THE STRUCTURE AND FUNCTION OF THE SHELL GLAND IN JAPANESE QUAIL UNDER DIFFERENT LEVELS OF DIETARY CALCIUM

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Faculty of Veterinary and Animal Sciences
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DECLARATION

I hereby declare that the thesis entitled **"THE STRUCTURE AND FUNCTION OF THE SHELL GLAND IN JAPANESE QUAIL UNDER DIFFERENT LEVELS OF DIETARY CALCIUM"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or other similar title, of any other University or Society.

Mannuthy,
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CERTIFICATE

Certified that this thesis entitled **"THE STRUCTURE AND FUNCTION OF THE SHELL GLAND IN JAPANESE QUAIL UNDER DIFFERENT LEVELS OF DIETARY CALCIUM"** is a record of research work done independently by **Mrs. P.T. Philomina**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Chapter 1

INTRODUCTION

Japanese quail (Coturnix coturnix japonica) has been recognized as the choicest model for production oriented research in birds. The quail is unique in being a prolific breeder with a short generation gap and high producer of good quality meat and eggs. They require less floor space and feed. Further, quails have greater physiological similarities with poultry. It is on the basis of the foregoing merits that they are preferred as research models for elucidating problems associated with poultry husbandry and production. Rearing quails for egg and meat production is gaining momentum not only in Kerala, but also in other parts of the country. If quail husbandry is to be an economically viable industry, a sound knowledge of the reproductive physiology of the bird is essential. Hammond (1960) has rightly pointed out that "physiology is the basis for animal production". Considerable work in these birds seems to have been done on various production aspects. However, literature available on the physiological aspects of egg production especially oviductal functions in Japanese quail is scanty and many lacunae exist in the knowledge of the mechanisms involved in the formation of an egg.

In avian egg, the shell is equally or more important than its contents due to reasons more than one. The keeping quality of egg, its transportation and marketability are all primarily dependent upon the quality of egg shell. The formation of a good quality egg shell depends basically on a normally functioning shell gland. Also essential is an adequate supply of dietary calcium and its proper utilization by the shell gland. Normal physiological performance of the oviduct being taken for granted, dietary calcium may become a limiting factor in the regulation of the egg shell quality in birds. The birds in pre-laying period show a tendency for an increased calcium retention, higher plasma calcium concentration and greater calcium storage in the medullary bone (Hurwitz and Griminger, 1960). This indicates that a precautionary preparation is undertaken by the prospective layers to meet the ensuing emergency of an increased demand for calcium during egg shell formation. An investigation into these physiological aspects based on the structural and functional characteristics in Japanese quail would certainly throw much light on the mechanisms involved in egg shell formation.

Review of Literature

Chapter 2

REVIEW OF LITERATURE

Calcite (calcium carbonate) is the most predominant constituent of egg shell and therefore, it plays a decisive role in the egg shell formation. Because of the high rate of calcium turn over in the shell gland, the laying bird must maintain an adequate buffer plasma concentration of calcium than nonlayers, lest a severe negative calcium balance will occur. The ability of hens to produce good quality egg shell depends on the steady availability of calcium from blood, skeleton and food. A thorough knowledge of the absorption of calcium from the gut, its deposition in the medullary bone and resorption therefrom are of practical importance to elucidate the mechanism of calcium utilization by shell gland for egg shell formation. Eventhough limited studies have been carried out on this in chicken, investigations on this aspect in Japanese quail seem to be meagre. Data available on biochemical mechanisms involved in egg shell formation in Japanese quail are sparse and scanty. Perusal of literature indicated that the information on the physiology of calcium utilization and shell formation is very limited. Therefore, in this review informations on physiological mechanism of calcium utilization and shell formation in chicken have been documented.

2.1 Body weight and anatomical studies

2.1.1 Body weight

Studies on the influence of dietary calcium on the body weight of birds are scanty especially in Japanese quails. Most of the published work encompasses mainly nutritional, production or managemental aspects. Hurwitz et al. (1969) noticed that dietary supplementation of calcium carbonate in laying hen led to a depression in feed intake resulting in lower body weight gain in spite of an improved feed conversion. Hurwitz and Bar (1971) found in White Leghorn pullets, body weight at the onset or after about six weeks of egg production was not significantly altered by pre-laying level of dietary mineral content ($P<0.05$). The obvious conclusion from this study was that the onset of egg laying caused a spurt in the increase in body weight gain resulting in, pullets with added weight gain during their first phase of egg production. Their dietary requirements for calcium during this period were therefore relatively large. Ahuja et al. (1978) studied the inheritance of the traits for growth and reproductive performance in Japanese quails. According to them sexual maturity was attained in six weeks at which time body weight was 90.55 g while at 16 weeks of age it was 143.64 g. Jues and Houghes (1978) observed that Coturnix quail gained mature body weight of 162 g at six weeks of age.

Sharma and Panda (1978) reported that the Japanese quails reared in battery cages attained body weight of 108 g at eight weeks of age. Tiwari and Panda (1978) recorded the average body weight of quail at 30, 50, 100, 150 and 200 days of age as 87.8, 111.6, 129.5, 136.5 and 136.9 g respectively. They further observed that at 51 days of age the first egg was laid by quails and 50 per cent of egg production was reached by 67 days. Reddy et al. (1980) could not establish any relationship between levels of dietary calcium and phosphorus and physiological responses like weight gain, bone ash content, food intake and food-weight gain ratio. Sato et al. (1981) reported that female quails had a higher body weight. At the sixth and eighth week of age the body weight registered was 109 g and 118 g respectively. Influence of sex on body weight difference was evident at significant levels from three weeks of age onwards. Sachdev and Ahuja (1986) noticed that egg production actually declined as the body weight at maturity increased beyond 200 g. Moreover, this drop in egg production was sharp in birds weighing more than 220 g. On the basis of this they concluded that 200 g body weight was the optimum level to be attained at sexual maturity for high egg production.

2.1.2 Oviduct development

At the onset of egg production the mineral metabolism in female birds undergoes profound changes. Nevalainen (1969) studied the effects of calcium deficient diet (0.13 per cent) in White Leghorn hens and reported decrease in the weight of ovary, oviduct and shell gland compared to the controls. Egg production finally ceased in such birds during the third week. However, the egg production gradually picked up when the birds were switched over to a normal layer diet containing 3 per cent calcium.

According to Shrivastava and Brahmakhatriya (1976) pre-laying dietary calcium levels were found not to influence significantly, either the length or the weight of oviduct or weight of the ovary when maintained on calcium deficient diet for 18-21 or 24-27 weeks of age. However, prelaying calcium levels of 1.05 and 2.2 per cent had significant beneficial effect ($P<0.05$) in comparison to the calcium level of 3.3 per cent on length of oviduct fed for 21-24 weeks of age. Dietary calcium level beyond certain level (2.2 per cent) did not influence the length of oviduct. Vohra et al. (1979) studied the effect of dietary deprivation of supplementary calcium in both female and male Coturnix and Leghorn hens. The dietary calcium deficiency did not influence ovarian and oviduct weight of Coturnix whereas they were significantly heavier in

normal chicken than in those maintained on calcium deficient diet.

2.1.3 Histological and histochemical studies

The general morphology and functional aspects of the avian oviduct were extensively studied and discussed but most of the studies were conducted on Gallus (Biswal, 1954; Breen, 1966; Solomon, 1970; Aitken, 1971; Gilbert, 1971; Draper et al., 1972; Hodges, 1974 and Bahr and Nalbandov, 1977). A perusal of the available literature revealed that there has not been a regular, detailed and systematic study carried out on the structure of the oviduct of Japanese quail (Coturnix coturnix japonica), especially on shell gland. Hoffer (1971) reported the ultrastructure and cytochemistry of isthmus of the oviduct of the Japanese quail. The ultrastructural study of magnum of quail oviduct was done by Daniel and Ulrich (1976). The histochemistry of utero-vaginal sperm host glands in Japanese quail was conducted by Renden et al. (1981). Study on the development of the oviduct in quail during sexual maturation in relation to plasma concentration of oestradiol and progesterone was done by Pageaux et al. (1984). A study in newly hatched Japanese quail on the development of arteries supplying the ovary and oviduct was carried out by Hashimoto et al. (1986). The histological study of different aspects of

oviduct of common mallard (Anas platyrhynchos) was done by Sharma and Duda, 1986; 1987 and 1992).

2.2. Egg production and shell quality

Berg et al. (1947) showed that egg production and shell quality in domestic fowl were greatly influenced by the level of dietary calcium during the laying period. However, supplementary calcium given during prelaying period was considered to be without any significant effect. According to them low dietary calcium intake leads to the production of thin shelled eggs followed by total cessation of production. Earlier belief was that a high rate of egg production was not necessarily accompanied by a loss of calcium from the calcium reserves. But Jenkins and Taylor (1960) conclusively showed that the dietary calcium level influenced decidedly the shell thickness. Furthermore, the shell strength of broiler type of laying chicken was shown to be greater at each increment of dietary calcium (1-6% calcium) in the diet (Arnon Jr., 1965). A comparison made between the shell thickness and egg weight of Bobwhite quail eggs and those of Coturnix quail eggs showed that the egg weight of Coturnix quail was comparatively lower and shell thickness was greater than Bobwhite quail eggs (Mahmoud and Coleman, 1967). Summers et al. (1970) found that the levels of prelaying dietary calcium did not have any

influence on egg size or quality of shell in hens. Woodard and Applanolp (1970) reported that rate of laying decreased sharply in Japanese quails after 26 weeks of age, maximum egg weight was obtained at the 16th week of age which remained rather constant thereafter. Hurwitz and Bar (1971) also reported that egg production and egg weight were not influenced significantly by the levels of prelaying dietary calcium. Meyer et al. (1971) supported the reports of Hurwitz and Bar (1971). But Scott et al. (1971) showed that inadequate calcium levels (2.5 per cent) in domestic hens led to a decrease in egg shell thickness and specific gravity as compared to the high dietary calcium level of 3.5 per cent. However, a dietary calcium level higher than 3.5 per cent was not accompanied by any improvement in shell thickness.

According to Garrett et al. (1972) average shell thickness of Japanese quail eggs was 0.215 mm and age of maximum egg production was between 60 and 150 days. Ademosun and Kalango (1973) noticed that increasing dietary calcium levels from 2 to 3.5 per cent resulted in increased egg production in laying hens, whereas it decreased at the highest level of 4.25 per cent calcium. The improvement in quality of egg shell consequent to increase in uptake of dietary calcium was revealed by increased shell weight per unit area of the shell. McCready et al. (1973) noticed that feeding lower

calcium absorption, metabolism of calcium, process of demineralisation in the bone marrow or its transfer to egg shell through the uterine mucosa. Hamilton (1978) found that the weight of White Leghorn eggs steadily increased between 165 and 490 days of age and that size of egg was a deciding factor in determining the quality of the shell in older hens. Roland, Sr. et al. (1978) showed that older hens were able to maintain specific gravity, shell and egg weight and serum calcium as high or even better than younger hens with an adequate dietary intake of calcium. The decline in shell quality with advancement of age in hen was considered not to be due to a decline in hen's ability to absorb calcium from the gastrointestinal tract or to its incapacity to utilise skeletal calcium. But Roland, Sr. (1979) confirmed that the decline in shell quality was not due to the inability of hens to maintain shell calcium deposition but to an increase in size of egg when shell deposition of calcium continued to remain fairly constant. Manley et al. (1980) found that increase in dietary calcium in laying turkey hens resulted in a slight numerical increase in egg production. Ousterhout (1980) studied the effects of calcium and phosphorus levels on egg weight and shell quality in laying hens and found that egg weight was highly significant and inversely related to dietary calcium levels. One per cent additional calcium depressed egg

weight approximately 0.4 g. Increase of 1 per cent dietary calcium increased shell weight approximately 0.05 g. Gilbert et al. (1981) reported that egg production, shell weight and calcium content of eggs were reduced on low dietary calcium level. Lowering the dietary calcium level from 3.2 to 2 or 1 per cent in domestic fowl influenced the specific gravity of eggs also (Hamilton and Cipera, 1981). Husseini et al. (1981) reported that increase in dietary calcium level improved the percentage weight of egg shell, shell thickness and specific gravity of hen eggs. However, the rate of egg production was not affected by a lower calcium level. Lennards and Roland, Sr. (1981b) showed that in hens the time of feeding calcium had a significant effect on their ability to maintain shell quality. Eventhough, most hens have sufficient skeletal calcium reserves for four to five normal shelled eggs, they cannot pass even a day without calcium in the diet to maintain peak shell quality. According to Proudfoot et al. (1982) a protein rich diet coupled with supplementary oyster shell-feeding in White Leghorn hens resulted in improved egg shell strength. Roland, Sr. and Farmer (1984) reported that utilization of skeletal calcium was inversely related to the availability of dietary calcium and that greater the percentage of skeletal calcium utilized for shell calcification, the poorer was the shell quality. Bolden and Jensen (1985) observed that lower the dietary calcium in layer

ration of domestic fowl, lesser the egg production and shell quality. According to Izat et al. (1985) age influenced the egg shell quality, the eggs from younger birds had higher specific gravity, the shell weight increased as age advanced but the percentage weight of shell decreased with ageing. NYS (1986) reported that the average time interval between oviposition increased as age advanced but neither the shell weight nor the rate or duration of shell deposition were influenced. The increase in egg weight with age accounted for the decrease in shell quality. Roland, Sr. (1986) showed that the quantity of calcium deposited on the shell increased slightly as the age advanced in hens. Rate of egg production might not be a factor in the daily requirement of calcium in individual hens. The calcium requirement for an individual hen for a particular egg on a particular day could increase with age (Roland, Sr. 1986). According to him the calcium requirement of the individual hen decreased as age advanced, the reason being the limited ability of hens to store calcium for future shell formation. Shrivastav and Panda (1986) studied the level and sources of calcium for egg production and shell quality in quails. According to them neither the sources of calcium nor its dietary level had any significant effect on shell weight or its specific gravity. However, shell thickness was significantly increased with 3.75 per cent

of dietary calcium compared to those receiving lower levels of calcium. Yannakopoulos and Tserveni-Gousi (1986) studied the quality and characteristics of quail eggs and found that egg weight and shell weight were significantly increased between 49 to 154 days of age. The egg shape index and specific gravity decreased slightly with age while the decrease in shell thickness was marked. Makled and Charles (1987) reported that specific gravity of White Leghorn eggs improved significantly with feeding oyster shell in combination with lime stone. Mohanty et al. (1987) found that average egg shell thickness of caged quails was 0.20 mm. Narbaitz et al. (1987) reported that with calcium deficient diet in hens the outer layers of shell (Cuticle and Spongy layer) were reduced in thickness or absent but mamillary layer was present even in thinnest soft shelled eggs. According to Proudfoot and Hulan (1987) supplemental source of calcium over and above normal, resulted in an improvement in egg shell strength. Singh and Panda (1987) reported that the average shell thickness of all lines of quail eggs tested was 0.173 mm. Mahapatra et al. (1988) found that the weight of egg was the only factor influenced by type of housing and all the other factors being not influenced by the pattern of housing. Funamoto and Vohra (1988) reported that Japanese quails continued their normal egg production and maintained egg weight but not shell thickness with calcium deficient diet (dietary calcium only

1.05 per cent). In contrast to Japanese quails, Leghorn hens virtually ceased egg production under the above conditions. If the diet did not contain enough calcium for optimal shell thickness, the shell thickness would be reduced. A dietary level of 1.05 per cent calcium is sufficient for maintaining optimal egg production and egg weight but not shell thickness in Japanese quail hens for which a higher percentage of dietary calcium was required. Hvidsten and Lund (1988) reported that increase in calcium intake in domestic hen suppressed intake of metabolizable energy, increased plasma calcium concentration, improved egg shell quality and albumen height. Narayanankutty et al. (1989) found that shell thickness and shape index of quail eggs were decreased with age. But shell weight was increased as age advanced. Shrivastav et al. (1989) noticed that in quail layers an increase in egg production was evident with each increment of dietary calcium from 2 to 2.8 per cent. They also found that the average egg weight, egg mass and egg shell quality as measured by shell weight, shell thickness and specific gravity were improved markedly by increasing the calcium content in the diet. Cheng and Coon (1990) reported that in domestic fowl egg production and egg weight were not affected by dietary calcium level but shell weight, shell weight per unit surface area, specific gravity, shell thickness etc. were

influenced. Nagarajan et al. (1991) found that the egg weight of Japanese quail increased and shell thickness decreased with age. The results of studies conducted by Bar et al. (1992) suggested that in old hens producing thin shelled eggs calcium absorption from the gastrointestinal tract occurred with a lower efficiency compared to those forming heavy shells or in pullets. The decrease in shell density in older hens was caused by a physiological calcium deficiency or by a defect in hen's ability to alter the rate of calcium mobilisation in response to calcium requirement. Clunies et al. (1992a) found that dietary calcium had a significant effect on egg production, average shell weight and daily egg shell quantity. In all kinds of treatments feeding a calcium deficient diet led to significant reduction in shell weight and average daily egg shell output. Clunies et al. (1992b) in a study in White Leghorn hen found that dietary calcium level had no significant effect on egg production and egg weight but an increase in calcium intake produced significant improvement in shell weight and shell calcium level. The above results showed that to increase shell weight and shell quality, calcium intake must be increased even when there was diminishing shell weight at higher levels of calcium intake.

Guinotte and NYS (1992) found that egg shell weight and breaking strength did not increase when marine shells were

used as a source of calcium in dwarf broiler breeder hens. Moreover, live weight, tibia strength, egg production and fertility were not affected by calcium supplementation. Keshavarz and Nakajima (1993) conducted a long term study involving layers to reevaluate the calcium and available phosphorus requirements of layers for optimum performance and egg shell quality. Their investigations reveled that increasing the dietary calcium from 3.5 to 4 per cent slightly but not significantly improved specific gravity of egg. Moreover, laying hens tolerated relatively high dietary level of calcium (6 g/hen/day) with no adverse effect on their performance.

2.3 Biochemical studies

2.3.1 Calcium and phosphorus profile in Japanese quail

According to Richardson (1935) calcium for egg shell formation is derived from the blood, with high calcium content, brought to the shell gland during egg shell calcification. The epithelium of the shell gland may not store any calcium for this purpose. Appreciable quantities of ingested radioactive calcium were found in the blood and bones of hen even after 21 days of heavy production. Sixty to 75 per cent of the egg calcium was derived directly from the diet while the remainder was from the body stores like the

medullary bone. Maintenance of such a store for calcium was therefore important for maximum performance of the laying hens (Driggers and Comar, 1949). Baldini and Zarrow (1952) reported slightly higher serum calcium levels in the Bobwhite quail compared to chicken and other species of birds. Polin and Sturkie (1957) recorded a decrease in the plasma diffusible calcium level during egg shell calcification with no change in its total calcium concentration. Further, they noticed that even with lower than normal dietary calcium level, the layers maintained a plasma level of 20 mg per cent throughout, including the interval stages of egg production. This indicated that the calcium requirement for egg shell formation was not of dietary source but the plasma calcium level was maintained by mobilization of calcium from the medullary bone. According to Mueller (1958) plasma calcium was influenced by dietary calcium. Winget and Smith (1958) observed fluctuation in blood calcium level during shell formation in domestic chicken. Hunsaker and Sturkie (1961) calculated the blood calcium level in the arterial and venous circulation of shell gland during egg shell formation and registered a decrease in total plasma calcium content across the uterus to the extent of 5.3 or 21.4 mg per cent of pre-uterine level compared to the stage when shell was not actually forming. On the other hand when egg shell formation

was not occurring the decrease was only 1.2 or 4.4 mg per cent. Resorption of medullay bone reserves would have to be resorted to meet the full demand of calcium. Taylor and Hertelendy (1961) accentuated the changes in blood calcium associated with shell formation and the levels after a period of 48 h on a low calcium diet. It was observed that a very large decrease in total, diffusible and non-diffusible calcium occur during the shell calcification. Level of plasma calcium was seen to be influenced by dietary calcium in laying pullets (Hurwitz, 1964). The plasma calcium concentration of laying hen on a diet containing 4.1 per cent calcium was 32.8 mg per cent, while that maintained on 1.2 per cent calcium was only 25.7 mg per cent. Dietary calcium promoted not only a greater pre-laying bone storage of calcium but also reduced the depletion of calcium from medullary reserves associated with early shell formation. Moreover, medullary bone formation actually started ahead of time, about two weeks of the start of shell calcification and the calcium for this purpose was largely derived from structural bone.

Schraer and Schraer (1965) noted that the calcium concentration in shell gland of domestic fowl with an egg *in situ* was 23.6 $\pm$ 2.1 mg/g of dry tissue whereas that of immature fowl without an egg in the uterus was 20.8 $\pm$ 5.6 mg/g

of dry tissue. However, in domestic fowl, Hurwitz and Bar (1967) could not produce any conclusive evidence to show whether calcium absorption or uterine calcium deposition was acting as a limiting factor in egg shell formation. ElJack and Lake (1967) estimated the amount of calcium in the shell gland fluid and found higher calcium around the time of egg laying. Snapir and Perek (1970a) demonstrated a positive correlation between production rate and total plasma calcium concentration within each one of the breeds of White Leghorn and White Plymonth Rock hens. A significant negative correlation was also evident between the concentration of uterine calcium and alkaline phosphatase concentration in both the breeds studied. At the same time it was noted that there was no relation between the calcium concentration in the uterus, the rate of egg production and the quality of egg shell formed. Snapir and Perek (1970b) further noticed neither an influence of age on the level of blood calcium nor any relationship between plasma calcium and uterine calcium contents in domestic fowl.

Bragg et al. (1971) found that the domestic hen appeared to utilize a greater percentage of dietary calcium at lower levels of calcium (2.25 per cent) in the diet as evidenced by 70 per cent of the dietary calcium being

preferentially utilized for egg shell formation. Ehrenspeck et al. (1971) concluded that a major pathway for calcium transport was an active transport mechanism across the shell gland. Morrissey and Wasserman (1971) reported that the degree of mineralization of skeleton was a prominent determinant of intestinal calcium absorption in the domestic chicken in the process of egg shell formation. Roland, Sr. et al. (1973) noticed a drop in serum calcium to 15 mg per cent level in those pullets which were maintained on a calcium deficient diet. Bar et al. (1976) found a rise in the concentration of calcium-binding protein (CaBP) both in the small intestine and shell gland mucosa of laying Japanese quail. Due to the similar dietary efficiency of quails for utilization of dietary calcium, they can be compared well with hen whose calcium absorption rate may be as high as 70-80 per cent (Navarrow and Munrillo, 1976). According to them the quails are very well equipped for laying, calcemia in layers being almost double that in non-laying and male quails. In the experimental work of Rao and Brahmakshatriya (1976) calcium level of blood plasma showed an insignificant rising trend in concentration as the level of dietary calcium increased along with improved storage of skeletal calcium. This could stand in good stead for sustained production and improved shell thickness. There were also changes in serum phosphorus levels of laying hens (Miller et al., 1977) which were related to

shell calcification, resorption and remineralisation of bones. The level of inorganic phosphate and calcium in the various regions of oviduct of domestic fowl were studied by Prabhakar et al. (1977) and they showed that the highest level of phosphate content was in the magnum of the oviduct. According to them shell glands do not store calcium to any significant extent.

Bar et al. (1978) found an increase in the intestinal absorption of calcium and phosphorus when sufficient Vitamin D derivative (one alpha - hydroxy cholecalciferol) was supplemented in the diet during the egg shell formation. They placed on record that calcification of shell and uterine concentration of CaBP (calcium-binding protein) were not related to the concentration of 1,25 dihydroxy cholecalciferol. Strong, Jr. and Nester (1978a) have noticed that while the total plasma calcium content of Coturnix quail was similar to that of chicken (19.4 $\pm$ 2 mg %) while, that of phosphorus was greater than in other avian species (7.99 mg%). A great increase in total calcium and phosphorus content of plasma at the onset of egg production was also reported by Strong, Jr. and Nester (1978b). Wasserman and Combs, Jr. (1978) estimated the calcium and phosphorus levels of plasma in Japanese quails and reported that plasma calcium concentration was similar to that reported for chicken whereas total plasma phosphorus

concentration was higher than other avian species. Luck and Scanes (1979) studied the relationship existing between reproductive activity and blood calcium level in calcium deficient quail hens. Quail layers when presented with a calcium deficient diet ceased to lay as the plasma concentration of ionised calcium decreased to less than 1.0 mM. Supplementation of the diet with calcium produced an immediate restoration of plasma ionised calcium level to normal, despite the fact that an interval of a few days was taken before the plasma total calcium concentration returned to normal with resumption of egg laying.

Cipera (1980) found that the total amount of calcium in both shell forming segments (isthmus and uterus) of hen's oviduct was found to be negligible, present only to the extent of a few milligrams. According to him shell gland was not storing calcium for shell formation to any degree. The calcium needed for shell formation must have been continuously supplied to the oviducal tissues, mostly from blood itself. Lennards and Roland, Sr. (1981a) could not establish any relationship between serum content of calcium and the hens ability to produce good quality egg shell, shell weight or egg weight. Blood calcium in the laying hens was seen to be affected by the reproductive state, photoperiodicity and pattern of intake of food (Parsons and Combs Jr., 1981).

Miles and Harms (1982) noticed a decrease in plasma phosphate concentration and an increase in specific gravity of egg in hens maintained on a higher level of dietary calcium. Farmer et al. (1983) observed that the dietary calcium was first taken up by bones and then only utilized by the shell gland for shell calcification. According to them skeletal system must have stored at least some of the calcium until it becomes required for egg shell calcification. However, Bolden and Jensen (1985) could not notice any significant difference in tibial ash, plasma phosphorus or egg weight in hens maintained on higher dietary calcium levels (3.5%). But the performance of older hens was reported to be affected by higher levels of dietary calcium (Keshavarz, 1986) and in younger hens on high dietary calcium, no significant effect was seen produced in their performance. According to this study absence of significant interaction between calcium and phosphorus on production parameters indicated that Ca-P ratio was not of crucial importance in laying hens compared to growing chicks. Vandevelde et al. (1986) showed that addition of oyster shell to the diet did not influence the concentration or pattern of the plasma characteristics during egg formation and did not reduce calcium mobilisation from medullary bone during shell formation. Ohashi et al. (1987) noted that medullary bone development, during reproduction under oestrogen control was a

unique feature which cope with the requirement of calcium for egg shell formation. Indications are obtained from the studies of Robert and Wideman, Jr. (1987) that avian plasma calcium and inorganic phosphate concentrations were co-regulated by parathormone (PTH) and Vitamin-D, acting directly or indirectly on the intestine, bone and kidneys. The shell gland removes calcium from the blood during egg shell calcification. When calcium absorption from gut does not balance that of shell gland removal of calcium or when shell calcification occurs during night time, when gut is more or less empty, blood calcium level decreases which in turn stimulates PTH secretion leading to demineralization of bones and release of calcium.

Arad et al. (1989) noticed a sharp increase in the calcium content of the uterine fluid during the early stages of shell calcification. Shrivastav et al. (1989) reported that serum calcium level in laying quail was influenced to a significant extent by dietary levels of calcium and phosphorus. A dietary level of 2.8 per cent calcium and 0.7 per cent total phosphorus gave maximum response in egg production. According to them the serum calcium level of laying quail with 2.8 per cent dietary calcium was 22.08 $\pm$ 1.46 mg per cent and that of serum phosphorus 14.66 $\pm$ 2.89 mg per cent. Egg shell quality as measured by shell weight,

shell thickness and specific gravity was found to be improved markedly by increasing the level of dietary calcium. The study indicated no significant difference in bone ash content of the laying quails fed diets containing 2 to 3 per cent of calcium and 0.55 to 0.85 per cent total phosphorus. So also at lower dietary calcium levels female coturnix preferred to cease egg production rather than lose bone calcium. The calcium deficient laying hens (0.36% dietary calcium) had significantly lower total and bound plasma calcium and inorganic phosphorus compared to control birds reared on 3.4 per cent dietary calcium (Ruschkowski and Hart, 1992).

2.4 Enzyme profile in japanese quail

Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities of the plasma of the domestic fowl in relation to dietary calcium metabolism were the subject of a number of investigations in the past. Common (1936) was the first to demonstrate a higher and more variable phosphatase activity in the serum of laying domestic fowl than in cocks. A still higher variation in the enzyme activity was evident if the hens were on a lower calcium diet. Since then much work has been carried out on the relationship between enzymes like ALP, ACP and egg production in birds.

Gutowaska et al. (1943) reported that there was no difference in the enzyme concentration of uterine tissue because of age. He further reported that the shell gland showed only minimum ALP activity during the laying cycle. Normally, dietary calcium directly supplied only part of the calcium needed for the formation of the egg shell, whereas the rest was withdrawn from the skeleton through the action of phosphatases (Driggers and Comar, 1949). According to Pritchard (1952) plasma ALP was thought to have its origin in the bones, to be associated with its osteoblastic activity. Histochemical studies of Pritchard (1952) have also supported this contention of origin of plasma ALP as from osteoblasts. Stutts et al. (1957) reported that plasma content of ALP in domestic hen was higher and more variable than that in cocks. Age of the bird did not show any significant influence on plasma phosphatase activity in either mature male or female birds.

Bell (1960) observed that the stimulation of the osteoblastic activity was triggered by the increased demand for calcium for egg shell formation resulting in the augmented drainage of bone calcium, which is mediated by a higher ALP activity in the bone. He also found that the medullary bone was rich in both osteoblasts and osteoclasts during the early stages of egg shell formation. As calcification progressed

osteoblasts declined in number and osteoclasts became more predominant. During the interval between laying and subsequent ovulation both osteoblasts and osteoclasts in the medullary bone varied in number. The variations in the activity of osteoblasts and osteoclasts in the medullary bone accounted for some of the fluctuations in the concentrations of the enzymes during laying period. He also concluded that egg shell formation in layers especially of heavy layers was accompanied by elevated osteoblastic activity as evidenced by highly variable levels of plasma ALP activity during the first month of lay.

According to Taylor et al. (1965) the level of plasma ALP activity was higher during active shell calcification than either just before or after oviposition. Wilcox and Cloud (1965) noticed that serum ALP content was greater in high producing hens in comparison with low and immature non-producers. According to them the ALP activity of oviductal regions varied and ALP activity was lower in fallopian tube compared to ovary in laying White Leghorn pullets. Govinda Rao et al. (1969) reported that serum ALP content of chicks in both sexes was higher than adult males and females. Serum ALP concentration was higher before laying than after one full year of production whereas, age did not show any influence on ALP profile of adult male birds. Paul and Snetsinger (1969)

observed that higher dietary calcium levels of White Leghorn hens were associated with lower plasma ALP concentration. A change in serum ALP zymogram specifically related to the onset of egg production in Japanese quail was noticed by Savage et al. (1970). No influence of age was evident in ALP activity and calcium content in the shell gland and isthmus of White Leghorn hens (Snapir and Perk, 1970a). Further more, the rate of egg production and shell quality were also seen not to be influenced by uterine calcium concentration and ALP activity (Snapir and Perek, 1970b). Solomon (1970) reported higher ACP activity in the shell gland compared to that in the blood which was reciprocal to that of ALP. There were fluctuations in the concentration of these two plasma enzymes which were the results of differences in the activity of osteoblasts and osteoclasts in the bone narrow. Tamaki and Tanabe (1970) noted a decline in plasma ALP activity with age in White Plymouth Rock hens. Jain et al. (1976) observed that as the number of serum ALP isoenzymes increased, the egg production also increased in young White Leghorn birds. Dimri et al. (1980) studied the serum ALP activity in Japanese quails and found that while the enzyme activity was higher in younger age groups in both the sexes, the females showed a higher value compared to adult males. There was a peak in the plasma ALP activity more or less coinciding with the initiation of egg laying in females. They also observed that

the progenies of sires with high serum ALP had higher growth rate compared with those with low serum ALP levels. Salevsky, Jr. and Leach, Jr. (1980) studied the ALP activity in the shell gland of single comb White Leghorn layers and found that it was relatively low (0.73 units/mg protein) as compared to the ACP activity (9.64 units/mg protein). The oviduct content of ACP was similar to that in prostatic gland but different from that of serum. However, they pointed out that the role of this enzyme in shell formation was unknown. Singh et al. (1983) noted that chicken having higher plasma ALP concentration grew faster, matured earlier and produced heavier eggs and plasma ALP activity was more in pullets selected for high production. Kansal and Gangwar (1984) reported a decrease in plasma ALP and ACP activities in layers as age advanced which may be due to greater demand, transportation and utilization of calcium for shell calcification from bone, consequent to lower blood calcium level. Nys and DeLaage (1984a) found that the uterine ALP activity in domestic hen was much lower than plasma ALP and uninfluenced by egg shell calcification. Hence this enzyme was considered to be less important in the transfer of calcium across the uterine wall. Nys and DeLagge (1984b) found that the activity of intestinal calcium-binding protein (CaBP) was regulated more by a feed-back mechanism of the calcium

requirement for egg shell calcification than by any direct action of gonadal hormones. Acid phosphatase and alkaline phosphatase activities of shell gland were not reported to be related to egg shell quality and dietary phosphorus levels (Klingensmith and Hester, 1985). Darshan and Panda (1987) estimated the phosphomonoesterase (ALP and ACP) activities of the shell gland in domestic hen and reported that the ALP content was lower (3.86 $\pm$ 0.526 Picomol P-nitrophenol formed/mt/mg protein) compared to ACP (203.194 $\pm$ 37.32 picomol P-nitrophenol formed/mt/mg protein). According to Kalita et al. (1993) ALP was one of the most common variants in the body and was a catalysing non-specific, multimolecular enzyme which act by releasing phosphate from many organic phosphomonoesters at an optimum pH (pH 9-10). All the metabolic processes in the body were under the direct influence of this enzyme and the level of this enzyme was influenced by sex. Plasma ALP was useful as a gene marker for spotting out the strains with high egg production traits.

Present Investigation

Chapter 3

PRESENT INVESTIGATION

This study envisages elucidation of some of the mechanisms involved in egg shell formation on the basis of structural and functional adaptations undertaken by the shell gland in Japanese quail. The influence of pre-laying and layer dietary calcium on the mineral (calcium and phosphorus) content of blood and shell gland, their enzyme profile with respect to alkaline phosphatase (ALP) and acid phosphatase (ACP) and the histological and histochemical peculiarities of the shell gland at different stages of egg production were the main topics of investigation. An attempt was also made to ascertain the influence of varying levels of dietary calcium (pre-laying and layer) on the body weight of birds, oviduct development and egg shell quality.

Observations to be made

1. Weight gain of birds from the third week of age, at fortnightly intervals and at the time of sacrifice.

2. Determination of the length and weight of oviduct and shell gland at the sixth, 16th and 24th week of age.

3. Anatomical and histochemical studies of the shell gland at the time of sacrifice of the birds.

4. Assessment of the quality of egg shell at different levels of dietary calcium and at various stages of egg production.

5. Determination of plasma and shell gland concentration of minerals (calcium and phosphorus) and enzyme (ALP and ACP) profile at different stages of egg production.

Materials and Methods

Chapter 4

MATERIALS AND METHODS

Six hundred and thirty, three week old, clinically healthy female Japanese quail birds of the same hatch and strain (egg type) from the Kerala Agricultural University Poultry Farm were selected at random. The study involved investigations in three phases of age groups viz. from third to sixth week (Phase I), sixth to sixteenth week (Phase II) and sixteenth to twenty fourth week (Phase III) of age.

4.1 Layout of the experiment

4.1.1 Phase I (3 to 6 weeks)

After wing banding and recording the initial body weight the female Japanese quail birds were divided into three groups (G-I, G-II and G-III) of 210 birds in each group in such a way that differences within the group and among the group were fairly similar. The birds were reared under standard management in cage system on starter ration (Panda, 1990) with three pre-laying dietary calcium levels: 0.5, 0.7 and 0.9 per cent for the groups G-I, G-II and G-III respectively (Table 1.1). At fortnightly intervals and at the time of sacrifice body weight of birds was recorded. At the sixth week of age blood samples were collected from thirty

Table 1.1 Composition and chemical analysis of experimental-Pre-laying ration

Ingredients	Rations parts per 100 kg		
	G I	G II	G III
Yellow maize	42.0	42.0	42.0
Groundnut cake	45.5	45.0	45.0
Gingelly oil cake	2.0	2.0	2.0
Fish meal	3.0	3.0	3.0
Rice polish	7.0	7.0	6.5
Salt	0.5	0.5	0.5
Shell meal	-	0.5	1.0
Total	100.0	100.0	100.0

For every 100 kg feed, added the following

Rovibe[1]: 75 g/100 kg; Rovi mix[2]: 25 g/100 kg

Choline chloride = 100 g/100 kg

Mineral mixture/100 kg feed; Ferrous sulphate 25 g, Manganese sulphate 25 g, Zinc sulphate 25 g, Copper sulphate 1.5 g and Potassium iodate 100 mg

1. Rovibe (Roche Products Ltd.). Guaranteed potency per gram. Vit Bl - 4 mg, B6 - 8 mg, Bl2 - 40 mg, Niacin - 60 mg, Calcium Pantothente - 40 mg and Vit E - 40 I.U.

2. Rovimix A, B2, D3 (Roche Products Ltd). Guaranteed potency per gram. Vit.A - 40,000 I.U., B2 - 20 mg, D3 - 5000 I.U.

Contd.

Table 1.1 (Contd.)

Chemical composition

	GI	GII	GIII
Metabolisable energy (KCal/kg)*	2835.0	2821.0	2814.0
Crude protein (percentage)**	24.0	24.0	24.0
Calcium (percentage)**	0.5	0.7	0.9
Phosphorus (percentage)**	0.68	0.67	0.67
Lysine (percentage)*	0.92	0.92	0.91
Methionine (percentage)*	0.45	0.45	0.45

* Calculated value

** Analysed value

birds in each group by cardiac puncture with heparin (1 mg/ 5 ml of blood) as the anticoagulant. After the collection of blood, the birds were sacrificed by decapitation and oviducts, cleared off all adhering tissues and fluids contained therein, were carefully removed and preserved for further studies.

4.1.2 Phase II (6 to 16 weeks)

The remaining birds in G-I, G-II and G-III were sub-divided group-wise into three sub-groups A, B, C (G-I A, G-II A, G-III A; G-I B, G-II B, G-III B; G-I C, G-II C, G-III C) and reared under standard managemental conditions on layer ration (Panda, 1990) with three different levels of dietary layer calcium levels viz. 2.5 per cent (A), 3 per cent (B) and 3.5 per cent (C) respectively (Table 1.2). At the 16th week of age blood samples were collected from 30 birds in each one of the nine sub-groups for biochemical and enzyme assay. The birds were then sacrificed, oviducts collected for further studies.

4.1.3 Phase III (16 to 24 weeks)

The remaining birds in each one of the nine sub-groups were reared on layer ration upto 24 weeks of age with the three layer dietary calcium levels as in the second phase.

Table 1.2 Composition and chemical analysis of experimental-Layer ration

Ingredients	Rations parts per 100 kg		
	A	B	C
Yellow maize	38.0	38.0	37.0
Groundnut cake	38.0	38.0	38.0
Gingelly oil cake	5.0	5.0	5.0
Fish meal	5.0	5.0	5.0
Rice polish	6.3	5.0	4.75
Salt	0.5	0.5	0.5
Shell meal	2.2	3.5	4.75
Mineral mixture[1]	3.0	3.0	3.0
Fat	2.0	2.0	2.0
Total	100.0	100.0	100.0

For every 100 kg feed, added the following

Rovibe[2] : 75 g/100 kg; Rovi mix[3] : 25 g/100 kg
Choline chloride 50 g/100 kg

1. Poultrymin (Aries Agro-Vet Industries Private Ltd.) Contained calcium (min) - 32.00%, Phosphorus (min) - 6.00%, copper (min) - 100 ppm, Cobalt (min) - 60 ppm, Manganese (min) - 2700 ppm, Iodine - 100 ppm, Zinc - 2600 ppm, Iron - 0.1% and Magnesium - 1000 ppm.

2 Rovibe (Roche Products Ltd.). Guaranteed potency per gram. Vit B1 - 4 mg, B6 - 8 mg, B12 - 40 mg, Niacin - 60 mg, Calcium Pantothente - 40 mg and Vit E - 40 I.U.

3. Rovimix A, B2, D3 (Roche Products Ltd). Guaranteed potency per gram. Vit.A - 40,000 I.U., B2 - 20 mg, D3 - 5000 I.U.

Contd.

Table 1.2 (Contd.)

Chemical composition of layer ration

	A	B	C
Metabolisable energy (KCal/kg)*	2770.0	2751.0	2716.0
Crude protein (percentage)**	22.0	22.0	22.0
Calcium (percentage)**	2.5	3.0	3.5
Phosphorus (percentage)**	0.68	0.65	0.64
Lysine (percentage)*	0.92	0.91	0.91
Methionine (percentage)*	0.46	0.46	0.46

* Calculated value

** Analysed value

Blood samples and oviducts were collected for biochemical enzymatic, structural and histo-chemical investigations.

4.2 Details of study

4.2.1 Body weight and anatomical studies

4.2.1.1 Body weight

Body weight of the birds was recorded at the initial stage of the experiment (three weeks of age) and thereafter at fortnightly intervals and finally at the time of sacrifice of the birds.

4.2.1.2 Anatomical studies

4.2.1.2.1 Measurements on oviduct

The total length and weight of the whole oviduct and that of the shell gland in particular were recorded as and when the birds were sacrificed.

4.2.1.2.2 Histological studies

Small pieces of the shell gland were preserved in 10% formalin for histological studies. Tissues were processed and stained by haematoxylin and eosin (Drury et al., 1967) and examined under the light microscope.

4.2.1.2.3 Histochemical studies

The pieces of shell gland tissue were preserved in cold acetone (4°C) for 24 h. Tissues were processed (Drury et al., 1967) stained for alkaline phosphatase (ALP) by calcium phosphate method of (Gomori, 1952) and for acid phosphatase (ACP) by lead nitrate method (Gomori, 1950) as explained by Drury et al. (1967) and examined under light microscope.

4.2.2 Egg production and shell quality

Eggs were collected daily after the onset of egg production. Egg shell quality traits were determined on the sixth, 16th and 24th weeks of age. Thirty eggs from each of the nine sub-groups were selected at random and weighed. The maximum width and length of the eggs were measured with the Vernier Calipers and their shape indices (S.I) were calculated by using the formula

$$S.I. = \frac{\text{Maximum width}}{\text{Maximum length}} \times 100$$

The specific gravity (sp. gr.) of the eggs was measured by floatation method using salt solutions ranging in specific gravity from 1.060 to 1.100. The eggs were then broken open, shell membrane was removed and thickness of shell without membrane was measured with 'AMES' thickness

micrometer. The shell thickness of at least three regions of shell, the broad, middle and narrow positions of the egg was measured and the mean shell thickness was calculated. The shell and the membrane were washed free of its contents, dried for 24 h in an oven (40°C), cooled and then weighed to the nearest 0.01 g.

4.2.3 Biochemical studies

4.2.3.1 Calcium and phosphorus profile in the plasma and shell gland of Japanese quail

4.2.3.1.1 Calcium

After collection, blood samples were centrifuged (2100 x g; 30 minutes) in a refrigerated centrifuge (0°C). The separated plasma was used for calcium estimation. The estimation of calcium content in blood plasma (1 in 100 with 0.1% lanthanum solution) and in shell gland ash (1 in 50 dilution of ashed sample of weighed shell gland in 0.1% lanthanum solution) was carried out using an Atomic Absorption Spectrophotometry (Perkin, Elmer, 2380 Atomic Absorption Spectrophotometry, 1982).

4.2.3.1.2 Phosphorus

Weighed samples of the shell gland were cut into small pieces and homogenised with cold double distilled water (DDW) over an ice bath using Potter Elvehjem tissue homogeniser (Bergmeyer, 1965). The tissue homogenates were filtered through double layers of muslin, the collected filtrates were stored at 4°C with final dilution made upto 25 ml with DDW.

Inorganic phosphorus content of the shell gland homogenate (1 in 25 dilution) and of plasma (0.5 ml) were estimated by the method of Fiske and Subba Row (1925) as given by Oser, 1965.

4.2.3.2 Enzyme profile in plasma and shell gland of Japanese quail

4.2.3.2.1 Alkaline phosphatase (ALP)

Alkaline phosphatase content was estimated in plasma diluted with DDW (1 in 4) and shell gland homogenate (1 in 25 dilution) employing the Kind and King's method (1954) as given by Stangen Immunodiagnostics, Kits Hyderabad.

4.2.3.2.2 Acid phosphatase (ACP)

Diluted plasma (1 in 2) and shell gland homogenate (1 in 100 dilution) were used for the estimation of ACP by the

King's method (King and Jagatheesan, 1959) using Stangen Immunodiagnostics, Kits, Hyderabad.

For both ALP and ACP the substrate used was disodium phenyl phosphate at pH 10 for ALP and pH 5 for ACP. A separate blank was run for each sample tested.

Statistical analysis of the data was carried out as outlined by Snedecor and Cochran, 1967.

Results

Chapter 5

RESULTS

5.1 Body weight and anatomical studies

5.1.1 Body weight

The initial body weight (at three weeks of age) of female Japanese quails was on an average 45.193 $\pm$ 0.180 g (Table 2.1). There was no significant influence of pre-laying dietary calcium at the fifth week of age (Table 2.2). However, birds of GIII group reared on the highest (0.9%) pre-laying dietary calcium showed highest body weight at fifth and sixth week of age (127.033 $\pm$ 0.419 g and 132.500 $\pm$ 0.864 g respectively). At the sixth, seventh, 15th, 16th and 19th week of age the influence of pre-laying dietary calcium was significant ($P<0.01$) and also at the 13th and 17th week of age ($P<0.05$) vide Table 2.2 and 2.3. After the 19th week of age, as age advanced, no influence of pre-laying dietary calcium was evident on the body weight (Table 2.3). Birds reared on the highest (0.9%) pre-laying dietary calcium (GIII group) recorded highest body weight from the fifth week to the 24th week of age except for the seventh and ninth week where birds reared on 0.7 per cent pre-laying dietary calcium (GII group) had the highest body weight (Table 2.1 and Fig.1.1).

Table 2.1 Influence of dietary calcium level on the body weight of female Japanese quail - different age groups - (Mean ± SE) - 30 birds/group and subgroup

Groups (pre-laying calcium)	Sub-groups (layer calcium)	Body weight in g													
		Age in weeks 3	5	6	7	9	11	13	15	16	17	19	21	23	24
GI (0.5%)	A (2.5%)	-	-	-	142.833 ±2.070	162.167 ±2.390	174.433 ±1.760	179.733 ±1.780	186.033 ±1.360	187.633 ±1.490	191.933 ±0.940	195.133 ±0.880	196.567 ±0.820	197.100 ±0.780	197.733 ±0.770
	B (3%)	-	-	-	142.267 ±1.260	155.133 ±1.700	165.533 ±1.580	176.600 ±1.630	184.000 ±1.320	188.033 ±1.320	190.267 ±1.002	193.700 ±0.800	195.800 ±0.790	196.267 ±0.798	199.000 ±0.500
	C (3.5%)	-	-	-	137.633 ±1.290	153.533 ±1.497	161.867 ±1.220	173.233 ±1.190	180.933 ±1.090	186.533 ±0.990	187.733 ±0.870	191.100 ±0.800	192.500 ±0.860	193.567 ±0.730	196.567 ±0.650
Group I Total Means		45.167 ±0.160	126.733 ±0.389	131.500 ±01.250	140.911 ±0.938	156.944 ±1.156	167.278 ±1.039	176.522 ±0.929	183.656 ±1.754	187.400 ±1.490	189.978 ±0.565	193.311 ±0.517	194.956 ±0.506	195.644 ±0.468	197.767 ±0.385
GII (0.7%)	A (2.5%)	-	-	-	144.233 ±1.760	164.567 ±1.860	170.067 ±1.900	176.433 ±1.470	182.133 ±1.070	182.233 ±1.740	188.967 ±0.760	193.433 ±0.711	194.500 ±0.720	195.600 ±0.610	197.600 ±0.550
	B (3%)	-	-	-	145.000 ±1.350	155.633 ±1.713	165.533 ±1.580	179.167 ±1.320	186.867 ±1.390	193.433 ±1.370	192.567 ±1.200	195.667 ±0.980	197.133 ±0.930	197.533 ±0.910	199.667 ±0.680
	C (3.5%)	-	-	-	140.100 ±1.260	154.333 ±1.420	163.133 ±1.670	174.700 ±1.639	184.067 ±1.450	189.700 ±1.340	189.633 ±1.300	192.567 ±1.162	194.133 ±1.160	194.633 ±1.110	196.933 ±0.840
Group II Total Means		45.080 ±0.510	126.833 ±0.980	131.400 ±0.970	143.111 ±0.871	158.178 ±1.071	166.600 ±0.939	176.767 ±0.868	184.356 ±0.778	188.456 ±0.655	190.389 ±0.655	193.889 0.570	195.256 ±0.561	195.922 ±0.530	198.067 ±0.417
GIII (0.9%)	A (2.5%)	-	-	-	143.300 ±1.200	160.833 ±1.560	166.033 ±1.370	175.933 ±1.330	184.833 ±1.060	189.033 ±1.310	191.300 ±1.950	194.600 ±0.770	195.833 ±0.800	197.300 ±0.650	199.200 ±0.460
	B (3%)	-	-	-	141.367 ±2.080	155.633 ±1.620	170.400 ±1.060	182.567 ±1.250	189.567 ±1.230	192.867 ±1.850	194.367 ±1.065	197.100 ±0.814	197.733 ±0.740	198.267 ±0.750	199.367 ±0.640
	C (3.5%)	-	-	-	141.667 ±1.660	153.333 ±1.660	165.867 ±1.830	179.200 ±1.460	186.067 ±1.260	190.400 ±1.180	191.000 ±1.070	194.700 ±0.800	195.300 ±0.750	195.467 ±0.730	197.133 ±0.560

Contd.

Table 2.1 (Contd.)

Groups (pre-laying calcium)	Sub-groups (layer calcium)	Body weight in g													
		Age in weeks 3	5	6	7	9	11	13	15	16	17	19	21	23	24
Group III	Total Means	45.333 ±0.161	127.033 ±0.419	132.500 ±0.864	142.111 ±0.965	156.600 ±0.980	167.433 ±0.860	179.233 ±0.821	186.822 ±0.707	190.766 ±0.666	192.222 ±0.610	195.467 ±0.471	196.289 ±0.451	197.011 ±0.425	198.567 ±0.325
Total Means	A (2.5%)	-	-	-	143.456 ±0.979	162.522 ±1.136	170.178 ±1.032	177.367 ±0.900	184.333 ±0.691	186.300 ±0.930	190.733 ±0.523	194.389 ±0.458	195.633 ±0.454	196.667 ±0.400	198.178 ±0.360
	B (3%)	-	-	-	142.878 ±0.931	155.467 ±0.959	167.511 ±0.733	179.444 ±0.850	186.811 ±0.790	191.444 ±0.733	192.400 ±0.650	195.489 ±0.530	196.889 ±0.480	197.356 ±0.480	199.344 ±0.350
	C (3.5%)	-	-	-	139.800 ±0.830	153.733 ±0.874	163.622 ±0.930	175.711 ±0.870	183.689 ±0.761	188.878 ±0.694	189.456 ±0.642	192.789 ±0.560	193.978 ±0.550	194.556 ±0.510	196.876 ±0.400
Overall Total Means		45.193 ±0.180	126.867 ±0.370	131.800 ±0.600	142.044 ±0.540	157.241 ±0.620	167.104 ±0.550	177.507 ±0.510	184.944 ±0.144	188.874 ±0.470	190.863 ±0.360	194.222 ±0.300	195.500 ±0.290	196.193 ±0.280	198.133 ±0.220
	CD*	0.640	1.310	2.070	2.500	2.800	2.430	2.330	1.990	2.030	1.640	1.410	1.370	1.280	0.990
	CD**	-	-	-	4.340	4.870	4.220	4.040	3.450	3.510	2.840	2.440	2.370	2.220	1.720

CD* = Critical difference for comparison of means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** = Critical difference for comparison of means 9 sub-groups each comprising of 30 birds

Table 2.2 ANOVA table of means - Influence of dietary calcium level on the body weight of female Japanese quail - (three to six week period)

Sources	df	M.S.S.		
		Age in weeks 3	5	6
Groups	2	0.497	0.700	11.1**
Error	87	3.08	12.908	32.439

** Significant at 1 per cent level (P<0.01)

Table 2.3 ANOVA Table of means - Influence of dietary calcium level on the body weight of female Japanese quail - (Seven to twenty four week period)

Sources	df	M.S.S.										
		Age in weeks 7	9	11	13	15	16	17	19	21	23	24
Groups	2	109.200**	61.937	17.670	202.415*	249.033**	266.848**	128.515*	112.044**	44.033	46.959	14.700
Sub-groups	2	347.544**	1950.448**	978.148**	314.937**	247.544**	595.470**	196.204**	165.900**	191.878**	191.570**	137.033**
Groups vs sub-groups	4	68.744	28.170	416.748**	242.120**	151.994*	255.754**	76.081*	48.178	38.811	18.431	7.383
Error	261	73.401	92.693	69.368	63.573	46.397	48.218	31.556	23.288	21.997	19.310	11.561

** Significant at 1 per cent level (P<0.01)

* Significant at 5 per cent level (P<0.05)

Significant ($P<0.01$) influence of layer dietary calcium was evident from the seventh week of age to the 24th week (Table 2.3 and Fig.1.2). At the seventh week, birds of subgroup C maintained on highest (3.5%) layer dietary calcium recorded significantly ($P<0.01$) lowest body weight of 139.8 $\pm$ 0.830 g (Table 2.1 and Fig.1.2). From the seventh to the 24th week of age birds of subgroup C maintained on the highest (3.5%) layer dietary calcium recorded the lowest body weight (Table 2.1 and Fig.1.2). Maximum body weight was found in subgroup B fed on 3 per cent layer dietary calcium especially from the 13th to 24th week of age (Table 2.1 and Fig.1.2). Among the subgroups A, B and C, subgroup B was unique in registering a uniformly increasing trend for gain in body weight from the 13th week to 24th week of age (Fig.1.2).

Interaction between pre-laying and layer dietary calcium was significant ($P<0.01$) at the 11th, 13th and 16th week of age and also at the 15th and 17th week of age ($P<0.05$). After the 17th week, interaction between pre-laying and layer dietary calcium was not significant (Table 2.3).

The overall mean body weight of female quails, at the age of first egg (sixth week of age) was 131.800 $\pm$ 0.600 g. The increase in body weight (growth rate) was marked upto the 16th week of age and thereafter it was only marginal. The

Fig.1.1 INFLUENCE OF PRE- LAYING DIETARY CALCIUM ON THE BODY WEIGHT OF JAPANESE QUAIL (VARIOUS PERIODS)

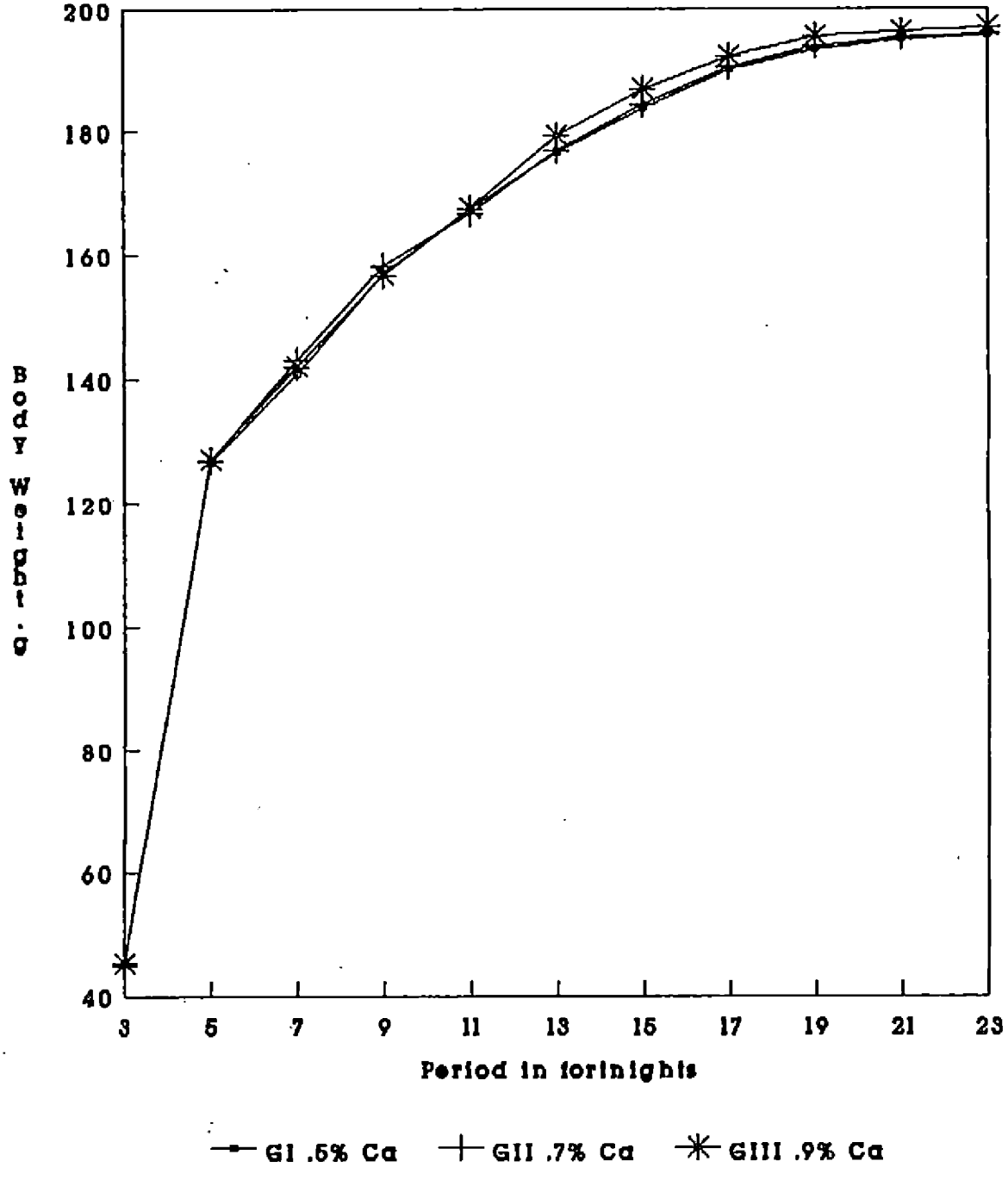
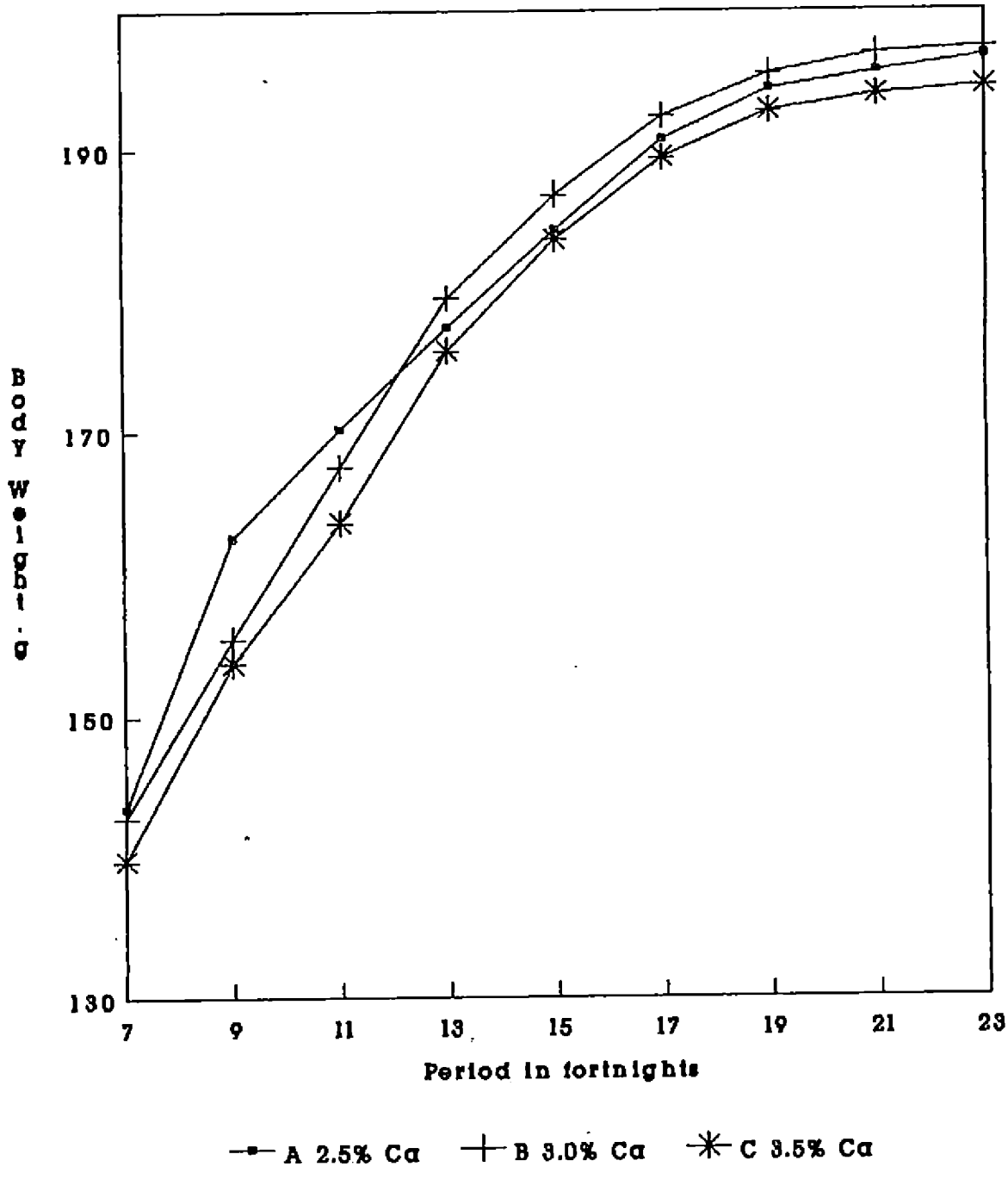


Fig.1.2 INFLUENCE OF LAYER DIETARY CALCIUM ON THE BODY WEIGHT OF JAPANESE QUAIL (VARIOUS PERIODS)



overall mean body weight at the 24th week of age was 198.133 ± 0.220 g (Table 2.1).

5.1.2 Anatomical studies

5.1.2.1 Oviduct development

5.1.2.1.1 General observations

As is the unique feature in the anatomical development of poultry, in Japanese quail also, only the left oviduct assumed full anatomical and physiological development, the right one remaining vestigeal and non-functional. The left oviduct as a whole was pinkish white in colour except for the shell gland which was dark brownish in appearance (Fig.2.1). Five regions were distinguishable in the oviduct on the basis of structure and function viz., the thin walled and funnel shaped infundibulum, the longest and thicker walled magnum, the isthmus which is the ovokeratin gland distinguished from the magnum by a narrow ring like readily distinguishable constriction, the expanded pouch or sac like, short dark brown shell gland (uterus) and the short muscular vagina which continued to the cloaca (Fig.2.1).

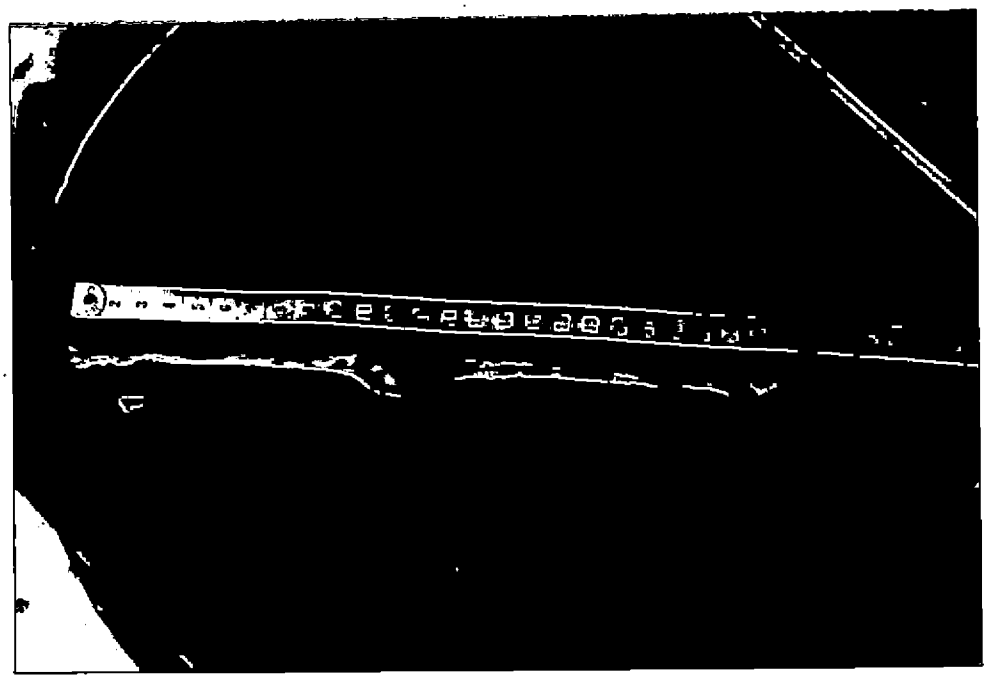
At the sixth week of age (age at first egg) the average length and weight of left oviduct were 28.66 ± 0.13 cm (Fig.2.2) and 4.874 ± 0.031 g respectively and that of shell

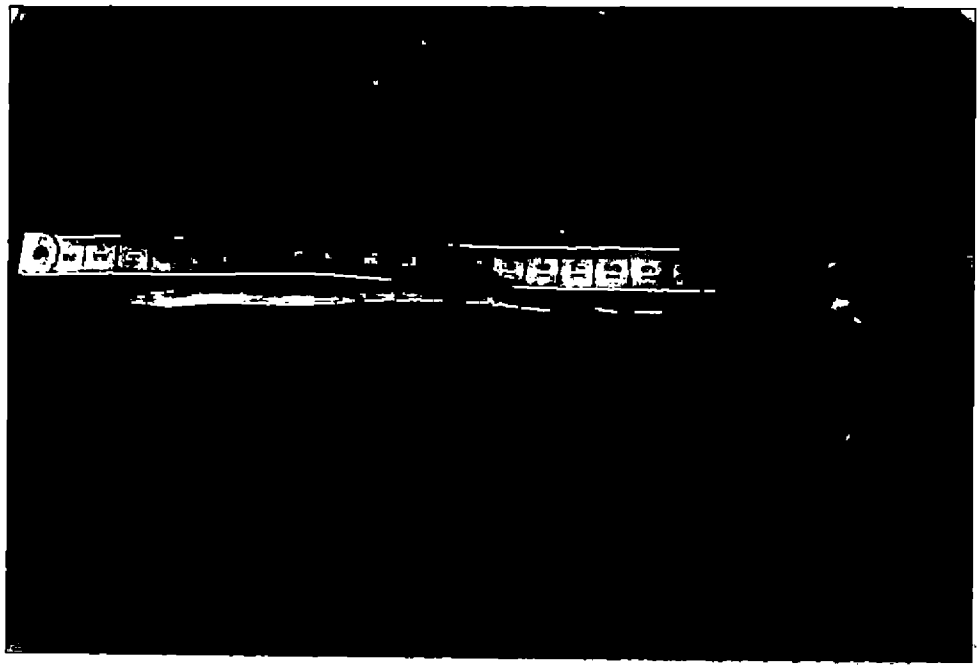
Fig.2.1 Quail oviduct, Anatomical description - (Sixth week)

1. Infundibulum
2. Magnum (with egg in situ)
3. Isthmus
4. Shell gland
5. Vagina

Fig.2.2 Quail oviduct - (Sixth week)

Length of oviduct = 28.60 cm
(with egg in shell gland)





gland were 2.93 ± 0.01 cm and 1.780 ± 0.007 g respectively (Table 3.1).

As growth advanced, at the 16th week of age the length and weight of oviduct increased to 29.98 ± 0.03 cm and 5.900 ± 0.010 g respectively (Table 3.3 and Fig.2.2, 3.1). The length and weight of shell gland were 3.09 ± 0.01 cm and 1.933 ± 0.004 g respectively (Table 3.3 and Fig.3.1, 3.2).

At the 24th week of age, length and weight of the oviduct increased to 31.90 ± 0.03 cm and 6.013 ± 0.010 g respectively (Table 3.4 and Fig.2.4, 3.1, 3.2). Length and weight of shell gland at this age were 3.12 ± 0.01 cm and 1.968 ± 0.004 g respectively (Table 3.4). As age advanced the overall increase in length and weight of oviduct was marked (Fig.3.1, 3.2). The length and weight of shell gland also increased at the 24th week of age (Table 3.4, Fig.3.1, 3.2).

5.1.2.1.2 Influence of dietary calcium on the development of oviduct

At the sixth week of age pre-laying dietary calcium did not show any significant ($P>0.01$) influence on the development of oviduct (Table 3.1, 3.2).

At the 16th week of age as the birds were maintained on layer dietary calcium, pre-laying dietary calcium did not

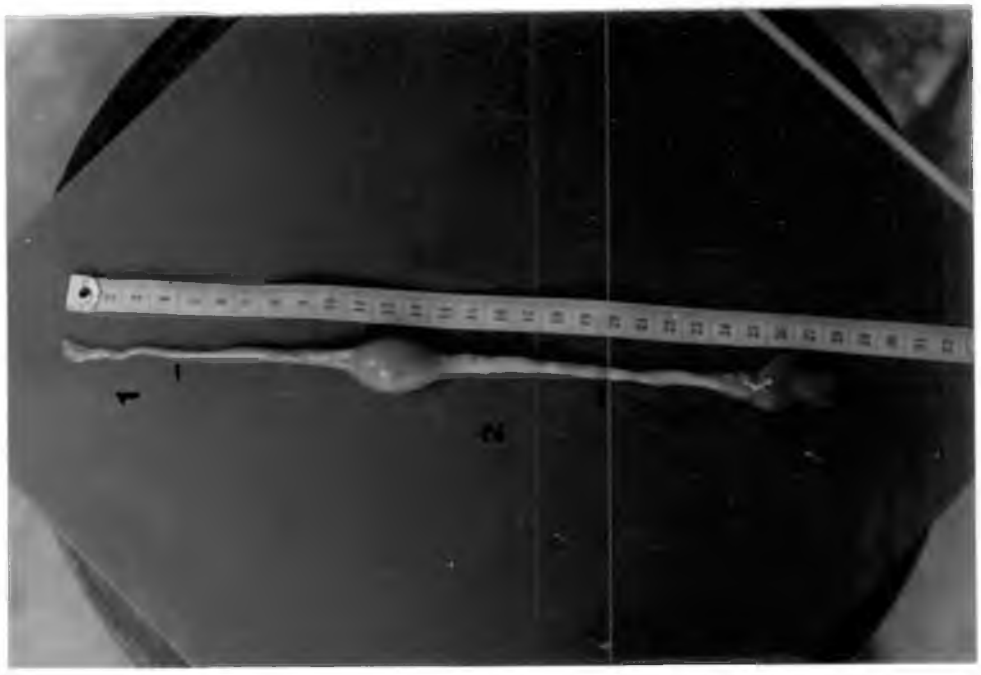


Fig.2.3 Quail oviduct - (16th week)

Length of oviduct = 29.98 cm
(with egg in shell gland)

Fig.2.4 Quail oviduct - (24th week)

Length of oviduct = 31.90 cm
(with egg in shell gland)

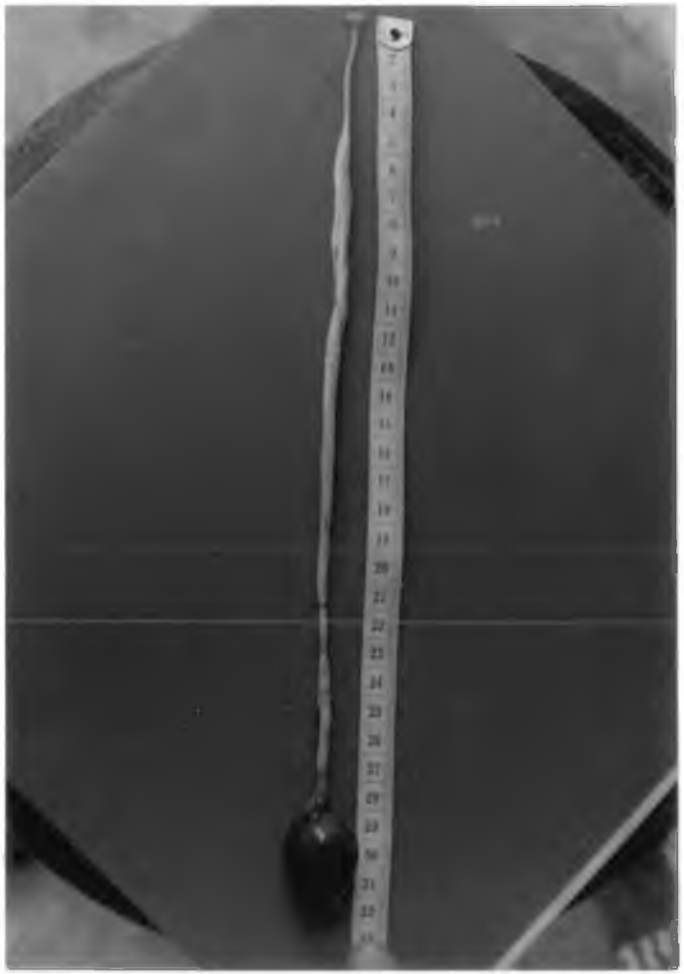


Fig.3.1 LENGTH OF OVIDUCT AND SHELL GLAND - JAPANESE QUAIL (DIFFERENT PERIODS)

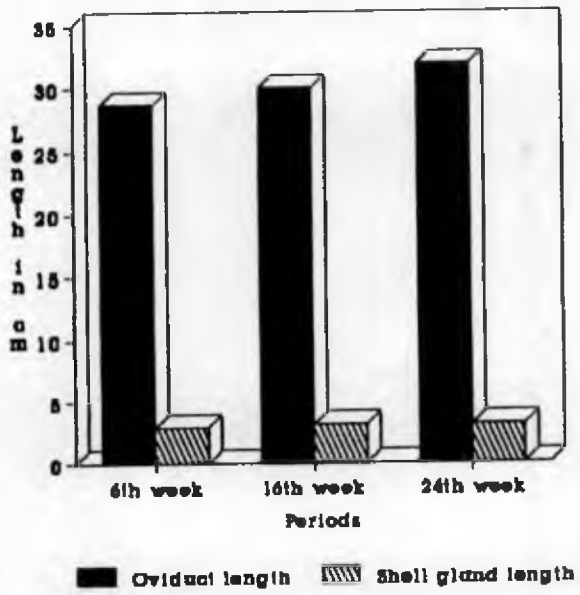


Fig.3.2 WEIGHT OF OVIDUCT AND SHELL GLAND - JAPANESE QUAIL (DIFFERENT PERIODS)

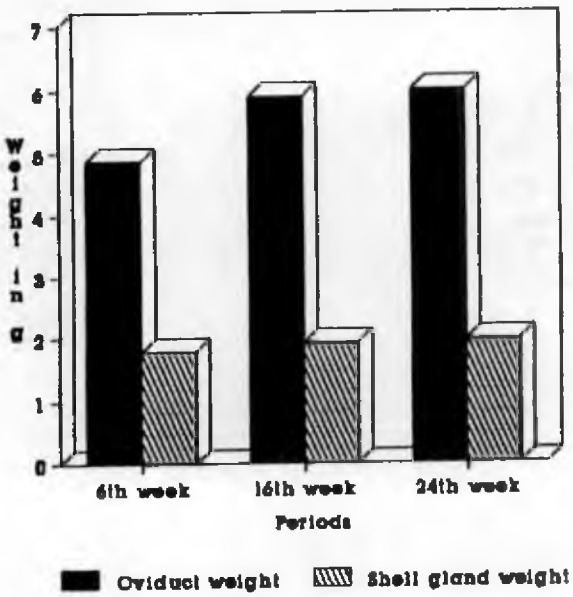


Table 3.1 Influence of dietary calcium level on the development of the oviduct of six week old female Japanese quail - (Mean ± SE) - 30 birds/group

Groups (Pre-laying dietary calcium)	Length in cm		Weight in g	
	Oviduct	Shell gland	Oviduct	Shell gland
GI (0.5%)	28.46 ± 0.15	2.91 ± 0.02	4.848 ± 0.040	1.764 ± 0.001
GII (0.7%)	29.01 ± 0.33	2.95 ± 0.02	4.856 ± 0.063	1.777 ± 0.001
GIII (0.9%)	28.51 ± 0.16	2.94 ± 0.02	4.917 ± 0.055	1.799 ± 0.001
Overall means	28.66 ± 0.13	2.93 ± 0.01	4.874 ± 0.031	1.780 ± 0.007
CD	0.460	0.040	0.099	0.020

CD = Critical difference for comparison of means

Table 3.2 ANOVA table - Influence of dietary calcium level on the development of oviduct of six week old female Japanese quail

Sources	df	MSS			
		Length		Weight	
		Oviduct	Shell gland	Oviduct	Shell gland
Groups (pre-laying dietary calcium)	2	2.76	0.014	0.043	0.009
Error	87	1.536	0.008	0.086	0.004

Table 3.3 Influence of dietary calcium level on the development of oviduct of 16 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)												Overall means			
	A (2.5)				B (3%)				C (3.5%)							
	Length in cm		Weight in g		Length in cm		Weight in g		Length in cm		Weight in g		Length in cm		Weight in g	
	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland
GI (0.5%)	30.01± 0.10	3.07± 0.01	5.840± 0.026	1.931± 0.011	30.00± 0.08	3.07± 0.02	5.987± 0.023	1.917± 0.014	29.97± 0.09	3.10± 0.01	5.875± 0.021	1.921± 0.012	30.00± 0.05	3.08± 0.01	5.900± 0.015	1.923± 0.007
GII (0.7%)	29.94± 0.06	3.09± 0.01	5.840± 0.043	1.966± 0.013	29.85± 0.09	3.09± 0.02	5.954± 0.024	1.928± 0.012	29.99± 0.07	3.11± 0.01	5.935± 0.016	1.955± 0.008	29.92± 0.04	3.10± 0.01	5.910± 0.018	1.950± 0.007
GIII (0.9%)	29.98± 0.10	3.09± 0.01	5.838± 0.034	1.942± 0.012	30.14± 0.08	3.09± 0.01	5.873± 0.034	1.903± 0.011	29.96± 0.07	3.12± 0.01	5.958± 0.014	1.937± 0.009	30.03± 0.05	3.10± 0.01	5.890± 0.020	1.927± 0.010
Overall means	29.97± 0.05	3.08± 0.01	5.839± 0.020	1.946± 0.007	30.00± 0.05	3.08± 0.01	5.938± 0.017	1.916± 0.007	29.97± 0.04	3.11± 0.01	5.922± 0.010	1.938± 0.006	29.98± 0.03	3.09± 0.01	5.900± 0.010	1.933± 0.004
CD*	-	-	-	-	-	-	-	-	-	-	-	-	0.135	0.021	0.043	0.018
CD**	0.233	0.04	0.088	0.030	0.233	0.04	0.088	0.030	0.233	0.04	0.088	0.030	-	-	-	-

CD* - Critical difference for comparison of overall means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 3.4 Influence of dietary calcium level on the development of the oviduct of 24 week old female Japanese quail-(Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)												Overall means			
	A (2.5)				B (3%)				C (3.5%)							
	Length in cm		Weight in g		Length in cm		Weight in g		Length in cm		Weight in g		Length in cm		Weight in g	
	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland	Ovi-duct	Shell gland
GI (0.5%)	31.80± 0.09	3.12± 0.01	5.999± 0.012	1.946± 0.012	32.05± 0.09	3.14± 0.02	6.037± 0.061	1.965± 0.006	31.78± 0.09	3.12± 0.01	5.989± 0.012	1.960± 0.005	31.88± 0.05	3.13± 0.01	6.008± 0.020	1.957± 0.010
GII (0.7%)	31.72± 0.09	3.11± 0.01	5.997± 0.009	1.989± 0.008	31.95± 0.09	3.09± 0.01	6.062± 0.046	1.939± 0.034	31.93± 0.09	3.13± 0.01	5.971± 0.015	1.968± 0.006	31.87± 0.05	3.11± 0.01	6.010± 0.020	1.965± 0.010
GIII (0.9%)	31.93± 0.08	3.11± 0.02	6.037± 0.027	1.981± 0.006	31.96± 0.09	3.13± 0.02	6.035± 0.036	1.991± 0.008	31.94± 0.08	3.13± 0.01	5.991± 0.009	1.969± 0.006	31.94± 0.05	3.12± 0.01	6.021± 0.020	1.981± 0.004
Overall means	31.82± 0.05	3.11± 0.01	6.011± 0.010	1.972± 0.006	31.99± 0.05	3.12± 0.01	6.045± 0.030	1.965± 0.010	31.88± 0.05	3.13± 0.01	5.984± 0.007	1.966± 0.003	31.90± 0.03	3.12± 0.01	6.013± 0.010	1.968± 0.004
CD*	-	-	-	-	-	-	-	-	-	-	-	-	0.140	0.020	0.050	0.020
CD**	0.240	0.042	0.082	0.046	0.240	0.042	0.082	0.046	0.240	0.042	0.082	0.046	-	-	-	-

CD* - Critical difference for comparison of overall means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 3.5 ANOVA table of means - Influence of dietary calcium level on the development of oviduct of female Japanese quail -(30 birds/sub-group)- 16th and 24th week of age

Sources	df	MSS							
		16 week of age				24 week of age			
		Length		Weight		Length		Weight	
		Oviduct	Shell gland	Oviduct	Shell gland	Oviduct	Shell gland	Oviduct	Shell gland
Groups (pre-laying dietary calcium)	2	0.239	0.010	0.009	0.018*	0.157	0.006	0.004	0.013
Sub-groups (layer dietary calcium)	2	0.017	0.024**	0.254**	0.022**	0.643	0.003	0.085*	0.001
Groups vs sub-groups	4	0.227	0.001	0.075**	0.002	0.256	0.007	0.011	0.012
Error	261	0.212	0.005	0.022	0.004	0.227	0.007	0.026	0.005

** Significant at 1 per cent level (P<0.01)

* Significant at 5 per cent level (P<0.05)

influence either the length or the weight of oviduct or the length of shell gland. However, the weight of the shell gland was influenced ($P<0.05$) by pre-laying dietary calcium at the 16th week of age (Table 3.5). Birds of GII group maintained on 0.7 per cent pre-laying dietary calcium recorded the maximum weight (1.950 $\pm$ 0.007 g) for the shell gland. Among all the subgroups, only the subgroups GII A, GII B and GII C showed the higher weight of shell gland viz., 1.966 $\pm$ 0.013 g, 1.928 $\pm$ 0.012 g and 1.955 $\pm$ 0.008 g respectively (Table 3.3). The length and weight of the shell gland and weight of the oviduct (Table 3.5) were seen significantly ($P<0.01$) influenced by layer dietary calcium. Maximum length of the shell gland, 3.11 $\pm$ 0.01 cm, was noticed for subgroup C (reared on 3.5 per cent layer dietary calcium) among all the subgroups. However, the maximum weight (1.946 $\pm$ 0.007 g) for the shell gland was observed for subgroup A maintained on 2.5 per cent layer dietary calcium (Table 3.3).

At the 24th week of age there was no significant influence of pre-laying dietary calcium on the development of oviduct (Table 3.5). However, layer dietary calcium marginally influenced ($P<0.05$) the weight of the oviduct (Table 3.5). Subgroup B reared at 3 per cent layer dietary calcium (Table 3.4) had maximum weight of oviduct (6.045 $\pm$ 0.030 g).

5.1.2.2 Histological and histochemical studies

5.1.2.2.1 Histological studies

Shell glands were collected for histological studies at the time of active egg shell formation with a calcifying egg in the shell gland. The wall of the shell gland of Japanese quail consisted of lining mucosa (lamina epitheliaris), glandular lamina propria, tunica muscularis (with inner circular and outer longitudinal muscle layers) and tunica serosa (Fig. 4.1). The surface mucosa of the shell gland was thrown into longitudinally oriented leaf shaped folds or rugae which were occasionally branched (Fig.4.1, 4.12).

In cross section the rugae were covered by a simple layer of columnar epithelium with alternating apical and basal cells giving a pseudostratified appearance (Fig.4.3, 4.4).

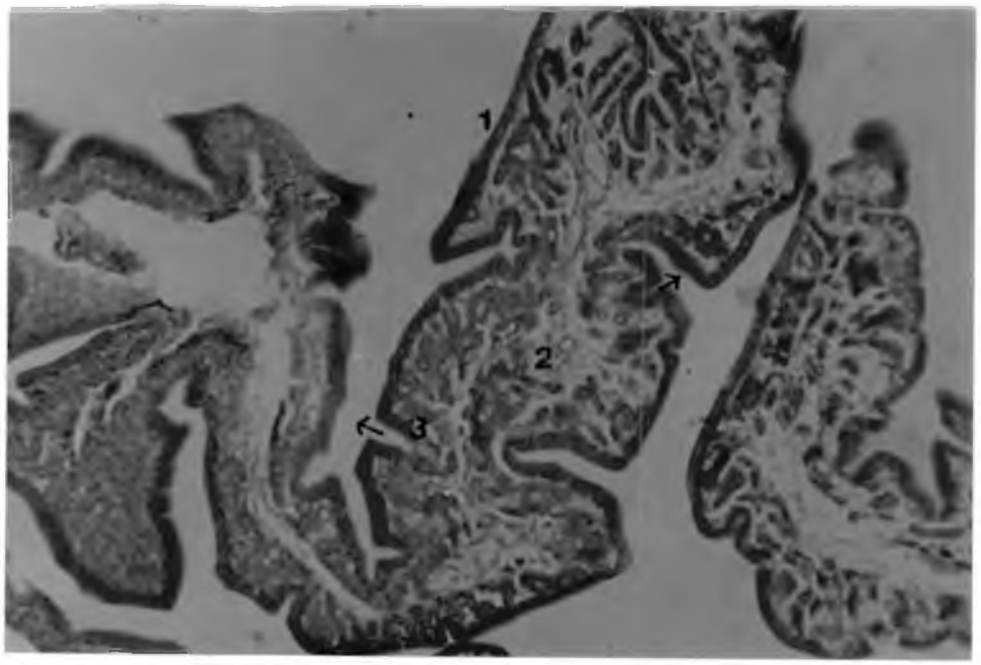
The apical cells were wedge shaped with apical nuclei, ciliated and with granules in the cytoplasm. Basal cells were non-ciliated, wider towards basal parts and had basally placed nucleus. The cells were narrow apically with striated border towards apical surface suggestive of microvilli (Fig. 4.3, 4.4). Next to this layer was a glandular lamina propria containing branched tubular glands which opened onto the surface of the folds of the mucosa by means of short ducts

Fig.4.1 **Quail shell gland - GII B sub-group (16th week) H&E x 250**

1. Lamina epitheliaris
2. Lamina propria
3. Duct of the tubular gland (↑)

Fig.4.2 **Quail shell gland - GII group (sixth week) H&E x 250**

1. Lamina epitheliaris
2. Lamina propria
3. Inner circular muscle
4. Outer longitudinal muscle
5. Tunica serosa
6. Connective tissue core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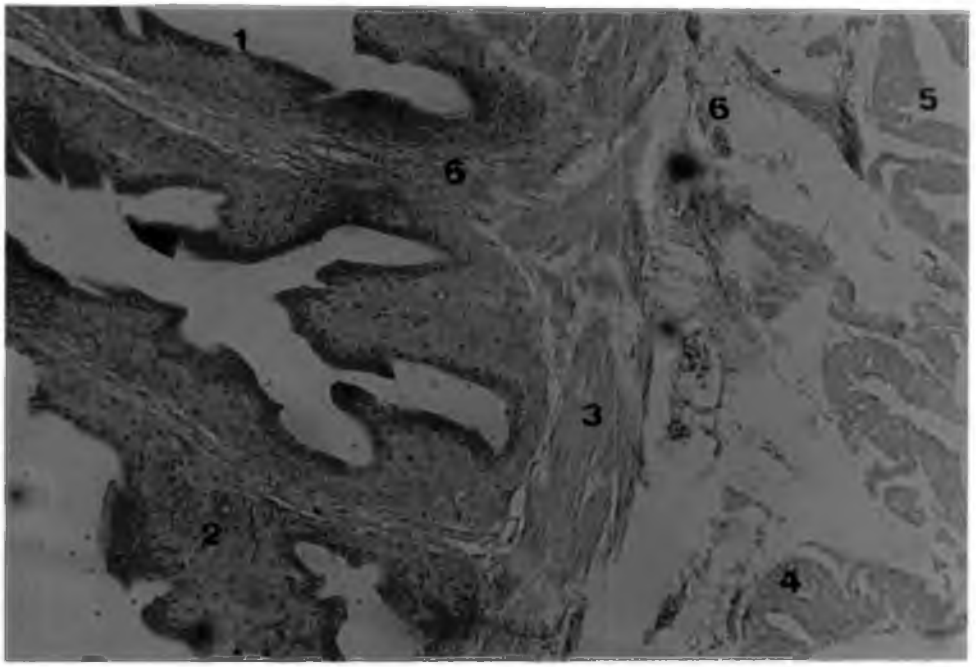
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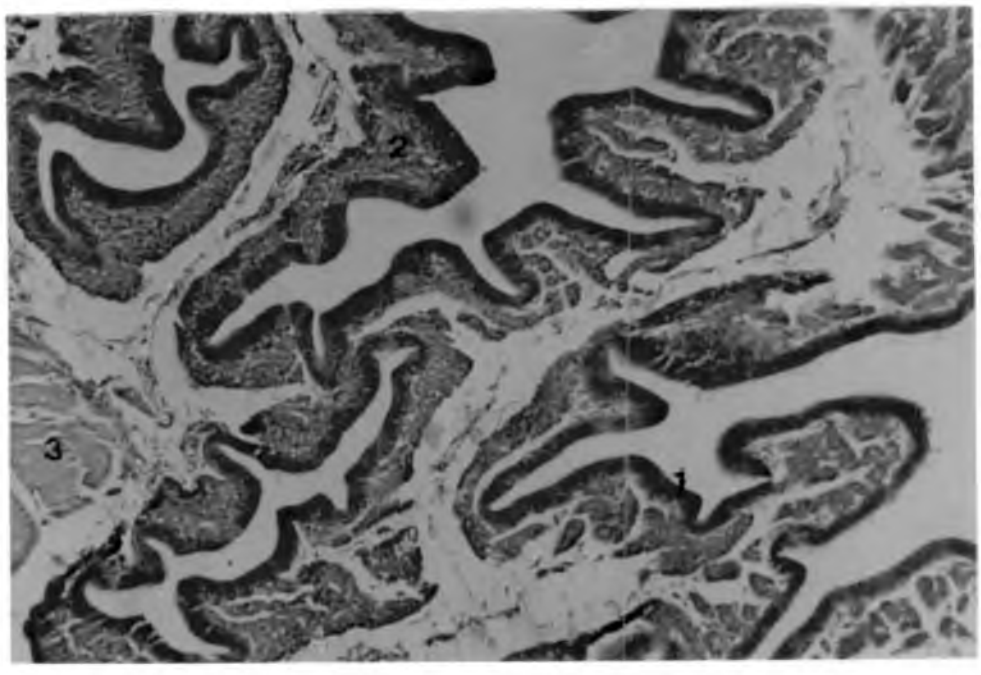
Fig.4.3 **Quail shell gland - GIII B sub-group (16th week) H&E x 250**

1. Lamina epitheliaris (Pseudostratified)
2. Lamina propria
3. Inner circular muscle

Fig.4.4 **Quail shell gland - GIII group (sixth week) H&E x 250**

1. Lamina epitheliaris (Pseudostratified - apical and basal cells)
2. Lamina propria
3. Central core of connective tissue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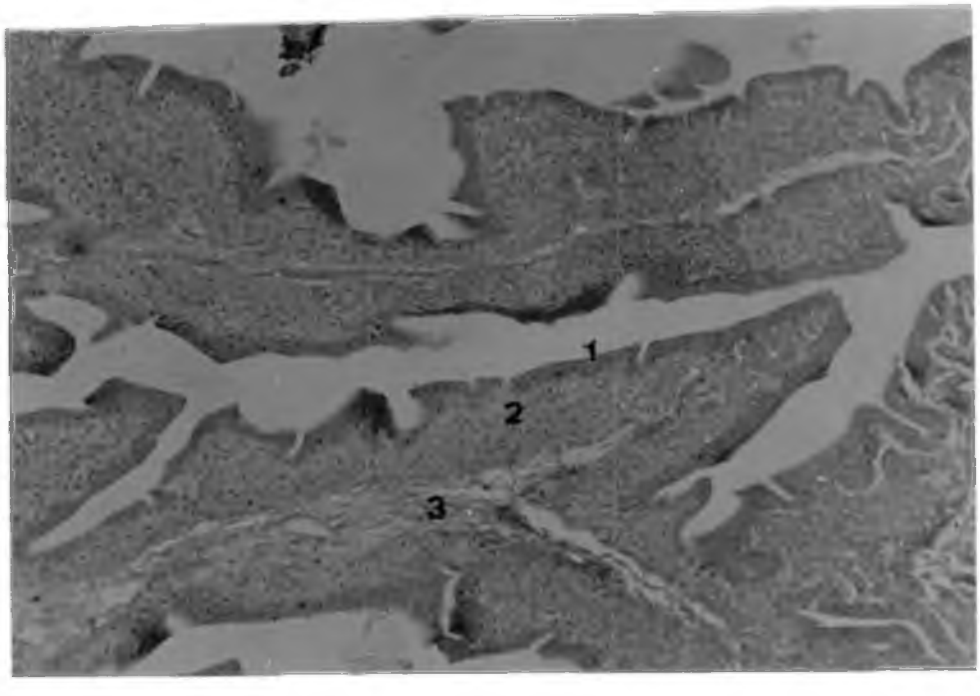
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lined by gland cells (Fig.4.1, 4.5, 4.7, 4.10). Invaginations of the surface epithelium formed short ducts through which underlying tubular glands communicated with the surface. These tubular glands of lamina propria were numerous and highly branched. The tubular gland ducts were closely packed together in cross section, they consisted of five to seven or more polygonal cells with large basal nucleus enclosing a lumen which was empty (Fig.4.10). These cells had pale staining granules. There was a central core of connective tissue extending into the lamina propria of the mucosal folds from the underlining tunica muscularis (Fig.4.2, 4.4). This connective tissue core contained fine Capillaries (Fig.4.6, 4.8). The mucosa was surrounded by two layers of smooth muscle (Fig.4.2,4.8), the thick inner circular muscle layer was separated from the thinner outer longitudinal layer, by a layer of connective tissue that was rich in large blood vessels (Fig.4.2, 4.6, 4.8). External to the tunica muscularis, a layer of flattered mesothelial cells formed the tunica serosa (Fig.4.2, 4.8).

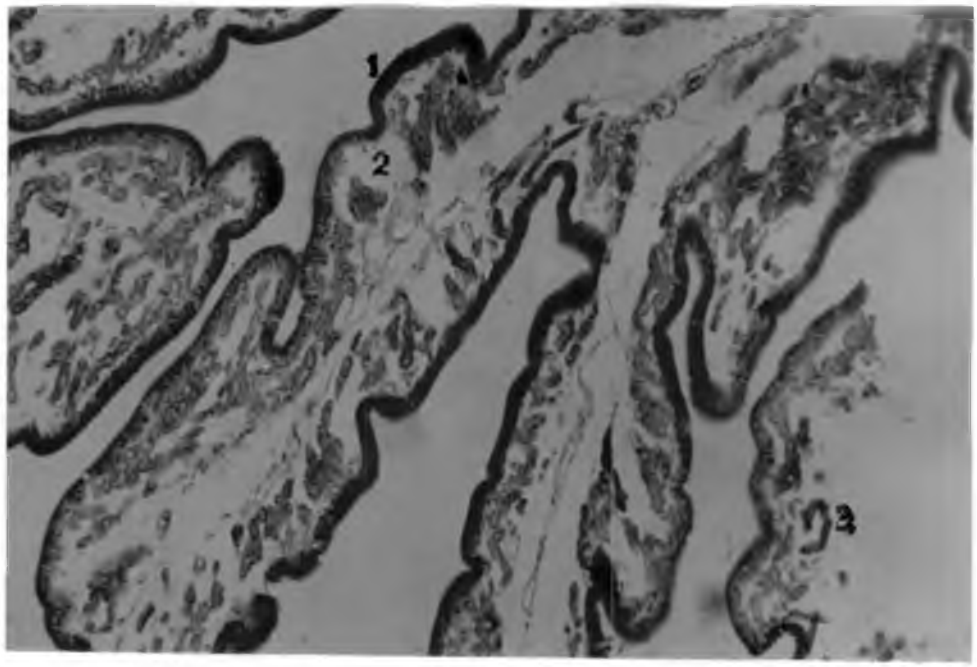
Comparison of the shell gland tissue of the quails reared under three pre-laying and layer dietary calcium levels, showed that the highest (3.5%) layer dietary calcium fed subgroup C having more vascularity in the connective tissue core of the lamina propria (Fig.4.10). If at all there

Fig.4.5 **Quail shell gland - GII A sub-group (16th week) H&E x 250**

1. Lamina epitheliaris
2. Lamina propria
3. Tubular gland duct cells of lamina propria

Fig.4.6 **Quail shell gland - GIII A sub-group (16th week) H&E x 250**

1. Lamina epitheliaris (Pseudostratified)
2. Lamina propria
3. Tunica muscularis (inner circular muscle layer)
4. Blood vessel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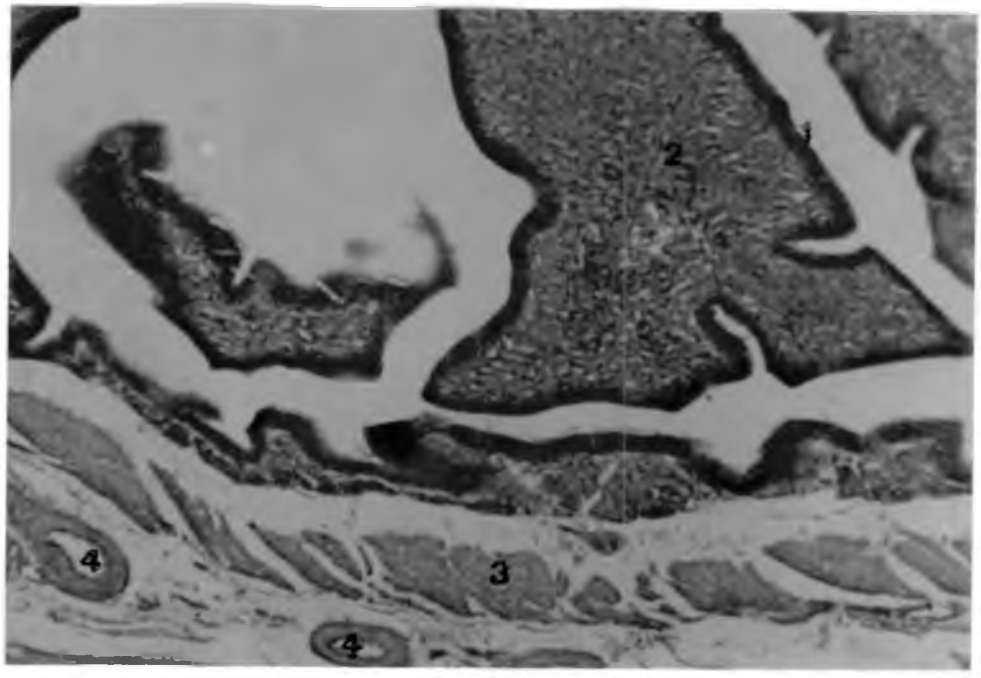
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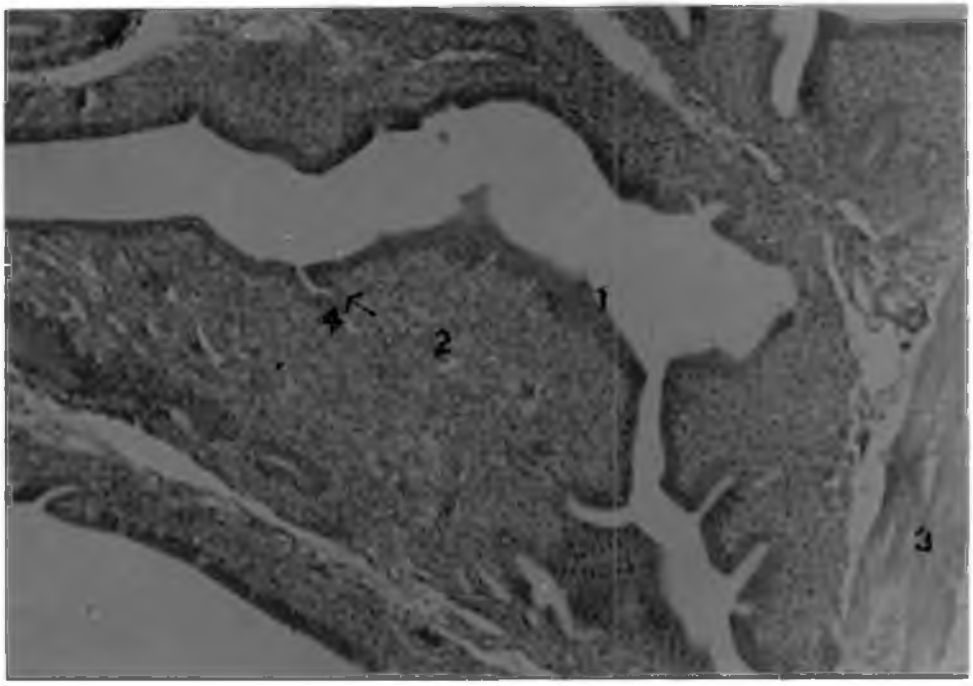
Fig.4.7 **Quail shell gland - GI group (sixth week)**
H&E x 250

1. Lamina epitheliaris
   (Pseudostratified with lesser folds)
2. Lamina propria
3. Tunica muscularis
   (inner circular muscle layer)
4. Duct of the tubular gland (↑)

Fig.4.8 **Quail shell gland - GI C sub-group (16th week)**
H&E x 250

1. Lamina epitheliaris
2. Lamina propria
3. Inner circular muscle layer
4. Outer longitudinal muscle layer
5. Blood vessel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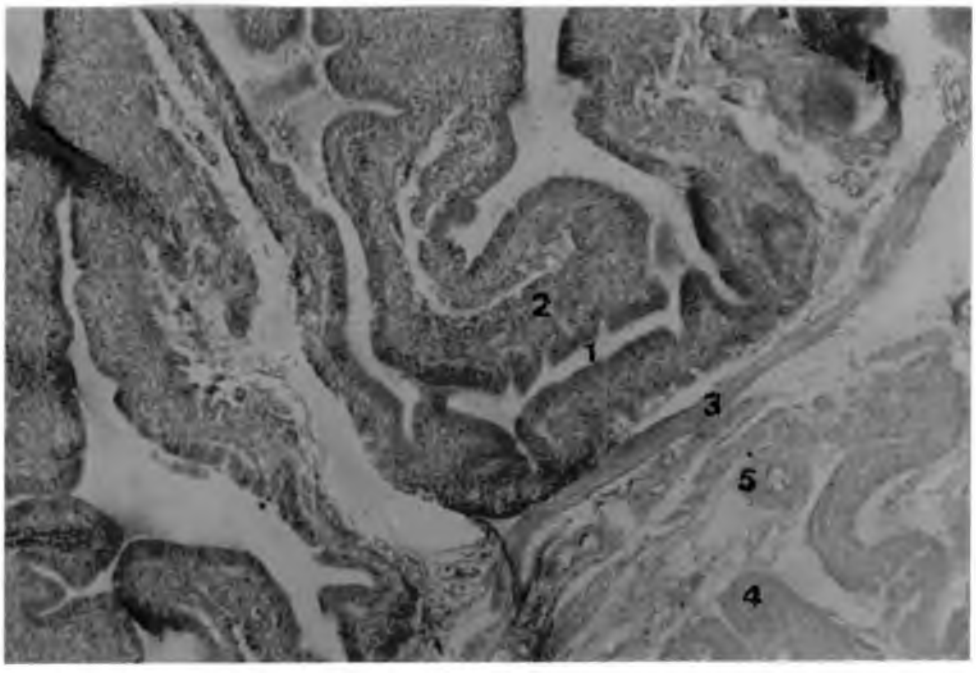
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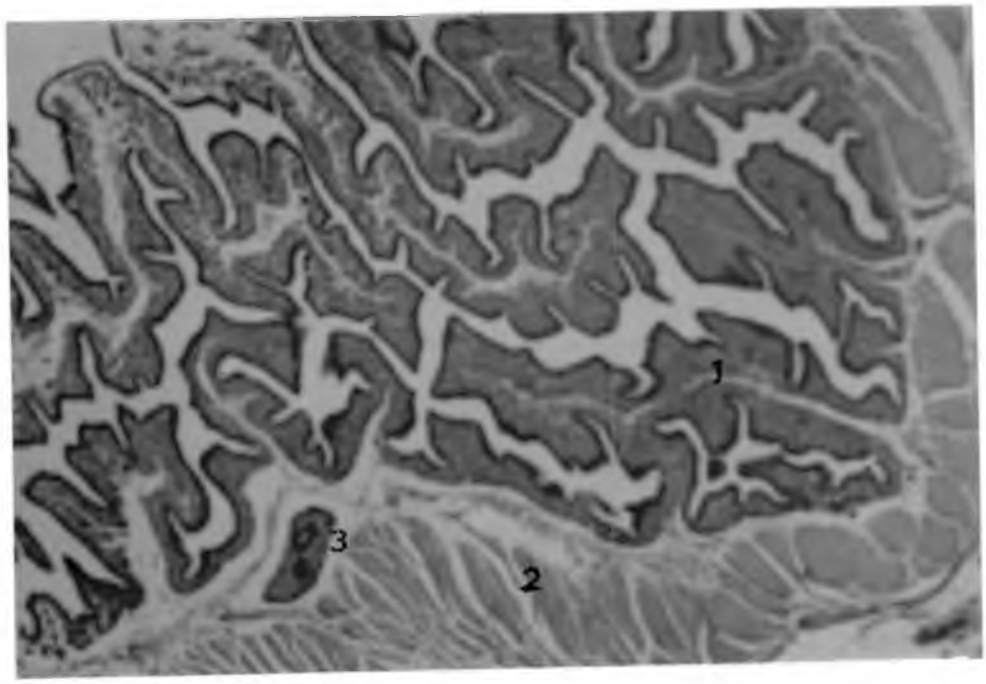
Fig.4.9 **Quail shell gland - GII C sub-group (16th week) H&E x 250**

1. Mucosal folds (numerous)
2. Inner circular muscle (thicker)
3. Blood vessel

Fig.4.10 **Quail shell gland - GIII C sub-group (24th week H&E x 250**

1. Lamina epitheliaris
2. Duct of tubular glands
3. Blood vessels in Lamina propria
4. Inner circular muscle (thicker)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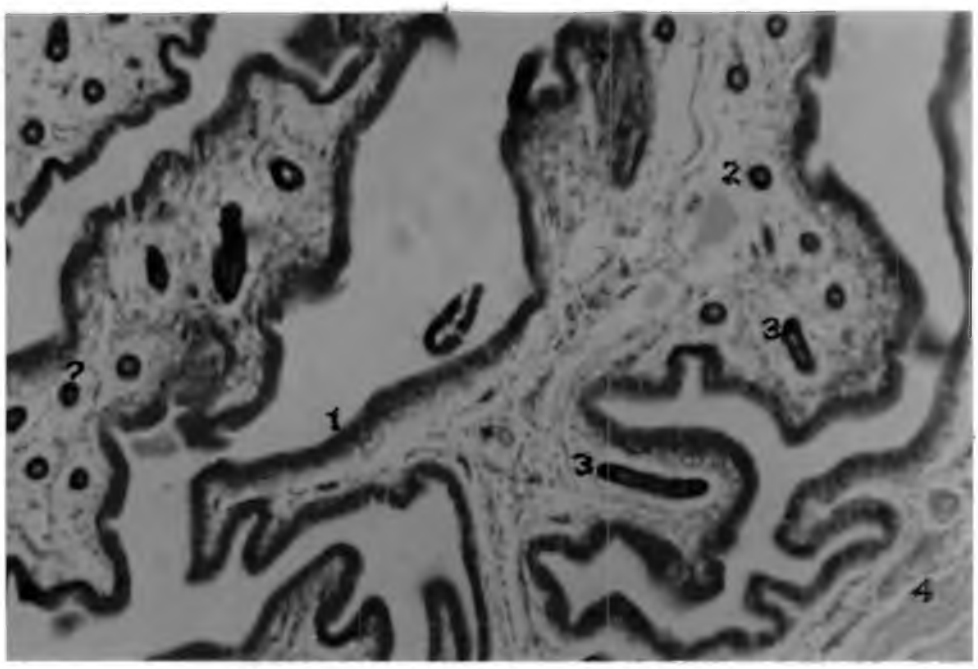
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was any influence of dietary calcium, it was revealed only in the augmented vascularity in the lamina propria.

Histological studies conducted on the shell gland tissues collected from quails of six, 16 and 24 weeks of age showed that birds of 16 and 24 weeks had more mucosal foldings than those of the six week old birds (Fig.4.2, 4.11, 4.12). Mucosal foldings were greatest for 24 week old birds. The inner circular muscle layer was thicker and penetrated into the folds of the mucosa (Fig.4.11, 4.12) with well developed vascular layer in between inner and outer muscle layers (Fig.4.6). Sixteen and 24 week old birds showed more mucosal foldings with better developed inner circular muscle layer and thinner longitudinal muscle layer (Fig.4.11, 4.12) compared to those of six week old birds.

5.1.2.2.2 Histochemical studies

5.1.2.2.2.1 Alkaline phosphatase (ALP)

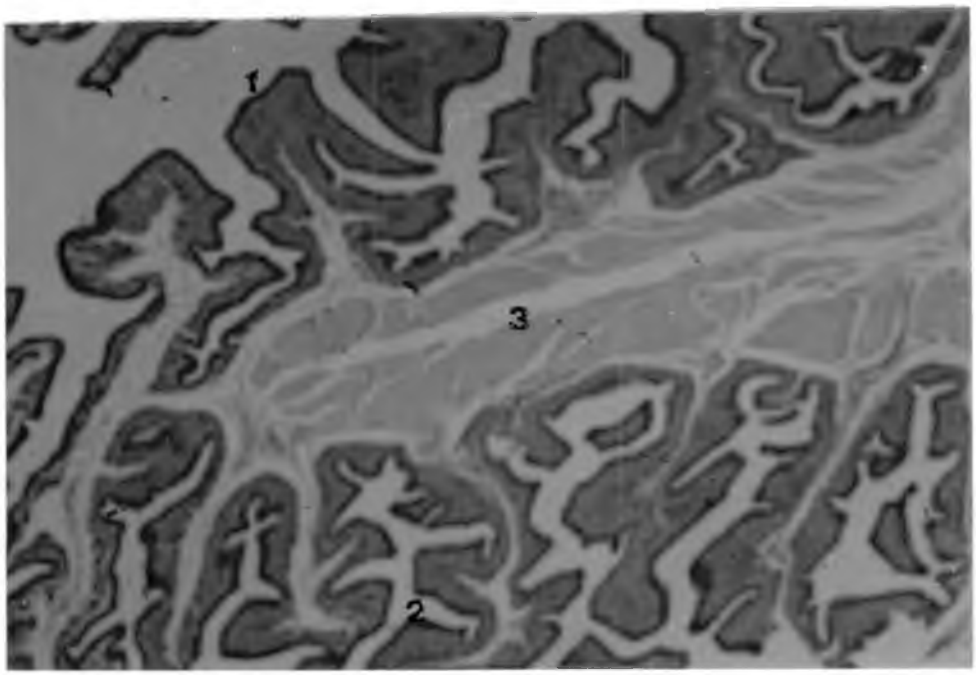
The intensity of the enzyme activity of ALP in the shell gland of Japanese quails was fairly weak (Fig.5.1, 5.2, 5.3) compared to ACP, only mild localisation of ALP was found in the epithelial lining or the mucosa (Fig.5.3). Comparison of shell gland tissues collected from various pre-laying and layer dietary calcium fed groups revealed that there was no significant difference between different groups and subgroups

Fig.4.11 **Quail shell gland - GI B sub-group (16th week) H&E x 250**

1. Lamina epitheliaris
2. Mucosal folds (numerous)
3. Inner circular muscle invading the mucosal folds

Fig.4.12 **Quail shell gland - GII B sub-group (24th week) H&E x 250**

1. Lamina epitheliaris
2. Mucosal folds (numerous)
3. Circular muscle invading the mucosal folds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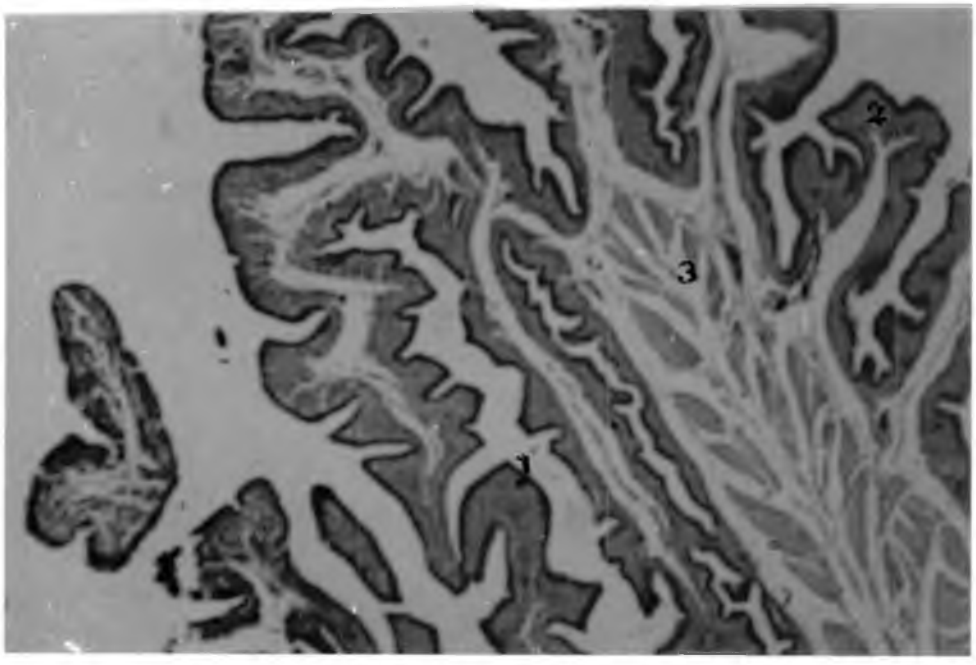
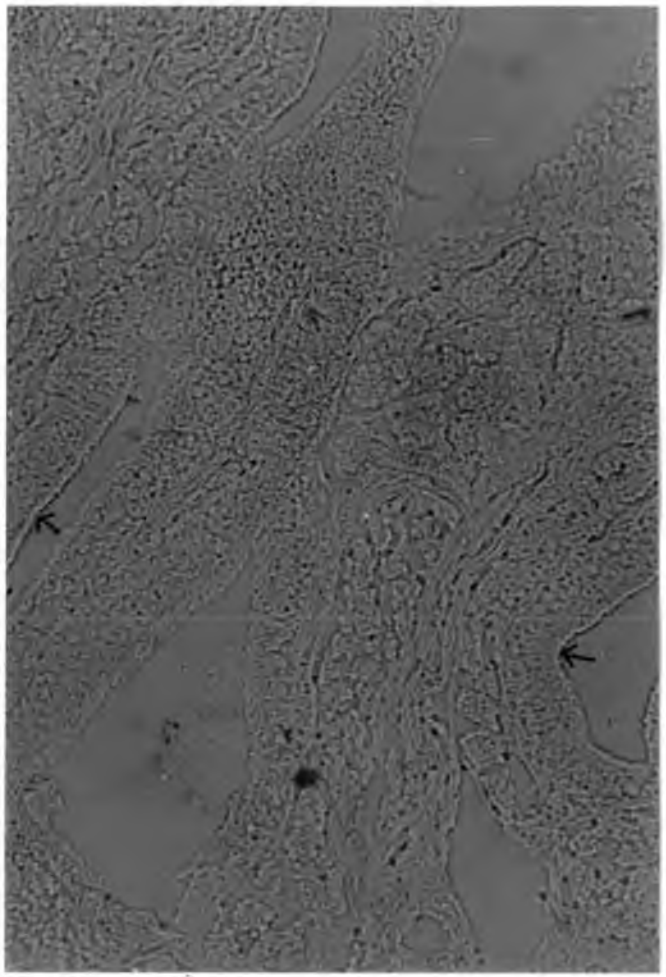

2
3
1

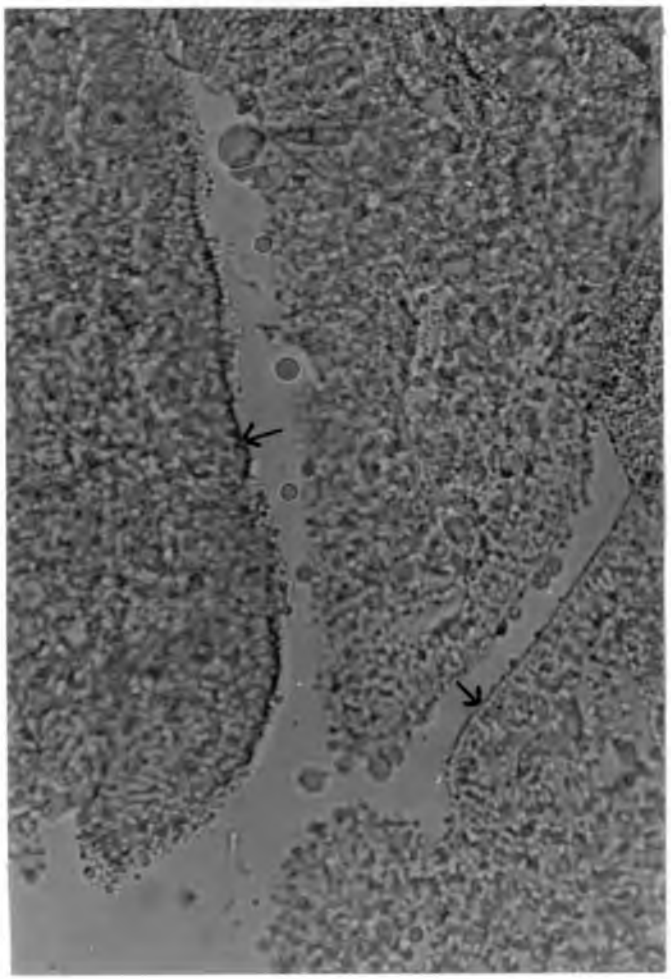
Fig.5.1 **Quail shell gland - GII group (sixth week)**

Alkaline phosphatase activity x 250

Fig.5.2 **Quail shell gland - GIII C sub-group (16th week)**

Alkaline phosphatase activity x 250





in their enzyme distribution. There was no difference on the comparative localisation of ALP in the shell gland tissue of six, 16 and 24 week old birds (Fig.5.1, 5.2, 5.3).

5.1.2.2.2.2 Acid phosphatase (ACP)

Histochemical localisation of the ACP in the shell gland of the Japanese quail revealed that an intense localisation of the enzyme occurred only in the epithelial lining of mucosal layer (Fig.5.4, 5.5, 5.6). Differences in the histochemical localisation of ACP were not appreciable in different groups and sub-groups reared on different dietary calcium levels (Fig.5.4, 5.5, 5.6, 5.7, 5.8). Similar reaction was observed for six, 16 and 24 week old quails (Fig.5.4, 5.5, 5.7).

5.2 Egg production and shell quality

5.2.1 Egg production

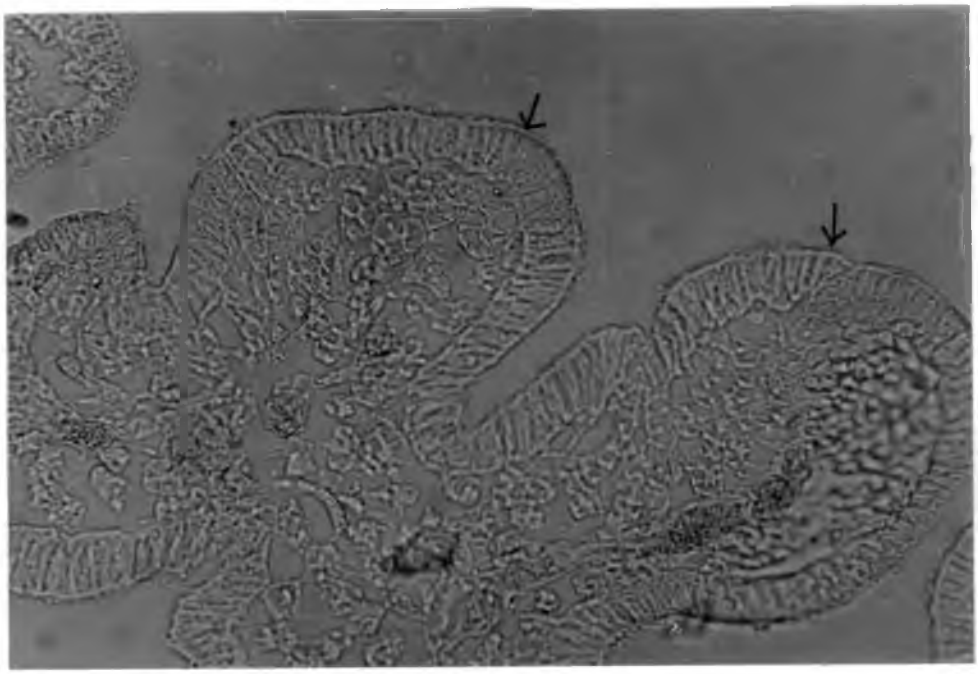
The egg production in quails started from 39 days to 42 days (Table 4.3) i.e., early sixth week of age and peak production was attained at the 16th week (83.219 $\pm$ 2.274 per cent) which continued even upto 24th week of age (Table 4.3 and Fig.6.2, 6.4).

Fig.5.3 **Quail shell gland - GII B sub-group (24th week)**

Alkaline phosphatase activity x 250

Fig.5.4 **Quail shell gland - GII group (sixth week)**

Acid phosphatase activity x 250



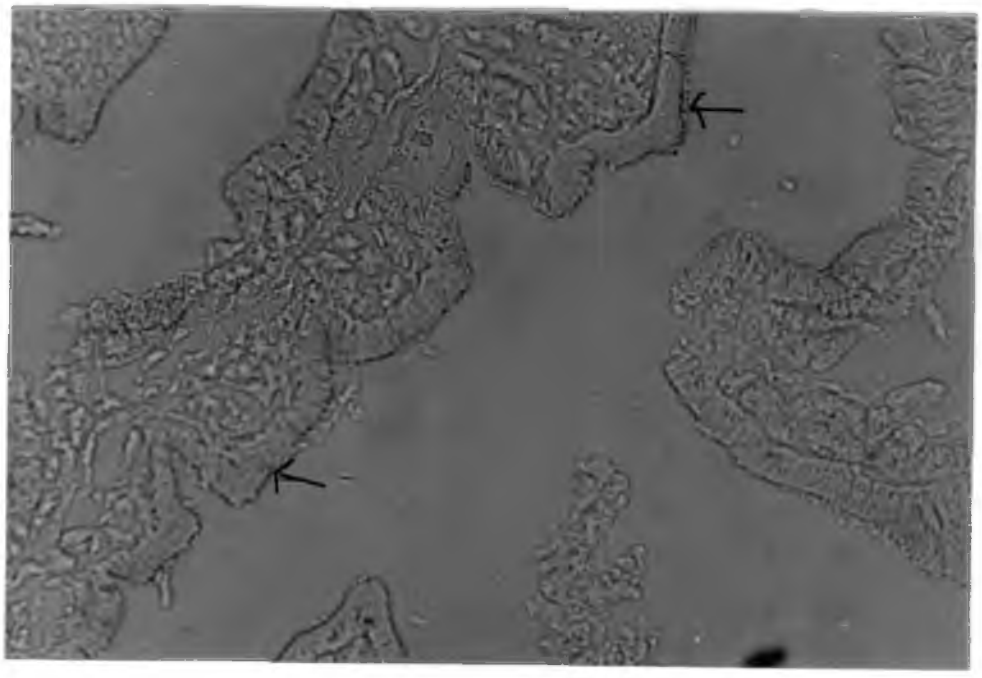
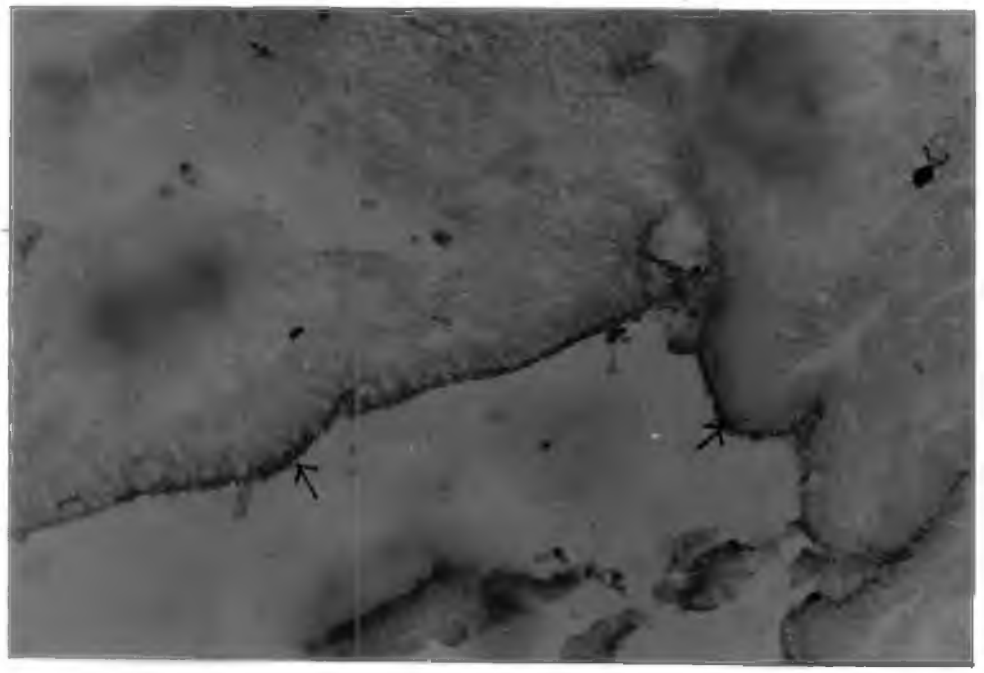


Fig.5.5 **Quail shell gland - GIII C sub-group (16th week)**

Acid phosphatase activity x 250

Fig.5.6 **Quail shell gland - GI B sub-group (16th week)**

Acid phosphatase activity x 100



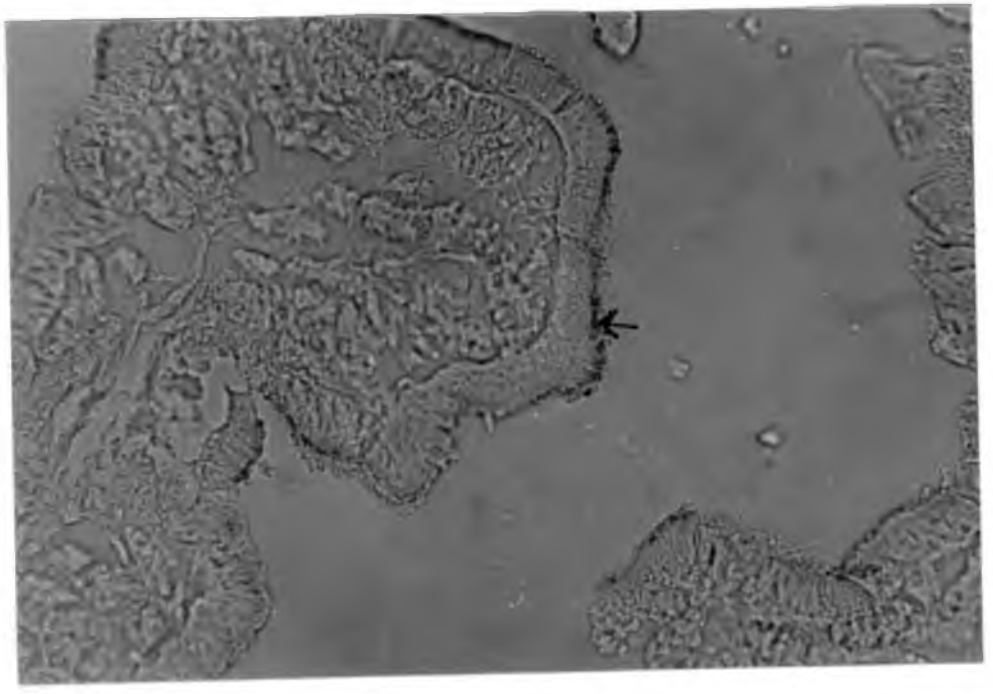
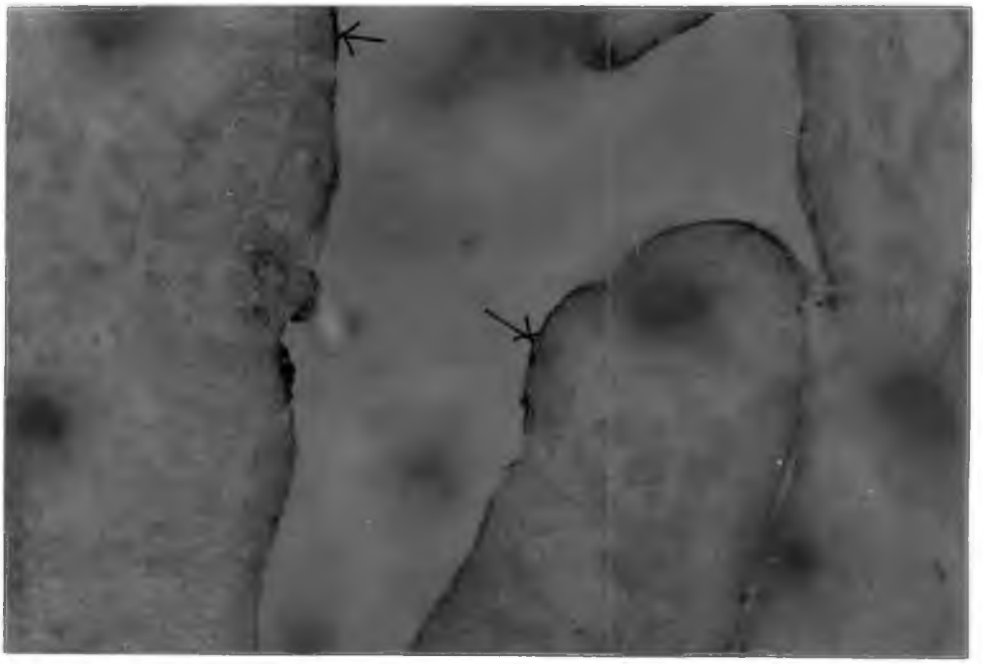


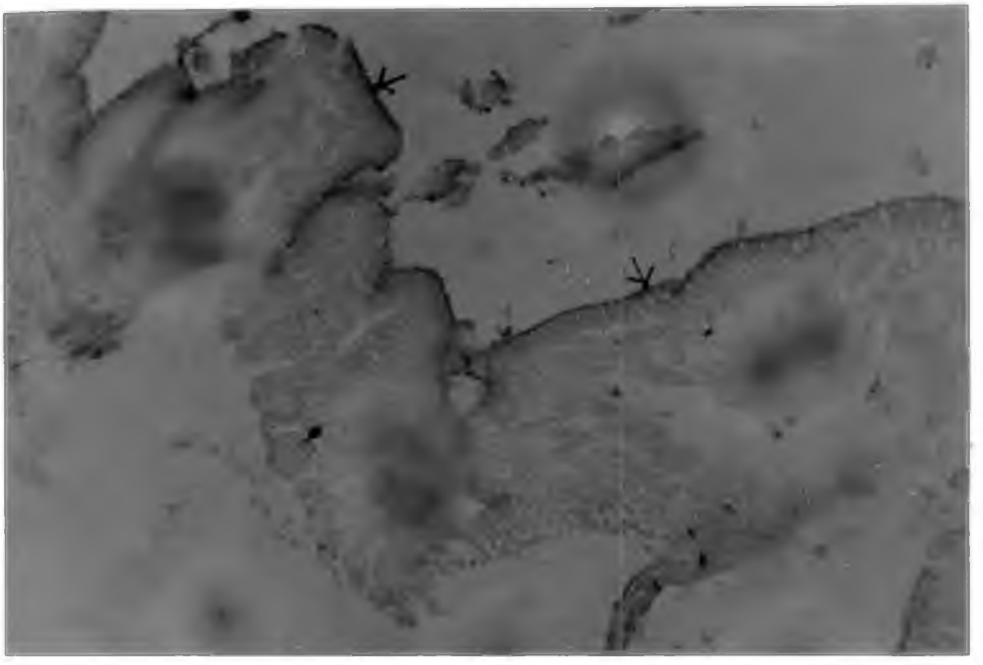
Fig.5.7 **Quail shell gland - GIII C sub-group (24th week)**

Acid phosphatase activity x 100

Fig.5.8 **Quail shell gland - GII C sub-group (16th week)**

Acid phosphatase activity x 100





The influence of pre-laying dietary calcium on egg production was evident in GI group reared on the lowest (0.5%) pre-laying dietary calcium recording the lowest (78.94 ± 1.97%) egg production (Table 4.1). At the same time the birds in GII group and GIII group reared on the higher pre-laying dietary calcium levels (0.7% and 0.9% respectively) recorded higher (80.89 ± 1.88% and 80.88 ± 1.77% respectively) rate of egg production (Table 4.1, Fig.6.4).

Comparison of the data on egg production using 'student t' test revealed that there was significant difference among different subgroups except for a few namely GII A vs GIII A, GII B vs GIII B and GI C, and GI C vs GII C (Table 4.2).

Layer dietary calcium significantly influenced the egg production in Japanese quails. The maximum egg production recorded was for birds of subgroup GIII B with an average value of 86.40 ± 2.89 per cent followed by GII B with 85.60 ± 2.95 per cent (Table 4.1, Fig.6.4). Birds maintained on the higher layer dietary calcium levels of 3 per cent (subgroup B) and 3.5 per cent (subgroup C) recorded maximum egg production, exceeding 80 per cent (Fig.6.1, 6.3). The overall mean egg production percentage in subgroup B and subgroup C were 84.64 ± 1.71 and 84.46 ± 1.57 respectively (Table 4.1). The lowest egg production (69.88 ± 2.42%) was for the birds of subgroup A

Fig. 6.1 EGG PRODUCTION AT DIFFERENT LEVELS OF DIETARY CALCIUM - JAPANESE QUAIL - (DIFFERENT PERIODS)

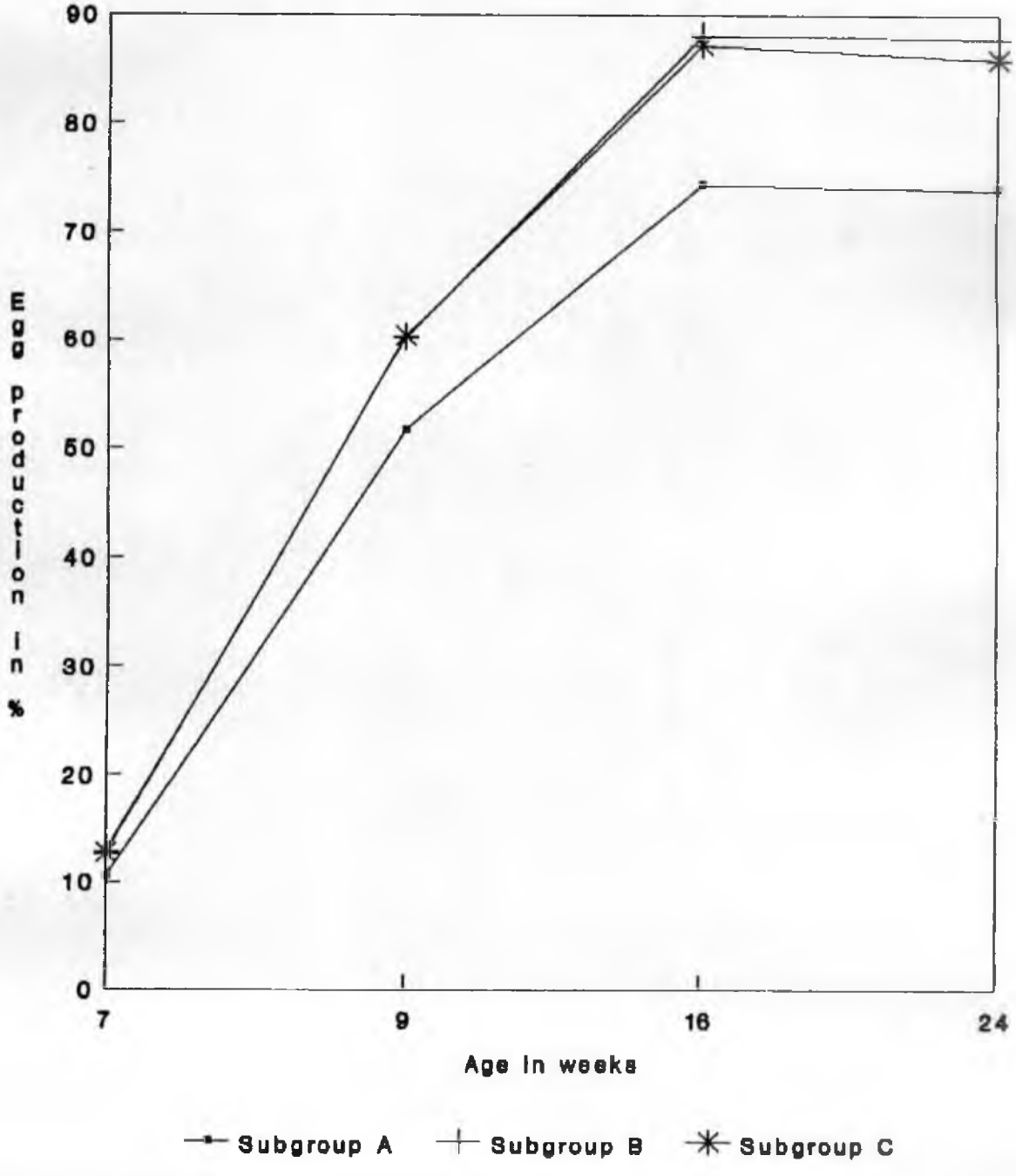


Fig.6.2 EGG PRODUCTION IN JAPANESE QUAIL - (DIFFERENT PERIODS)

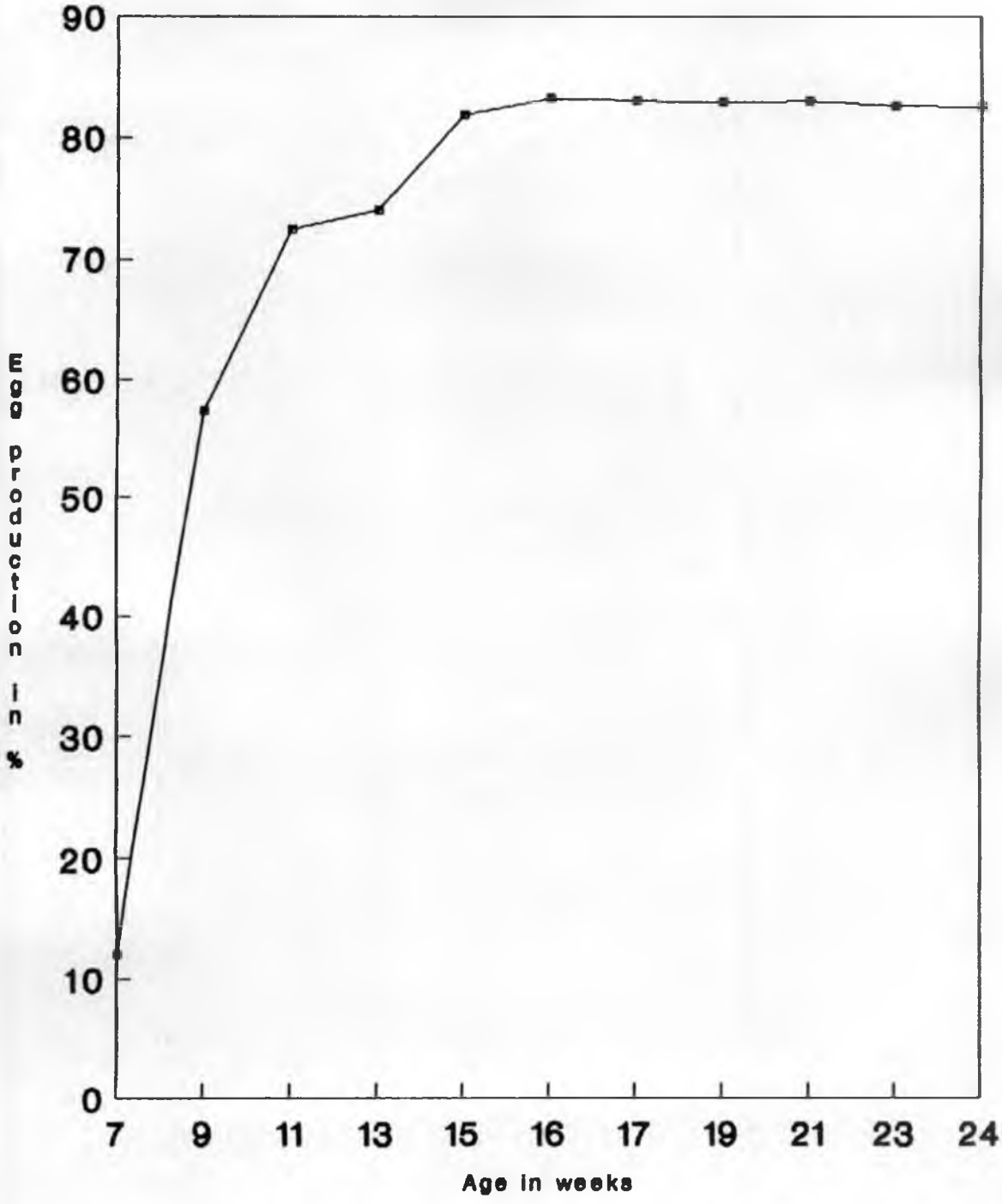


Fig. 6.3 EGG PRODUCTION AT DIFFERENT LAYER LEVELS OF DIETARY CALCIUM - JAPANESE QUAIL (DIFFERENT PERIODS)

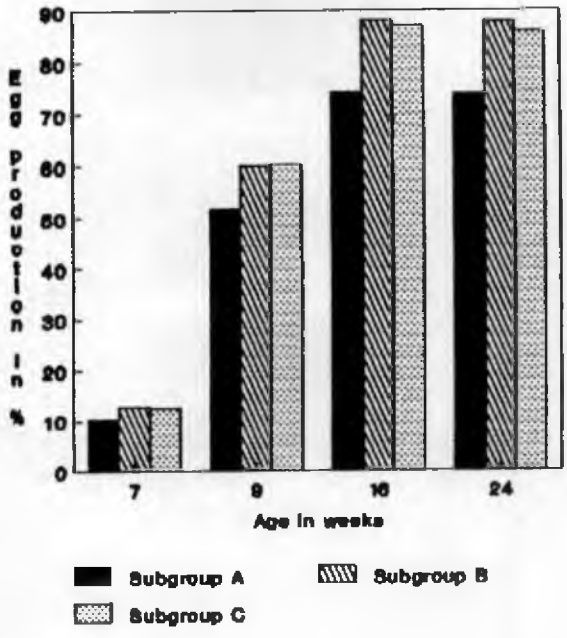


Fig.6.4 EGG PRODUCTION AT DIFFERENT LEVELS OF DIETARY CALCIUM - JAPANESE QUAIL (6TH TO 24TH WEEK)

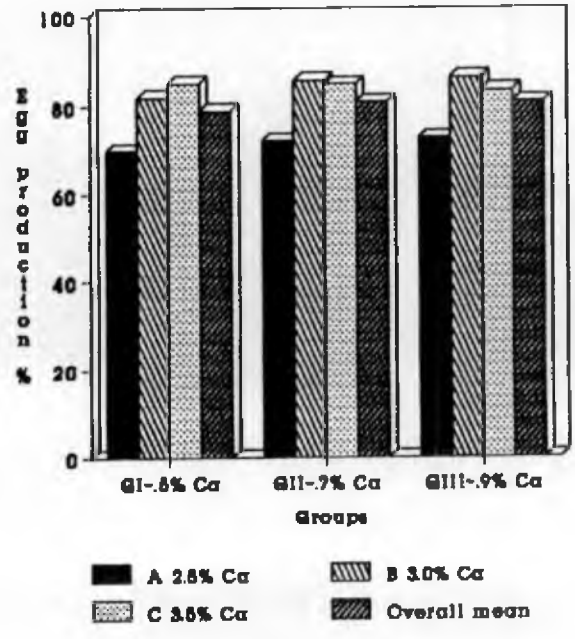


Table 4.1 Influence of dietary calcium level on egg production in Japanese quail in percentage - Mean ± SE) - (30 birds/subgroup) from six to 24 week period

Groups (pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)			
	A (2.5%)	B (3%)	C (3.5%)	Overall means
GI (0.5%)	69.88± 2.42	81.85± 3.12	85.08± 2.95	78.94± 1.97
GII (0.7%)	72.19± 2.39	85.60± 2.95	84.81± 2.80	80.89± 1.88
GIII (0.9%)	72.78± 2.14	86.40± 2.89	83.47± 2.62	80.88± 1.77
Overall means	71.62± 1.32	84.64± 1.71	84.46± 1.57	80.24± 1.82

Table 4.2 Table of 't' values of comparison of sub-groups Egg production in Japanese quail under different levels of dietary calcium

Sl. No.	Sub-groups comparison	't' values	Sl. No.	Sub-groups comparison	't' values
1.	IA VS 2A	4.498**	19.	3A VS 1C	11.970**
2.	IA VS 3A	5.965**	20.	" 2C	12.710**
3.	" 1B	12.470**	21.	" 3C	13.480**
4.	" 2B	18.920**	22.	IB VS 2B	5.410**
5.	" 3B	22.750**	23.	" 3B	9.180**
6.	" 1C	18.150**	24.	" 1C	6.180**
7.	" 2C	18.440**	25.	" 2C	5.150**
8.	" 3C	18.860**	26.	" 3C	2.230*
9.	2A VS 3A	1.470	27.	2B VS 3B	1.190
10.	" 1B	9.870**	28.	" 1C	1.520
11.	" 2B	18.900**	29.	" 2C	2.240*
12.	" 3B	16.860**	30.	" 3C	4.600**
13.	" 1C	15.904**	31.	3B VS 1C	3.630**
14.	" 2C	18.970**	32.	" 2C	3.340**
15.	" 3C	19.850**	33.	" 3C	5.810**
16.	3A VS 1B	7.560**	34.	1C VS 2C	0.762
17.	" 2B	13.070**	35.	" 3C	4.200**
18.	" 3B	13.310**	36.	2C VS 3C	4.910**

** Significant at 1 per cent level (P<0.01)

* Significant at 5 per cent level (P<0.05)

Table 4.3 Summary of egg production in Japanese quail reared under different levels of dietary calcium

Parameter	Sub-groups									Overall mean
	GI A	GII A	GIII A	GI B	GII B	GIII B	GI C	GII C	GIII C	
Age at first egg in days	42	39	41	40	40	40	41	39	41	40.33
Percentage of production at ninth week (Mean ± SE)	50.143 ±0.459	51.714 ±0.892	53.000 ±0.787	58.429 ±0.528	61.714 ±0.714	60.286 ±0.714	60.429 ±0.297	59.571 ±0.528	60.857 ±0.937	57.349 ±1.482
Percentage of production at 16th week (Mean ± SE) (Peak production)	73.000 ±0.333	75.000 ±0.289	74.667 ±0.373	87.667 ±0.373	88.000 ±0.441	88.556 ±0.530	87.222 ±0.846	88.667 ±0.441	85.889 ±0.309	83.219 ±2.274
Percentage of production at 24th week (Mean ± SE) (Peak production)	73.286 ±0.421	73.714 ±0.474	74.714 ±0.522	86.429 ±0.812	87.714 ±0.680	89.429 ±0.841	85.714 ±0.778	86.286 ±0.808	85.857 ±0.553	82.571 ±2.202

maintained on the lowest pre-laying (0.5%) and layer (2.5%) dietary calcium levels. All the birds of subgroup A (fed on the lowest, 2.5 per cent layer dietary calcium level) had the lowest (71.62 ± 1.32%) egg production (Table 4.1, Fig.6.4).

5.2.2 Quality of egg shell

5.2.2.1 Egg weight

Analysis of data for egg quality traits of six week old birds revealed that the mean egg weight was significantly ($P<0.01$) influenced by pre-laying dietary calcium (Table 5.4). Layer dietary calcium also influenced the egg weight significantly ($P<0.01$). There was interaction of pre-laying and layer dietary calcium ($P<0.05$) on the egg weight of Japanese quails.

The egg weight at the sixth week of age was significantly highest (9.496 ± 0.063 g) in the highest pre-laying dietary calcium fed group, GIII (Table 5.1). The egg weight of birds of subgroup B reared on 3 per cent layer dietary calcium was the highest 9.402 ± 0.074 g than subgroup A and subgroup C reared on 2.5 per cent and 3.5 per cent layer dietary calcium levels respectively.

At the 16th week of age birds were on good egg production and both pre-laying and layer dietary calcium

Table 5.1 Influence of dietary calcium level on the egg shell quality traits of six week old Japanese quail - (Mean ± SE) - 30 eggs/sub-group

Groups (pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)															Overall means				
	A (2.5%)					B (3%)					C (3.5%)									
	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr
GI (0.5%)	8.975± 0.186	0.787± 0.004	0.182± 0.001	78.990± 0.430	1.072± 0.001	9.480± 0.118	0.789± 0.006	0.182± 0.001	78.840± 0.360	1.072± 0.002	9.073± 0.148	0.774± 0.007	0.181± 0.001	78.760± 0.430	1.073± 0.001	9.176± 0.090	0.783± 0.003	0.181± 0.004	78.860± 0.230	1.072± 0.002
GII (0.7%)	9.318± 0.100	0.796± 0.007	0.189± 0.001	79.700± 0.460	1.075± 0.001	9.012± 0.134	0.784± 0.004	0.187± 0.001	79.220± 0.350	1.075± 0.001	8.739± 0.125	0.792± 0.004	0.188± 0.001	78.820± 0.440	1.075± 0.001	9.023± 0.073	0.791± 0.003	0.188± 0.005	79.244± 0.241	1.075± 0.001
GIII (0.9%)	9.466± 0.090	0.818± 0.004	0.189± 0.001	79.570± 0.450	1.078± 0.001	9.716± 0.101	0.807± 0.004	0.190± 0.001	79.430± 0.470	1.078± 0.001	9.307± 0.124	0.807± 0.005	0.190± 0.001	79.410± 0.410	1.078± 0.001	9.496± 0.063	0.811± 0.003	0.190± 0.001	78.800± 0.270	1.078± 0.001
Overall means	9.253± 0.077	0.800± 0.003	0.186± 0.001	78.750± 0.270	1.075± 0.001	9.402± 0.074	0.793± 0.003	0.186± 0.001	79.160± 0.230	1.075± 0.001	9.040± 0.080	0.791± 0.003	0.186± 0.001	79.000± 0.250	1.075± 0.001	9.230± 0.050	0.796± 0.002	0.186± 0.006	78.970± 0.144	1.075± 0.001
C.D*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.200	0.010	0.0013	0.690	0.002
C.D**	0.350	0.016	0.002	1.200	0.004	0.350	0.016	0.002	1.200	0.004	0.350	0.016	0.002	1.200	0.004	-	-	-	-	-

CD* = Critical difference for comparison of means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** = Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

EW = Egg weight; SW = Shell weight with shell membrane; ST = Shell thickness without shell membrane; S.I = Shape index; Sp.gr. = Specific gravity

Table 5.2 Influence of dietary calcium level on the egg shell quality traits of 16 week old Japanese quail - (Mean ± SE) - 30 eggs/sub-group

Groups (pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)															Overall means				
	A (2.5%)					B (3%)					C (3.5%)									
	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr
GI (0.5%)	11.261± 0.100	0.825± 0.009	0.209± 0.002	77.670± 0.520	1.078± 0.001	11.500± 0.110	0.940± 0.012	0.217± 0.001	79.710± 0.290	1.080± 0.001	11.650± 0.090	0.975± 0.011	0.228± 0.001	79.260± 0.420	1.083± 0.001	11.470± 0.059	0.913± 0.009	0.218± 0.001	78.880± 0.260	1.080± 0.001
GII (0.7%)	11.389± 0.110	0.876± 0.009	0.285± 0.001	78.990± 0.620	1.079± 0.001	11.730± 0.090	0.958± 0.011	8.219± 0.001	79.410± 0.400	1.080± 0.001	11.558± 0.072	0.966± 0.009	0.229± 0.001	80.420± 0.450	1.084± 0.0001	11.559± 0.053	8.933± 0.007	0.218± 0.001	79.610± 0.290	1.081± 0.001
GIII (0.9%)	11.647± 0.120	0.894± 0.011	0.209± 0.001	78.530± 0.390	1.079± 0.001	11.947± 0.073	0.966± 0.011	0.223± 0.001	79.860± 0.470	1.080± 0.001	11.704± 0.074	0.954± 0.009	0.220± 0.001	80.980± 0.430	1.084± 0.001	11.766± 0.054	0.938± 0.007	0.220± 0.001	79.792± 0.267	1.081± 0.001
Overall means	11.432± 0.070	0.865± 0.006	0.208± 0.001	78.400± 0.300	1.078± 0.001	11.726± 0.060	0.935± 0.007	0.220± 0.001	79.660± 0.230	1.080± 0.001	11.637± 0.050	8.985± 0.006	0.230± 0.001	80.218± 0.260	1.088± 0.001	11.598± 0.033	0.928± 0.004	0.220± 0.006	79.426± 0.160	1.081± 0.001
C.D*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.140	0.016	0.001	0.726	0.0003
C.D**	0.245	0.028	0.002	1.260	0.001	0.245	0.028	0.002	1.260	0.001	0.245	0.028	0.002	1.260	0.001	-	-	-	-	-

CD* = Critical difference for comparison of means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** = Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

EW = Egg weight; SW = Shell weight with shell membrane; ST = Shell thickness without shell membrane; S.I = Shape index; Sp.gr. = Specific gravity

Table 5.3 Influence of dietary calcium level on the egg shell quality traits of 24 week old Japanese quail - (Mean ± SE) - 30 eggs/sub-group

Groups (pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)															Overall means				
	A (2.5%)					B (3%)					C (3.5%)									
	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr	E.W g	S.W g	S.T mm	S.I	Sp.gr
GI (0.5%)	12.441± 0.100	0.869± 0.013	0.200± 0.001	76.580± 0.390	1.074± 0.001	12.517± 0.091	0.912± 0.008	0.210± 0.001	78.440± 0.410	1.077± 0.002	12.469± 0.103	0.946± 0.007	0.220± 0.001	79.780± 0.460	1.079± 0.001	12.476± 0.057	0.909± 0.006	0.210± 0.001	78.270± 0.280	1.077± 0.001
GII (0.7%)	12.327± 0.120	0.901± 0.007	0.197± 0.007	77.060± 0.420	1.074± 0.001	12.704± 0.096	0.911± 0.009	0.213± 0.001	78.910± 0.370	1.078± 0.001	12.462± 0.086	0.946± 0.006	0.221± 0.001	79.210± 0.480	1.079± 0.001	12.498± 0.060	0.919± 0.005	0.210± 0.003	78.390± 0.260	1.077± 0.001
GIII (0.9%)	12.576± 0.120	0.908± 0.007	0.204± 0.001	76.880± 0.370	1.074± 0.002	12.558± 0.100	0.922± 0.008	0.215± 0.001	79.520± 0.390	1.077± 0.001	12.435± 0.090	0.946± 0.007	0.225± 0.001	79.490± 0.420	1.080± 0.001	12.523± 0.060	0.925± 0.005	0.215± 0.001	78.630± 0.260	1.077± 0.001
Overall means	12.448± 0.067	0.893± 0.006	0.200± 0.002	76.840± 0.230	1.074± 0.004	12.593± 0.060	0.915± 0.005	0.213± 0.001	78.960± 0.230	1.077± 0.002	12.455± 0.053	0.946± 0.004	0.222± 0.001	79.491± 0.260	1.079± 0.001	12.499± 0.034	0.918± 0.003	0.212± 0.001	78.430± 0.150	1.077± 0.001
C.D*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.160	0.013	0.004	0.665	0.006
C.D**	0.273	0.023	0.007	1.150	0.001	0.273	0.023	0.007	1.150	0.001	0.273	0.023	0.007	1.150	0.001	-	-	-	-	-

CD* = Critical difference for comparison of means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** = Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

EW = Egg weight; SW = Shell weight with shell membrane; ST = Shell thickness without shell membrane; S.I = Shape index; Sp.gr. = Specific gravity

Table 5.4 ANOVA table of means – Influence of dietary calcium level on egg shell quality traits of Japanese quail – Six, 16 and 24 week period

Sources	df	M.S.S.														
		Sixth week					16th week					24th week				
		E.W	S.W	S.T	S.I	Sp.gr	E.W	S.W	S.T	S.I	Sp.gr	E.W	S.W	S.T	S.I	Sp.gr
Groups (pre-laying calcium)	2	5.246**	0.018**	0.002**	5.164	0.001**	2.070**	0.016**	0.0002**	20.987*	0.000012**	0.050	0.006*	0.001**	3.077	0.00001
Sub-groups (Layer calcium)	2	2.989**	0.002	0.0000004**	3.768	0.0000004**	2.037**	0.273**	0.01**	78.295**	0.001**	0.599	0.064**	0.010**	176.973**	0.001**
Groups vs sub-group	4	1.473*	0.001	0.000024**	18.188*	0.000001**	0.375	0.015**	0.00013**	8.595	0.000004**	0.356	0.004	0.0001	4.996	0.000004
Error	261	0.464	0.001	0.0000185	5.622	0.00053	0.234	0.003	0.0000168	6.179	0.00000079	0.290	0.002	0.00018	5.182	0.00000435

** Significant at 1 per cent level ($P<0.01$)

* Significant at 5 per cent level ($P<0.05$)

EW = Egg weight; SW = Shell weight with shell membrane; ST = Shell thickness without shell membrane; S.I = Shape index; Sp.gr. = Specific gravity

levels influenced significantly ($P<0.01$) the egg weight (Table 5.4). Eggs collected from birds of GII and GIII groups reared on higher pre-laying dietary calcium levels (0.7% and 0.9% respectively) averaged the weight of egg as 11.559 $\pm$ 0.053 g and 11.766 $\pm$ 0.054 g respectively (Table 5.2). The layer dietary calcium level of 3 per cent influenced the weight of eggs more than the 3.5 per cent level. Egg weight of subgroup B (fed on 3%) was maximum at 11.726 $\pm$ 0.060 g (Table 5.2) and subgroup A (fed on 2.5%) was the minimum at 11.432 $\pm$ 0.070 g.

At the 24th week of age neither pre-laying nor layer dietary calcium levels influenced the egg weight (Table 5.4). The overall mean egg weight at the sixth week of age (age at first egg) was 9.230 $\pm$ 0.050 g which was low (Table 5.1). At the 16th week of age egg weight increased to 11.598 $\pm$ 0.033 g (Table 5.2) and at 24th week to 12.499 $\pm$ 0.034 g (Table 5.3 and Fig.7.1).

5.2.2.2 Shell weight (with shell membrane)

The influence of pre-laying dietary calcium was significant ($P<0.01$) on the shell weight of quail eggs at the sixth week of age (Table 5.4). Maximum shell weight recorded (0.811 $\pm$ 0.003 g) was for GIII group reared on the highest (0.9%) pre-laying dietary calcium (Table 5.1). There was no significant ($P>0.01$) influence of layer dietary calcium or

Fig. 7.1 EGG QUALITY OF JAPANESE QUAIL EGGS AT DIFFERENT PERIODS EGG WEIGHT (g)

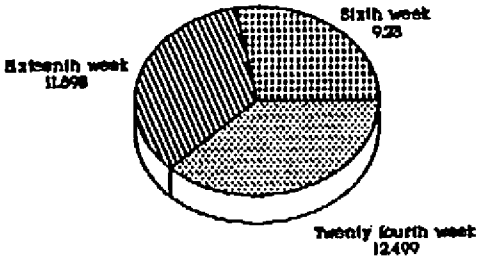


Fig.7.2 EGG QUALITY OF JAPANESE QUAIL EGGS AT DIFFERENT PERIODS SHELL WEIGHT (g)

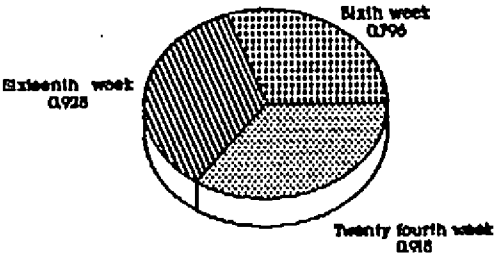
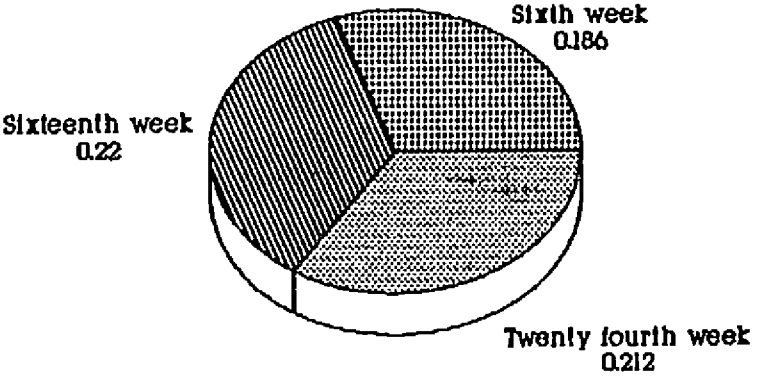


Fig.7.3 EGG QUALITY OF JAPANESE QUAIL EGGS AT DIFFERENT PERIODS SHELL THICKNESS (mm)



interaction between pre-laying and layer dietary calcium on the shell weight at the sixth week of age (Table 5.4).

At the 16th week of age both pre-laying and layer dietary calcium levels influenced the egg shell weight significantly ($P<0.01$). There was also interaction of pre-laying and layer dietary calcium levels (Table 5.4). Birds of GIII group fed on highest (0.9%) pre-laying dietary calcium recorded the highest overall mean egg shell weight (0.938 ± 0.007 g) and birds of subgroup C reared on highest layer dietary calcium (3.5%) recorded highest shell weight (0.965 ± 0.006 g) indicating that dietary calcium significantly (<0.01) increased the shell weight of quail eggs (Table 5.2).

At the 24th week of age also there was significant influence of pre-laying ($P<0.05$) and layer ($P<0.01$) dietary calcium on the egg shell weight. Maximum shell weight (0.925 ± 0.005 g) was recorded for GIII group reared on maximum (0.9%) pre-laying dietary calcium. Subgroup C reared on highest (3.5%) layer dietary calcium had the highest shell weight, 0.946 ± 0.004 g (Table 5.3).

The egg shell weight at sixth week of age in quails was 0.796 ± 0.002 g (Table 5.1), which increased to 0.928 ± 0.004 g at 16th week of age (Table 5.2). However, at the 24th

week of age, the shell weight was decreased to 0.918 ± 0.003 g (Table 5.3, Fig.7.2).

5.2.2.3 Shell thickness (without shell membrane)

Analysis of data for egg shell quality traits of six week old birds (Table 5.1, 5.4) revealed that shell thickness was significantly ($P<0.01$) influenced by pre-laying dietary calcium levels. Highest (0.190 ± 0.001 mm) shell thickness was recorded for the GIII group maintained on the highest (0.9%) pre-laying dietary calcium and the lowest 0.181 ± 0.004 mm for the GI group fed on the lowest (0.5%) pre-laying dietary calcium.

At the 16th and 24th week of age both the pre-laying and layer dietary calcium levels significantly ($P<0.01$) influenced the egg shell thickness (Table 5.4). Subgroup GIII C (reared on the highest, 0.9% pre-laying and 3.5%, layer dietary calcium) recorded the highest value of 0.230 ± 0.001 mm (Table 5.2) at the 16th week of age. At this age, GIII group (fed on 0.9%, highest, pre-laying dietary calcium) presented an overall mean value of 0.220 ± 0.001 mm and subgroup C (fed on the highest, 3.5% layer dietary calcium) had an overall mean value of 0.230 ± 0.001 mm (Table 5.2).

At the 24th week of age birds of GIII group (fed on 0.9%, highest, pre-laying dietary calcium) had the thickest

shell of 0.215 ± 0.001 mm. So also subgroup C (reared on the highest, 3.5% layer dietary calcium) showed an over all mean shell thickness of 0.222 ± 0.001 mm (Table 5.3).

The shell thickness was influenced by age (Fig.7.3). The overall mean shell thickness at sixth, 16th and 24th week of age were 0.186 ± 0.006 mm, 0.220 ± 0.006 mm and 0.212 ± 0.001 mm respectively (Table 5.1, Table 5.2, Table 5.3 and Fig.7.3). However, the shell thickness increased markedly from the sixth to 16th week of age and thereafter actually a marginal reduction in shell thickness occurred, as the birds attained 24th week of age.

5.2.2.4 Shape index

At the sixth week of age pre-laying and layer dietary calcium did not influence ($P>0.01$) the shape index of quail eggs. There was significant ($P<0.05$) interaction between pre-laying and layer dietary calcium levels (Table 5.4). Maximum shape index 79.244 ± 0.241 recorded at the sixth week of age was for GII group maintained on 0.7 per cent pre-laying dietary calcium (Table 5.1). Birds of sub-group B fed on 3.0 per cent layer dietary calcium had the highest overall mean of 79.160 ± 0.230 (Table 5.1). Better shape index was recorded for sub-group GII A, GII B, GIII B and GIII C with mean values

as 79.700 ± 0.460, 79.220 ± 0.350, 79.430 ± 0.470 and 79.410 ± 0.410 respectively (Table 5.1).

At the 16th week of age there was significant difference in the shape index produced as a result of the influence of pre-laying ($P<0.05$) and layer dietary calcium ($P<0.01$) levels (Table 5.4). Higher shape index (79.792 ± 0.267) recorded was for the birds of GIII group fed on the highest (0.9%) pre-laying dietary calcium and for sub-group C (80.218 ± 0.260) reared on the highest (3.5%) layer dietary calcium (Table 5.2).

At the 24th week of age (Table 5.4) pre-laying dietary calcium had no influence on the shape index of quail eggs. Layer dietary calcium exerted a significant ($P<0.01$) influence. Maximum shape index (79.491 ± 0.260) was found for the highest (3.5%) layer dietary calcium fed sub-group C (Table 5.3).

The overall mean shape index recorded for quail eggs at the sixth, 16th and 24th week of age were 78.970 ± 0.144, 79.426 ± 0.160 and 78.430 ± 0.150 respectively (Table 5.1, Table 5.2, Table 5.3, Fig.7.4) indicating maximum shape index attained at the 16th week of age. However, from the 16th to 24th week of age shape index was lowered as age advanced.

Fig.7.4 EGG QUALITY OF JAPANESE QUAIL EGGS AT DIFFERENT PERIODS - SHAPE INDEX

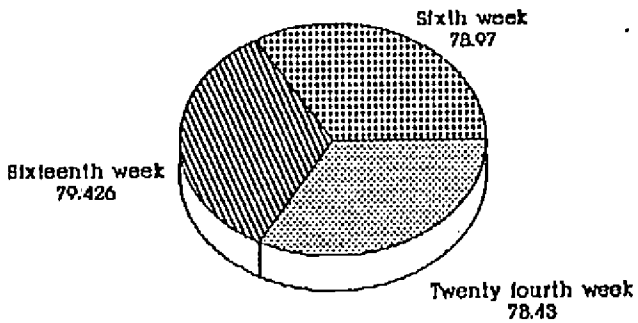
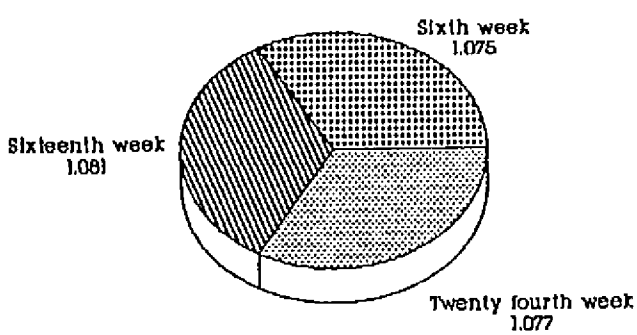


Fig.7.5 EGG QUALITY OF JAPANESE QUAIL EGGS AT DIFFERENT PERIODS SPECIFIC GRAVITY



5.2.2.5 Specific gravity

Pre-laying dietary calcium significantly ($P<0.01$) influenced the specific gravity of quail eggs at the sixth week of age (Table 5.4). The maximum specific gravity (1.078 $\pm$ 0.001) was recorded for the eggs collected from GIII group reared on the highest (0.9%) pre-laying dietary calcium, and minimum specific gravity 1.702 $\pm$ 0.002 for the GI group reared on the lowest (0.5%) pre-laying dietary calcium (Table 5.1).

At the 16th week of age pre-laying and layer dietary calcium levels significantly ($P<0.01$) influenced the specific gravity of quail eggs (Table 5.4). The highest specific gravity (1.083 $\pm$ 0.001) was recorded for sub-group C reared on highest (3.5%) layer dietary calcium, and the lowest, (1.078 $\pm$ 0.001) for the sub-group A fed on the lowest (2.5%) layer dietary calcium (Table 5.2). The influence of layer dietary calcium was marked while that of pre-laying level was only nominal (Table 5.2).

At the 24th week of age specific gravity of quail eggs was not influenced by pre-laying dietary calcium, whereas layer dietary calcium significantly ($P<0.01$) influenced it (Table 5.4). The maximum overall mean specific gravity 1.079 $\pm$ 0.001 recorded was for eggs collected from the birds of subgroup C reared on the highest (3.5%) layer dietary calcium

and the lowest (1.074 ± 0.004) for the lowest (2.5%) layer dietary calcium fed subgroup A.

The influence of age on the specific gravity of eggs was evident (Fig.7.5). At the sixth, 16th and 24th week of age the overall mean specific gravity of quail eggs were 1.075 ± 0.001, 1.081 ± 0.001 and 1.077 ± 0.001 respectively (Table 5.1, 5.2, 5.3). From the sixth to 16th week, the specific gravity markedly increased whereas from 16th to 24th week it was lowered.

5.3 Biochemical studies

5.3.1 Calcium and phosphorus profile in the plasma and shell gland of Japanese quail

5.3.1.1 Plasma calcium

At the sixth week of age the quails reared on the three different pre-laying dietary calcium levels (GI, GII and GIII) had significantly ($P<0.01$) different plasma calcium concentration (Table 6.1 and Table 6.2). The mean plasma calcium concentration was the highest (23.269 + 0.075 mg/dl) in birds of GIII group reared on the highest (0.9%) pre-laying dietary calcium level. It was the lowest (21.031 ± 0.135 mg/dl) in GI group reared on the lowest (0.5%) level of pre-laying dietary calcium (Table 6.1 and Fig.8.1). The overall

Table 6.1 Influence of dietary calcium level on the mineral profile of six week old female Japanese quail - (Mean ± SE) - 30 birds/group

Groups (Pre-laying calcium levels)	Mineral profile in Japanese quail			
	Plasma (mg/dl)		Shell gland (mg/g)	
	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
GI (0.5%)	21.031 ± 0.135	11.393 ± 0.087	11.045 ± 0.037	1.900 ± 0.008
GII (0.7%)	22.360 ± 0.088	11.063 ± 0.057	10.973 ± 0.106	1.907 ± 0.007
GIII (0.9%)	23.269 ± 0.075	10.677 ± 0.092	10.999 ± 0.012	1.903 ± 0.009
Overall means	22.220 ± 0.110	11.040 ± 0.060	11.006 ± 0.040	1.903 ± 0.010
CD	0.288	0.225	0.183	2.305

CD = Critical difference for comparison of means

Table 6.2 ANOVA table of means - Influence of dietary calcium level on the mineral profile of six week old female Japanese quail

Sources	df	MSS			
		Plasma minerals		Shell gland minerals	
		Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
Groups	2	37.992**	3.854**	4.004	4.578
Error	87	0.315	0.192	0.127	2.020

** Significant at 1 per cent level (P <0.01)

Table 6.3 Influence of dietary calcium level on the plasma mineral profile of 16 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	(mg/dl)		(mg/dl)		(mg/dl)		(mg/dl)	
	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
GI (0.5%)	21.590± 0.211	10.896± 0.254	25.007± 0.277	11.716± 0.117	30.083± 0.338	10.461± 0.177	25.560± 0.403	11.024± 0.122
GII (0.7%)	21.131± 0.191	10.224± 0.210	28.775± 0.437	11.556± 0.234	30.150± 0.285	11.191± 0.160	26.685± 0.459	10.991± 0.131
GIII (0.9%)	21.467± 0.182	12.172± 0.192	26.613± 0.292	10.770± 0.212	30.113± 0.403	10.694± 0.187	26.064± 0.415	11.212± 0.134
Overall means	21.396± 0.113	11.098± 0.150	26.798± 0.260	11.347± 0.120	30.115± 0.200	10.782± 0.110	26.103± 0.250	11.080± 0.071
CD**	-	-	-	-	-	-	1.851	1.306
CD*	0.840	0.550	0.840	0.550	0.840	0.550	-	-

CD** - Critical difference for comparison of overall means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD* - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 6.4 Influence of dietary calcium level on the shell gland mineral profile of 16 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	mg/g tissue		mg/g tissue		mg/g tissue		mg/g tissue	
	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
GI (0.5% Ca)	10.417± 0.235	1.850± 0.044	10.201± 0.133	1.916± 0.013	10.941± 0.196	1.896± 0.028	10.520± 0.115	1.887± 0.018
GII (0.7% Ca)	10.785± 0.125	1.938± 0.030	11.908± 0.229	1.869± 0.025	11.333± 0.216	1.869± 0.028	11.342± 0.123	1.892± 0.016
GIII (0.9% Ca)	11.931± 0.201	1.935± 0.016	10.464± 0.128	1.904± 0.018	12.625± 0.133	1.845± 0.025	11.674± 0.131	1.895± 0.121
Overall means	11.044± 0.130	1.907± 0.020	10.858± 0.130	1.896± 0.010	11.633± 0.130	1.870± 0.015	11.180± 0.080	1.891± 0.009
CD**	-	-	-	-	-	-	1.232	0.069
CD*	0.510	0.070	0.510	0.070	0.510	0.070	-	-

CD** - Critical difference for comparison of overall means of 3 groups and 3 subgroups each comprising of 90 birds

CD* - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 6.5 Influence of dietary calcium level on the plasma mineral profile of 24 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	mg/dl		mg/dl		mg/dl		mg/dl	
	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
GI (0.5% Ca)	21.490± 0.123	11.157± 0.159	26.052± 0.283	11.574± 0.146	30.500± 0.235	10.627± 0.181	26.014± 0.410	11.119± 0.102
GII (0.7% Ca)	21.778± 0.163	11.487± 0.186	25.381± 0.134	11.882± 0.148	30.474± 0.213	10.896± 0.192	25.878± 0.391	11.422± 0.110
GIII (0.9% Ca)	21.782± 0.151	11.814± 0.167	25.435± 0.165	11.909± 0.192	30.147± 0.186	11.013± 0.184	25.788± 0.375	11.579± 0.110
Overall means	21.683± 0.080	11.486± 0.100	25.623± 0.120	11.788± 0.090	30.374± 0.120	10.845± 0.110	25.893± 0.230	11.373± 0.063
CD*	-	-	-	-	-	-	0.307	0.276
CD**	0.530	0.480	0.530	0.480	0.530	0.480	-	-

CD* - Critical difference for comparison of overall means of 3 groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 6.6 Influence of dietary calcium level on the shell gland mineral profile of 24 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	mg/g tissue		mg/g tissue		mg/g tissue		mg/g tissue	
	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
GI (0.5% Ca)	11.315± 0.189	1.927± 0.024	11.335± 0.131	1.977± 0.006	11.019± 0.120	1.937± 0.015	11.223± 0.090	1.947± 0.009
GII (0.7% Ca)	11.460± 0.137	1.957± 0.048	11.286± 0.132	1.977± 0.008	11.407± 0.136	1.859± 0.017	11.384± 0.080	1.931± 0.020
GIII (0.9% Ca)	11.050± 0.115	1.974± 0.013	11.171± 0.107	1.973± 0.007	11.411± 0.129	1.898± 0.014	11.211± 0.070	1.948± 0.010
Overall means	11.275± 0.090	1.953± 0.020	11.264± 0.071	1.976± 0.004	11.279± 0.080	1.898± 0.009	11.270± 0.050	1.942± 0.007
CD*	-	-	-	-	-	-	0.217	0.034
CD**	0.374	0.060	0.374	0.060	0.374	0.060	-	-

CD* - Critical difference for comparison of overall means of 3 main groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 6.7 ANOVA table of means - Influence of dietary calcium level on the mineral profile of 16 week and 24 week old female Japanese quail

Sources	df	MSS							
		16 weeks of age				24 weeks of age			
		Plasma		Shell gland		Plasma		Shell gland	
		Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus	Calcium	Inorganic phosphorus
Groups (pre-laying dietary calcium)	2	28.727	1.283	31.795	0.001	1.195	4.932**	0.861	0.009
Sub-groups (layer dietary calcium)	2	1743.274**	7.213	14.756	0.032	1704.274**	20.883**	0.025	0.143**
Groups vs sub-groups	4	40.133**	19.978**	17.788**	0.056	2.488	0.027	1.084	0.027
Error	261	2.749	1.169	1.003	0.021	1.085	0.905	0.545	0.013

** Significant at 1 per cent level (P<0.01)

* Significant at 5 per cent level (P<0.05)

mean plasma calcium concentration at the sixth week of age in female quails was 22.220 ± 0.110 mg/dl.

The data for plasma calcium content in 16 and 24 week old layers were analysed by analysis of variance (Table 6.7), to decipher the effects of interaction due to pre-laying and layer dietary calcium levels.

At the 16th week of age the interaction between the groups reared on pre-laying dietary calcium and subgroups reared on layer dietary calcium was significantly ($P<0.01$) different (Table 6.7). In the two way table of means between groups and sub-groups (Table 6.3) birds of sub-group GII B reared on 0.7 per cent pre-laying and 3 per cent layer dietary calcium had the greatest interaction (Fig.8.3) with the highest plasma calcium concentration of 28.775 ± 0.437 mg/dl. In general all the birds of sub-group A reared on 2.5 per cent dietary layer calcium GI A, GII A, GIII A showed the lowest plasma calcium concentration with an overall mean value of 21.396 ± 0.113 mg/dl (Table 6.3), while the birds of sub-groups reared on 3 per cent (B) and 3.5 per cent (C) higher dietary calcium (GI B, GII B, GIII B; GI C, GII C, GIII C) had higher plasma calcium concentrations (Fig.8.3). The over all mean values of sub-group B and sub-group C recorded higher plasma calcium content of 26.798 ± 0.260 mg/dl and 30.115 ± 0.200 mg/dl respectively (Table 6.3, Fig.8.3).

Fig.8.1 CALCIUM AND PHOSPHORUS CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL(6TH WEEK)

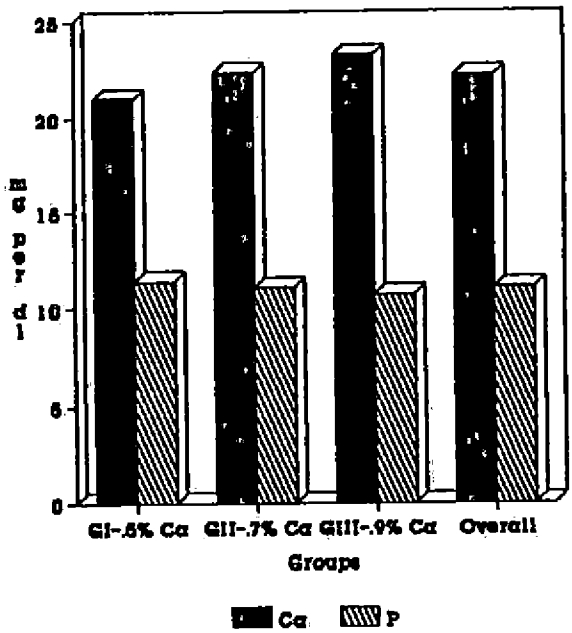


Fig.8.2 CALCIUM AND PHOSPHORUS CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL(6TH WEEK)

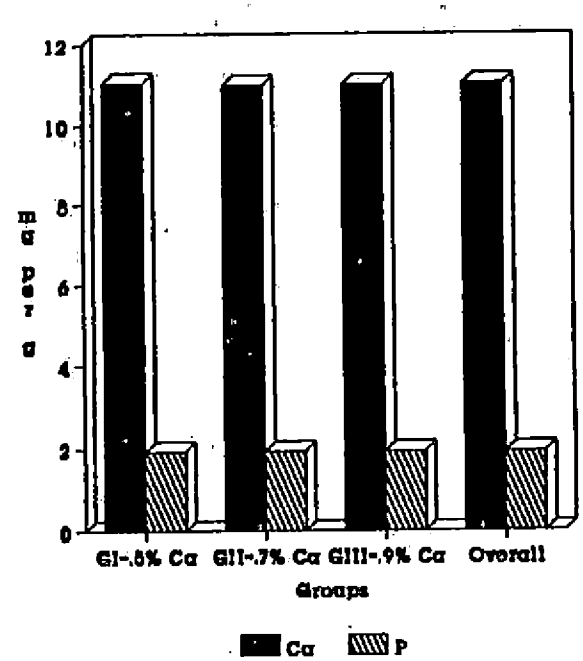


Fig.8.3 CALCIUM CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

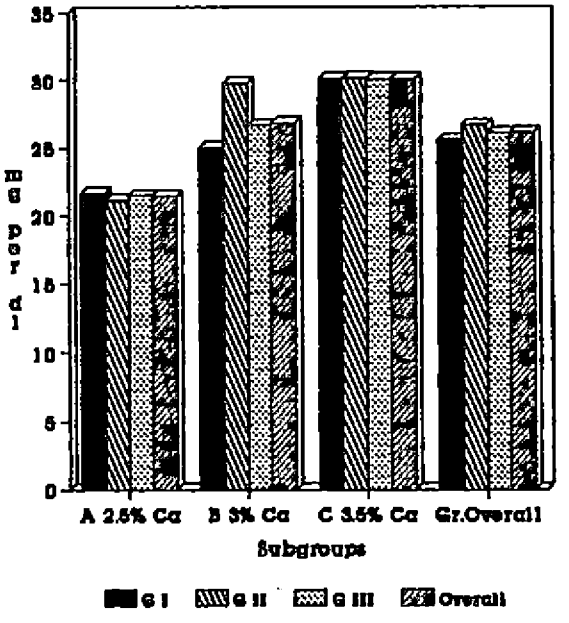


Fig.8.4 PHOSPHORUS CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

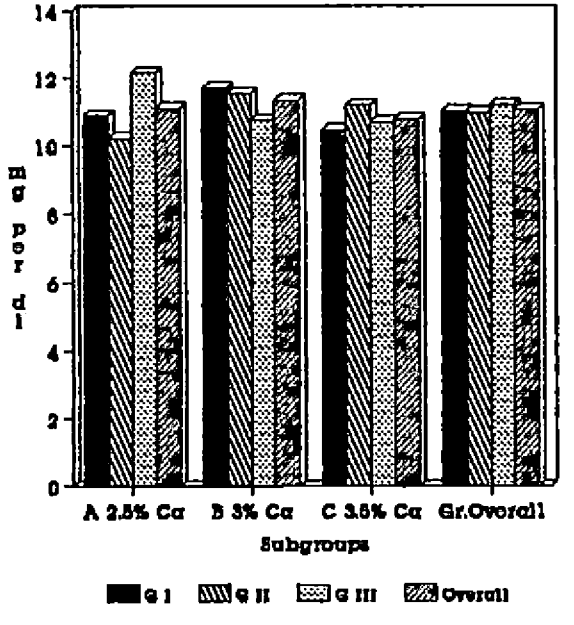


FIG. 8.3 CALCIUM CONTENT OF PLASMA
UNDER DIFFERENT DIETARY CATIONS
FRAPS-JAPANESE QUAIL (10TH WEEK)

FIG. 8.4 PHOSPHORUS CONTENT OF PLASMA
UNDER DIFFERENT DIETARY CATIONS
FRAPS-JAPANESE QUAIL (10TH WEEK)

Fig.8.5 CALCIUM CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

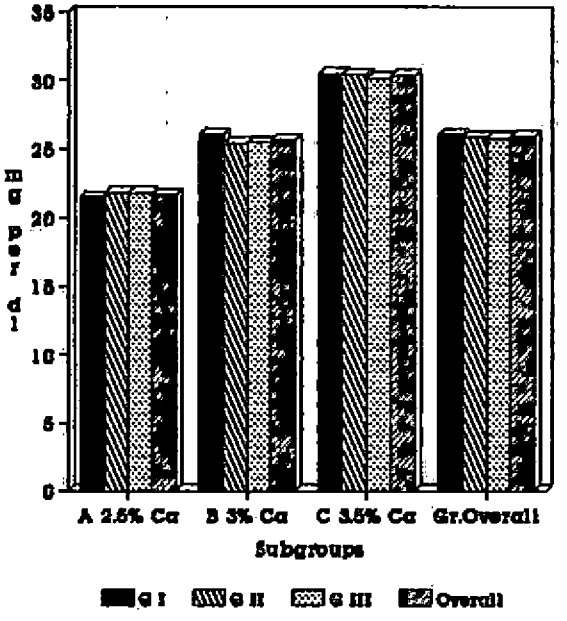


Fig.8.6 PHOSPHORUS CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

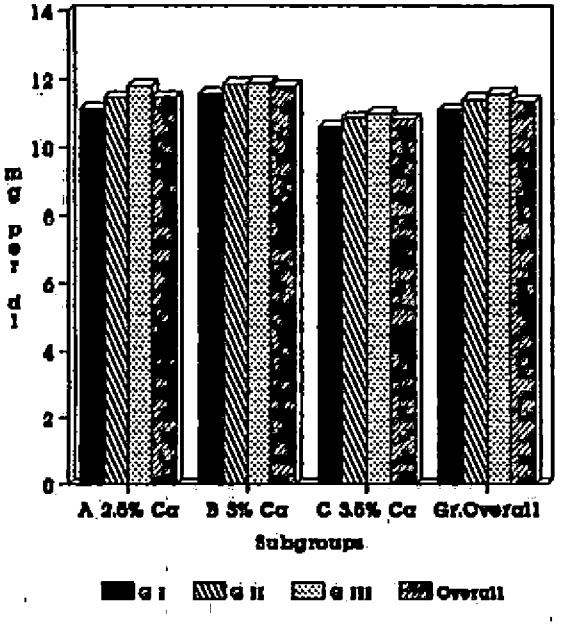


Fig.3.6 CALCIUM CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

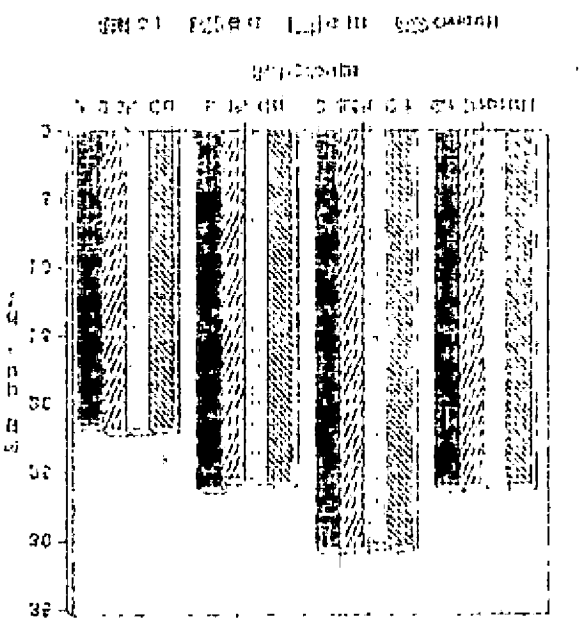


Fig.3.5 PHOSPHORUS CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

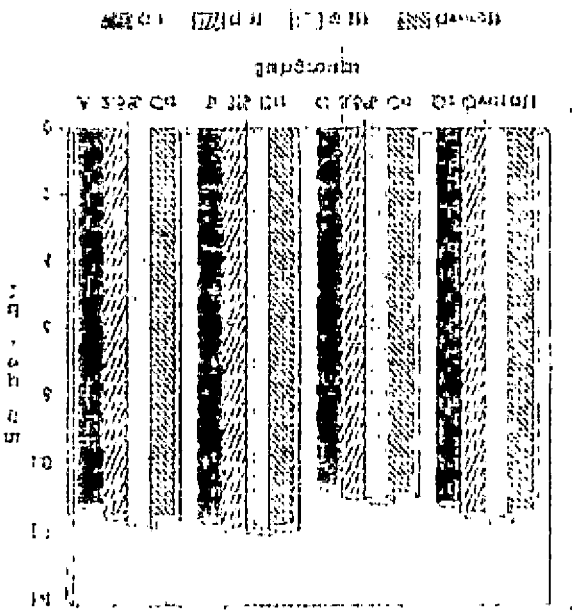


Fig.8.7 PLASMA CALCIUM CONTENT (mg/dl) AT DIFFERENT PERIODS JAPANESE QUAIL

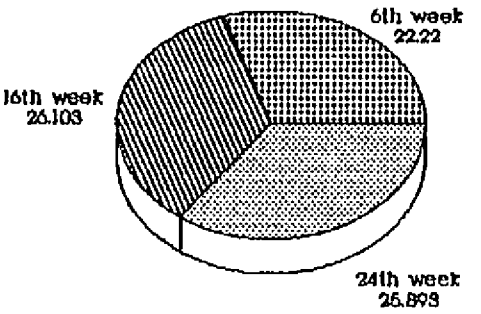
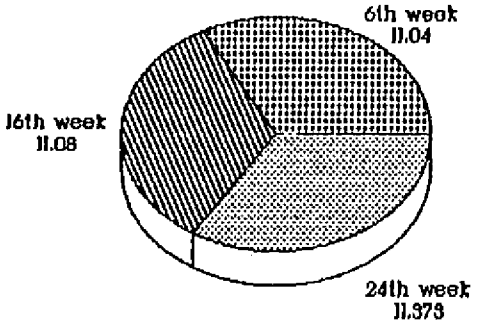


Fig.8.8 PLASMA PHOSPHORUS CONTENT(mg/dl) AT DIFFERENT PERIODS - JAPANESE QUAIL



At the 24th week of age the birds reared on different layer dietary calcium ration showed significant ($P<0.01$) difference between one another because of variation of calcium in the layer diet (Table 6.7, Fig.8.5). Pre-laying dietary calcium did not influence the plasma calcium content at the 24th week of age. The highest overall mean plasma calcium level (30.374 ± 0.120 mg/dl) was recorded in sub-group C reared on 3.5 per cent (highest) layer dietary calcium, which was significant ($P<0.01$) indicating the influence of layer dietary calcium content on the Plasma Calcium Content (Table 6.5 and Fig.8.5). The Plasma Calcium content was the lowest 21.490 ± 0.123 mg/dl, in the sub-group GI A which was reared on the lowest pre-laying (0.5%) and lowest layer (2.5%) dietary calcium levels. The plasma calcium concentration was uniformly higher in all the birds in sub-group B and C reared on 3 and 3.5 per cent (higher)layer dietary calcium levels. Out of these, the sub-group C reared on the highest layer dietary calcium (3.5%) was showing the highest value of Plasma Calcium Content (overall mean being 30.374 ± 0.120 mg/dl) among all the sub-groups (Table 6.5 and Fig.8.5).

The overall mean plasma Calcium Content of six week old quails reared on different pre-laying calcium levels was 22.220 ± 0.110 mg/dl (Table 6.1). At the 16th and 24th week

of age, birds reared on the lowest layer dietary calcium (2.5%) had the plasma calcium level of 21.396 ± 0.113 mg/dl and 21.683 ± 0.080 mg/dl respectively (Table 6.3 and Table 6.5). The highest layer dietary calcium (3.5%) fed sub-group C at 16th and 24th week of age had the plasma calcium concentration of 30.115 ± 0.200 mg/dl and 30.374 ± 0.120 mg/dl respectively (Table 6.3 and Table 6.5). This may indicate that the dietary calcium was the only factor influencing the level of plasma calcium, and that age did not have any influence. The overall mean plasma calcium concentration at 16th and 24th week of age in Japanese quail were 26.103 ± 0.250 mg/dl and 25.893 ± 0.230 mg/dl respectively (Table 6.3, 6.5 and Fig.8.7).

5.3.1.2 Plasma phosphorus

The plasma inorganic phosphate concentration in six week old Japanese quail reared on the highest pre-laying dietary calcium 0.9 per cent (GIII group) was the lowest (10.677 ± 0.092 mg/dl, Table 6.1). Dietary calcium significantly ($P<0.01$) influenced the plasma inorganic phosphate content (Table 6.2). The overall mean plasma inorganic phosphate concentration of female Japanese quail was 11.040 ± 0.060 mg/dl at sixth week of age (Table 6.1 and Fig.8.1).

The analysis of the data from the 16 week old birds in a completely randomised design (CRD) with interaction of groups and sub-groups revealed a pattern of observation similar to that in the sixth week of age i.e. the lowest inorganic phosphate concentration in the sub-group C which was reared on the highest level of layer dietary calcium (3.5%), the overall mean value being 10.782 $\pm$ 0.110 mg/dl (Table 6.3 and Fig.8.4). The interaction of pre-laying and layer dietary calcium was significant ($P<0.01$) at this age (Table 6.7). The plasma inorganic phosphate content of female Japanese quails at the 16th week of age was 11.080 $\pm$ 0.071 mg/dl (Table 6.3 and Fig.8.4).

At the 24th week of age there was significant ($P<0.01$) influence of pre-laying and layer dietary calcium levels on the plasma inorganic phosphate concentration (Table 6.7). As in the case of 16 week old birds, the 24 week old birds also showed lowest concentration of plasma inorganic phosphate (10.845 $\pm$ 0.110 mg/dl) in the highest level of layer dietary calcium (3.5%) fed sub-group C (Table 6.5 and Fig.8.6).

The data revealed no influence of age on the plasma inorganic phosphate concentration as the values at the sixth, 16th and 24th week of age were 11.040 $\pm$ 0.060 mg/dl, 11.080 $\pm$ 0.071 mg/dl and 11.373 $\pm$ 0.063 mg/dl respectively (Tables 6.1, 6.3, 6.5 and Fig.8.8).

5.3.1.3 Shell gland calcium

At the sixth and 24th week of age the calcium concentration of the shell gland did not show any significant variation due to varying levels of pre-laying and layer dietary calcium (Tables 6.2, 6.7 and Fig.8.2, 8.11). However, at the 16th week of age there was a significant ($P<0.01$) interaction of pre-laying and layer dietary calcium on the shell gland calcium content (Table 6.7). Concentration in Japanese quail was comparatively lower ranging from 10.201 $\pm$ 0.133 mg/g (GI B subgroup at the 16th week of age) to 12.625 $\pm$ 0.133 mg/g (GII C subgroup at the 16th week of age, Tables 6.1, 6.4 and 6.6) than that of plasma calcium levels ranging from 21.031 $\pm$ 0.135 mg/dl (GI group, at the sixth week of age) to 30.500 $\pm$ 0.235 mg/dl (GI C sub-group, at the 24th week of age, Tables 6.1, 6.3 and 6.5).

The data also revealed the absence of any influence of age on the shell gland calcium concentration in Japanese quails (Fig.8.13). The shell gland calcium concentration at the sixth, 16th and 24th week of age were 11.006 $\pm$ 0.040 mg/g, 11.180 $\pm$ 0.080 mg/g and 11.270 $\pm$ 0.050 mg/g respectively (Tables 6.1, 6.4, 6.6 and Fig.8.13).

Fig.8.9 CALCIUM CONTENT OF SHELL - GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

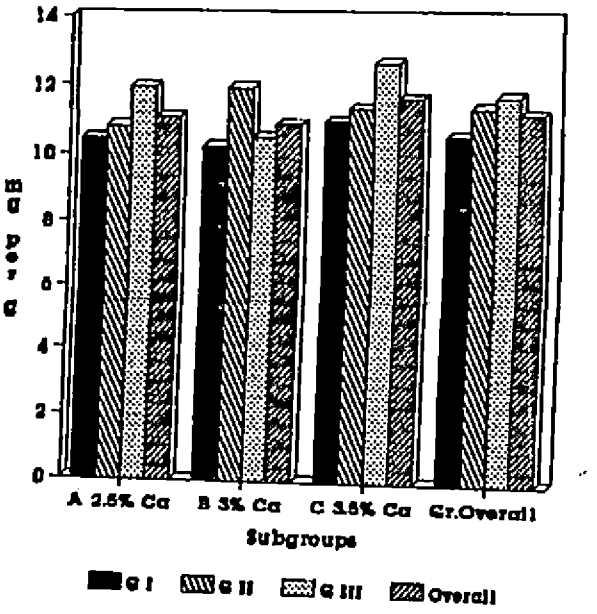


Fig.8.10 PHOSPHORUS CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

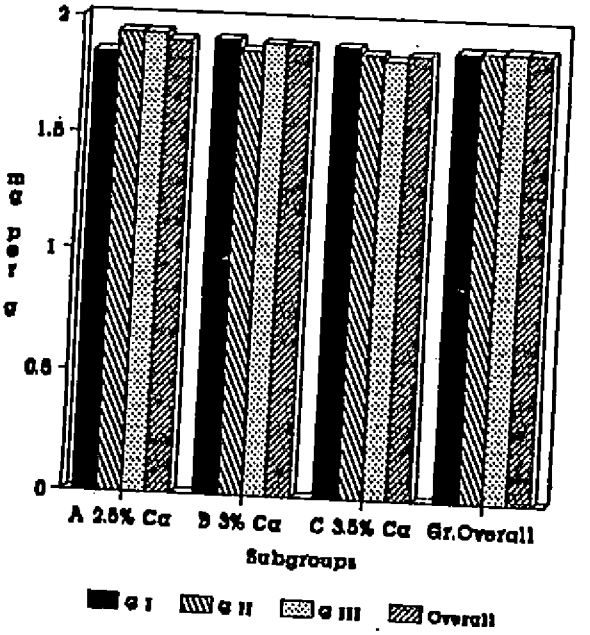


Fig.8.11 CALCIUM CONTENT OF SHELL-GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

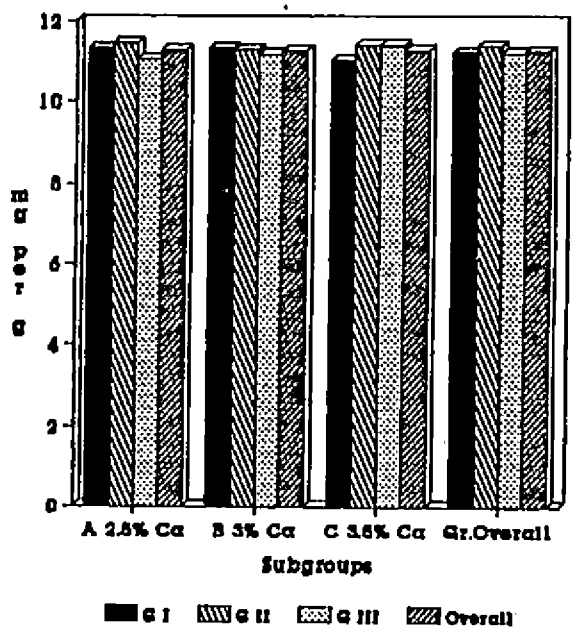


Fig.8.12 PHOSPHORUS CONTENT OF SHELL-GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

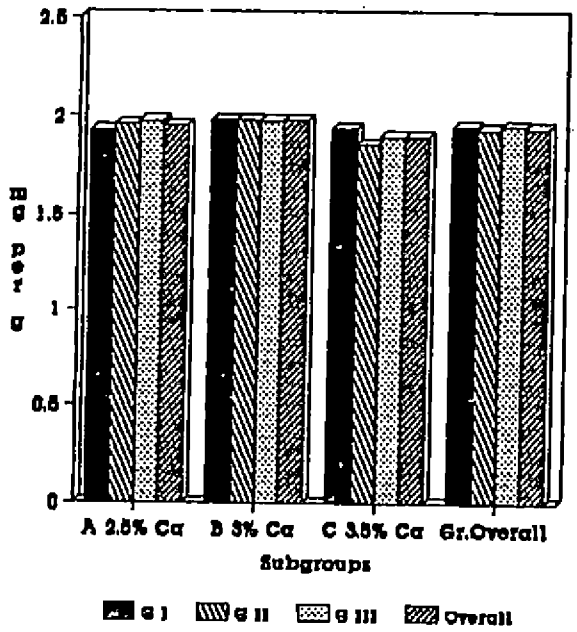


Fig.8.13 SHELL GLAND CALCIUM CONTENT (mg/g) AT DIFFERENT PERIODS JAPANESE QUAIL

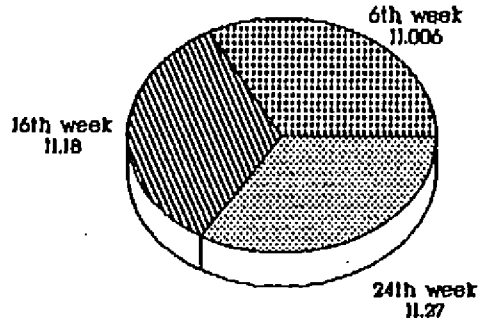
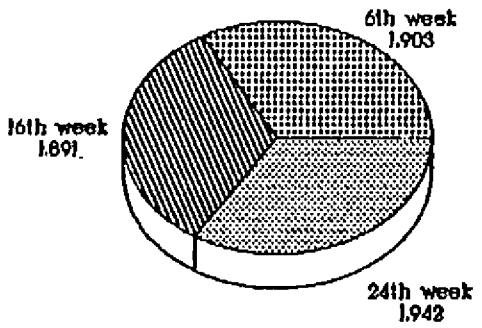


Fig.8.14 SHELL GLAND PHOSPHORUS CONTENT (mg/g) AT DIFFERENT PERIODS JAPANESE QUAIL



5.3.1.4 Shell gland phosphorus

At the sixth week of age shell gland content of inorganic phosphorus was not seen influenced by the varying levels of pre-laying dietary calcium (Tables 6.1, 6.2 and Fig. 8.2). The average value of shell gland inorganic phosphate at the sixth week of age was 1.903 $\pm$ 0.010 mg/g (Table 6.1 and Fig.8.2).

At the 16th week of age also there was neither a significant influence of either pre-laying or layer dietary calcium levels nor an interaction of the same calcium levels on the shell gland inorganic phosphate concentration (Table 6.7 and Fig. 8.10). The overall mean inorganic phosphorus content of shell gland at the 16th week of age was 1.891 $\pm$ 0.009 mg/g (Table 6.4).

At the 24th week of age shell gland inorganic phosphorus content was significantly ($P<0.01$) higher due to layer dietary calcium (Table 6.7). The shell gland inorganic phosphorus content was lower (1.859 $\pm$ 0.017 mg/g and 1.898 $\pm$ 0.014 mg/g respectively) in the highest calcium fed sub-groups GII C and GIII C (Table 6.6 and Fig.8.12). Furthermore, there was no significant difference due to pre-laying dietary calcium and interaction between pre-laying and layer dietary calcium at the 24th week of age on the shell gland inorganic

phosphate content (Tables 6.6 and 6.7). The overall in organic phosphate of shell gland at the 24th week of age was 1.942 ± 0.007 mg/g (Table 6.6).

There was no influence of age on the shell gland inorganic phosphorus concentration and at the sixth, 16th and 24th week of age the overall mean values were 1.903 ± 0.010 mg/g, 1.891 ± 0.009 mg/g and 1.942 ± 0.007 mg/g respectively (Tables 6.1, 6.4, 6.6 and Fig.8.14). The inorganic phosphorus content of the shell gland of the Japanese quail ranged from 1.845 ± 0.025 mg/g (GIII C - 16th week of age) to 1.977 ± 0.006 mg/g (GI B and GII B - 24th week of age) vide Tables 6.1, 6.4 and 6.6.

5.3.2 Enzyme profile in plasma and shell gland of Japanese quail

5.3.2.1 Plasma alkaline phosphatase (ALP)

On analysis of data the plasma ALP concentration of the birds in GI group fed with the lowest (0.5%) pre-laying dietary calcium was significantly ($P<0.01$) higher (98.912 ± 0.340 KA units/dl) compared to those of GII group on medium (0.7%) pre-laying dietary calcium (91.173 ± 0.340 KA units/dl) and GIII group on highest (0.9%) pre-laying dietary calcium (89.330 ± 0.220 KA units/dl) vide Tables 7.1, 7.2 and Fig.9.1. This indicated that higher the dietary calcium level lower was

Table 7.1 Influence of dietary calcium level on the enzyme profile of six week old female Japanese quail - (Mean ± SE) - 30 birds/group

Groups (Pre-laying dietary calcium)	Plasma enzymes (KA units/dl)		Shell gland enzymes KA Units/g tissue	
	ALP	ACP	ALP	ACP
GI (0.5% Ca)	98.912 ± 0.340	16.401 ± 0.240	0.642 ± 0.033	19.271 ± 0.115
GII (0.7% Ca)	91.173 ± 0.340	14.854 ± 0.170	0.661 ± 0.024	19.752 ± 0.170
GIII (0.9% Ca)	89.330 ± 0.220	14.395 ± 0.160	0.660 ± 0.024	19.892 ± 0.142
Overall means	93.138 ± 0.473	15.217 ± 0.142	0.654 ± 0.015	19.639 ± 0.090
CD*	0.620	0.380	0.060	0.280

CD* = Critical difference for comparison of means of groups

Table 7.2 ANOVA table of means - Influence of dietary calcium level on the enzyme profile of six week old female Japanese quail

Sources	df	MSS			
		Plasma enzymes		Shell gland enzymes	
		ALP	ACP	ALP	ACP
Groups	2	775.489**	33.126**	0.003	3.181**
Error	87	2.839	1.112	0.021	0.619

** Significant at 1 per cent level (P <0.01)

Table 7.3 Influence of dietary calcium level on the plasma enzyme profile of 16 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium levels)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	KA units/dl		KA units/dl		KA units/dl		KA units/dl	
	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
GI (0.5% Ca)	97.482± 0.201	13.751± 0.139	87.193± 0.189	11.554± 0.130	79.737± 0.126	10.824± 0.043	88.138± 0.778	12.043± 0.147
GII (0.7% Ca)	92.504± 0.453	12.759± 0.092	85.636± 0.148	10.937± 0.114	79.377± 0.096	10.276± 0.064	85.839± 0.590	11.324± 0.123
GIII (0.9% Ca)	89.887± 0.241	11.986± 0.082	83.593± 0.194	10.877± 0.064	78.913± 0.122	10.044± 0.041	84.131± 0.489	10.969± 0.092
Overall means	93.291± 0.380	12.832± 0.098	85.474± 0.190	11.123± 0.070	79.342± 0.080	10.381± 0.050	86.036± 0.380	11.445± 0.075
CD*	-	-	-	-	-	-	0.350	0.145
CD**	0.600	0.250	0.600	0.250	0.600	0.250	-	-

CD* - Critical difference for comparison of overall means of 3 groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 7.4 Influence of dietary calcium level on the shell gland enzyme profile of 16 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium levels)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	KA units ALP	tissue ACP	KA units ALP	tissue ACP	KA units ALP	tissue ACP	KA units ALP	tissue ACP
GI (0.5% Ca)	0.612± 0.020	20.218± 0.159	0.592± 0.016	20.179± 0.156	0.608± 0.018	20.600± 0.158	0.604± 0.010	20.332± 0.092
GII (0.7% Ca)	0.577± 0.021	19.588± 0.147	0.596± 0.010	20.131± 0.090	0.599± 0.022	20.274± 0.186	0.591± 0.011	19.997± 0.089
GIII (0.9% Ca)	0.589± 0.020	19.974± 0.164	0.615± 0.021	20.406± 0.156	0.604± 0.015	20.487± 0.110	0.603± 0.011	20.289± 0.080
Overall means	0.592± 0.012	19.927± 0.100	0.601± 0.010	20.239± 0.080	0.604± 0.011	20.454± 0.090	0.599± 0.006	20.206± 0.050
CD*	-	-	-	-	-	-	0.039	0.231
CD**	0.053	0.400	0.053	0.400	0.053	0.400	-	-

CD* - Critical difference for comparison of overall means of 3 groups and 3 sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 7.5 Influence of dietary calcium level on the plasma enzyme profile of 24 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium levels)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	KA units/dl		KA units/dl		KA units/dl		KA units/dl	
	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
GI (0.5% Ca)	79.757± 0.198	12.527± 0.086	68.925± 0.436	10.702± 0.096	65.447± 0.297	9.874± 0.048	71.376± 0.672	11.034± 0.126
GII (0.7% Ca)	79.316± 0.170	12.441± 0.073	69.732± 0.309	10.367± 0.105	64.211± 0.275	9.590± 0.049	71.086± 0.678	10.799± 0.135
GIII (0.9% Ca)	79.508± 0.110	11.940± 0.069	69.032± 0.185	10.091± 0.080	64.397± 0.252	9.271± 0.032	70.979± 0.679	10.434± 0.124
Overall means	79.527± 0.100	12.303± 0.051	69.230± 0.190	10.387± 0.060	64.685± 0.170	9.578± 0.040	71.147± 0.390	10.756± 0.075
CD*	-	-	-	-	-	-	0.428	0.114
CD**	0.740	0.200	0.740	0.200	0.740	0.200	-	-

CD* - Critical difference for comparison of overall means of 3 groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 7.6 Influence of dietary calcium level on the shell gland enzyme profile of 24 week old female Japanese quail - (Mean ± SE) - 30 birds/subgroup

Groups (Pre-laying dietary calcium levels)	Sub-groups (Layer dietary calcium)						Overall means	
	A (2.5% Ca)		B (3% Ca)		C (3.5% Ca)			
	KA units/g tissue		KA units/g tissue		KA units/g tissue		KA units/g tissue	
	ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
GI (0.5% Ca)	0.603± 0.014	20.526± 0.187	0.569± 0.007	20.598± 0.182	0.589± 0.007	20.506± 0.170	0.587± 0.066	20.543± 0.103
GII (0.7% Ca)	0.573± 0.011	20.675± 0.157	0.575± 0.006	20.722± 0.143	0.574± 0.005	20.732± 0.200	0.574± 0.004	20.710± 0.096
GIII (0.9% Ca)	0.575± 0.012	20.493± 0.161	0.577± 0.008	20.536± 0.144	0.576± 0.004	20.793± 0.131	0.576± 0.005	20.607± 0.084
Overall means	0.584± 0.010	20.564± 0.100	0.574± 0.004	20.619± 0.090	0.580± 0.003	20.677± 0.100	0.579± 0.003	20.620± 0.060
CD*	-	-	-	-	-	-	0.013	0.270
CD**	0.023	0.470	0.023	0.470	0.023	0.470	-	-

CD* - Critical difference for comparison of overall means of 3 groups and 3 layer sub-groups each comprising of 90 birds

CD** - Critical difference for comparison of means of 9 sub-groups each comprising of 30 birds

Table 7.7 ANOVA table of means - Influence of dietary calcium level on the enzyme profile of female Japancese quail - 16th and 24th week of age

Sources	df	MSS							
		16th week of age				24th week of age			
		Plasma		Shell gland		Plasma		Shell gland	
		ALP	ACP	ALP	ACP	ALP	ACP	ALP	ACP
Group	2	363.879**	26.956**	0.005	2.986**	3.806	8.234**	0.004	0.635
Sub-group	2	4399.016**	142.164**	0.003	6.320**	5204.656**	176.256**	0.002	0.286
Group vs sub-group	4	92.862**	2.780**	0.005	0.761	8.376**	0.159	0.003	0.303
Error	261	1.410	0.247	0.011	0.623	2.143	0.152	0.002	0.856

** Significant at 1 per cent level (P <0.01)

* Significant at 5 per cent level (P <0.05)

the plasma ALP content. The overall mean concentration of ALP at the sixth week of age in Japanese quail was 93.138 ± 0.473 KA Units/dl (Table 7.1 and Fig.9.1).

From the ANOVA table (Table 7.7) it was observed that the pre-laying and layer dietary calcium levels significantly ($P<0.01$) influenced the plasma ALP concentration at the 16th week of age. There was significant ($P<0.01$) interaction of pre-laying and layer calcium levels on the plasma ALP content.

At the 16th week of age (Table 7.3 and Fig.9.3) layer quails showed more or less a parallel decrease in the plasma ALP concentration when the dietary calcium level was more, both in the pre-laying and layer rations. The overall mean for GIII group reared on highest pre-laying dietary calcium (0.9%) was 84.131 ± 0.489 KA units/dl whereas for GI group reared on the lowest pre-laying dietary calcium (0.5%) was the highest being 88.138 ± 0.778 KA units/dl (Table 7.3 and Fig.9.3). The lowest pre-laying dietary calcium level of 0.5 per cent seemed to cause a marked increase in the plasma ALP content which also showed a tendency to decrease in concentration as pre-laying dietary calcium levels were increased. The overall mean plasma ALP concentration (93.291 ± 0.380 KA units/dl) was highest for the sub-group A, reared on the lowest layer dietary calcium (2.5%) whereas, it was

lowest (79.342 ± 0.080 KA units/ dl) for the sub-group C reared on the highest layer dietary calcium (3.5%). At higher layer dietary calcium (3% and 3.5%) levels sub-group B and C showed a tendency to decrease the plasma ALP concentration (Table 7.3 and Fig.9.3). Analysis of the results of interaction between pre-laying and layer calcium levels on the plasma ALP activity indicated a significantly ($P<0.01$) increasing trend in the concentration of enzyme as the level of pre-laying and layer calcium levels decreased. The highest value for plasma ALP activity was recorded in quails of GI A sub-group reared on lowest pre-laying (0.5%) and layer (2.5%) dietary calcium levels (97.482 ± 0.201 KA units/dl).

At the 24th week of age layer dietary calcium significantly ($P<0.01$) influenced plasma ALP concentration (Table 7.7). There was significant interaction ($P<0.01$) between pre-laying and layer dietary calcium on plasma ALP content. An inverse relationship seemed to exist between higher concentration of plasma ALP activity and lower dietary calcium level at the 24th week of age also. The plasma ALP concentration was the lowest (64.685 ± 0.170 KA units/dl) in the highest layer dietary calcium (3.5%) fed sub-group C and highest (79.527 ± 0.100 KA units/dl) in the lowest layer dietary calcium (2.5%) fed sub-group A (Table 7.5 and Fig.9.5). Even when there was an interaction between

Fig.9.1 ALP AND ACP CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS - JAPANESE QUAIL (SIXTH WEEK)

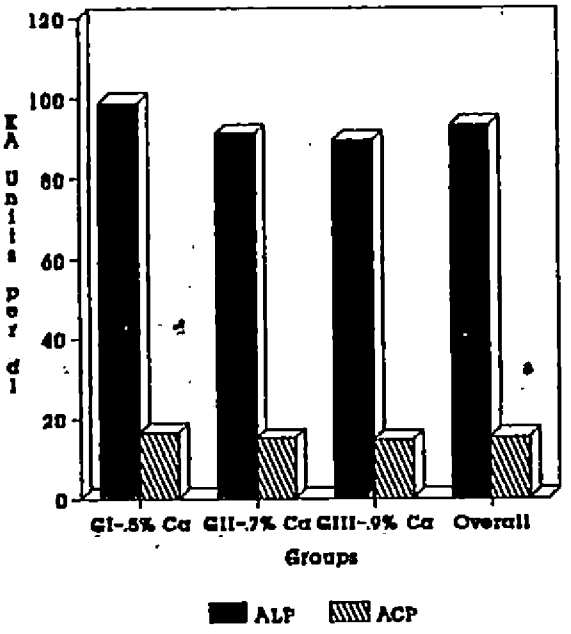


Fig.9.2 ALP AND ACP CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL(SIXTH WEEK)

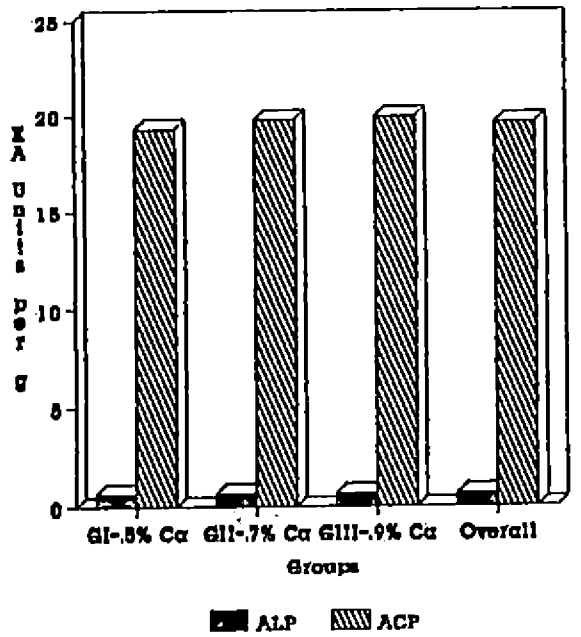


Fig.9.3 ALP CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

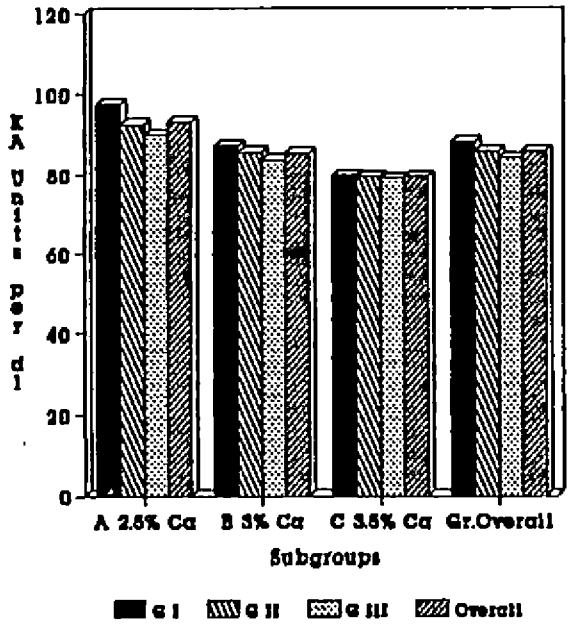


Fig. 9.4 ACP CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

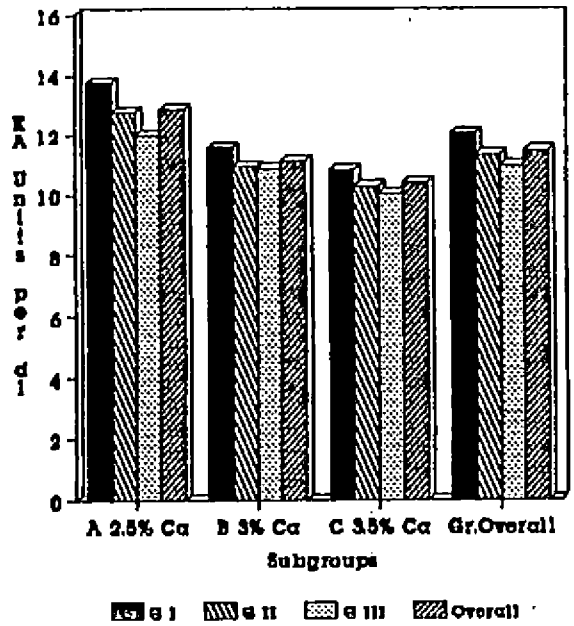


Fig.9.5 ALP CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

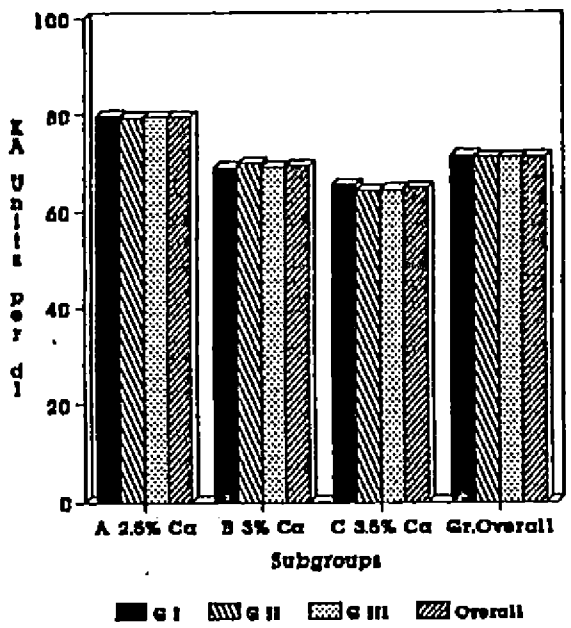
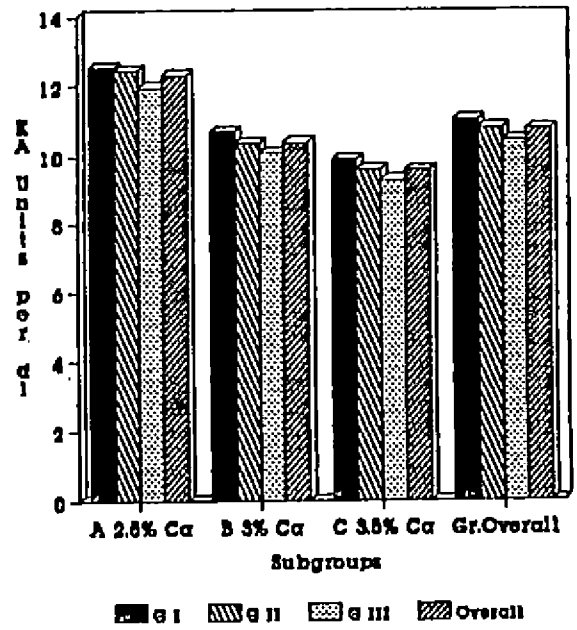


Fig. 9.6 ACP CONTENT OF PLASMA UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)



pre-laying and layer dietary calcium on the plasma ALP concentration, the influence of pre-laying dietary calcium was insignificant. The influence of pre-laying dietary calcium on plasma ALP content appeared to be waning out as age advanced.

Plasma ALP concentration was the lowest (Table 7.5 and Fig.9.5) at the 24th week of age (71.147 $\pm$ 0.39 KA units/dl) as compared to that at the 6th and 16th week of age where the concentrations were 93.138 $\pm$ 0.473 KA units/dl (Table 7.1) and 86.036 $\pm$ 0.380 KA units/dl respectively (Table 7.3 and Fig.9.7).

5.3.2.2 Plasma acid phosphatase (ACP)

At the sixth week of age plasma ACP concentration of GI group fed on lowest pre-laying dietary calcium (0.5%) was the highest, 16.401 $\pm$ 0.240 KA units/dl (Table 7.1 and Fig.9.1). There was significant ($P<0.01$) difference in the plasma ACP content of quails maintained on different levels of pre-laying dietary calcium (Table 7.2). Birds fed on higher pre-laying dietary calcium 0.7 and 0.9 per cent (GII and GIII groups) showed significant decrease in their plasma ACP content, the values being 14.854 $\pm$ 0.170 KA units/dl and 14.395 $\pm$ 0.160 KA units/dl respectively (Table 7.1 and Fig.9.1). As in the case of plasma ALP, the ACP content was also inversely proportional to the level of dietary calcium.

Fig.9.7 PLASMA CONTENT OF
ALP(KA UNITS/dl) AT DIFFERENT PERIODS
JAPANESE QUAIL

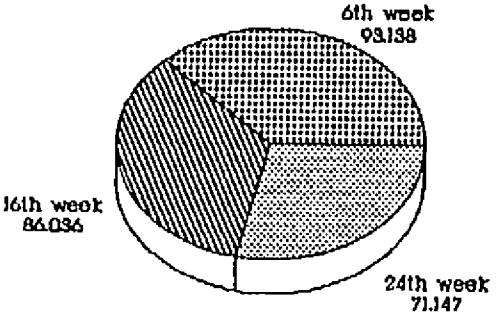
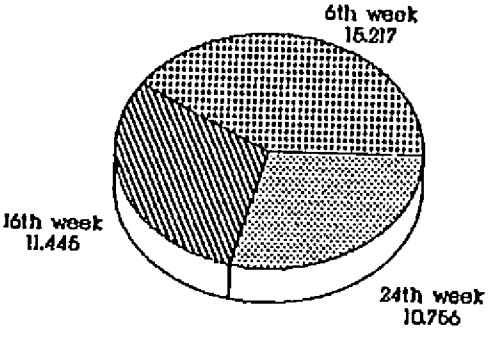


Fig.9.8 PLASMA CONTENT OF
ACP(KA UNITS/dl) AT DIFFERENT PERIODS -
JAPANESE QUAIL



The mean plasma ACP at the sixth week of age in quails was 15.217 ± 0.142 KA units/dl (Table 7.1).

At the 16th and 24th week of age plasma ACP content was influenced significantly ($P<0.01$) by the pre-laying and layer dietary calcium levels (Table 7.7). At the 16th week of age there was also significant ($P<0.01$) interaction between the pre-laying and layer dietary calcium levels. As in the sixth week of age, all the age groups including the 16th and 24th week of age, birds in GI group reared on the lowest pre-lying dietary calcium (0.5%) had high plasma ACP content of 12.043 ± 0.147 KA units/dl and 11.034 ± 0.126 KA units/dl respectively (Tables 7.3, 7.5 and Fig.9.4, 9.6). Conversely birds fed on highest pre-laying dietary calcium (0.9%), GIII group had the lowest plasma ACP content of 10.969 ± 0.092 KA units/dl at 16th week and 10.434 ± 0.124 KA units/dl at the 24th week of age. At the lowest layer dietary calcium (2.5%) fed birds of subgroup A had the highest plasma ACP content of 12.832 ± 0.098 KA units/dl, at the 16th week of age and subgroup C maintained on the highest (3.5%) layer dietary calcium had the lowest value of 10.381 ± 0.050 KA units/dl (Table 7.3 and Fig.9.4). At the 24th week of age the lowest layer dietary calcium (2.5%) fed sub-group A had the highest plasma ACP content of 12.303 ± 0.051 KA units/dl and subgroup C reared on the highest layer dietary calcium (3.5%) had the

lowest value of 9.578 ± 0.040 KA units/dl (Table 7.5 and Fig.9.6). In the case of plasma ACP activity also the tendency to show the highest plasma enzyme content at the lowest dietary calcium level was evident. At the sixth, 16th and 24th week of age the plasma ACP concentration in female Japanese Quail were, 15.217 ± 0.142 KA units/dl, 11.445 ± 0.075 KA unit/dl and 10.756 ± 0.075 KA units/dl respectively (Table 7.1, 7.3, 7.5 and Fig.9.8). As age advanced plasma ACP concentration was seen to be lowered.

5.3.2.3 Shell gland alkaline phosphatase (ALP)

At the sixth week of age shell gland ALP content in quails seemed to be uninfluenced by pre-laying dietary calcium (Tables 7.1, 7.2 and Fig.9.2). Shell gland ALP content of GI, GII and GIII groups were 0.642 ± 0.033 KA units/g, 0.661 ± 0.024 KA units/g and 0.660 ± 0.024 KA units/g respectively (Table 7.1).

At the 16th and 24th week of age it was found that shell gland ALP content was not influenced by either pre-laying or layer dietary calcium levels (Tables 7.7 and Fig.9.9, 9.11). There was no interaction between the two calcium levels (Table 7.7). The shell gland ALP content at the sixth, 16th and 24th week of age were 0.654 ± 0.015 KA units/g, 0.599 ± 0.006 KA units/g and 0.579 ± 0.003 KA units/g

Fig.9.9 ALP CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

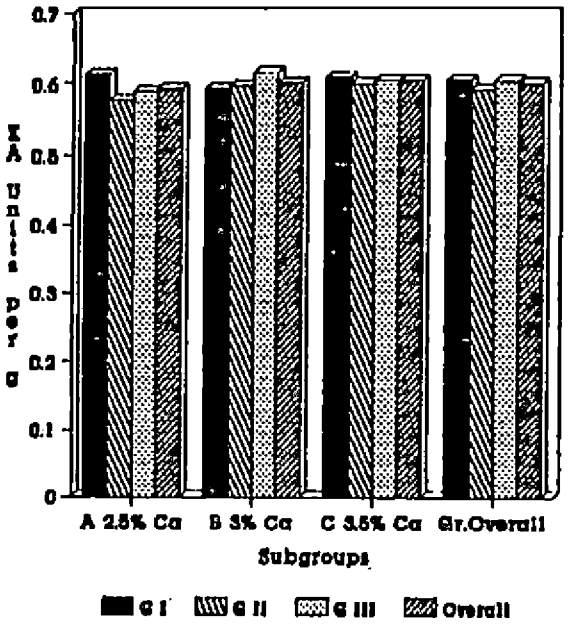


Fig.9.10 ACP CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (16TH WEEK)

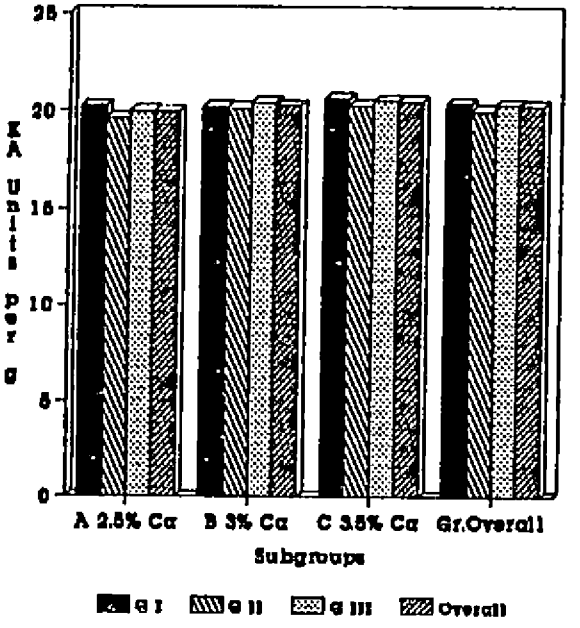


Fig.9.11 ALP CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)

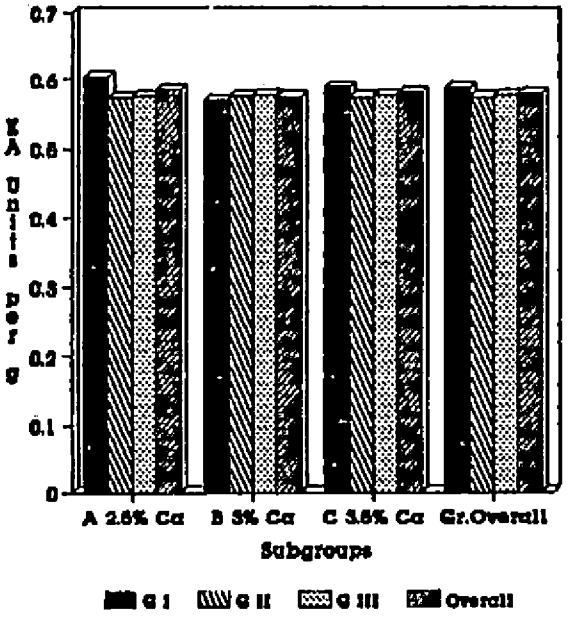
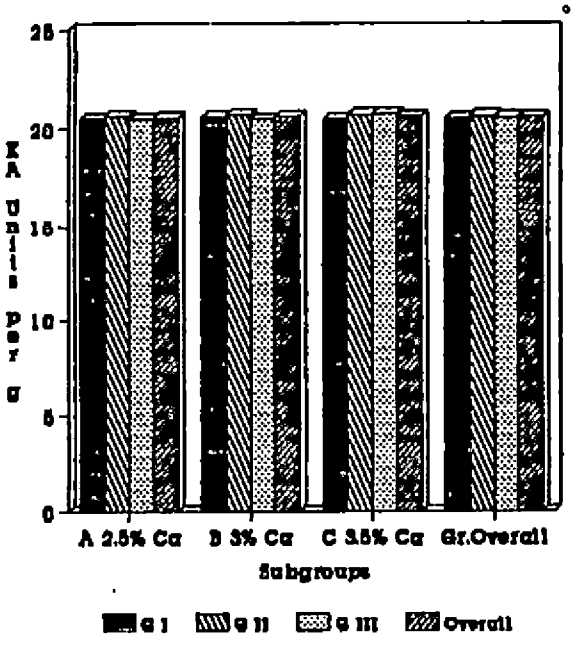


Fig.9.12 ACP CONTENT OF SHELL GLAND UNDER DIFFERENT DIETARY CALCIUM LEVELS-JAPANESE QUAIL (24TH WEEK)



respectively (Table 7.1, 7.4, 7.6 and Fig.9.13). The ALP concentration of shell gland was not influenced by age.

5.3.2.4 Shell gland acid phosphatase (ACP)

The shell gland ACP content at the sixth week of age was significantly ($P<0.01$) influenced by pre-laying dietary calcium (Table 7.2). Highest shell gland ACP concentration was recorded for GIII group (19.892 $\pm$ 0.142 KA units/g) reared on highest (0.9%) pre-laying dietary calcium (Table 7.1 and Fig.9.2). At the 16th week of age also shell gland ACP content was significantly ($P<0.01$) influenced by pre-laying and layer dietary calcium levels (Table 7.7). At the 16th week of age highest layer dietary calcium (3.5%) fed subgroup C had the highest (20.454 $\pm$ 0.090 KA units/g) shell gland ACP concentration, the lowest layer dietary calcium (2.5%) fed subgroup A had the lowest (19.927 $\pm$ 0.100 KA units/g) shell gland ACP content (Table 7.4 and Fig.910). At the 24th week of age shell gland ACP content was uninfluenced by dietary calcium (Table 7.7). At this age quail layers of sub-group A, sub-group B and sub-group C had the shell gland ACP content of 20.564 $\pm$ 0.100 KA units/g; 20.619 $\pm$ 0.090 KA units/g and 20.677 $\pm$ 0.100 KA units/g respectively (Table 7.6 and Fig.9.12). The over all mean shell gland ACP concentration at the sixth, 16th and 24th week of age were 19.639 $\pm$ 0.090 KA units/g; 20.206 $\pm$ 0.050 KA units/g and 20.620 $\pm$ 0.060 KA units/g respectively (Tables 7.1, 7.4, 7.6 and Fig.9.14).

Fig.9.13 SHELL GLAND CONTENT OF ALP (KA UNITS/g) AT DIFFERENT PERIODS JAPANESE QUAIL

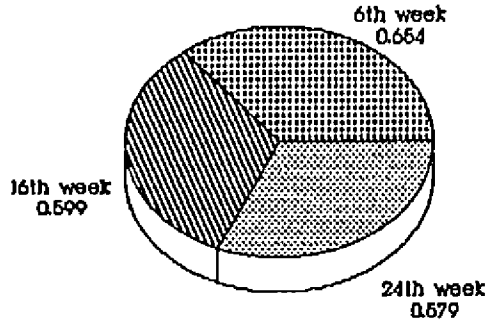
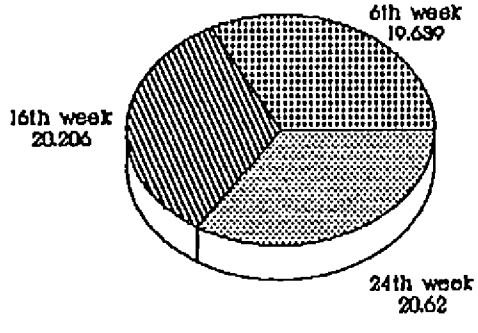


Fig.9.14 SHELLGLAND CONTENT OF ACP(KA UNITS/g) AT DIFFERENT PERIODS JAPANESE QUAIL



Discussion

Chapter 6

DISCUSSION

6.1 Body weight and Anatanical studies

6.1.1 Body weight

From the fifth to 24th week of age highest pre-laying dietary calcium (0.9%) fed GIII group recorded highest body weight except at seventh and ninth week of age, when the birds of GII group (fed on 0.7% pre-laying dietary calcium) recorded the highest body weight (Table 2.1 and Fig.1.1). Increase in the pre-laying dietary calcium evinced a tendency to induce an increase in the body weight of young growing quails. After the 19th week of age, as age advanced, no influence of pre-laying dietary calcium was evident on the body weight. Interaction between pre-laying and layer dietary calcium was also absent after the 17th week of age (Table 2.3). It was also noticed that the birds put on more body weight upto the 16th week. After 19th week of age, pre-laying dietary calcium was uninfluential and only layer dietary calcium level was responsible for the gain in body weight (Table 2.1 and Fig.1.2). However, in the early weeks of growth, higher pre-laying dietary calcium level, 0.9 per cent was required for

optimal growth and development (Table 2.1). Hurwitz and Bar (1971) reported an increase in the body weight of domestic fowl at the onset of lay, pullets attained considerable body weight during their first phase of egg production and their dietary requirement during this period was relatively large. According to them, younger layers apparently put on weight at a rate typical of mature hens even at the age of first egg. In the present study also body weight gain was marked in the early weeks of age especially upto the 16th week, thereafter only marginal increase in the body weight was noticed (Fig.1.1, 1.2).

Immediately after switching over to the layer dietary calcium levels at the sixth week of age, the layer calcium levels significantly ($P<0.01$) influenced the body weight/ growth from the seventh week upto 24th week of age (Fig.1.2). An overall review of the results on the body weight (Table 2.1) showed that lowest body weight registered was for the subgroup C fed on the highest (3.5%) layer dietary calcium (Fig.1.2). At the same time subgroup B maintained on 3 per cent layer dietary calcium showed better body weight than all the other subgroups. Layer dietary calcium levels higher than 3 per cent did not improve the body weight in almost all the age groups (Table 2.1). This indicated that optimum layer dietary calcium level that can be recommended for quail ration

was 3 per cent. However, Hurwitz (1964) could not record any difference in body weight in calcium deficient hens, whereas Rao and Brahmakshatriya (1976) reported similar findings as that of the present investigation. According to them the higher calcium containing diet, 3.3 per cent in layer ration actually depressed the body weight gain in White Leghorn pullets.

It was also observed that quails attained sexual maturity at the sixth week of age with an average body weight of 131.800 ± 0.600 g (Table 2.1). Ahuja et al. (1978) recorded the age of sexual maturity in quails at the sixth week, with an average body weight 90.550 g only. It was also recorded in the present study that the body weight gain was steadily on the increase upto the 16th week of age and thereafter it was only marginal especially from the 19th week onwards. The body weight at the 24th week of age was 198.133 ± 0.220 g (Table 2.1). Sachdev and Ahuja (1986) recorded a body weight of 200 g which was considered to be optimum for good egg production at sexual maturity. In the present study also the production was good in birds with an average body weight of 198.133 ± 0.220 g at the 24th week of age.

The following conclusions can be drawn from the above investigation:-

1. Female Japanese quails attained sexual maturity at the age of six weeks by which time they registered an average body weight of 131.800 ± 0.600 g.

2. Gain in body weight was at a faster rate upto 16th week of age, becoming marginal thereafter, especially after the 19th week.

3. At the 24th week of age, the performance of female Japanese quails was very good/excellent with an average body weight of 198.033 ± 0.220 g.

4. Pre-laying dietary calcium had only negligible influence on the growth after the 19th week of age.

5. Pre-laying dietary calcium levels ranging from 0.7 per cent to 0.9 per cent and layer dietary calcium level of 3 per cent were optimal for growth and egg production in Japanese quails.

6.1.2 Anatomical studies

6.1.2.1 Oviduct development

The quail oviduct was a highly convoluted hollow muscular organ having five distinct regions based on the structure and function namely infundilulum, magnum, isthmus, shell gland and vagina. The oviduct as a whole was pinkish

white in colour except for the area of shell gland which was dark brown (Fig.2.1). Similar observations had been made by Woodard and Mather (1964) and Hoffer (1971) in Japanese quail.

At the sixth week of age (age at first egg) when quails attained sexual maturity the oviduct had become longer and heavier (length of 28.66 ± 0.13 cm and weight of, 4.874 ± 0.031 g respectively, Table 3.1 and Fig.2.2). The shell gland was a dark brown, short and pouch like structure with an average length of 2.93 ± 0.01 cm and weight of 1.780 ± 0.007 g (Table 3.1).

At the 16th week of age when birds were on peak egg production the length and weight of the oviduct increased (29.98 ± 0.03 cm and 5.900 ± 0.010 g respectively; Table 3.3 and Fig.2.3). Likewise the length and weight of shell gland also increased (3.09 ± 0.01 cm and 1.933 ± 0.004 g respectively). At the 24th week of age length and weight of oviduct further increased (31.90 ± 0.03 cm and 6.013 ± 0.010 g respectively; Table 3.4 and Fig.2.4). Similar was the case with the length and weight of shell gland also (3.12 ± 0.01 cm and 1.968 ± 0.004 g respectively). As growth advanced from the sixth to 24th week of age marked increase in length and weight of oviduct was observed. Eventhough there was a marked increase in length and weight of shell gland from the sixth to

16th week, the growth from the 16th to 24th week was only marginal.

Pre-laying dietary calcium had not materially influenced the development of oviduct especially at the sixth week of age. Shrivastava and Brahmakshatriya (1976) found no influence of pre-laying dietary calcium in White Leghorn pullets either on the length or in weight of the oviduct when the birds were maintained for 18-21 weeks or 24-27 weeks. Pre-laying dietary calcium showed no influence on the oviduct development at the 24th week of age. But at the 16th week of age layer dietary calcium produced significant ($P<0.01$) difference in length and weight of shell gland and weight of oviduct (Table 3.5).

The maximum overall mean length of oviduct ws recorded (30.00 ± 0.05 cm) at the 16th week of age for the birds of subgroup B reared on 3 per cent layer dietary calcium (Table 3.3). Maximum length of shell gland was registered in the birds of subgroup GIII C with a mean value of 3.12 ± 0.01 cm. At the 24th week of age layer dietary calcium had only marginal influence ($P<0.05$) on the weight of the oviduct, the lower value for subgroup C being 5.984 ± 0.007 g (Table 3.4). Similar was the response of layer dietary calcium on body weight too. The absence of any marked increase in body weight due to highest layer dietary calcium (3.5%) after the 16th

week of age might have been due to negligible gain in body weight after the age of maximum weight gain at this age. It may also be possible that by the 16th week of age, the birds might have attained sufficient growth and production so that any further increase in the level of dietary calcium might not have produced any added effect either on the body weight or oviductal weight. So also the data (Table 3.4) on the influence of dietary calcium on the weight of oviduct revealed that the maximum weight of the oviduct was attained by the diet containing 2.5 to 3 per cent of layer dietary calcium levels. Further increase in the layer dietary calcium level (3.5%) did not produce any appreciable increase in its weight gain indicating that the optimum level of layer dietary calcium for oviduct development was between 2.5 to 3 per cent.

Shrivastava and Brahmakshatriya (1976) also found that the dietary calcium level beyond 2.2 per cent did not influence the length of the oviduct. The importance of adequate levels of dietary calcium was highlighted from the observations of Nevalainen (1969) who reported significantly lower weights of ovary, oviduct and shell gland in White Leghorn hens when the ration was deficient in calcium (0.13%). However, Vohra et al. (1979) could not find any influence of dietary calcium on ovarian and oviduct weight of Japanese quails, whereas significantly heavier ovary and oviduct were

noted in chicken reared on normal ration compared to those of calcium deficient diet.

It can be inferred from the above studies that:

1. The oviduct of Japanese quail was pinkish white in colour except for the area of shell gland where it was dark brown in appearance.

2. Based on the anatomical features the oviduct of Japanese quail could be identified as infundibulum, magnum, isthmus, shell gland (uterus) and vagina as in chicken.

3. The whole length and weight of the quail oviduct at the age at first egg (sixth week of age) were on an average 28.66 $\pm$ 0.13 cm and 4.874 $\pm$ 0.031 g respectively.

4. The length and weight of the shell gland at the age at first egg were on an average 2.93 $\pm$ 0.01 cm and 1.780 $\pm$ 0.007 g respectively.

5. The pre-laying dietary calcium had negligible influence on the growth and development of the oviduct at the sixth, 16th and 24th week of age. However at the 16th week shell gland weight was marginally ($P<0.05$) influenced by prelaying dietary calcium. Maximum weight for the shell gland at the 16th week of age was for birds reared on 0.7 per cent pre-laying dietary calcium.

6. Layer dietary calcium level of 2.5 to 3 per cent was sufficient for optimal development of the shell gland at the 16th week of age since maximum weight achieved at this age was for 2.5 per cent layer dietary calcium fed group.

7. No additional effect was produced by providing more than 3 per cent of layer dietary calcium on the development of the oviduct at the 24th week of age.

8. The length and weight of the oviduct at the 16th week of age were 29.98 ± 0.03 cm and 5.900 ± 0.010 g respectively and at the 24th week of age were 31.90 ± 0.03 cm and 6.013 ± 0.010 g respectively.

9. The length and weight of the shell gland at the 16th week of age were 3.09 ± 0.01 cm and 1.933 ± 0.004 g respectively and at the 24th week, the values were 3.12 ± 0.01 cm and 1.968 ± 0.004 g respectively.

6.1.3 Histological and histochemical studies

6.1.3.1 Histological studies

Shell glands were collected for the present investigation from actively laying birds (with egg undergoing calcification in the shell gland). The walls of quail shell gland consisted of lining mucosa (tunica epitheliaris)

glandular lamina propria, layers of smooth muscle (tunica muscularis), connective tissue and tunica serosa, similar in structure to that of domestic fowl (Hodges, 1974) except for the arrangement of layers of smooth muscle in quails, where the inner circular muscle layer was thicker and outer longitudinal layer was thinner (Fig.4.2).

The lining mucosa was thrown into the longitudinally oriented branched leaf shaped rugae (Fig.4.3, 4.11, 4.12).

The surface epithelium could be described as pseudo-stratified columnar as in the case of domestic fowl (Aitken, 1971) since it consisted of two types of cells, the apical cells with apical nucleus which were ciliated and basal cells with basal nucleus which were non-ciliated but provided with microvilli. The apical and basal cells were responsible for imparting the pseudostratified appearance for the epithelial layer (Fig.4.2, 4.3). A central core of loose connective tissue invaded the mucosal folds (Fig.4.4). Just beneath the surface epithelium was located the glandular lamina propria consisting of highly branched tubular glands as in the case of the domestic fowl (Schraer and Schraer, 1971 and Hodges, 1974). The ducts of the tubular glands opened into the surface of the folds of columnar epithelium (Fig.4.1, 4.7, 4.10). Vascular tissue invaded the glandular lamina propria

(Fig.4.10). This vascular supply was probably for more calcium translocation to the shell gland lumen. There was a central core of connective tissue extending into the lamina propria from the tunica muscularis (Fig.4.2). This connective tissue was also rich in blood capillaries (Fig.4.10). Tunica muscularis was having two layers of smooth muscle tissue, thicker inner circular and thinner outer longitudinal muscle layer (Fig.4.2). But in the shell gland of the domestic fowl (Hodges, 1974) noticed the outer longitudinal muscle layer thicker than inner circular muscle layer. The layer of connective tissue core located in between the circular and longitudinal muscle layers was highly vascular (Fig.4.6). External to the longitudinal muscle layer was a thin layer of tunica serosa consisting of flattened mesothelial cells (Fig.4.2). It was identical, to what described by Hodges (1974) in domestic fowl. Perusal of the available literature on structure of the oviduct revealed the paucity of information of a regular detailed and systematic study on this aspect especially that of shell gland in Japanese quail.

Influence of dietary calcium on the histology of the shell gland was also studied. Comparison of different groups and subgroups revealed more vascularity in the connective tissue core of the glandular lamina propria, in highest (3.5%) layer dietary calcium fed subgroup (Fig.4.10). No other

appreciable difference in any other group/subgroup was evident. The increased vascularity in the glandular lamina propria is suggestive of increased blood calcium transfer to the calcifying egg shell in the shell gland lumen. It can be suggested that tubular glands in the lamina propria may be involved in the calcium transfer since the highest vascularity was seen in this layer in highest calcium fed birds. This may also suggest that calcium was not stored in the shell gland but was transferred from blood to the shell gland lumen at the time of calcification. Sturkie (1976) could not pin point the type of cells which transport calcium, elaborate carbonate and organic matrix precursors in the shell gland. It was also not certain whether the movement of calcium across the shell gland was an active transport. Hodges (1974) was of the opinion that tubular glands (lamina propria) elaborate a watery calcium containing fluid which give rise to the inorganic part of the egg shell. It can be concluded from the results of this study that tubular glands may be involved in the calcium transport across the wall of the shell gland since this layer was highly vascular especially when birds were fed on highest layer dietary calcium.

The comparative histological picture of the shell glands of birds from the sixth, 16th and 24th week of age revealed the occurrence of more mucosal folds in birds of the

higher age groups (16 and 24 weeks of age) with the greatest number of folds in the 24th week (Fig.4.2, 4.11, 4.12). So also inner circular muscle layer was thicker and extending into the mucosal foldings extensively with rich vascular supply. Further the outer longitudinal muscle layer was very thin. As birds grew older, they put on more weight, had more egg production and further development of the oviduct. This was reflected more on the histological structure. The egg weight was found to be progressively increasing as age advanced from the sixth to 24th week of age. This was in consonance with the histological findings of increased surface area for secretion, provided by the enhanced mucosal foldings, thereby increasing the transport of egg shell materials across the walls of shell gland.

From the above histological studies it can be concluded that:

1. Histologically the wall of the shell gland consisted of tunica epitheliaris, lamina propria, tunica muscularis, connective tissue core and tunica serosa.

2. Tunica muscularis in Japanese quail (Coturnix coturnix japonica) was peculiar in having thicker inner circular and thinner outer longitudinal muscular layers in contrast to those of domestic fowl.

3. Only the highest layer dietary calcium (3.5%) fed birds of subgroup C showed rich vascularity in their connective tissue core of lamina propria.

4. As age advanced, at the 16th and 24th week of age:-

   a. The foldings in the lining mucosa increased in number and complexity

   b. Inner circular muscle thickness increased and even invaded the mucosal foldings and outer longitudinal muscle thickness decreased

   c. Vascularity increased both in the inner and outer muscle layers.

6.1.3.2 Histochemical studies

6.1.3.2.1 Alkaline phosphatase (ALP)

The histochemical localisation of ALP in the shell gland tissue especially epithelial lining of the mucosal layer was very weak compared to ACP (Fig.5.1, 5.2, 5.3). Brown and Badman (1962) noticed that ALP was localised in the secretary epithelial layers of the oviductal mucosa and that it was concerned with the excretion of various components of the egg shell. Gutowska et al. (1943) and Salevsky, Jr. and Leach, Jr. (1980) reported that the ALP activity of the shell gland

fluid was relatively low as compared to the ACP activity. It was also found that in homogenates of oviduct tissue of White Leghorn hens, ALP activity was minimal while all sections of oviduct were rich in ACP (Salevsky, Jr. and Leach, Jr., 1980). Romanoff and Romanoff (1949) suggested that phosphatase content of the uterus was low indicating that the uterus excreted calcium rather than mobilising it for egg shell formation. Comparison of histo-chemical studies on the shell gland for ALP localisation in birds reared on different levels of pre-laying and layer dietary calcium revealed that there was no significant difference, indicating that dietary calcium was uninfluential in histo-chemical localisation of ALP activity of the shell gland. Similar line of observation was recorded in the quantitative estimation of the shell gland ALP content of 16 and 24 week old birds reared under different pre-laying and layer dietary calcium levels. Age also did not produce any significant variation with the ALP activity of shell gland. Quantitative determination of the shell gland ALP, in the present investigation was also not influenced by age. The shell gland ALP content was very low in the sixth, 16th and 24th week of age (0.654 $\pm$ 0.015 KA units/g; 0.599 $\pm$ 0.006 KA units/g and 0.579 $\pm$ 0.003 KA units/g respectively). This discrete localisation (lower concentration) together with insignificant relationship with dietary calcium and age suggested that this enzyme may not be involved directly in

shell calcification and may be more concerned with calcium mobilisation and deposition in bone, similar to that reported in domestic fowl (Solomon, 1970). Aitken (1971) was unable to localise ALP in the epithelial lining of avian shell gland. Nys and DeLaage (1984a) found that uterine ALP activity in domestic fowl was uninfluenced by egg shell calcification and that it's level in uterine tissue suggested that it is unlikely to be involved in the calcium transport in the uterus.

6.1.3.2.2 Acid phosphatase (ACP)

Acid phosphatase (ACP) was localised in the lamina epitheliaris of the shell gland mucosa. Compared to ALP, ACP was localised moderately (Fig.5.4, 5.5, 5.6). In the quantitative estimation of the shell gland content of ALP and ACP, the ACP content was comparatively higher.

No variation in the intensity of reaction was noticed between the birds maintained on the different levels of dietary calcium on the ACP activity (Fig.5.4, 5.5, 5.6). However, in the quantitative estimation there was a positive correlation with the shell gland ACP concentration and dietary calcium levels at the sixth and 16th week of age. The intensity of histochemical staining reaction was similar at the sixth, 16th and 24th week of age, indicating that the

enzyme level was not influenced by age (Fig.5.4, 5.5, 5.7, 5.8). The quantitative estimation of the shell gland ACP content was also not influenced by age (Fig.9.14). Solomon (1970) reported that ACP was present in the cytoplasm of both cells of surface epithelium and glandular cells of the shell gland. It varied appreciably in amount depending on the position of an egg in the oviduct. The activity was maximal when soft shelled egg was present in utero and diminishing with increasing calcification of the shell, to reach a minimum at oviposition. Simikiss (1969) reported the intra-cellular pH at the time of shell mineralisation as 6.22. It can be hypothesised that the acidic intracellular conditions were in fact an essential inhibitory mechanism developed to prevent intracellular deposition of calcium in the form of calcium carbonate as described by Solomon (1970). Salevsky, Jr. and Leach, Jr. (1980) reported that homogenates of White Leghorn hen oviduct was rich in ACP and poor in ALP. According to them shell gland ACP was different from plasma ACP, oviduct ACP was inhibited by sodium arsenite and tartaric acid, similar to prostatic ACP. Its role in shell formation is not well delineated, but, it can be instrumental in preventing intra-cellular deposition of calcium carbonate and in the transfer of calcium across the shell gland mucosa from blood to the egg shell in the uterus.

The following conclusions could be drawn from the histo-chemical studies of ALP and ACP:-

1. Localisation of ALP in the shell gland was comparatively weak compared to that of ACP.

2. Histochemical localisation of ACP in the mucosal layer of the shell gland was remarkably intense.

3. Neither dietary calcium levels nor age influenced the histochemical localization of ALP and ACP in the shell gland.

6.2 Egg production and shell quality

6.2.1 Egg production

The age at first egg in Japanese quail in the present investigation was found to be 39 to 42 days (early sixth week). Wilson et al. (1961) found that Coturnix reached sexual maturity at an age as early as 35 to 42 days. However, Tiwari and Panda (1978) reported that quail started laying at 51 days of age and maximum egg production (70%) was attained at 100 days after sexual maturity (150-200 days). Peak production was observed in the present investigation at the 16th week of age and the trend continued till the 24th week (Table 4.3 and Fig.6.1, 6.2). Woodard and Applanolp (1970) reported that the rate of laying decreased sharply in Japanese

quail after 26th week of age only. According to McFarland and Franti (1972) the age of maximum egg production in Japanese quail was between 60 and 150 days.

In Japanese quails dietary calcium (both pre-laying and layer) levels exerted a significant influence on the egg production (Fig.6.3, 6.4). The lowest (0.5%) pre-laying dietary calcium fed GI group recorded the lowest egg production whereas GII and GIII groups reared on higher pre-laying dietary calcium levels (0.7% and 0.9% respectively) had more or less identical, higher egg production (Table 4.1 and Fig.6.3, 6.4). Robson et al. (1976) also found that lower (0.5%) dietary calcium level markedly reduced the egg production in Japanese quail. The egg production in Japanese quails of subgroup GI A maintained on both the lowest pre-laying and layer levels recorded the lowest value among all the other subgroups, which indicated that dietary calcium (both pre-laying and layer) levels significantly influenced the egg production (Table 4.1).

There was significant interaction between different subgroups due to different pre-laying and layer dietary calcium levels (Table 4.2). Even when, birds of subgroup GI A (maintained on the lowest, pre-laying, 0.5 per cent, and layer, 2.5 per cent dietary calcium) had lowest egg

production, subgroups GII A and GIII A (reared on higher, 0.7% and 0.9% prelaying and lowest, 2.5%, layer dietary calcium levels) recorded higher egg production which was more or less identical (Table 4.1). This indicated that there was interaction of pre-laying and layer dietary calcium levels and 0.7 per cent pre-laying dietary calcium level was optimum for better egg production. It was also seen that subgroup GI A recorded lowest egg production while subgroup GI B and GI C reared on the same lowest pre-laying (0.5%) and higher (3% and 3.5% respectively) layer dietary calcium levels recorded more than 80 per cent (higher) egg production (Table 4.1). This indicated that if proper layer dietary calcium level was maintained, the influence of pre-laying dietary calcium became insignificant.

Birds of all subgroups of B and C maintained on 3 and 3.5 per cent (higher) layer dietary calcium levels recorded an egg production exceeding 80 per cent (Table 4.1). Birds of subgroup A reared on 2.5 per cent (lowest) layer dietary calcium recorded the lowest egg production indicating layer dietary calcium significantly improved the egg production in Japanese quails. Navarro and Murillo (1976), and Shrivastav et al. (1989) observed similar effects in Japanese quails. There were reports (Berg et al. 1947; Hurwitz and Bar, 1971;

Gilbert et al. 1981; Bolden and Jensen, 1985a and Clunies et al. 1992a)on similar lines about the influence of dietary calcium on egg production in domestic fowl. However, Husseini et al. (1981), Cheng and Coon (1990) and Guinotte and Nys (1992) reported that the rate of egg production in domestic fowl was not influenced by lower levels of dietary calcium. Funamoto and Vohra (1988) reported that Japanese quails continued normal egg production and maintained egg weight but not the shell thickness with a dietary calcium level of 1.05 per cent. They also observed a cessation of egg production in White Leghorn hens under the same conditions.

The overall mean egg production in higher layer dietary calcium fed subgroup B (3%) and subgroup C (3.5%) was higher and almost identical (Table 4.1). The rate of egg production was augmented when the level of layer dietary calcium was increased from 2.5 to 3 per cent, where as only negligible difference was noticed between subgroup B (fed on 3% layer dietary calcium) and subgroup C (reared on 3.5% layer dietary calcium) indicating layer dietary calcium level higher than 3 per cent was without any added advantage and influence on egg production (Fig.6.1, 6.3, 6.4). Shrivastav et al. (1989) reported that egg production in quails increased with each increment of dietary calcium from 2 to 2.8 per cent, without any added advantage above this level. Ademosun and

Kalango (1973) noticed that increase in dietary calcium levels from 2 to 3.5 per cent in layer ration of domestic fowl increased egg production whereas the production decreased at the highest dietary calcium level of 4.25 per cent.

The conclusions drawn from the study were:-

1. The age at first egg ranged from 39 to 42 days for the stock of quails used.

2. By the 16th week of age quails reached peak production.

3. Even at the 24th week of age, production continued to be high.

4. Dietary calcium positively influenced egg production.

5. For optimum egg production a pre-laying dietary calcium level of 0.7 per cent and a layer calcium level of 3 per cent were ideal.

6.2.2 Egg shell quality

6.2.2.1 Egg weight

The highest (0.9%) pre-laying dietary calcium fed GIII group recorded highest egg weight at the sixth and 16th week of age (Table 5.1 and 5.2). Pre-laying dietary calcium did not influence the egg weight at the 24th week of age.

However, Hurwitz and Bar (1971) could not find any significant influence on the egg weight of chicken due to various pre-laying dietary calcium levels.

Layer dietary calcium significantly improved the egg weight at the sixth and 16th week of age, but not at the 24th week. Birds reared on higher (3%) layer dietary calcium had highest egg weight both at the sixth and 16th week of age indicating that layer dietary calcium of 3 per cent was sufficient for optimum egg weight. Dietary calcium levels higher than this did not produce any beneficial effect. Vohra et al. (1979) noticed higher egg weight in Japanese quail when fed on higher layer dietary calcium levels. Shrivastav and Panda (1986) recorded an improvement in egg weight numerically from 10.1 g to 10.5 g as the level of dietary calcium, in quail ration was increased from 3 to 3.75 per cent. Shrivastav et al. (1989) in another study found that egg weight and mass were increased with each increment of dietary calcium. But Cheng and Coon (1990), Clunies (1992a) and Keshavarz and Nakajima (1993) could not find any significant influence of dietary calcium on egg weight of domestic fowl. The influence of dietary calcium on egg weight was waning out as age advanced, since the egg weight at the 24th week of age was not influenced by dietary calcium.

As age advanced (from the sixth to 24th week) the weight of egg increased markedly as was observed by Tiwari and Panda (1978) and Yannakopoulos and Tserveni-Gousi (1986). Nagarajan et al. (1991) also found weight of quail eggs increased with the advancement of age. Similar were the observations made in domestic fowl by Perek and Snapir (1970), Hamilton (1978), Roland, Sr. (1979) and Nys (1986).

6.2.2.2 Shell weight (with shell membrane)

From the sixth week of age, the influence of dietary calcium on the egg shell weight was evident. The highest (0.9%) pre-laying dietary calcium fed GIII group had highest shell weight at the sixth, 16th and 24th week of age. Highest layer dietary calcium (3.5%) fed subgroup C exhibited highest shell weight at the 16th and 24th week of age (Table 5.2 and 5.3). The egg shell weight progressively increased with the increase in dietary calcium levels and consequently the egg shell quality also. However, Shrivastav and Panda (1986) did not find any significant influence of dietary calcium on the shell weight of quail eggs, so also Hurwitz and Bar (1971) in chicken eggs. But Shrivastav et al. (1989) noticed an increase in egg shell weight in quails due to dietary calcium levels. Similar observations were made by Peterson (1960), Ademosun and Kalango (1973), Gilbert et al. (1981), Husseini et al. (1981) and Roland, Sr. (1986) in domestic fowl.

Clunies et al. (1992a, b) noted that calcium deficient diet in White Leghorn hen resulted in a significant ($P<0.05$) reduction in shell weight and average daily shell output. However, they also noticed a diminishing trend in shell weight at higher levels of calcium intake. In this study also there was marked improvement in shell weight between 2.5 and 3.5 per cent dietary layer calcium levels. But the difference between 3 and 3.5 per cent calcium fed subgroups were only marginal.

The influence of age on the shell weight was evident from the sixth to 16th week of age. The shell weight markedly increased from the sixth to 16th week of age (Fig.7.2). But from the 16th to 24th week, there was a marginal drop. At the same time the egg weight increased from the sixth to 24th week of age which might have led to the increase in the egg volume leading to thinning of the shell and therefore the drop in egg shell weight. The deposition of shell calcium was fairly constant even when the size of the egg was increased (Hamilton, 1978) and since the size of an egg is a deciding factor in determining the quality of shell in older hens the egg shell quality was reduced. So with the advancement of age even with proper dietary calcium, egg shell weight decreased along with an increase in egg weight. Izat et al. (1985) also noticed that shell weight increased as age advanced but the percentage weight of shell decreased with aging. The increase

in egg weight with age accounted for decrease in shell quality (Nys, 1986). Yannakopoulos and Tserveni-Gousi (1986) found that shell weight was increased with age ranging from six to 22 weeks and the decline in shell quality was due to increase in egg weight with a concomitant percentage decrease in shell weight.

6.2.2.3 Shell thickness (with shell membrane)

Shell thickness of quail eggs was increased with an increase in the dietary calcium (pre-laying and layer) levels especially at the 16th and 24th week of age. Reading et al. (1976), Robson et al. (1976), Shrivastav and Panda (1986) and Shrivastav et al. (1989) observed an improvement in quail egg shell thickness as the dietary calcium level was increased. Funamoto and Vohra (1988) found that in a calcium deficient diet (1.05%) Japanese quails continued to lay normal eggs but without maintaining optimum shell thickness. Berg et al. (1947), Jenkins and Taylor (1960) and Hertelendy and Taylor, 1961 found very low levels of dietary calcium resulting in production of thin shelled eggs in domestic fowl. Scott et al. (1971) found that highest shell thickness was produced in chicken eggs when the dietary calcium level was high and that inadequate calcium levels (2.5%) led to a decrease in egg shell thickness. In the present investigation also much

variation was evident in shell thickness between 2.5 and 3.5 per cent of layer dietary calcium. However, only marginal difference was evident in the shell thickness as the birds were maintained on a ration containing layer calcium level between 3 and 3.5 per cent. Therefore, for optimum egg shell thickness a layer dietary calcium level of 3 to 3.5 per cent can be recommended.

It was also found that as age advanced from the sixth (early production) to 16th week (peak production), shell thickness was markedly increased (Table 5.1, 5.2 and Fig.7.3), but at the 24th week with the same layer dietary calcium level, shell thickness was found to be marginally lowered (Table 5.3). Yannakopoulos and Tserveni-Gousi (1986) found that shell thickness of quail eggs significantly increased between 49 to 154 days (seven to 21 weeks) of age and thereafter it was lowered. In the present investigation also shell thickness was found to be increased upto the 16th week of age and as age advanced, towards the 24th week the shell thickness was lowered (Fig.7.3). The average shell thickness in quail eggs was found to decrease with age (Narayanankutty et al., 1989 and Nagarajan et al., 1991). The reduction in egg shell thickness with advancing age may either be due to the lower efficiency of calcium transport across the gastro-intestinal tract or to the lowered rate of calcium

mobilisation from the bones in response to calcium requirement.

6.2.2.4 Shape index

At the sixth week of age pre-laying and layer dietary calcium had no significant influence on the shape index of quail eggs. However, there was an interaction ($P<0.05$) between pre-laying and layer dietary calcium levels on the shape index of eggs (Table 5.4). Maximum shape index was recorded for the eggs collected from birds of GII group fed on 0.7 per cent pre-laying and sub-group B, reared on 3 per cent layer dietary calcium levels (Table 5.1). At the 16th week of age highest dietary calcium (pre-laying and layer) levels fed GIII group and sub-group C registered highest shape index (Table 5.2). However, at the 24th week, pre-laying dietary calcium did not produce any significance, whereas highest layer dietary calcium fed sub-group C had highest value (Table 5.3). A perusal of the available literature on the influence of dietary calcium on shape index of quail eggs revealed only negligible information on this account.

The shape index increased from the sixth to 16th week of age, but afterwards, from the 16th to 24th week, it was lowered (Fig.7.4). The influence of increasing age on lowering the shape index in quails was also reported by

Yannakopoulos and Tserveni-Gousi (1986) and Narayanankutty et al. (1989).

6.2.2.5 Specific gravity

Highest pre-laying dietary calcium fed GIII group recorded highest specific gravity at the sixth and 16th week of age (Table 5.1, 5.2). At the 24th week of age pre-laying dietary calcium had negligible influence on the specific gravity of quail eggs (Table 5.4).

Both at the 16th and 24th week of age highest (3.5%) level of layer dietary calcium fed sub-group C recorded maximum specific gravity (Table 5.2, 5.3). Robson et al. (1976) reported that lower dietary calcium (0.5%) markedly reduced the specific gravity of quail eggs. However, Shrivastav and Panda (1986) could not find any significant influence of dietary calcium on the specific gravity of quail eggs. Pepper et al. (1968), Scott et al. (1971), Hamilton and Cipera (1981), Makled and Charles (1987), Cheng and Coon (1990) and Keshavarz and Nakajima (1993) reported an increase in the specific gravity of eggs, in chicken reared on higher levels of dietary calcium. Shrivastav et al. (1989) found an improvement in specific gravity of quail eggs, as the dietary calcium level was increased.

Specific gravity tended to increase from the sixth to 16th week of age, as age advanced; however, from the 16th to 24th week a lowering tendency was recorded (Fig.7.5). Roland, Sr. et al. (1978) found older hens were able to maintain specific gravity as high as or better than younger hens with an adequate intake of dietary calcium. However Izat et al. (1985) and Yannakopoulos and Tserveni-Gousi (1986) reported that younger birds laid eggs with high specific gravity. Yannakopoulos and Tserveni-Gousi (1986) found the specific gravity of quail eggs decreased with the advancement of age. The findings of the present investigation were in line with the above reports.

6.2.2 Shell quality - general discussion

The shell quality traits as egg weight, shell weight, shell thickness shape index and specific gravity were inter-related and influenced by dietary calcium levels. The thickness of the individual egg shell is a function of calcium metabolism, relative efficiency of assimilation and secretion of calcium and other materials involved in shell formation. The egg shell thickness depends on the relative amount of shell present to that of egg surface area. For the shell thickness to increase there must be an increase in shell weight/material and decrease in egg surface area or a combined effect of the two changes. The egg shape in hens is affected

by uniformity in shell thickness. The shell thickness may influence specific gravity of the egg. There seems to be a close relationship existing between specific gravity and egg shape. Eggs with greater shape indices had stronger shells as reflected by egg shape and specific gravity (Yannakopoulos and Tserveni-Gousi, 1986).

Both the higher pre-laying and layer dietary calcium levels significantly improved the shell quality traits. The highest pre-laying and layer dietary calcium levels fed birds produced eggs with maximum egg weight, shell weight and shell thickness, shape index and specific gravity. The relevance of pre-laying dietary calcium disappeared with the advancement of age as optimum layer dietary calcium level was maintained. Hurwitz and Bar (1971) found some improvement in shell quality traits due to the high pre-laying dietary calcium levels. Hurwitz (1964) found that the size of the pre-laying stores of calcium in the body could indeed be manipulated by dietary levels of calcium. Berg et al. (1947) found that quality of hen eggs was greatly influenced by dietary calcium during laying period but supplementary calcium when given during the pre-laying period was without any statistically significant effect. The ability of the hen to withstand the challenge of calcium deprivation is a function of the size of the calcium stores. Therefore, at least theoretically, it is most

desirable for the pullet to accumulate as much mineral as possible in her skeletal stores during the pre-laying storage period itself (Hurwitz and Bar, 1971). The comparative difference between 3 and 3.5 per cent layer dietary calcium level on shell quality parameters was marginal. Better shell quality was achieved by 3 per cent layer dietary calcium indicating that the 3 per cent layer dietary calcium level was sufficient for maintaining ideal egg shell quality. Increasing the layer dietary calcium level above 3.5 per cent in the ration was found to produce no further improvement in the egg shell thickness and specific gravity of chicken eggs (Scott et al., 1971). The results of the present investigation indicated that to ensure maximum shell quality the quail layers may be reared on 0.7 to 0.9 per cent pre-laying dietary calcium followed by a layer ration containing 3 per cent of calcium.

At the 24th week, many of the shell quality traits such as shell weight, shell thickness, shape index and specific gravity were all lowered with an increase in the weight of the egg. It is probable that as age advanced only the egg weight increased but not the shell quality, since the transfer of calcium across the shell gland might have reached its maximum by the time of peak production viz. by about the 16th week of age. The potential for intestinal absorption and

uterine transfer of calcium might have reached the maximum by the time of peak production as noted by Bar et al. (1992). Peterson (1965) also reported that reduced shell quality with increase in age was due to the reduced ability of birds to absorb and retain calcium in the body or utilize the calcium derived from the bones for shell formation. So also Nagarajan et al. (1991) found that the egg weight in Japanese quail increased and shell thickness decreased with age. From the foregoing it may be pointed out that:-

1. Higher pre-laying and layer dietary calcium levels improved the egg shell quality.

2. The influence of pre-laying dietary calcium level on shell quality traits was more marked at the sixth and 16th week of age. As age advanced layer dietary calcium had more significance.

3. Pre-laying dietary calcium level of 0.7 to 0.9 per cent and layer dietary calcium level of 3 per cent were sufficient for maintaining optimum shell quality.

4. As age advanced shell quality was lowered with an increase in egg weight.

6.3 Biochemical studies

6.3.1 Calcium and phosphorus profile in the plasma and shell gland of Japanese quail

6.3.1.1 Plasma calcium

During the pre-laying period pullets experience profound changes in their mineral metabolism. It has been shown that during this period pullets show increased calcium retention, higher plasma calcium levels and greater storage of calcium in the medullary bone (Hurwitz and Griminger, 1960). Rao and Brahmakshatriya (1976) observed that calcium retention and storage could be manipulated by dietary levels of calcium in pre-layers of domestic chicken. Hurwitz (1964) also reported that the plasma calcium level of pullets in the high calcium fed group had a higher calcium content than that of the two low calcium fed lot. The results of the present investigations are in agreement with the above observations. The total plasma calcium concentration of six week old Japanese quail birds reared on the three levels of pre-laying dietary calcium were found to be significantly ($P<0.01$) different. Birds reared on the highest pre-laying dietary calcium (0.9 per cent), G III group had the highest (23.269 ± 0.075 mg/dl) plasma calcium concentration (Table 6.1 and Fig.8.1).

At the 16th week of age, the interaction in between pre-laying dietary calcium fed groups and layer dietary calcium fed subgroups was significant ($P<0.01$). Birds of GII B reared on 0.7 per cent pre-laying and 3 per cent layer dietary calcium had 28.775 $\pm$ 0.437 mg/dl of plasma calcium content (Table 6.3 and Fig.8.3). Birds of GII C subgroup reared on 0.7% pre-laying and 3.5 per cent layer dietary calcium had 30.150 $\pm$ 0.285 mg/dl. These results indicated that in Japanese quail maximum calcium retention was achieved in birds reared on an optimal level of 0.7 per cent pre-laying dietary calcium. Hurwitz and Bar (1967) reported that calcium retention and storage were influenced by dietary levels of calcium in pre-layers. There was significant ($P<0.01$) influence of layer calcium on the plasma calcium content (Table 6.7), subgroup C had the maximum plasma calcium content of 30.115 $\pm$ 0.200 mg/dl) (Table 6.3 and Fig.8.3).

At the 24th week of age there was no evidence of any influence of pre-laying dietary calcium on the plasma calcium content ($P<0.01$), however, calcium fed during the laying period significantly ($P<0.01$) influenced the plasma calcium content (Table 6.7). The highest plasma calcium concentration of 30.374 $\pm$ 0.120 mg/dl was recorded for the birds reared on the highest calcium level of 3.5 per cent layer dietary calcium and all the subgroups reared on 3.5 per cent layer

dietary calcium recorded values above 30 mg/dl (Table 6.5 and Fig.8.5). Furthermore, at the 16th and 24th week of age both the subgroups B and C reared on higher layer dietary calcium also recorded higher plasma calcium concentration (Tables 6.3 and 6.5). Similar pattern of observations were made in Japanese quail by Shrivastav et al. (1989) and in domestic fowl by Mueller (1958), Hurwitz (1964), Paul and Snetsinger (1969), Roland, Sr et al. (1973) and Rao and Brahmakshatria (1976).

In the present study there was highly significant increase in the plasma calcium concentration of the birds reared on higher dietary calcium either during pre-laying or layer periods. This can be due to increased absorption from the gastro intestinal tract and the consequent higher concentration in the blood. The fact that a preferential and direct utilization of calcium absorbed from the intestine for egg shell formation was highlighted by Navarro and Murillo (1976). They observed the unique feature in Japanese quail layers having a high propensity for egg production, to have proportionaly high blood calcium concentration compared to non-layers and males. According to them most of the calcium of dietary origin was transferred by means of blood for egg shell formation indicating that the egg gets more than 80 per cent of the dietary calcium absorbed. According to

Bragg et al. (1971) due to the high nutritive efficacy of utilization of calcium, the quail can be compared with the domestic hen in which case also calcium absorption rate may be as high as 70 to 80 per cent in layers.

The plasma calcium content of quail layers was not influenced by the age. There were no variation in the plasma calcium concentration at the 16th and 24th week of age when the birds were reared on rations with similar layer dietary calcium levels (Tables 6.3 and 6.5). Snapir and Perek (1970b) also noticed that there was no influence of age on the level of blood calcium in the laying domestic fowl. According to them the ability of hens to produce quality shells depended primarily on the availability of calcium from the ingested food and then from the skeleton. Birds usually consumed calcium in response to depletion of calcium or anticipated requirement of calcium for egg shell formation.

6.3.1.2 Plasma inorganic phosphorus

The plasma inorganic phosphorus concentration (10.677 ± 0.092 mg/dl) was the lowest in six week old birds of GIII group reared on the highest (0.9 per cent) pre-laying dietary calcium level (Table 6.1 and Fig.8.1). Similarly at the 16th week of age also (Table 6.3 and Fig.8.4) the highest layer dietary calcium (3.5 per cent) fed subgroup C had the lowest

plasma inorganic phosphate content (10.782 ± 0.110 mg/dl). There was significant ($P<0.01$) interaction between pre-laying and layer dietary calcium levels on the plasma inorganic phosphate content at the 16th week of age (Table 6.7). However, at the 24th week of age there was no significant interaction but only significant ($P<0.01$) influence of pre-laying and layer dietary calcium levels on the plasma inorganic phosphate content (Table 6.7). Birds of subgroup C reared on the highest layer dietary calcium (3.5 per cent) had the lowest plasma inorganic phosphate concentration of 10.845 ± 0.110 mg/dl (Table 6.5). The variation in plasma inorganic phosphate concentration was of lesser magnitude with higher levels of dietary calcium. However, Bolden and Jensen (1985b) could not notice any significant difference in the plasma inorganic phosphate content when maintained on higher dietary calcium level (3.5 per cent). This pattern of distribution of inorganic phosphate in the blood plasma indicated the operation of a mechanism of action of higher plasma calcium concentration resulting from higher level of dietary calcium intake by lowering the rate of bone resorption leading to lower plasma concentration of inorganic phosphorus. The lesser variation in the plasma inorganic phosphorus can be due to reduced resorption of bone when the blood calcium level is relatively high, the resorption of bone from the medullary bone can be reduced by the operation of the negative feed back

mechanism. Bloom et al. (1958) reported that medullary bone resorption during the advanced stages of shell calcification appeared to be a normal feature of bone metabolism in hens, even when, a ration high in calcium was fed. Calcium and phosphorus soon after their release from the medullary bone appear in the blood. The addition of dietary calcium increases the calcium concentration in the blood to a higher level. Part of this calcium may go to the shell gland for egg shell calcification and another part may go to medullary bone for cyclic bone formation and resorption which is a normal feature in layers. The reason behind higher variation in blood calcium compared to the relatively lesser variation in inorganic phosphorus can be due to the fact that while calcium and phosphorus are released simultaneously from the bone - medullary bone, calcium is being continuously withdrawn for egg shell calcification, while, phosphorus is not required for this process. It may be remembered at this juncture that calcium is being continuously absorbed from the diet at an increasing concentration which can also supplement any loss of calcium from the blood for egg shell calcification. Sturkie (1965 and 1976) and Vohra et al. (1979) reported that calcium absorption of layers fed on higher calcium diet was more efficient, the variation in plasma calcium in layers due to dietary calcium being a reflection of its higher absorption

from gastro intestinal tract and utilization of calcium for egg shell calcification. Furthermore, plasma inorganic phosphorus is liable for more urinary excretion (Paul and Snetsinger, 1969) which will eliminate phosphorus in a steady way so that its blood concentration remains less variable. Moreover, Levinsky and Davidson (1957) reported that parathormone secreted in higher concentration during active egg shell calcification is associated with augmented renal excretion of inorganic phosphorus. However, Shrivastav et al. (1989) reported an insignificant increase in serum phosphorus level along with the increase in the level of dietary calcium. Age did not seem to influence the level of plasma inorganic phosphate concentration (Fig.8.8).

6.3.1.3 Shell gland calcium

In Japanese quails there was no variation in the shell gland calcium concentration at the sixth and 24th week of age due to different levels of pre-laying and layer dietary calcium (Tables 6.2, 6.7 and Fig.8.2, 8.11). However, there was an interaction of pre-laying and layer dietary calcium levels on the shell gland calcium content at the 16th week of age (Table 6.7). At the sixth, 16th and 24th week of age the shell gland calcium concentration was 11.006 $\pm$ 0.040 mg/g, 11.180 $\pm$ 0.080 mg/g and 11.270 $\pm$ 0.050 mg/g respectively (Tables 6.1, 6.4, 6.6 and Fig. 8.13). The values indicated

the absence of any influence of age in the shell gland calcium content of Japanese quail layers. Snapir and Perek (1970a) and Prabhakar et al. (1977) reported that shell gland calcium content in domestic fowl was independent of plasma or dietary calcium levels. Age also did not influence the level of uterine calcium. Furthermore, Snapir and Perek (1970b) found that uterine calcium level was not influenced by the breed, rate of egg production or shell quality. The calcium concentration of the shell gland in Japanese quail layers was very low ranging from 10.201 $\pm$ 0.133 mg/g to 12.625 $\pm$ 0.135 mg/g of shell gland (Tables 6.1, 6.4 and 6.6). This revealed that shell gland did not store calcium to any appreciable extent which is similar to the findings of McCallion (1953) who reported that shell gland of domestic hen did not store calcium for shell formation. During the shell calcification a steady supply of this ion was maintained from the diet and through the mobilisation from the bone (Bloom et al., 1958; Prabhakar et al., 1977 and Taylor and Hertelendy, 1960). Cipera (1980) also observed that the calcium concentration of shell gland was very low. Therefore, it is apparent that there is no appreciable storage of calcium in the shell gland for egg shell formation. Hence it can be stated that calcium has to be continuously supplied to the oviductal tissue directly from the blood. Simkiss and Taylor (1971) reported

that calcium used for the formation of egg shell is derived from the blood since there are no stores of calcium in the oviduct. Bar and Hurwitz (1973) found that calcium concentration in the intestine changed with physiological need for calcium whereas the shell gland calcium remained relatively constant. Sturkie (1976) reported that shell gland does not store significant quantities of calcium and that the ion has to be extracted continuously from the blood. Farmer et al. (1983) hypothesised that calcium was utilized directly from the blood for egg shell calcification without first being deposited in the bones. Skeletal system stored some of the dietary calcium until it was needed for egg shell calcification. The results obtained during the course of this investigation indicated that the dietary calcium primarily go for egg shell formation during the active stages of shell calcification. During the active calcification stage the utilization of calcium by the shell gland from the mobilisation of skeleton (medullary bone) is comparatively less than that of the dietary calcium. Soon after the absorption the dietary calcium is utilised for shell calcification bypassing partly the skeletal storage of calcium. The results of the present investigation was in line with the above hypothesis. At higher dietary calcium levels the plasma calcium concentration was high with low inorganic phosphorus. Under this condition the shell gland stored only

negligible amounts of calcium with no appreciable alteration due to dietary calcium, which indicated that egg shell formation in quail layers occur preferably by direct utilization of dietary calcium. There seems to be no appreciable storage of calcium in the shell gland tissue, calcium may actually be transferred directly from the blood to the shell gland through their wall.

6.3.1.4 Shell gland phosphorus

At the sixth and 16th week of age dietary calcium did not influence ($P>0.01$) the shell gland inorganic phosphorus concentration in Japanese quails (Tables 6.1 and 6.7). However, at the 24th week of age layer dietary calcium produced significant ($P<0.01$) influence on shell gland, inorganic phosphorus concentration. In the birds of 24th week of age shell gland inorganic phosphate concentration was lowest in highest calcium fed subgroups GII C and GIII C (Table 6.6). There was no interaction between pre-laying and layer dietary calcium at the 16th and 24th week of age (Table 6.7). The low concentration of inorganic phosphate in shell gland as compared to that of plasma indicated the absence of storage of this mineral in shell gland. Bachra et al. (1963) reported that precipitation of calcium as calcium carbonate was prevented by the presence of low concentration of

phosphate ions, at the same time it also prevented calcium from being precipitated as calcium phosphate. Paul and Snetsinger (1969) reported that only little phosphorus is used up for shell formation, leading to its being built up in plasma and eventually being excreted via the kidneys. The present investigation revealed that the shell gland inorganic phosphate concentration was only in traces (1.845 $\pm$ 0.025 to 1.977 $\pm$ 0.008 mg/g tissue) as per Tables 6.1, 6.2 and 6.3 and not influenced by age (Fig.8.14). Prabhakar et al. (1977) reported that the inorganic phosphate level of shell gland of domestic hen did not show any significant variation between puberant Vs layers.

The following conclusions can be drawn from the results of the studies on the influence of dietary calcium on the mineral profile of female Japanese quail:-

1. The concentration of plasma calcium in Japanese quail layers was significantly influenced by an increase in dietary (both pre-laying and layer) calcium level.

2. As age advanced, at the 16th and 24th week of age pre-laying dietary calcium had only negligible influence on plasma calcium content when adequate level of dietary calcium was maintained in the layer ration.

3. Plasma calcium concentration was high in laying quails ranging from 21.131 ± 0.191 mg/dl to 30.500 ± 0.235 mg/dl.

4. Plasma inorganic phosphate content was influenced by the level of pre-laying dietary calcium at the sixth week of age.

5. At the 24th week of age both the pre-laying and layer dietary calcium levels influenced plasma inorganic phosphate concentrations.

6. At the highest level of dietary calcium, 3.5 per cent, the plasma inorganic phosphate concentration was the lowest.

7. Age did not influence the plasma calcium and inorganic phosphate level during the laying period.

8. Plasma inorganic phosphorus content of laying Japanese quail was ranging from 10.224 ± 0.210 to 12.172 ± 0.192 mg/dl.

9. Shell gland calcium concentration was not influenced by dietary calcium at the sixth, 16th and 24th week of age except for the interaction between pre-laying and layer dietary calcium levels at the 16th week of age during the laying period.

10. Level of the shell gland calcium was not influenced by plasma calcium content.

11. Shell gland concentration of calcium in Japanese quail was lower than that of plasma.

12. At all the age groups studied except for the 24th week (with significant influence of layer calcium on the shell gland inorganic phosphate concentration) pre-laying and layer calcium levels did not reveal any influence on shell gland inorganic phosphate concentration.

13. Shell gland concentration of inorganic phosphate was lower (1.845 $\pm$ 0.025 mg/g to 1.977 $\pm$ 0.008 mg/g) than that of plasma.

14. Age did not influence the shell gland inorganic phosphorus concentration during the laying period.

6.3.2 Enzyme profile in plasma and shell gland of Japanese quail

6.3.2.1 Plasma alkaline phosphatase (ALP) and acid phosphatase (ACP)

The results of the study revealed that the plasma enzyme profile of ALP and ACP of the birds aged six, 16 and 24 weeks were exhibiting a negative correlation with dietary

calcium levels (Tables 7.2 and 7.7). The plasma ALP and ACP content decreased with each increment of dietary calcium, both in the pre-laying and layer calcium levels (Tables 7.1, 7.3, 7.5). Similar observations were made in domestic fowl by Hurwitz and Griminger (1961) and Paul and Snetsinger (1969) and in Japanese quail for plasma ALP by Consuegra and Anderson (1967).

In the immediate pre-laying weeks increased mineralization of the skeleton was observed (Hurwitz and Griminger, 1960 and Hurwitz, 1964), with the intention of preparing the bones as a source of calcium for egg shell formation in the pre-laying period itself. Moreover, Hurwitz and Bar (1971) reported that calcium retention and storage can be manipulated by dietary calcium levels. Female birds develop medullary bone during the laying period which is an evolutionary adaptation related to production of eggs with calcified shells. Cyclic changes occur in the medullary bone during egg shell formation where intense bone formation and resorption occur in a definite pattern. It was on the basis of this fact that different pre-laying and layer dietary calcium levels were tried in female Japanese quails.

The results of the study revealed that both 0.7 and 0.9 per cent pre-laying dietary calcium levels had no

great significant difference in influencing the level of plasma enzymes (ALP and ACP) at the sixth week of age. The highest concentration of plasma ALP and ACP was recorded in the GI group (Table 7.1) reared on 0.5 per cent, lowest pre-laying dietary calcium. So also the birds at 16th week of age, which were reared on 0.5 per cent pre-laying dietary calcium recorded highest enzyme level of ALP and ACP (Table 7.3). The variation in the enzyme content was negligible at the 16th and 24th week of age with higher pre-laying dietary calcium levels (0.7 and 0.9 per cent). Hence it may be concluded that 0.7 per cent pre-laying dietary calcium was sufficient in Japanese quail, if proper layer dietary calcium was maintained during the laying period. As age advanced pre-laying dietary calcium had only negligible influence on plasma ALP concentration. At the 24th week of age plasma ACP content was significantly ($P<0.01$) higher in lower pre-laying as well as layer calcium fed birds, while ALP content was only influenced by layer calcium levels. However, there was an interaction between pre-laying and layer dietary calcium levels on the plasma ALP activity. Rao and Brahmakshatriya (1976) reported that calcium retention and storage can be manipulated by dietary levels of pre-laying calcium in White Leghorn pullets. The plasma ALP and ACP in fowls originate in the bones (medullary bone) from osteoblasts and osteoclasts respectively (Hurwitz and Griminger, 1961). Histochemical

studies by Pritchard (1952) strongly suggested the place of origin of plasma ALP as osteoblasts. According to Bell (1960) the variations of these enzymes in the plasma reflected the operation of a mechanism for calcification and resorption in bones. The fluctuation in the concentration of plasma ALP and ACP showed that utilization of dietary calcium for shell calcification was mediated through the activity of these enzymes on medullary bone. Driggers and Comar (1949) found a definite peak in the plasma ALP activity to precede the onset of lay in domestic fowl. There can be considerable turn over of skeletal calcium in the laying bird and very active resorption and recalcification in the medullary bone to cause an increase in plasma phosphatases activity during the early periods of egg production. If high producing hens do not receive adequate dietary calcium, a higher proportion of calcium is likely to be withdrawn from the bones for shell calcification. Such a withdrawal is rapidly followed by medullary bone formation Driggers and Comar (1949) and Jowsey et al. (1956) demonstrated that dietary calcium supplied less than half the requirement of shell calcium while the remainder was derived from the bone for the production of a given egg. Vohra et al. (1979) reported that calcium absorption of layers fed on higher calcium diet was more efficient; the variation in the plasma calcium in layers due to dietary calcium being a

reflection of its higher absorption and utilization for egg shell formation. A portion of calcium absorbed from the diet preferentially goes for shell calcification. Immediately after absorption at the higher dietary calcium level the calcium concentration shoots up in the plasma where the ALP and ACP concentrations are lower. The activity of plasma phosphatases is associated with bone formation and resorption. Hurwitz and Griminger (1961) observed a decrease in plasma ALP activity in hens as the dietary calcium level was increased. On the other hand if a hen continues to lay on a low calcium diet there can be a very rapid break down of medullary bone so as to meet the increased calcium demand for egg shell formation. This naturally resulted in an increase in the level of plasma phosphatases due to higher osteoblastic and osteoclastic activity. The results of the present study revealed higher plasma ALP and ACP activity because of increased osteoblastic and osteoclastic activity respectively, under the lower pre-laying and layer dietary calcium regimen. The laying birds under these conditions utilize the medullary bone calcium in addition to dietary calcium for egg shell formation. Bloom et al. (1958) and Hurwitz and Bar (1969) reported that medullary bone resorption during the advanced stages of shell calcification appeared to be a normal feature of bone metabolism in laying hens, even when a ration high in calcium was fed. So it may be expected that the effect on

medullary bone can be all the more pronounced when the level of dietary pre-laying and layer calcium levels are below normal leading to a higher ALP and ACP activity. Calcium and phosphorus soon after their release from medullary bone appear in the blood. The addition of dietary calcium to this blood calcium (medullary bone) increases the calcium concentration in the blood to a higher level. Part of this dietary calcium goes to the shell gland for shell formation and another part to medullary bone (if dietary calcium level is in excess) for cyclic bone reformation and resorption which is a normal feature in layers. The higher concentration of plasma phosphatases observed in the laying quails under lower levels of (pre-laying and layer) dietary calcium reflected a stimulating effect of osteoblastic recalcification process called into action by the drainage of skeletal calcium for egg shell formation.

It is revealed that the plasma ALP and ACP content was highest during the sixth week of age followed by 16th and 24th week. This indicated that the plasma enzyme levels were influenced by age. Similar observations were made in domestic fowl by Tanabe and Wilcox (1960); Sturkie (1965); Dimri et al. (1980) and Kansal and Gangwar (1984). However, the observations of Snapir and Perek (1970a) were at variance with this. The explanation for the variation in enzyme levels due

to age may be found in the alterations produced in homeostasis with respect to mobilisation of calcium between gut, blood, bone and oviduct, so as to establish a proper level of osteoblastosis and osteoclastosis to meet the requirement of calcium for egg shell formation.

During the early stages of egg production the body mechanisms might not have come to stability resulting in higher rate of osteoblastosis and osteoclastosis leading to a higher plasma phosphatase level. Moreover, a higher plasma ALP activity in younger laying quails may be expected due, to the presence of a higher level of endogenous oestrogen (Tanabe and Wilcox, 1961) especially at the onset of lay which is responsible for medullary bone formation.

As age advanced a stabilization of phosphatases might have been established to meet with the exingencies of calcium turnover. This may be one of the reasons for the increase in concentrations of plasma phosphatases seen in laying birds which continue to lay eggs with normal shells at a higher rate. Egg laying poses a major challenge to the regulation of calcium metabolism. Because of the high rate of calcium transfer to the egg shell, the ovulating bird must maintain high plasma concentration of calcium than a non-laying bird (Simkiss, 1961) for which higher plasma phosphatase level is a must.

6.3.2.2 Shell gland alkaline phosphatase (ALP) and acid phosphatase (ACP)

Salevsky, Jr. and Leach, Jr. (1980) and Darshan and Panda (1987) observed that the ALP activity of the shell gland was relatively lower compared to ACP activity. Results obtained in the present investigation also concur with this. It is also found that the level of shell gland ALP was lower than that of plasma. However, shell gland ACP content was higher than that of plasma. Prabhakar et al. (1975), NYS and DeLaage (1984a) reported that in the domestic foul shell gland ALP activity was lower than the ACP activity. The shell gland concentration of ALP and ACP was seen to be uninfluenced by the level of dietary calcium especially at the 24th week of age.

Nys and DeLaage (1984a) reported that the shell gland ALP content was uninfluential in shell calcification. Salevsky Jr. and Leach, Jr. (1980) reported that oviduct and prostatic ACP were considered to be similar which were inhibited by sodium arsenate, while serum ACP was not. The role of shell gland ACP in shell formation has not yet been vividly explained. It may be involved in the transfer of calcium ions across the shell gland mucosa since its concentration in the shell gland was comparatively high, even surpassing that of ALP and the pH in shell gland fluid is

towards the acidic side. At the same time the ACP concentration along with that of ALP was seen increased in the plasma during active shell calcification. This was a reflection of osteoblastic and osteoclastic activity. Such an activity need not be occurring in shell gland, hence their variation in shell gland was negligible. Shell gland ALP activity was very low, indicating that it may not be involved in the calcium transport through the shell gland mucosa to the egg shell.

Shell gland phosphatases were not influenced by age. In the case of shell gland ALP and ACP the stabilisation in the production of the enzymes might have been reached by the time the bird attained the age of six weeks for the process of egg formation. Thereafter there may not be any additional requirement of the enzymes for egg shell formation.

It can be concluded from the results of the study that:-

1. Plasma concentration of ALP and ACP was significantly higher in the lowest pre-laying and layer dietary calcium fed female quails during the laying period at the sixth, 16th and 24th week of age.

2. The lowest pre-laying (0.5%) and layer (2.5%) dietary calcium induced a marked increase in the concentration of plasma ALP in quail layers.

3. The plasma concentration of ALP and ACP were influenced by age. Younger age group had more plasma phosphatase level.

4. Plasma concentration of ALP was significantly higher than ACP, in layers.

5. Shell gland ALP content was independent of both pre-laying and layer dietary calcium levels.

6. Shell gland ACP concentration was marginally higher in the highest pre-laying dietary calcium fed group. It was also significantly influenced by the pre-laying and layer dietary calcium levels at the 16th week of age.

7. Shell gland concentration of ALP was markedly lower than its ACP concentration at all age groups studied.

8. Shell gland phosphatases were not influenced by age.

Summary

Chapter - 7

SUMMARY

An investigation into the structural and functional role of the shell gland of Japanese quail (Coturnix coturnix japonica) in the egg shell formation under different dietary (pre-laying and layer) calcium regimen was carried out at the Department of Physiology and Biochemistry, College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy.

Six hundred and thirty, three-week old, healthy female Japanese quails from the same hatch and strain were divided into three groups and maintained on starter ration with three pre-laying calcium levels (0.5, 0.7 and 0.9%) upto the sixth week of age. At the sixth week of age the birds were subdivided into nine subgroups and reared on standard farm conditions on layer ration with three layer calcium levels (2.5, 3 and 3.5%). Birds were reared upto the 24th week of age. Body weight was recorded initially, fortnightly and at the time of sacrifice (sixth, 16th and 24th week of age).

The study involved three phases of investigations. Phase one represented third to sixth week of age, phase two, sixth to 16th week and phase three from 16th to 24th week.

During each phase, 30 birds from each of the three groups/nine subgroups were sacrificed for collection of blood and oviduct for further study.

Birds started laying from 39 to 42 days (early sixth week). Egg production was recorded daily and studies on the egg shell quality were carried out on the sixth, 16th and 24th week of age.

The studies on the oviduct involved measurement of the length and weight of the oviduct and shell gland, histology and histochemical localisation of ALP and ACP of the shell gland and quantitative estimation of minerals (calcium and inorganic phosphorus) and enzymes (ALP and ACP). Plasma concentration of minerals (calcium and inorganic phosphorus) and enzymes (ALP and ACP) were also estimated.

The data from the above studies were statistically analysed.

Quails attained sexual maturity at the sixth week. The age at first egg ranged from 39 to 42 days. Peak egg production was attained at the 16th week and the trend continued even at the 24th week. Pre-laying dietary calcium ranging from 0.7 to 0.9 per cent and layer dietary calcium level of 3 per cent were optimal for growth and production in

quails. Growth rate was faster upto the 16th week of age but was marginal thereafter.

Pre-laying dietary calcium did not influence the development of the oviduct while 2.5 per cent layer dietary calcium level was optimum. The development of the oviduct was faster upto the 16th week of age (peak egg production), with only marginal variation especially in the length and weight of the shell gland, thereafter.

Histologically the wall of the shell gland was identical with that of the domestic fowl except for the tunica muscularis. This consisted of thicker inner circular and thinner outer longitudinal muscle layers. Older birds (16 and 24 weeks) showed numerous mucosal foldings. The inner circular muscle layer became thicker, even invading the lamina propria and was provided with rich blood supply. Highest (3.5%) layer dietary calcium fed quails showed increased vascularity in their shell gland connective tissue core of the lamina propria.

Egg shell quality traits like the egg weight, shell weight, shell thickness, specific gravity and shape index improved with increased levels of dietary calcium. As age advanced from the 16th to 24th week shell quality lowered because of increase in egg weight with concurrent decrease in

shell weight, shell thickness, specific gravity and shape index. Pre-laying dietary calcium level of 0.7 to 0.9 per cent and layer level of 3 per cent were sufficient for maintaining optimum shell quality.

Increase in the dietary calcium (pre-laying and layer) levels positively influenced the plasma calcium concentration. Dietary calcium level was inversely related to the plasma inorganic phosphate concentration. As age advanced the influence of pre-laying dietary calcium on plasma calcium content became insignificant. Both calcium and inorganic phosphate content of the plasma was uninfluenced by age.

The shell gland concentration of calcium was not influenced by dietary calcium and age. While the inorganic phosphate content of the shell gland was not influenced by age, at the 24th week of age it was influenced by layer dietary calcium levels. The shell gland concentration of calcium and inorganic phosphorus was lower compared to those in plasma.

Plasma concentration of ALP and ACP was inversely related to pre-laying and layer dietary calcium levels. Plasma concentration of ALP was much higher than that of ACP, and both of them decreased with age. Shell gland concentration of ACP was more than that of ALP. Histochemical

localisation of the shell gland ALP activity was weaker than that of ACP. Qualitative (histochemical) and quantitative concentration of ALP and ACP in the shell gland were independent of the age. Shell gland ALP concentration was not influenced by dietary calcium both in histochemical localisation and in quantitative estimation. The shell gland ACP concentration was marginally higher in quails reared on highest pre-laying dietary calcium.

Body weight, egg production, shell quality, plasma calcium and ALP concentration were not influenced by pre-laying dietary calcium at the 24th week of age.

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THE STRUCTURE AND FUNCTION OF THE SHELL GLAND IN JAPANESE QUAIL UNDER DIFFERENT LEVELS OF DIETARY CALCIUM

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ABSTRACT

Literature available on the physiological aspects of egg production in Japanese quail is scanty and many lacunae exist in the knowledge of various mechanisms involved in the formation of egg. Under the modern practice of intensive quail husbandry it is often found that the egg shells of Japanese quail are becoming thinner, more fragile and easily broken. Consequently, safety in transportation and marketability of these eggs are considerably reduced. Hence it was thought worthwhile to investigate some of the factors involved in egg production, more especially the egg shell formation. In the present investigation attempts were made to elucidate the mechanism of the egg shell formation with respect to the structural and functional peculiarities of the shell gland in Japanese quail (Coturnix coturnix japonica) under different dietary levels of pre-laying and layer calcium. The study envisaged histological, histochemical peculiarities of the shell gland; the mineral (calcium and inorganic phosphorus) and enzyme (ALP and ACP) profile of the plasma and shell gland. The influence of dietary calcium on the body weight, development of the oviduct, egg production and egg shell quality was also investigated.

Japanese quails attained sexual maturity early at the sixth week of age, by which time they attained a body weight of 131.8 ± 0.600 g and started egg production. The birds grew at a faster rate in the early weeks, came into peak production at the 16th week of age, and the trend continued even at the 24th week of age, with a body weight of 198.033 ± 0.220 g for good production. The length and weight of the oviduct increased from the sixth to 24th week of age. The variation in length and weight of the shell gland from the 16th and 24th week was marginal. Growth rate was almost marginal from the 16th week onwards.

Histologically the quail shell gland showed similar structure to that of the domestic fowl except for the tunica muscularis. The inner circular muscle layer was thicker and outer longitudinal layer was thinner. As age advanced, at the 16th and 24th week of age the mucosal foldings were numerous. The inner circular muscle layer became thicker and even this layer invaded the lamina propria and the vascularity in the connective tissue core of the tunica muscularis increased.

The egg quality traits such as egg weight, shell weight, shell thickness, shape index and specific gravity markedly improved as age advanced from the sixth to 16th week. From the 16th to 24th week the egg quality was lowered with an

increase in egg weight, lowering of shell weight, shell thickness and specific gravity.

Dietary calcium significantly influenced the body weight, egg production, shell quality, histology of the shell gland and mineral and enzyme profile of plasma.

Birds exhibited optimum growth, and production at higher pre-laying and layer dietary calcium levels. Pre-laying dietary calcium level of 0.7 to 0.9 per cent and layer level of 3 per cent were found to be optimum in quail ration. As far as the oviduct development is concerned, pre-laying dietary calcium had negligible influence, whereas layer dietary calcium level of 2.5 per cent was just sufficient for optimum development of oviduct/shell gland. Histologically the only difference noticed in highest (3.5%) layer dietary calcium fed quails was the increased vascularity in their connective tissue core of the lamina propria of the shell gland.

There was a positive correlation between dietary calcium (pre-laying and layer) level and plasma calcium concentration at the sixth, and 16th week of age. But at the 24th week of age only layer dietary calcium induced such a relationship. As age advanced, pre-laying dietary calcium became insignificant.

In the case of plasma inorganic phosphate concentration, there existed a negative correlation to that of pre-laying and layer dietary calcium levels at all the age groups of quails. It may be emphasized in this context that the extent of the negative correlation existing between plasma inorganic phosphate and pre-laying and layer dietary calcium levels was considerably lower. The concentration of calcium and inorganic phosphorus in the plasma and shell gland were uninfluenced by the age. The shell gland concentration of calcium and inorganic phosphorus was lower compared to their plasma level. These minerals were not stored in the shell gland for shell calcification. At the time of calcification calcium was transferred from the plasma (blood) to the shell gland and then to the shell. Dietary calcium significantly influenced the plasma concentration.

Plasma enzyme concentration of ALP and ACP was negatively correlated with dietary calcium (pre-laying and layer) levels, whereas the shell gland ALP concentration was uninfluenced by the dietary calcium and age and its concentration was very low compared to those of plasma ALP and shell gland ACP. Shell gland ALP was unimportant in shell formation. Histochemical localisation of the shell gland ALP was in confirmation with that of quantitative estimation. Shell gland ACP content was comparatively higher than ALP,

which was also in agreement with histochemical localisation. Shell gland ACP may be involved in the transfer of calcium from the plasma to the shell through the shell gland mucosa, since its level in the shell gland was higher and influenced by dietary calcium. Variation in the plasma concentration of ALP and ACP as influenced by dietary calcium levels were related to the cyclic medullary bone formation and resorption which is a normal feature in laying birds.

Variations in the dietary calcium induced changes in the concentration of plasma minerals and enzymes supported the view that dietary calcium is important for proper shell formation. Higher level of dietary calcium improved the egg shell quality, egg production and body weight. Pre-laying dietary calcium level of 0.7 to 0.9 per cent and layer level of of 3 per cent were found to be optimum in quail ration.