kg (10-500 mg/kg range) or inorganic iodide (Potassium Iodide 1000mg iodine/kg feed) for inducing feather loss and cessation of egg production in avian females.

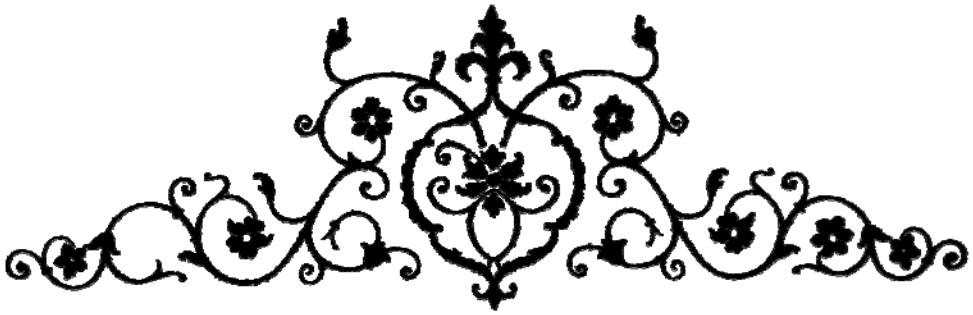
In the current experiment, no significant changes were found either with respect to egg weight or shell thickness before or after moulting. In case of shell thickness, there was a numerical increase in mean shell thickness in the groups which were treated with feed withdrawal along with thyroxine in water. However, there was significant reduction in shell quality in the unmoulted birds during this time. This indicates that in case of moulting by both methods, there were actual improvements in the shell quality.

The stoppage of production was earlier in the birds which were fasted and given thyroxine in water, compared to other groups. This indicates the possibility of usage of this method to reduce the period of fasting required during moult induction.

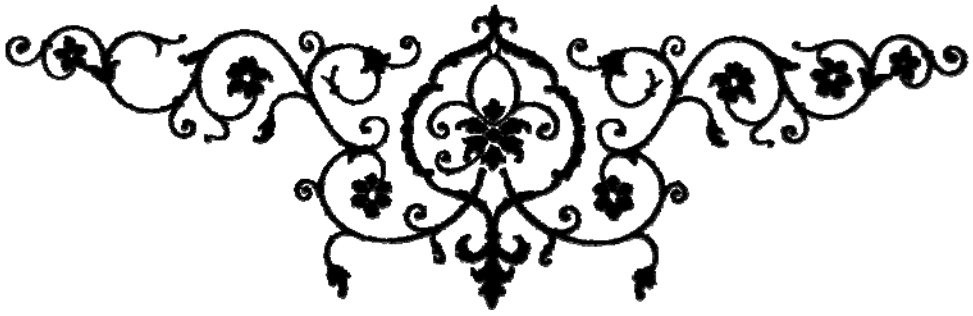
It was also evident from the current experiment that when thyroxine is used for moulting, there was no significant reduction in body weight compared to fasting treatment along with thyroxine in water. The onset of production was late in case of thyroxine fed chickens. This may be due to negative effect of high thyroxine in these birds. However, massive feather loss was observed in thyroxine fed birds. Probably the moulting caused by thyroxine is more superficial though it could bring about cessation of production. The possibility of incomplete reproductive regression is also indicated by the early attainment of 50% egg production in thyroxine fed birds. More studies are required on the changes in reproductive organs during moulting by high level of thyroxine in feed.

The results of this experiment indicated that feeding thyroxine may stand for a humane induced moulting method which can effectively cause moulting

and cessation of egg production without deleterious effects on post-moult egg quality. Nevertheless, further studies are needed for the economic implications of this moulting method as the cost of thyroxine may be a prohibitive factor. Further, studies are also to be conducted on dosage optimization of thyroxine and residual properties when alternative moulting programmes are devised with synthetic thyroxine compounds.



SUMMARY & CONCLUSION



Summary & Conclusions

Forced moulting is a common management practice in commercial poultry production. It provides greater flexibility to the farmer for anticipated fluctuations in chick cost, feed cost and egg prices. Although the forced moulting is in practice for last 50 years, of late, it attained criticism because of the feed withdrawal practices resulting in animal suffering and public health problems. Therefore, efforts have been made to explore the alternative methods to feed withdrawal. However, such alternative methods developed so far could nto produce results comparable to the traditional method. The design of a viable, animal friendly and hazard-free method of moulting necessitates a thorough understanding of the molecular and physiological processes in the chicken. The present work is an attempt towards understanding the role of the various hormones/receptors implicated in the digestive and reproductive system of birds during moulting. Besides, attempts were also made to manipulate the purchase moulting and regeneration using progesterone and estrogen analogs and thyroxine.

Experiment I

This experiment was designed to understand the role of a few hormones and receptors in the digestive system and reproductive system of chicken during moulting and recovery stages using gene expression studies. The birds were moulted by feed withdrawal for 13 days and were provided cracked maize for the next 14 days. Thereafter, they were provided with a layer diet for 9 days. During the treatment period, 3 birds each were sacrificed on days 0, 1, 2, 3, 4, 6, 9, 12, 14, 16, 20, 26, 28, 32, and 36 days and samples from reproductive (magnum and uterus) and digestive (liver, jejunum and proventriculus) tracts were collected. The mRNA expression profiles of estrogen receptor-α, progesterone receptor and adiponectin receptor-I were studied in reproductive system using real-time PCR methodology. In the digestive tract, adiponectin receptor-I, ghrelin and its receptor, glucagon receptor, insulin receptor, insulin-like growth factor-I and its receptor and leptin were studied. The results of this experiment indicated

that there is a differential expression of genes in different areas of reproductive and digestive tracts during experimental period.

The expression of adiponectin receptor mRNA in the oviduct indicated that, in the uterus, it might have a role in the loss of uterine lipids causing a better calcium deposition in the subsequent production cycle.

Variations were found in the expression level of ER-α mRNA in magnum and uterus in the current study. However, the results did not corroborate with the reported changes in the plasma estradiol during fasting. It appears that ER-α mRNA expression levels in oviduct were not related to the plasma estradiol levels. No significant change in progesterone expression was found in magnum whereas changes in expression were found in uterus. These changes in expression of estrogen and progesterone receptors in the reproductive tissues suggest that there is a differential expression of steroid hormone receptor mRNA in different parts of oviduct of chicken during moulting.

A notable change in ghrelin mRNA expression was found in proventriculus. These changes may be more or less related with diet modifications. It was already reported that the composition of diet can cause variation in the level of ghrelin expression in stomach. With respect to cGHSR expression, significant reduction from initial expression was found on day-12. In jejunum, the results indicated that on progression of fasting, the cGHSR mRNA levels come down, and then resumes to initial levels on refeeding.

In liver, no significant differences in leptin mRNA expression were noticed on most of the experimental days with respect to initial days.

In the current study, a significant downregulation in expression of glucagon receptor mRNA was noticed on day-3 of moulting in liver. Thereafter, normal levels were maintained throughout the experimental period except for a slight reduction on day-9. A similar trend was exhibited by insulin receptor, IGF-I and IGFR. It was reported already that there is a significant reduction in liver weight on day-3. These results suggested that the weight changes and yolk

synthesis in liver may be associated with expression of glucagon receptor, insulin receptor, IGF-I and IGFR.

In general, the pattern of expression of various genes in different tissues varied from no changes in expression to significant changes in expression associated with fasting and refeeding. The alterations in expression of various genes in liver especially glucagon and insulin receptors and IGF-I and IGFR were probably related with liver weight changes and yolk synthesis. In jejunum and proventriculus, the expression of IGF-I and IGFR was associated with fasting and refeeding. Ghrelin and glucagon receptor expression in proventriculus may also be probably associated with fasting and refeeding. More studies are required to find further association of different genes in moulting so that the molecular mechanism of induced moulting may be elucidated.

Experiment II

This experiment was designed to understand the effect of supplementing a progesterone and estrogen analog, thyroxine, vitamin E, and shell grit during the post-moult period compared to the classical post-moult diet of cracked maize. The birds were moulted by feed withdrawal for 14 days and subsequently divided in to three groups and then fed with (1) a diet containing cracked maize only, (2) cracked maize along with thyroxine, vitamin E and shell grit or (3) cracked maize along with steroid hormone analog, thyroxine, vitamin E and shell grit for a period of 14 days. Thereafter, layer diet was provided.

The results indicated that the thyroxine treatment was ineffective at improving the reproductive organ weight. Rather, it might have caused a delay in reproductive recovery.

It was concluded from this experiment that the supplementation of thyroxine, progesterone and estrogen analog and other components did not help in improving the performance significantly compared to the traditional post-moult diet. Thus, it may be assumed that further interventions with supplemented steroid hormones and thyroxine are not required to improve the moulting procedure as far as post-moult diet is concerned. The present post-

moult diet of cracked maize is sufficient for good growth of reproductive organs and egg production/quality.

Experiment III

The experiment was designed to evaluate the ability of a high dose of thyroxine to induce a moult compared to a fasting group provided with a small dose of thyroxine. After moult induction, the birds were provided with a conventional or enriched post-moult diet to understand the production status and egg parameters of moulted birds.

No significant changes were found either with respect to egg weight or shell thickness before or after moulting. This indicates that in case of moulting by both methods, there were actual improvements in the shell quality.

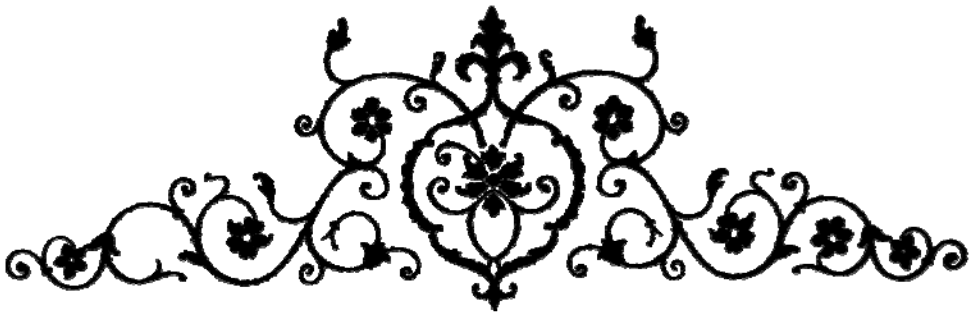
The stoppage of production was earlier in the birds which were fasted and given thyroxine in water, compared to other groups. This indicates the possibility of usage of this method to reduce the period of fasting required during moult induction.

It was also evident from the current experiment that when thyroxine is used for moulting, there was no significant reduction in body weight compared to fasting treatment along with thyroxine in water. The onset of production was late in case of thyroxine fed chickens. This may be due to negative effect of high thyroxine in these birds. However, massive feather loss was observed in thyroxine fed birds. Probably the moulting caused by thyroxine is more superficial though it could bring about cessation of production. The possibility of incomplete reproductive regression is also indicated by the early attainment of 50% egg production in thyroxine fed birds.

The results of this experiment indicated that feeding thyroxine may stand for a humane induced moulting method which can effectively cause moulting and cessation of egg production without deleterious effects on post-moult egg quality.

Conclusions

1. There is a differential expression of various hormone and receptor genes in different areas of reproductive and digestive tracts during moulting and post-moult periods.
2. Interventions at rejuvenation phase with supplemented steroid hormones and thyroxine as followed at the current regime are not required as far as rest diet is concerned.
3. Use of thyroxine may help in reducing the period of fasting required during moult induction.
4. Moulting with 40mg/kg feed thyroxine caused moulting and cessation of egg production, but failed to reduce body weight significantly.
5. Moulting by thyroxine could improve the shell quality similar to fasting treatment.
6. Thyroxine feeding may serve as a humane method of induced moulting.



MINI ABSTRACT

Mini Abstract

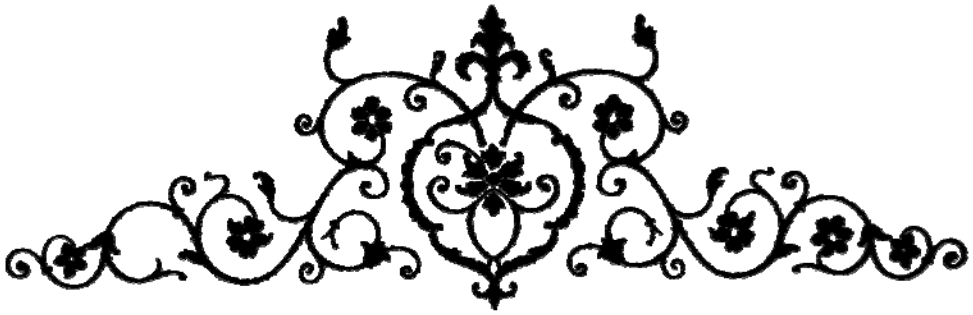
Induced moulting accelerates both natural feather loss and the process of regression and rejuvenation of reproductive organs at the end of the production cycle. One of the most commonly practiced methods of induced moulting is feed withdrawal, which encounters stiff resistance from animal-welfare organizations and has public-health implications also. To develop a humane and effective alternative method of moulting, in-depth understanding of the molecular and physiological events of moulting is necessary. To move ahead on the above lines, three experiments were carried out using RIR birds at the end of first year of lay.

In the experiment-I, birds were moulted by feed withdrawal (13d) followed by feeding of cracked maize (14d) and then a layer feed (9d). During the treatment period, birds were sacrificed on day-0, 1, 2, 3, 4, 6, 9, 12, 14,16, 20, 26, 28, 32 and 36 and samples from reproductive (magnum and shell gland) and digestive (proventriculus, liver and jejunum) tracts were collected. Using real-time PCR methodology, the gene expression profiles were studied for estrogen receptor-α, progesterone receptor and adiponectin receptor-1 in the reproductive system and adiponectin receptor-1, leptin, ghrelin and its receptor, glucagon and insulin receptors, insulin-like growth factor-I and its receptor in the digestive tract. The results of this experiment indicated that there is a differential expression of genes in different areas of reproductive and digestive tracts during experimental period.

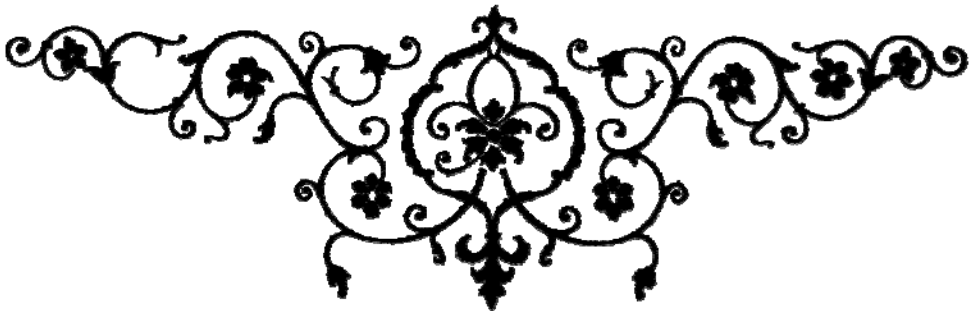
The experiment-II was conducted to study endocrine interventions at the rejuvenation phase of moulting. After 14d of fasting, the treatment groups were offered cracked maize supplemented with antioxidant and shell grit with either thyroxine or steroid hormone analog for a period of 14d. Thereafter, a few birds from each of the group were sacrificed and visceral organ (ovary, oviduct, liver and proventriculus) weights were recorded. Remaining birds were continued with *ad lib* layer feed. Egg production and quality parameters were studied. The results of this experiment indicated that the interventions at rejuvenation phase using current regime did not serve any additional useful purpose.

The experiment-III was undertaken to study the use of thyroxine during induction phase of moulting. In one of the treatment groups, low level of thyroxine was administered in water (5mg/l) along with feed withdrawal. In another treatment group, feed fortified with thyroxine (40mg/kg) was offered. Two control groups (unmoulted and conventional moulted) were also run simultaneously. After moult induction phase, each of the two treatment groups was further divided into two subgroups. One was supplied with normal and another with hormone enriched post-moult diet for 14d followed by *ad lib* layer feed. Egg production and quality parameters were monitored. The results of this experiment indicated that thyroxine supplementation in feed may constitute an animal-welfare friendly method of moulting without adverse changes in egg quality.

It is concluded that (1) gene expression of certain hormones and/or receptors are associated with moulting process, (2) no additional benefit is obtained on supplementation with hormones, antioxidant and shellgrit during rejuvenation phase, and (3) thyroxine is valuable in shortening the induction period without the need for fasting.



HINDI ABSTRACT



लघु सारांश

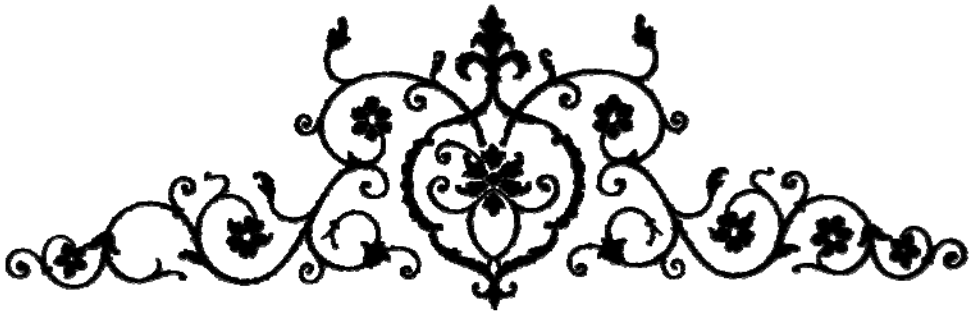
मुर्गियों में अण्डा उत्पादन चक्र के अन्त में प्राकृतिक रूप से उनके पखं गिरना तथा प्रजनन अगों की अवनति तथा पुर्नयुवाकरण को त्वरित करता है। उत्प्रेरित निर्मोचन के आहार विहीन पद्धति एक बहु प्रचलित विधि है। किन्तु इस पद्धति का पशु कल्याण संगठनो द्वारा तथा निर्मोचन की जन स्वास्थ समस्याओं से घोर विरोध होता है। एक मानवीय एवं कारगर विधि विकसित करने के लिए निर्मोचन के दौरान आण्विक व दैहिकी प्रतिक्रियाओं की गहरी जानकारी होना परम आवश्यक है। उस दिशा में आगे बढतें हुए प्रस्तुत शोध को आर आई आर पक्षियों को उत्पादन चक्र के अन्त में तीन प्रयोगों द्वारा सम्पन्न किया गया।

प्रथम प्रयोगों में– पक्षियो की 13 दिन तक आहार विहीन रख कर निर्मोचित किया गया तथा उसके बाद 14 दिन तक टूटी हुई मक्का तत्पश्चात लेयर आहार (9 दिन) दिया गया । इस प्रयोग विधि में पक्षियों का 0, 1, 2, 3, 4, 6, 9, 12, 14, 16, 20, 26, 28 , 32 तथा 36 दिन पर वध करके उनके प्रजनन अगं (मैगनम तथा शेल ग्रन्थि) तथा पाचन अगं (प्रावेन्ट्रीकुलस, यकृत एवं जिजूनम) के नमूनो को एकत्रित किया गया। रीयल टाइम पी0 सी0 आर0 विधि द्वारा विभिन्न जीन अभिव्यक्ति प्रोफाइल का अध्ययन किया गया। जिसमें इस्ट्रोजन रिसेप्टर α , प्राजेस्ट्रान रिसेप्टर, ऐडियोनेक्टिन रिसेप्टर–1, लेपटिन, घ्रेलिन तथा इन दोनो के रिसेप्टर, ग्लूकागोन, इन्सूलिन, इन्सूलिन समान वृद्विकारक –1 एवं उनके रिसेप्टर की अभिव्यक्ति का अध्ययन किया गया। प्राप्त परिणाम दर्शाते है कि विभिन्न पुनरूत्पादन तथा पाचन अगों में जीन अभिव्यक्ति में भिन्नता पायी गयी।

द्वितीय प्रयोग निर्मोचन की पुर्नयुवाकरण अवस्था मे अन्तः स्त्रवी हस्तक्षेप के अध्ययन के लिये किया गया। आहार विहीन पद्वति से 14 दिन तक निर्मोचन के पश्चात पक्षियों को टूटी हुई मक्का मे आक्सीडेन्ड रोधी एवं सेल ग्रीट मिलाकर थायरोक्सीन या स्टीरायड हारमोन अनुरूप के साथ चौदह दिन तक दिया। तत्पश्चात् प्रत्येक वर्ग से कुछ पक्षियों का वध करके अन्तः अंग (अण्डाशय, अण्डवाहिनी, यकृत तथा प्रावेन्ट्रीकुलस) का भार लिया गया । शेष पक्षियों को भरपेट लेयर दाने पर रखा गया । जिनमें अण्डा उल्पादन व अण्डों के गुणों का अध्ययन किया गया। इस प्रयोग के परिणाम दर्शाते है कि अन्तः स्त्रवी हस्ताक्षेप पुर्नयुवाकरण अवस्था मे जारी वातावरण मे कोई अतिरिक्त लाभ नही पहुचँाते।

तृतीय प्रयोग उत्प्रेरक निर्मोचन की अवस्था के दौरान किया गया। प्रथम वर्ग मे निम्न स्तरीय थायराक्सीन (5 एम जी/ली. पानी) तथा आहार विहीनता को साथ मे लागू किया। दूसरे वर्ग ने भोजन की 40 एम.जी. /के.जी. थायराक्सीन को भोजन को सशक्त कर पक्षियों को दिया। दो नियन्त्रित वर्ग (निर्मोचन रहित तथा पारंपरिक विधि से निर्मोचित)साथ ही रखे गये। निर्मोचन के उत्प्रेरक अवस्था बाद उपरोक्त दोनों वर्गो मे से प्रत्येक को फिर दो भागों मे बांटा गया । एक वर्ग को सामान्य तथा दूसरे को हारमोन मिश्रित आहार निर्मोचन के 14 दिन बाद तक दिया गया तत्पश्चात पेट भर लेयर दाना दिया। अण्डा उत्पादन व गुणवत्ता मापकों पर आकडें एकत्रित किये गये। परिणाम दर्शाते हैं कि दाने में थायराक्सीन मिश्रण बिना अण्डों की गुणवत्ता के परिवर्तन के निर्मोचन विधि अधिक प्रभावी ढगं से पशु कल्याण हिताय विकसित की जा सकती है ।

अध्ययन से यह निष्कर्ष प्राप्त हुए –(1) कुछ हारमोन या रिसेप्टर की जीन्स की अभिव्यक्ति स्तर निर्मोचन क्रिया से सम्बन्ध रखते है ,(2) हारमोन्स, आक्सीडेन्ट रोधी, तथा शेल ग्रिट के मिश्रण पुर्नयुवाकरण निर्मोचन अवस्था में देने से कोई अतिरिक्त लाभ नही है, (3) पक्षियों को बिना भुखा रखे थायराक्सीन द्वारा निर्मोचन अवधि कम की जा सकती है।



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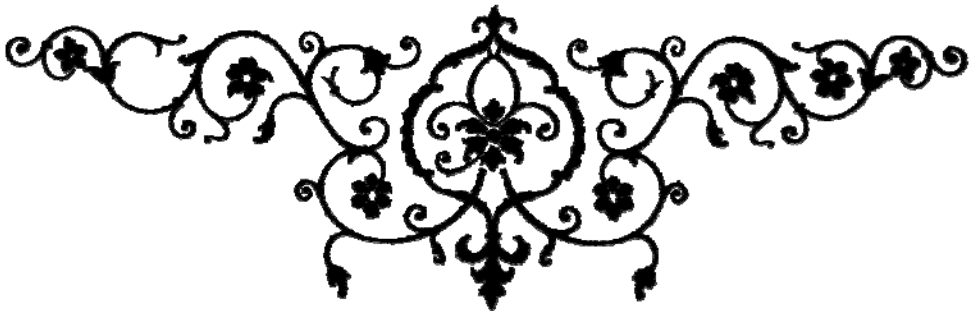

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ANNEXURE

Composition of Grower and Layer feeds used in experiments

Component	**Grower (%)**	**Layer (%)**
Maize	52	60
De-oiled Rice Bran	23.46	4
Soybean	7.2	15.665
Sunflower	11	5
Fishmeal	3	4
Mineral Mix (ISI)	1.2	1.2
Oyster Shell	0	3
Marble Chips	1	3.5
Limestone	0.5	2.5
DCP	0	0.4
Salt	0.2	0.2
Trace Mineral Premix	0.05	0.1
Vitamin Premix	0.15	0.15
B-complex	0.015	0.015
Choline Chloride	0.03	0.03
Sodium Bicarbonate	0.075	0.075
Vitamin C	0.01	0.01
Star Blend (Vit-E and Se)	0	0.01
Toxin Binder	0.06	0.075
Protexin	0	0.02
Coccidiostat	0.05	0
Crude Protein	15.41	16.24
Metabolizable Energy	2600	2635
Calcium	1.17	3.75
Available Phosphorus	0.42	0.40
Lysine	0.79	0.87
Methionine	0.37	0.35

Vitae

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