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# Physiological studies on calcium level in poultry blood and eggs

A thesis presented

*By*

**Samir Ahmed Abo-Eleyon**

B.V.Sc

(Cairo University)
1982

For the degree of M.V.Sc (physiology)

*To*

*Alexandria University*
*Faculty of veterinary medicine*
*Department of Animal Physiology*

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# Under the supervision

*Of*

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**2000**

بسم الله الرحمن الرحيم

# قرار لجنة الحكم والمناقشة

قامت لجنة الحكم والمناقشة بفحص الرسالة وترى انها اشتملت على بحثا هادفا ومواضيع لها اهميتها فى مجال الفسيولوجيا كما قامت اللجنة بمناقشة المتقدم مناقشة مستفيضة ووجدت ان الطالب ملما الماما كاملا بكل ما جاء بها.

**لـــــــذلك**

قررت اللجنة ترشيح السيد ط.ب/ سمير احمد ابو العيون للحصول على درجة الماجستير فى العلوم الطبية البيطرية ( تخصص فسيولوجيا)

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CONTENTS

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# Introduction

Late embryonic mortality in chickens eggs is one of the most serious problems in poultry industry, where the hatching process represented the final stage in a long chain of efforts and money waste.

There are many factors which interfere with each other to produce this problem, some of them are related to the parent stocks e.g (diseases and nutritional factors).

The others related to the egg handling from the laying till the hatching time which includes (time of egg sitting after laying , period of egg storage before sitting , conditions of storage , requirements of incubation, water loss, egg turning during incubation and shell quality.

The shell thickness, which plays an important role in the success of the hatchability process, where the more thicker shell would unable the chick embryo from pipping the egg during the hatching process.

The shell is broken by hydraulic pressure created by infiltration of lymph and serous fluid into the chick muscles , rather than by excitation ,contraction and coupling of the skeletal muscle ***(Samil ,1964)***.

The activation of the muscle complexus during pipping and hatching results from concentration of the calcium ions, regulating actomyocin ATP ase activity. Actomyocin ATP ase activity is stimulated by the calcium ***(Christensen and Bieller, 1982)***.

## INTRODUCTION

There are two sources of calcium for chick embryos( the egg shell and egg content). But the primary source after 15 days of incubation is the shell ***(Johnston and Comar, 1955)***, where as 75-80 % of the calcium in the newly hatched chicks is derived from the egg shell during the normal incubation ***(Romanoff, 1967 and Crooke and Simkiss, 1974)***.

The shell consists of 98.4 % solid material and only 1.6 % water . Of the solids , 95.1 % is inorganic matter , mainly calcium carbonate in the form of calcite ***(Romanoff and Romanoff, 1949)***. About 99 % of the calcium is deposited in the bones, the other 1 % is in the blood or soft tissues.. Although calcium makes up only a small percent of the body fluids , it is necessary for egg shell formation `blood clotting , enzyme systems , calcification of tissues and for regulation of irritability of the nerves and muscles .

The aim of the present work was to study some factors affecting calcium transfer from the shell to the chick embryo during incubation.

For this purpose eggs were treated during incubation with calcitonin , vitamin $D_3$ and calcium channel blockers verapamil hydrochloride (isoptin)$^{R}$ .

Calcium and phosphorus were determined in egg shell after hatching blood and bones of chicks embryos. The hatchability of the treated eggs was correlated with the different treatments employed.

# Review of literature

## I) Calcium distribution in the body

The majority of the calcium of the body (99%) is present in the inorganic matrix of bone as hydroxyapaetite, most of the remaining calcium (0.9%) is sequestered in the plasma membrane and endoplastic reticulum of the cells. Extracellular fluid contains 0.1% of the body calcium mass, with a total Ca. concentration of about 2.5 mmol/litre ***(Brown., 1994)***.

Approximately 50% of the extracelullar calcium (1.2 mmol/litre) is in the ionized form ($Ca^{++}$) which is biologically active form of calcium ***(Hazewinkel,1991 and Chew et al., 1992)***.

### *Calcium transfer from egg to chick embryo*

Egg shell of infertile egg has been studied by a number of workers including ***(Tyler,1961. and Hunt and Voisey, 1966)***, but relatively little work has been carried out on the shell of fertile incubated egg.

***Romanoff (1929)*** reported that calcium was probably passed to the embryo from the shell through the shell membranes .

***Driggers et al .(1951)*** using radioactive calcium found that the only possible mechanism for the $Ca^{45}$ to have richen the yolk sac , which was drown into the chicks prior to hatching , was by diffusion from the shell and to much lesser extent from egg white .

***Nozaki et al. (1954)*** studied the transference of $Ca^{45}$ in the hens egg during the development of the embryo. This study was carried out by injection of $Ca^{45}$ into the albumin or the yolk of the eggs prior to incubation . Their results indicated that, during the early and middle stage , most of the calcium comes from the albumin and the yolk is used for embryo development .During the late stage, most of the calcium comes from the shell .These workers used the first and the second eggs laid by hens within three days after oral administration of $Ca^{45}$ to study the utilization of the shell calcium by the embryo. They found that in the final stage of developed embryo 25% of the total amount of calcium was from the yolk , and 75% was from the shell .

***Jonston and Comar (1955)*** labeled the albumin calcium and found that shell calcium was starting to be mobilized at about the tenth to eleventh day of incubation.

***Edwards and Mraz (1961)*** reported that $Ca^{45}$ were transferred from the shell to the embryo .

The primary source of calcium (over 80%) for the developing embryo is dissolution of the shell via the chorioallantoic circulation (***Johnston and Comar, 1955)*** and the only source of calcium in late incubation is the shell. ***(Nozaki et al., 1954; Johnston and Comar., 1955 and Ono and Wakausgi., 1984)***.

***Crooke and Simkiss (1974)*** recorded that 75-80% of the calcium in the newly hatched chicks is derived from the egg shell.

During normal incubation , active calcium transport from the shell begins by 12-14 days ***(Johnston and Comar., 1955)***.

## *Vit D & Calcium transfer from egg shell to chick embryo:*

***Tuan and Scott (1977)*** stated that without adequate vitamin $D_3$, the onset of the calcium transport from the egg shell via the Chorioallantoic membrane is attenuated because of lack of vitamin $D_3$ metabolites .

***Sund et al. (1978)*** showed that the developing chicks embryo can utilize 1,25-$(OH)_2D_3$ because injecting the metabolitis into eggs prevented rickets and allow normal development and hatchability .

***Kubota et al. (1981)*** found that the embryonic chicks renal 25-hydroxy-1- hydroxyylase does not become active until the $8^{th}$ day of incubation .Therefore, 1,25-$(OH)D_3$ can be converted to 1,25-$(OH)_2D_3$ at this stage to support calcium transport for the skeletal calcification .

## II) Egg shell:

***Muir et al. (1976)*** recorded that substantial economic losses are incurred in the poultry industry from broken or cracked egg shells.

Efforts were made to improve egg shell quality by feeding different sources of calcium, different size particles of calcium sources, or a combination of the two ***(Scott et al., 1971).***

Feeding calcium in particulate form has been found to increase egg shell thickness and or egg shell quality***( Chenge and Coon, 1990a and Guinotte and Nys, 1991).***

### a) Calcium in egg shell :

***Bradfield (1951)*** recorded that the shell of an average egg contains 1.5-2 g calcium ,most of which is deposited during 15 hours prior to oviposition .

***Romanoff and Romanoff (1949)*** stated that the shell consists of 98.4% solid material and only 1.6% water .Of the solids 95.1% is inorganic matter, mainly calcium carbonate in the form of calcite ,3.3% is protein in nature and there is a trace of lipid materials .

***Taylor and Kirkley (1967)*** showed that the mean weight of the shell calcium was 2.04 g. compared with the mean calcium retention on laying days of 1.6 g. the birds were in negative calcium balance on most laying days .

***Scott et al. (1969)*** found that the high proceeding laying hens need enough calcium to produce strong egg shell .Each large egg contains about 2 - 2.2 g. of calcium .

Calcium absorption from the intestinal tract of laying hens is relatively poor, only 50-60% of the dietary calcium intake is available for egg shell formation , calcium retention depend to some extent upon the level of the calcium in the diet. Therefore, a mature hen laying an egg each day requires more than 4 g. of calcium per day for egg shell formation of maximum breaking strength ***(Scott et al.,1969).***

***Sturkie (1976)*** reported that each egg laid contains approximately 2 grams of calcium .The ingestion, absorption and turnover of calcium must therefore be very high , in order to supply the calcium required for shell formation .

***Guyer et al. (1980)*** stated that the production of shell for a 60 gm egg requires about 2.4 g of calcium. This calcium must come ultimately from the gut and be transferred by the blood to the shell gland .

***Soares (1984)*** reported that the average table egg contains 2g. of calcium ,the skeleton of the hens body weight 2 kg contains approximately 20 g. of calcium .Therefore ,each egg contains about 10% of the total body calcium .

***b) Effect of shell calcification on calcium metabolism and serum calcium level***

***Tyler (1946)*** suggested that during shell formation calcium from the digestive tract is not able to meet the requirement for shell calcification .It was postulated that any difference was made up by bone minerals resorption ***(Taylor., 1970)***.

***Hertelendy and Taylor (1961)*** reported that egg shell calcification is associated with a fall in the plasma calcium. Furthermore, it has been shown that this fall is related to the actual process of calcification , and not to a reduction in the rate of calcium absorption from intestine .

***Simkiss (1967)*** reported that during calcium depletion, hens can mobilize as much as 38% of their bone mineral.

***Simkiss and Taylor (1971)*** observed that the shell gland has a maximal calcium transport of 100-150 mg/hour , at this rate the blood calcium would be depleted within a period less than 30 min. if increased intestinal absorption and bone turnover did not occur.

***Sturkie (1976)*** reported that the blood of laying hen contains about 20-30 mg. calcium .Since shell formation involves the withdrawal of 100-150 mg/hr. the concentration of calcium in blood would be zero within 8-18 min. if it is not replenished continuously through intestinal absorption and mobilization from the bone. The relative importance of these two organs as sources of egg shell calcium seems to depend on the concentration of calcium in the food.

***Farmer et al. (1986)*** concluded that the skeletal calcium utilization is directly related to time and level of calcium intake. The greater the dependent on skeletal calcium, the less the quantity of calcium deposition on the egg shell, the utilization of skeletal calcium for shell formation ranged from 28-96%.

***c) Time required for shell calcification:***

***Bradfield (1951)*** reported that the main period of shell calcification lasts about 16 hours.

***Roland et al. (1973)*** stated that the process of egg shell formation occupies about 21 hours, but the rate of calcium deposition is slow for the first 5 hours.

***d) Effect of oviposition time on shell quality .***

***Roland et al. (1973)*** studied the effect of oviposition time on shell quality, in general, the afternoon oviposition, the egg was laid with better egg shell, with the possible exception of the first egg in the sequence .These results are in agreement with ***Burmestar et al. (1939)*** who reported that shell thickness of egg laid by the same hen vary from day to day .

***Wilhelm (1940)*** stated that the last egg in the sequence had a thicker shell than the eggs intervening between the first and the last egg. Because the last remains in the uterus longer ***(Berg, 1945)***.

***e) Calcium level in laying hens ration:***

***Pre-laying period***

***Hughes and Wood-Gush (1971)*** stated that pullets should be fed diets containing no more than 1.2% calcium until they are 18-20 weeks of age .

***Hurwitz (1976)*** recommended that a pre-laying diet containing approximately 2g. calcium /kg should be feed for at least 2 weeks before the onset of egg production.

***Wideman et al. (1985)*** reported that in practice , maintaining such a feeding regime of 3 diets containing 10, 20 and 35-40 g. calcium /kg respectively, for growing , prelaying and laying periods is complicated and may be unsafe. It may lead to feed laying hens a pre laying diets containing 20g. calcium/kg, insufficient to support egg production, alternatively to feeding pullets that begins to lay late a diet rich in calcium (35-40g/kg), thereby causing kidney lesions and disturbances in calcium hemostasis or Vit D metabolism. On the other hand, ***Bar et al. (1998)*** found that feeding pullets with prelaying diets containing 3.9% calcium did not affect the performance or shell quality during the whole productive periods, whether, the birds started to lay early or late, the dietary treatment did not cause renal damage .

***Laying period.***

It is known that the requirements of laying hens for calcium differ according to the hens age and the percent of egg production ***(Nagwa ,1986)***.

***Table (1)* Absolute dietary levels of calcium needed at different rates of production *(Scott et al.,1969)*.**

| Production | **Dietary calcium needed per day** | |
|---|---|---|
| | Young pullets (22-40) weeks of age | Mature hens after 40 weeks of age) |
| % | Gms | Gms |
| 100 | 3.3 | 3.7 |
| 90 | 3.0 | 3.3 |
| 80 | 2.7 | 3.0 |
| 70 | 2.3 | 2.6 |

***f) Calcium and phosphorus sources in poultry rations :***

***Leeson and Summers (1997)*** reported that calcium is usually supplied in the form of Limestone or Oyster shell. Both ingredients are highly soluble and are approximately 38% calcium in composition .Whatever, source is used large particle size appears to be more beneficial .This is because such particles are retained in the upper digestive tract and dissolved more slowly providing a more uniform and sustained release of calcium .While ,the dicalcium phosphate is commonly used in poultry diets because it is available to the birds by 100% and containing around 23% calcium and 20% phosphorus.

***The Histochemical structure of egg shell***

***Romanoff and Romanoff (1949)*** recorded that the hard, calcareous shell has an average thickness of approximately

0.3mm. The shell consists of 98.4% solid material and only 1.6 % water. Of the solids 95.1% is inorganic matter, mainly calcium carbonate in the from of calcite , 3.3 % is protein in nature and there is a trace of lipid material. The in organic content of the shell is distributed either in definite concentrations, in the mammillary cores and the cuticle , or in a more diffuse form, in shell matrix throughout the greater part of the shell substance.

***Simons (1971)*** reported that the egg shell consists from three layers , of distinct structure and chemical composition , the mamillary , the palisede and the cuticle layer.

***1)The mammillary layer***

The mammillary layer comprises about one third to one fifth of the total thickness of the shell. It consists of numerous roughly conical knobs, the mammillae, whose apiece are embedded in the outer shell membrane and whose irregular bases are fused together to from the foundation of the palisade layer ***(Simons, 1971)***.

***Simkiss (1967)*** found that centrally within the tip of each mammilla is a mass of protein material , the mammillary core which appears to be the center where calcification starts during the formation of the shell. Around the core are fibrous rings ***(Terepka, 1963)***.

The mammillary core can be considered to be small masses of organic matter attached to the outer layer of the shell membrane, which become embedded within the shell **(Simkiss, 1968 and Bellairs and Boyde, 1969)**.

***Simons and Wiertz (1963)*** recorded that the sides of the maimmillae are covered with 0.1-0.5um thick membrane, within the mammilla the matrix consists of a very fine fibrous meshwork with a maximum fibril diameter of 0.008 um and a mesh size of 0.1 um.

***Simons (1971)*** found that minute vesicle are embedded in the meshwork of the matrix (0.8um. maximum diameter).

***Simons and Wiertz (1963)*** found that within the mamillary base a much larger (9 um maximal diameter) irregularly branched cavities which appear by the electron microscope called Sajner's ring.

The chemical nature of the matrix has been investigated by a number of authors ***Simkiss and Tyler (1957)*** have shown that it consists of a protein-acid mucopolysaccharide complex. At least 70% of the matrix consists of a non-collagenous protein , hydroxy prolin being absent ***( Baker and Balch , 1962 and Frank et al.1965)***. Of the remainder, 11% is polysaccharide in nature, with chondriotin sulphates A and B comprising 35% of the total polysaccharide ***(Baker and Balch, 1962)***. The presence of some sialic acid has been reported ***(Frank et al.1965)***.

***Simkiss and Tyler (1957)*** reported that the mamillary core composed from protein, carbohydrate and fat

***Robinson and king (1968)*** recorded that the histochemical structure studies shows that the main part of the core contained neutral mucopolysacchraide and that it was surrounded by substances believes to be sialomucins.

Biochemical investigation by ***(Cooke and Balch, 1970)*** confirm the presence of a neutral mucopolysaccharide in the cores and describe the sialic acid containing material as being applied to the outer surface of the core.

***2)The palisade layer***

The palisade layer, otherwise termed the spongy layer in the older literature, it is continuation of the bases of the mammillae and form the greater part of the thickness of the shell. It is penetrated by 7000 to 17,000 pores, which are funnel-shaped cavities arising between the mammillae basely and opening beneath the cuticle on the surface of the shell. The pores may be 15-65um in diameter at the mouth and 6-23 um at the inner end ***(Tyler, 1956; Simkiss, 1967).***

The matrix of the palisade layer is structurally composed of fibrils up to 10 um long and 0.01um thick running parallel to the surface of the egg shell. Associated with these fibrils, mostly attached to them or lying along their axis, are vesicles about 0.4um in diameter, termed vesicular holes by ***Simons (1971)***

***Simkiss and Tyler (1957)*** reported that the matrix is composed of a mucopolysacchride bound to protein and, similar to chemical constitution to the mammillary matrix.

The mineral content of the palisade layer is essentially a continuation of the crystal columns which originated in the mammillary layer. The crystals are almost pure calcium carbonate in crystalline form of calcit **(Romanoff and Romanoff, 1949 and Cain and Heyn, 1964).**

*3) The cuticle:-*

The cuticle is the outermost layer of the egg shell, it is a thin transparent coating consisting predominantly of organic matter, which was considered to be mucin by ***(Moran and Hale, 1936)***. The thickness of the cuticle varies in different eggs and in different parts of the shell of any one egg

***Simons and Wiertz (1963)*** found that the thickness of the cuticle varies in different eggs and indifferent part of the shell of any one egg, it was 5- 10um ***( Romanoff and Romanoff, 1949)*** and between 8.3 and 12.8um in one egg and between 1.7 and 2.3um in another ***(Simons and Wiertz, 1963)***.

***Romanoff and Romanoff (1949)*** reported that the cuticle has been divided into two layers .And the electron micrographs of ***Simons and Wiertz (1963)*** tend to confirm this as the outer quarter of its thickness is much more compact than the remainder.

***Simons and Wiertz (1970)*** found that the cuticle has a vesicular structure with air space between the vesicles. The greater part of the cuticle consists of round to oval vesicles with diameters up to 1um. They may be empty or partially filled peripherally or centrally with granular material.

***Baker and Balch (1962)*** reported that the cuticle is composed mainly of a protein which is different in composition from the protein of the shell matrix. The remainder of the content is mainly polysaccharide (***Baker and Balch , 1962 and Cooke and Balch, 1970)*** and possibly a little lipid material ***(Simkiss., 1958)***.

## IV) Calcium in the bone

### *Structure and maintenance of the skeleton*

The skeleton of the domestic fowl is composed of three different types of bone tissue, namely compact cortical bone found in the diaphysis of the long bones cancellus bone found in the vertebrae and epiphysis of the long bone and specialized medullary bone in the marrow cavities of certain bones ***(Newman and leeson ,1997)***. Medullary bone acts as an important calcium reserve for shell calcification.

**Newman and *Leeson(1997)*** recorded that the formation and destruction of bone material in the skeleton is carried out by two specialized types of cells osteoclasts and osteoblasts. Osteoclasts are responsible for bone resorption and the subsequent release of calcium and phosphorous from the skeleton to supply these essential minerals to other organ systems or for excretion. Osteoblasts are responsible for the deposition of organic matrix which will eventually become mineralized bone tissue ***(Hodges ., 1974).***

### *Medullary bone formation*

***Taylor (1965)*** reported that in maturing chicken , medullary bone developed 10 to 14 days before the first egg was laid and the retention of dietary calcium markedly replaced before laying began .

***Simkiss (1967)*** recorded that most of the increase in the skeletal weight during 10 days before sexual maturity was caused by the formation of a new type of bone , which occurred only in female birds. Because this new bone was most easily observed in the marrow cavity of the femur and tibia , it has been called medullary bone .

***Taylor (1970)*** stated that the increase in skeletal weight by about 20 % during 10 days before pullets started to lay indicating that most of the additional mineral was incorporated into bone.

***Diatery calcium levels and calcium contents of bone:***

***Hurwitz and Bar (1971)*** recorded that raising the calcium in the pre-laying diets during sexual maturation was found to increase the calcium content of the bone.

***Chenge and Coon (1990b)*** found that increasing dietary calcium concentration above normal values have been shown to result in greater medullary bone formation.

***Frost and Roland (1991)*** reported that tibia breaking strength, tibia weight and bone mineral content increase significantly with increasing dietary calcium ,in Dekalb.XL 25 weeks old, pullets.

***Keshavarz and Nakajima(1993)*** found that increasing the daily intake of calcium to up to 5.5% of intake had no effect on percentage of bone ash or bone calcium. Where as ***Farmer et al. (1986)*** and ***Willson and Duff(1991)*** reported that the nutritional deficiencies of calcium have been shown to result in bone loss.

***Dietary calcium content and bone ash.***

**Itho and Hatano (1964)** recorded that the levels of dietary calcium , phosphorus and vitamin D regulate bone ash content. In many cases , the tibia has been used for analysis they studied the comparison of calcium metabolism in various bones (femur, tibia, metatarsus and toes, wing bones ,upper trunk bones in (one ,two and three weeks of age )in varying status of vitamin D supplementation . The data stated that in every case values for the femur were more, similar to the total skeleton than those were from any other bone sample. Consequently, the femur appeared to furnish a good index of calcium metabolism in the chick skeleton.

***Rogler and Parker (1972)*** showed that tibia ash was significantly increased by increasing the calcium level . Also , they declared that the breaking strength of tibia was good measure of dietary calcium level as was tibia ash.

***Keshavarz (1987)*** recorded that the feeding of non laying pullets with diet containing 35 g. calcium /kg increase bone ash in the young layer .

The increasing dietary calcium concentration above normal values have been shown to result in small increases in bone ash and breaking strength ***(Chenge and Coon, 1990$_{a,b}$ and Guinotte and Nys, 1991)*** .

***Frost and Roland (1991)*** reported that tibia ash increased significantly with increasing dietary calcium , in Dekalb. Xl pullets , 25 weeks old. On the other hand. ***Clark (1969)*** reported that the removal of calcium from the diet had no significant effect on the ash percentage of the femur.

***Vitamin D and calcium content of the bone:-***

***Maynard et al. (1979)*** recorded that vitamin D plays an important role in the absorption and deposition of calcium in the bones . There exists a dynamic equilibrium between the various forms of calcium, in turn the total concentration of calcium of the blood is governed by dietary and hormonal agencies influencing absorption and the accumulation or release of calcium from the skeletal sources ***(Maynard et al, 1979)***.

Bone mineralization of the pullets , as judged by tibia breaking strength and severity of rickets scores was improved by increase vitamin D3 of turkey hen diet ***(Stevens and Blair, 1984)***.

***Soars et al. (1988)*** found that hydroxy cholecalciferol increase tibia breaking strength in 40-week-old hen.

***Frost and Roland (1990)*** stated that supplementing the diet of 72. week-old hens with 0.5 or 1 ug of 1,25, DHCC /kg significantly and lineary increased tibial bone density and breaking strength. On the other hand ***Renni et al. (1997)*** recorded that feeding of 5ug 1,25-DHCC/kg to laying hens does not have any net effect no bone structure .

***Dietary vitamin D and bone ash.***

Increasing the level of vitamin $D_3$ (7500 I.u/kg of diet) with high calcium had no effect on growth and produced only a small increase in the ash content of the tibia ***(Motzak et al., 1965).***

***Boris et al. (1977)*** found that all of the metabolites and analogies of cholecalciferol increased tibia ash weight .

***Bar et al.(1978)*** found that bone ash was reduced by dietary calcium ,phosphorus or cholecalciferol restriction .

***Vohra et al. (1979)*** showed that the dietary deficiencies in calcium or vitamin D resulted in reduced female tibia ash of quail and Loghorn hens. On the other hand. ***Chang and McGinnis (1967)*** stated that a deficiency of vitamin D did not affect the percentage bone ash of laying hens .

## V) Calcium in the blood

### *Calcium levels in the blood*

***Maynard et al.(1979)*** found that the plasma calcium level of most species is maintained at approximately 10mg/dl under the normal circumstances although, the level can be 3-4 times higher during egg production. Over half of this is in the soluble ionized form while about 40% is protein bound.

***Serum calcium in laying hens:***

***Charles and Hogben (1933)***found that serum calcium concentration during egg formation was about 20% higher than that during non laying period.

***Greenberg et al. (1936)*** showed that a great increase in serum calcium level from 12 mg /100ml to reach 31.6mg /100ml at two days before the onset of laying followed by a gradual decrease to 23mg /100ml two weeks after laying.

***Sturkie (1954)*** reported that the plasma calcium of the laying hens ranged from 20 to 25mg /100ml.

***Ghany et al (1961)*** studied the effect of sexual maturity and egg laying capacity on blood constituents, in Fayoumi and Rhode Island red chicken . They found that blood calcium level showed a significant increase in the two breeds at the onset of laying.

***Simkiss ,(1967)*** recorded that the concentration of the serum calcium increased from 10mg /100ml to 16-30mg/100ml during 10 days before pollet started to lay. The serum calcium level varied according to the stage of egg calcification ***(Paul and snetsinger., 1969 and Miller et al., 1978).***

The plasma calcium level of most species is maintained at approximately 10mg /dl under normal circumstances, although level can be 3-4 times higher during egg production.***(Maynard et al., 1979)***.

## Factors affecting serum calcium in laying birds

***a)Calcium serum and age.***

At 50 weeks of age serum calcium in the female geese ranged between 32 and 50mg with a mean of 39 . 2mg ***(Hunt et al., 1964 )*** . While , ***El -For (1984)*** found that total calcium in the female turkey increased enormously from 28 weeks up to the maximum value at 40-44 week of age.

***b) Serum calcium level and time of oviposition :***

***Paul and Snetsinger (1969)*** observed that the highest plasma calcium level was attained. 1 hour post oviposition failing slowly towards the latter stage of shell calcification .

***Sloan et al. (1974)*** found a positive correlation between serum calcium level and the time of oviposition . On the other hand, the results of ***Miller et al.(1977)*** indicated that serum calcium did not change during the 10 hours post oviposition.

***Sloan (1976)*** reported that the serum level at 4 hours before and after oviposition was significantly lower than that at time of laying .

***Bacon et al. (1980)*** reported that turkey hens in reproductive pause had relatively low level of both calcium binding protein and total plasma calcium compared to laying hens .

***c) Blood calcium and egg production :***

***Urist et al. (1960)*** stated that the active ovulating female bird had a double or thrice calcium level to that observed in sexually immature female.

***Hunsaker and Sturki(1961)*** reported that during egg shell formation , the decrease in total plasma calcium across the uterus was greater than that when the uterus did not contain an egg with a shell being formed .

***Snapir and Perk (1970)*** found positive correlation between egg production and total plasma calcium .

***Solmon (1971)*** reported that a maximum level (23.5mg/100ml )of plasma calcium was found with the egg in the magnum , two hours after oviposition, and a minimum level (10.2mg/100ml) 14 hours after ovulation. The difference between these maximum and minimum values was significant ($P< 0.05$). During the latter half of shell calcification .

***Nagwa (1986)*** showed that mean calcium value during shell calcification was 21.5 ± 1.7 mg/100ml compared with 26.9 ± 1.8 mg/100ml before calcification.

***d) Serum calcium & Vit D3:***

***Dukes (1955)*** recorded that the main action of vitamin $D_3$ appears to increase the absorption of calcium by increasing the permeability of the intestine to calcium salts. The resulting in increases in serum calcium level leads to decrease parathyroid activity .

***Hurwitz and Bar (1972)*** reported that the vitamin D deficiency resulted in a marked hypocalcemia .On the other hand , ***Ramp et al.(1974)*** found that serum calcium of vitamin D deficient chicks decreased one week after the beginning of the treatment and continued to decrease progressively afterwards.

***Deluca(1980)*** showed that plasma calcium controlled directly and indirectly vitamin $D_3$ endocrine system .

## VI) Regulation of plasma calcium

***Freeman (1984)*** recorded that calcium uptake and utilization are primarily controlled by three hormones, parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxy cholecalciferol [1,25-$(OH)_2$ $D_3$]. These compounds function together in a feed back loop to maintain plasma calcium concentration at the level needed to achieve optimum skeletal tissue integrity and prevent muscle tetany with avoiding hypercalcemia and possible soft tissue calcification .While, ***Soares (1984)*** reported that the control of calcium metabolism in the avian includes four endocrine system, calcitonin, parathyroid hormone, 1,25-dihydroxy vitamin D and estrogen.

***Nys et al. (1986)*** Dissociated the roles of sex steroids in calcium metabolism as they concluded that the increases in the intestinal and uterine calcium were of insignificant value, a result which is agreed by Soares (1984) who reported that the insignificant changes in calcium level at 5-18 hr after laying.

## *1)* Parathyroid hormone(PTH)

### *Biological effects of PTH*

***Kaneko et al. (1997)*** stated that the most important biological effects of PTH are to

Elevate the blood calcium concentration by the following mechanism.

1- Increase tubular reabsorption of calcium , resulting in diminished calcium loss into urine.

2- Increase the rate of skeletal remodeling and the net rate of bone resorption.

3- Increase osteolysis and the number of osteocalstes on bone surfaces .

4- Stimulate increased intracellular calcium .

5- Accelerate the formation of the principle active vitamin D metabolite 1,25-$(OH)_2$ $D_3$ by the kidney .

***Effect of PTH on the kidney .***

The parathyroid hormone stimulates renal synthesis of 1,25-$(OH)_2$ $D_3$ so it enhances the reabsorption of calcium from the distal convoluted tubules ***(Luck and Scans ,1979; Luck et al., 1980and Freeman,1984)*** and this result is agreed by ***(Yanagawa and Lee, 1992)*** who stated that PTH enhances the renal reabsorption of calcium on the distal convoluted tubule through the direct effect of PTH on this portion.

***Effect of PTH on gastrointestinal tract .***

***Favus (1992)*** recorded that parathyroid hormone has been shown to promote the absorption of calcium from the gastrointestinal tract. In animals under a variety of experimental condition the increased intestinal calcium transport is due principally to an indirect effect of PTH by its action of stimulating the renal synthesis of the biologically active metabolite of vitamin [1,25$(OH)_2D_3$],however ,PTH also may play a minor role by direct stimulating calcium absorption by intestinal epithelial cells ***(Favus , 1992).***

***Response of the bone to P.T.H.***

***Parsons and Robinson (1971)*** recorded that the administration of the parathyroid hormone (PTH) causes an initial decline followed by a sustained increase in blood calcium which is considered to be the result of a sequestration of calcium phosphate in bone and soft tissue.

***Parfitt (1977)*** stated that the response of bone to parathyroid hormone (PTH) is biphasic .The immediate effects are the result of increasing the activity of exiting bone cells. This rapid effect of (PTH) depends upon the continuos presence of hormone and results in an increased flow of calcium from deep in bone to bone surface through the action of an osteocyte-osteoblast 'pump' in order to make fine adjustments in the blood calcium concentration. The later effects of parathyroid hormone on bone are potentially of greater magnitude of response and not dependent upon the continuos presence of hormone. Osteoclasts are primary responsible for the long-term action of PTH in increasing bone resorption and overall bone remodeling ***(Canalis et al., 1994).***

The decrease in ionic calcium during shell formation causes stimulation of (PTH) secretion, the increase in circulating PTH results in an immediate increase in bone resorption, supplying the calcium needed in circulation ***(Luck and Scans , 1979 and Luck et al., 1980).***

***Georgievskii(1981)*** reported that PTH acts to increase ionic calcium concentration by causing increased osteoclast population in the bone ,which stimulates the resorption of the calcium from skeleton .

***High et al. (1981)*** recorded that the subsequent increase in blood calcium results from an interaction of PTH with receptors on osteoblasts that stimulate increase calcium release from bone and direct an increase in osteocalstic bone resorption .

***Freeman (1984)*** stated that the ionic concentration of calcium in extracellular tissue regulates the release of PTH. If the hypothalamus detects low level of ionic calcium (hypocalcemia )PTH will be secreted .The target organs for this hormone are the skeleton and the kidneys.

***Canalis et al. (1994)*** reported that important action of parathyroid hormone on bone is to mobilize calcium from skeletal reserves into extracellular fluids.

**2) Calcitonin**

***Calcitonin secretion :***

***Care (1992)*** recorded that the concentration of calcium ion in plasma and extracellular fluids is the principle physiological stimulus for secretion of calcitonin by C. Cells. Calcitonin is secreted continuously under conditions of normocalcemia.

***Chattopadhyay et al. (1996)*** Stated that when blood calcium concentration increases, the intracellular $Ca^{++}$ concentration in C cells increases resulting in enhancing calcitonin secretion, C- cells, express the same $Ca^{++}$ sensing receptor as parathyroid chief cells, the receptors is responsible for sensing the extracellular calcium ions ($Ca^{++}$) concentration and likely contributes to the regulation of calcitonin secretion along with a voltage-sensitive $Ca^{++}$ channel .

***Kaneko et al., (1997)*** reported that secretion rate of calcitonin increases greatly in response to an elevation in blood calcium.

***Action of calcitonin :***

***Georgievskii (1981)*** reported that calcitonin depressed calcium levels by reducing the production of [1,25$(OH)_2D_3$].

***Chambers and Moor (1983)*** recorded that the action of calcitonin on inhibiting bone resorption stimulated by PTH and other factors is from blockage of osteoclastic osteolysis.

***Freeman (1984)*** Stated that the secretion of calcitonin is stimulated by hypercalcemia. If the blood ionic calcium concentration in the bird become excessive ,calcitonin depressed calcium levels by decreasing gut absorption and bone demineralization .

***Mcdowell (1989)*** found that calcitonin prevents [1,25$(OH)_2D_3$] and PTH from causing the body calcium level to rise to the degree that could cause extreme bone resorption or calcification of soft tissue .

***Heerche(1992)*** found that the hypocalcimic effects of calcitonin are primarily the result of decreased entry of calcium from the skeleton into plasma due to a temporary inhibition of parathyroid hormone- stimulates bone resorptions .

## 3)Vitamin D3

### *Forms of vitamin D*

The third major hormone involved in the regulation of calcium metabolism and skeletal remolding is cholecalciferol (vitamin $D_3$) or irradiated ergocalciferol ($D_2$) although these compounds have been considered to be vitamins for a long time , they can equally be considered hormones ***(Bell., 1985)***.

***Holick and Clark (1978)*** reported that cholecalciferol from endogenous sources is synthesized in the skin from 7-dehydroxy cholecalciferol by a photochemical reaction in the presence of ultraviolet irradiation.

***Belsey et al. (1974)*** reported that ergocalciferol or vitamin $D_2$ having only about 5 % of the activity of vitamin $D_3$ there is some indications that this due to a rapid turnover rate of vitamin $D_2$ forms, because there is less efficient binding to plasma transport protein.

***Valinietis and Bauman (1981)*** reported that birds can efficiently metabolize only the cholecalciferol ($D_3$) form of Vitamin D. Plant sources of this vitamin (ergocalciferol) or vitamin $D_2$ are essentially non functional.

### *Metabolism and activation of vitamin D*

***Lund and Deluca (1966)*** demonstrated that further metabolism of vitamin $D_3$ to 25-hydroxy vitamin $D_3$ was necessary for normal activity of this vitamin .

***Tucker et al. (1973)*** reported that the liver is the site for synthesis of vitamin D metabolism , although in the chicken the intestine and the kidney are capable for synthesis of 25-OH- $D_3$ to some degree .

***Deluka(1974)*** recorded that the rate limiting step for activation of vitamin D-endocrine system is catalyzed by the 25-OH-D-1- α- hydroxylase .

***Deluca (1976)*** recorded that within the microsomal fraction of the liver ,the enzyme vitamin $D_3$-25-hydroxylase is capable of hydroxylating $D_3$ at the 25 position.

***Lawson et al. (1969)*** stated that further metabolism of 25-OH-D3 occurs in the kidney of chicken

***Wasserman and Corradino (1973)*** reported that the liver cells hydroxylate position 25 in the molecule to give 25-hydroxy-cholecalciferol (25-OH-CC) which is the main circulating cholecalciferol metabolite. This hydroxylation step does not appear to be linked by any feed back mechanism to calcium metabolism. An additional hydroxylation in position 1 of the molecule is carried out in the kidney ,yielding [1,25$(OH)_2$CC] 1,25 dihydroxy cholecalciferol. This hydroxylation step appear to be feed back linked to calcium metabolism .

***Tenenhouse (1990)*** recorded that depending on the need of the bird, hydroxylation may take place at either the C-1 (active) or the C-24 (nonactive metebolite) site, if the body demand for calcium is high, the 25(OH) $D_3$ is converted to the active metabolite 1,25$(OH)_2D_3$. If Ca levels are adequate, the C-24 hydroxylation will be increased , creating greater amounts of the non-active 24,25,$(OH)_2D_3$ metabolite.

***Armbrecht et al.(1992)*** recorded that the first step in the metabolic activation of vitamin D is the conversion of cholecalciferol to 25-hydroxycholecalciferol(25-OH-CC) in the liver. The enzyme responsible for controlling this reaction is a hepatic microsomal enzyme , referred to as calceferol - 25-hydroxylase, associated with the endoplasmic reticulum. This first metabolite of cholecalciferol (25-OH-CC) is transported to the kidney and undergoes further transformation to a more polar and active metabolite ***Armbrecht et al.,1992).***

***Vitamin D and Ca .absorption :***

***Maynard et al. (1979)*** recorded that vitamin D plays an important role in the absorption and deposition of calcium in the bone. There exists a dynamic equilibrium between the various forms of calcium , in turn the total concentration of calcium of the blood is governed by dietary and hormonal agencies influencing absorption and the accumulation or release of calcium from skeletal sources.

***Rasmussen et al. (1979)*** reported that in hypocalcemic state , 1,25$(OH)_2D_3$ is known to stimulate increased active and passive absorption of calcium and phosphorus along the entire intestinal tract .

***Wasserman and Corradino (1975)*** reported that 1,25 dihydroxy cholecalciferol is transported to the intestine where it stimulates calcium absorption, the increased absorption of calcium is associated with the synthesis of cholecalciferol dependant calcium- binding protein (CaBP), which is bound to be involved in calcium absorption .

***Deluca (1980)*** reported that the vitamin $D_3$-endocrine system are direct or indirect controlled by plasma calcium, where hypocalcemia stimulates the activity of 1- α-hydroxylase thereby increasing the production of $1,25(OH)_2D_3$.

***Freeman (1984)*** stated that 1,25 dihydroxy cholecalciferol [$1,25(OH)_2D_3$] enhances uptake of calcium by promoting the synthesis of calcium binding-protein (Ca BP), a compound specific to the active absorption of calcium , and by increasing the permeability of the intestinal membrane allowing increased Ca-transport across the intestinal epithelium .

***Soares (1984)*** stated that vitamin D is further metabolized to the hormone $1,25(OH)_2D$, which has a major role in regulating calcium homeostasis by stimulating intestinal absorption as well as bone mobilization of calcium .

***Action of vitamin D***

***Garabedian et al. (1974)*** stated that $1,25\ (OH)_2D_3$ and PTH act on the osteoclasts of the skeletal system to release calcium and phosphorus into circulation .

***Mcdowell (1989)*** reported that [1,25$(OH)_2D_3$] can also leads to the mobilization of calcium reserves in the bone tissue if the ionic calcium level can not be maintained at a level which will support production and prevent muscle tetany through dietary absorption and reduce excretion .

***Finkelman and Butler (1985)*** stated that the active metabolites of cholecalciferol also acts on bone , in addition to its indirect effect on mineralization of bone matrix, vitamin D is necessary for osteoclastic resorption and calcium mobilization from bone.

**4) Estrogen**

***Effect of estrogen :***

***Castillo et al. (1977)*** reported that the relationship between 17 B-estradial (E2) and calcium are complex .

***Asem et al. (1987)*** recorded that calcium and estrogen are intricately associated with each other .

***Williams and Frolik (1991)*** stated that gonadal steroids are important in the maintenance of skeletal homestasis by way of regulation both proliferative and resorptive events as well as mineralization .

***Kenny (1982)*** recorded that estrogen induced hypercalcemia. And also ***Castillo et al., (1977)*** reported that estrogen was shown to increase serum calcium and renal activity of 1-α-hydroxylase.

***Rath et al. (1996)*** stated that esteroids caused increase serum calcium level , also progesterone and megasterol produced an elevations in serum level of calcium although the effect was significantly smaller than that produced by estradiol .

***Navickis et al. (1979)*** reported that estrogens enhancing calcium transport in the shell gland. Moreover, estrogens may influence egg shell quality by elevating protein bound calcium in plasma.

## PHOSPHORUS

***Plasma phosphorus levels :***

Phosphorus levels usually rang from 3.5-4.5 mg/dl blood , most of which is present in the cells with 4-9 mg/dl in plasma ***(Maynard et al., 1979).***

***Transefer of phosphorus from diet to egg :***

***Oneil et al. (1948)*** showed that radioactive phosphorus was deposited in the yolk and albumin of an egg soon after the oral administration of the isotope, and the egg which were laid subsequent to that time showed increasing amounts up to the fifth day , after which time the total amount of $P^{32}$ decreased .

***Transefer of phosphorus to chick embryo :***

***Driggers et al. (1951)*** recorded that the greatest concentration of $P^{32}$ was found in the chicks hatched from eggs laid 6 days after administration, where this period allowing for the entire yolk and white to receive the maximum dose of this element .

***Phosphorus metabolism:***

***Hurwitz and Bar (1965)*** reported that the large amount of P are excreated during the process of shell formation. In this context P balance would provide available indication of bone mineral balance in hens feed diets containing different amount of calcium. The mobilization of bone mineral calcium for shell formation leads to elevated levels of plasma phosphorus ***(Miles et al., 1984)***. This is due to the high Ca:P ratio of egg shell compared with hydroxyappetite of the bone .

***Dietary phosphorus requirement for laying hens:***

***Atkinson et al. (1967)*** reported that the P requirement of the turkey hen was between 0.6 and 0.8 % of the diet. ***National Research Council(1971)*** suggested that the requirement for turkey breeder hens is 2.5% calcium and 0.75 % phosphorus.

***Waldroup et al. (1974)*** found that 2.5 percent calcium and 0.3 percent inorganic phosphorus are sufficient to maintain egg production, fertility and hatchability and egge shell thickness of caged turkey breeder hens .

***National Research Council(1984)*** Recommends 0.32% available phosphorus or 320mg /hen per day throughout the productive cycle .

***The relationship between phosphorus and bone:***

***Frost and Roland (1990)*** reported that reducing of dietary phosphorus significantly decreased tibia weight, tibia ash and bone mineral content.

***Keshaverz and Nakajima(1993)*** recorded that a constant levels of 3.5 - 4 percent of calcium in the diet and 0.4 percent available phosphorus was effective in the maintaining shell quality and bone parameters.

The nutritional management of the bird is important in maximizing the mineralization of the skeleton (***Whitehead ., 1994 and Lesson et al.,1995)*** .If excess calcium is present in the diet leading to an imbalance in the ratio of the calcium to phosphorus ,it will be excreted as $Ca_3(PO_4)_2$, causing a metabolic deficiency of phosphorus ***(Leeson and Summers .,1997).***

***Regulation of the phosphorus metabolism:***

***Baxter and Deluca(1976)*** reported that low serum phosphorus stimulates the production of 1,25-dihydroxy vitamin $D_3$ in chicks.

The release of calcium will cause a concurrent release of phosphorus from the skeleton .To prevent excess phosphorus levels in the blood , PTH stimulates the excretion of the inorganic phosphorus liberated from the skeleton by increasing the secretion of phosphorus in the distal convoluted tubule of the kidney ***(Georgievskii., 1981).***

*Scott et al. (1982)* stated that 1,25-$(OH)_2D_3$ enhances uptake of phosphorus by promoting the synthesis of calcium binding protein , a compound specific to the active absorption of calcium and by increasing the permeability of the intestinal membrane allowing increase calcium and phosphorus transport across the intestinal epithelium .Low phosphorus level may be able to stimulate renal synthesis of [1,25(OH)2D3] independent the action of PTH ***(Scott et al.,1982)***.

## VII) Egg incubation and egg hatchability

### *Definition of hatchability*

Hatching was defined by ***George (1978)*** as the events that makes the termination of the embryonic life and involves a complex sequence of events :invitation of pulmonary respiration , pipping of the egg shell , and emergence of the hatching.

## 1)Egg storage:

***Storage condition and hatchability***

***a) Storage time***

All available data are in agreement in showing that hatchability is reduced as the length of the holding period is increased, especially after storage for one week. These findings are well documented by the reports of ***( McDonald, 1960)***.

***Walter (1963)*** stated that hatchability was reduced when the eggs were stored more than one week , the greatest reduction occurred after the eggs were held 2 weeks , the hatching time is lengthened when hatching eggs are stored for long periods. These effects have been found in growth chicken (***Kosin ., 1950)*** .

***Mather and Laughlin (1979)*** found decrease in the rate of embryonic development after long-term storage.

***b) Storage temperature***

***Proudfoot (1968)*** observed that optimum hatchability can be achieved with a storage temperature of approximately 15 °C. for eggs held for longer periods.

***Reinhart and Hurnik (1976)*** stated that constant temperatures of 15. to 16 °C. and 10 °C. to 11 °C. for short and long time pre-incubation storage respectively are optimum for all treatment groups combined , malposition and malformation were responsible for approximately 15% of total embryonic mortality.

***Proud foot and Hulan (1983)*** recorded that temperature and relative humidity have been the two most common variables used to manipulate the storage environment of hatching eggs.

***c) $Co_2$ and $O_2$ exchange***

The chicken egg must exchange adequate levels of oxygen and carbon dioxide ($CO_2$) in order to hatch. (***Rahn et al., 1979 and Tazawa , 1980)*** .

***Walsh et al . (1995)*** stated that temperature and $CO_2$ appeared to have independent mode of action . the presence of $CO_2$ may be beneficial in maintaining albumen quality and acid base balance appropriate for embryo survival during storage period of about 14 days but may be detrimental for shorter storage period due to increase albumen quality , which may result in reduced vital gas exchange .

*d)Egg position at storage :*

***Proudfoot(1967)*** found that eggs stored with the narrow end-up hatched better than those in the reverse position, if the storage period was less than seven days but for longer incubation periods, this storage position tend to depress hatchability. While ***Oluyemi and George (1972)*** stated that the hatchability of White Rock eggs was found to be significantly ($P<0.05$) affected by storage period but not by storage position.

*e) Water loss during storage :*

***Landauer (1967)*** found that evaporative water loss from the egg was likely lead to a decline in the hatching quality of eggs during storage .

***Hinton (1988)*** concluded that one requirement for successful long-term storage was the prevention of water loss from the egg.

***Walsh et al. (1995)*** stated that the effects of these storage variables (Temperature, $CO_2$ & R.H) on early embryonic mortality have generally been explained through altered water loss, embryonic developmental stage, or albumin quality, however, clear mechanisms are not apparent, generally, water loss increases with the length of egg storage. During pre-incubation storage , water from the egg is lost through evaporation at a rate that is influenced by the temperature and relative humidity of the storage environment ***(Walsh et al., 1995)***.

## 2) Pre-incubation requirements:

### *a) Time of setting:*

***Hutt and Pilkey (1930)*** suggested that incubation of the eggs four to five hours directly after laying could result in the development of the embryo beyond the critical stage associated with pre-gastrulation .

***Funk et al. (1950)*** reported that storage of the chicken eggs for one or two days resulted in greater hatchability than setting the eggs on the day of laying.

### *b) Pre-incubation warming .:*

Warming the eggs at the day after they are laid proved to be the most effective time for pre-incubation ***(Kan et al.,1962)***.

### *Warming procedure:*

The usual warming procedure is to subject the eggs to a period of five hours at 99.5 °F at the day after they are laid, after that the eggs are returned to the egg holding room for the reminder of the storage period ***(Milby and Sherwood, 1960)***.

### *Egg warming and hatchability :*

***Kosin (1956)*** reported that the pre-incubation warming of turkey eggs advanced early embryonic development, improve hatchability and shortened incubation time.

***Becker and Bearse. (1958)*** stated that the pre-incubation warming of chicken egg increases hatchability.

***Milby and Sherwood(1960)*** showed that pre-incubation warming increased hatchability of turkey eggs produced from hens whose hatchabilities were below the average of the population .

***Kan et al. (1962)*** stated that eggs held up to 2 weeks ,warming tended to yield more rapid hatching of chicks. On the other hand, held egg up to 3–4 weeks warming delayed hatchability.

## 3) Egg incubation requirements :

***Rahn(1981)*** reported that three conditions required for successful hatching of turkey eggs. The first condition required the incubating embryo must be consume approximately 100 ml of oxygen for each gram of initial egg mass .The second requires the fractional concentration of oxygen and carbon dioxide in the air cell of the egg to be 14% and 6% respectively. The third requirement is that the incubating egg will have lost 15% of its initial mass as water. If turkey eggs fail to meet any of these requirements , they will not hatch.

***Oxygen and temperature***

Temperature accelerates growth and metabolism in developing chicken embryos ***(Romanoff et al., 1938),*** and incubation oxygen has been observed to do the same ***(Metcalfe et al.,1981)***.

Temperature and oxygen availability may accelerate growth through different physiological mechanisms, the two treatments affected hatchability differently, However, when oxygen was supplemented and incubation temperatures were increased, they acted synergistically to improve hatachability of turkey embryos ***(Christensen and Bagley, 1988)***.

## ***Relative humidity***

***Robertson (1961)*** reported that a relative humidity (RH) of 50% during incubation was optimum. He suggested that eggs of different weights may have different humidity requirement for optimum hatchability.

***Kirk et al. (1980)*** reported that an increase or decrease from a(RH) of 53% depressed hatchability of eggs from young chicken broiler breeders (28 to 44 weeks of age). Also they noted that hatchability was depressed in an older flock (44 to 60 weeks of age ) as relative humidity above 44%.

***Peebles et al. (1987)*** found that at day 0 to 17 of incubation the egg weight loss percentage was increased when the incubating relative humidity was lowered from 55 to 50 %, the hatching percentage of 38 weeks fertile eggs was improved at the higher humidity , the higher humidity also decreased late dead and increased pipped embryonic moralities, where the changes in the humidity affect the vital gas exchange.

## *Turning of the egg during incubation :*

### *a) Effect of egg turning :*

In natural incubation , the hen moves the eggs many times , up to 96 times in 24 hrs. ***(Landauer, 1967).*** Egg turning during artificial incubation has been reported to reduce malpositions ***Robertson (1961a,b)*** to prevent abnormal adhesion of the embryo or embryonic membranes to the shell membrane ***(Robertson, 1961b. and Orlov, 1962)***

### *b) Time of egg turning :*

***Byerly and Olsen (1936)*** concluded that turning of eggs during the last week of incubation was unnecessary. ***North (1984)*** suggested that turning during the 3rd week of incubation is of questionable value.

***Proudfoot et al.,(1981)*** found that the broiler eggs could be transferred from turning to hatching trays after 13 days of incubation without significantly affecting hatchability. On the other hand ***Wilson and Wilmering(1988)*** found that the hatchability of the broiler eggs was significantly reduced by the cessation of turning on day 13th compared with results on day 19th .

### *c) Water loss during incubation:*

***Rahn and Ar(1980)*** found that the rate of the water loss from egg during incubation is directly related to the rate of the embryonic development, prepipping temperature and oxygen consumption rate .

***d)Egg weight losses during incubation :***

Egg weight losses during incubation have been found to influence the hatchability in chickens ***(Tullett and Burton., 1982)*** .

***Hays and Spear (1951)*** noted that egg weight loss could vary between 6.5 and 12 percent without significantly affecting the hatchability, however, there was a decline in hatchability with weight loss greater than 12 percent.

Eggs from different avian species, independent of egg mass or incubation time , lose 15 % of initial mass during natural incubation ***(Rahn et al.,1979).***

The chicken egg must loss 12 to 15 percent of its primary weight as metabolic water during incubation. ***(Ar and Rahn, 1980 and Tazawa, 1980).*** The loss is required to maintain the same relative percent of water in egg composition at the end of incubation as when the egg was laid ***(Rahn, 1981).***

***(Christensen and McCorkle, 1982a)*** reported that a higher incidence of late embryonic mortality occurred in turkey eggs that lost less weight during incubation while, ***Christisen and McCorkle (1982b)*** reported that turkeys embryos from eggs that lost only 9.6 percent of their initial egg mass died late in the incubation period , whereas eggs that lost 10.6 percent of their initial mass hatched. They suggested that turkey embryos may die late in the incubation period because they lose insufficient water vapor.

***Meir et al. (1984)*** reported that 12 percent loss in initial egg mass gave optimum hatchability of turkeys eggs

***e)Egg position during incubation :***

***El.Ibiary et al. (1966)*** found that egg set, during incubation, with the narrow end down hatch better than those oriented on the opposite direction. The same results confirmed by ***Oluyemi and Goerge (1972)*** who reported that egg hatchability was significantly ($P<0.01$) improved when eggs incubated with small end down.

## 4) Hatchability:

Hachability of eggs is of major concern to hatchery man. These are two independent factors, fertility and hatchability , but a decrease in either one or both of them will reduce the economic returns .

***Mechanism of hatching :***

***Christensen and Biellier (1982)*** stated that the mechanism of hatching is little understood, but it is accepted that the "egg tooth" breaks the shell, little interest has been shown in the muscular power that propels the egg tooth.

***Watterson et al. (1964),*** observed that the pipping muscle (musculus complexus), undergoes marked alteration in structure before and during hatching.

***Fisher (1958)*** reported that a possible cause of failure to break the shell was the failure of approximately 25% of the muscle fibers in the M.complexus to contract.

***Taylor* (1963)** suggested that an alteration in calcium to magnesium ratio (Ca : Mg ratio) is of significance for considerable muscular contraction necessary for successful hatching in chicks .Low Ca : Mg ratios are generally thought to induce mild anaesthesia and reduce muscular contraction.

***Mountcastle(1974)*** recorded that ionic calcium stimulates muscular contractions while Mg inhibits contraction . ***Christensen and Biellier(1982)*** stated that an increase plasma Ca:Mg ratio may be a physiological mechanism to provide increased muscular activity for the embryo in breaking the shell and emerging from it and so it plays a role in the hatchability .

Muscular relaxation may have a resulted in insufficient locomotor activity to the embryo to free itself from the shell ***(Christinsen and Edens. , 1985)***.

***Factors affecting hatchability :***

***a) Vitamin $D_3$ and hatchability :***

***Soars et al. (1995)*** reported that an interesting observation from mature fowl studies has been shown that several metabolites of Vit $D_3$ were required for normal hatchability of eggs.

***Sunde et al. (1978)*** showed that hens fed 1,25-$(OH)_2D_3$ as the only source of vitamin $D_3$ could not support normal hatchability of the eggs even the fertility was normal .

***Henry and Norman (1978 and 1984)*** suggested that 24,25-$(OH)_2D_3$ and 1,25 $(OH)_2$ $D_3$ both were needed for normal reproduction in chickens . This is because hens fed diets containing only 1,25$(OH)_2D_3$ as the vitamin $D_3$ source produced no hatchable egg but when both 24,25-$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were fed together , maximal egg fertility and hatchability were observed .

***Hart and Deluca (1985)*** recorded that exclusive feeding of 1-α-hydroxylated vitamin $D_3$ forms or 24,25-$(OH)_2D_3$ do not support hatchability whereas, both 25-$(OH)_2D_3$ or vitamin $D_3$ support normal hatchability when either is the sole dietary source of vitamin $D_3$ for chicken ***(Hart et al., 1984).***

***Soars et al.* (1995)** stated that 25-$(OH)D_3$ is the most active form of vitamin $D_3$ that can be metabolized to fully support normal embryonic development and hachability, when fed as the only source of Vitamin $D_3$ and this is probably because 25-(OH)-$D_3$ has a relatively high affinity for vitamin $D_3$ binding protein and is successfully transported into the fertile egg.

***b) Calcium in breeder diets and hatchability :***

***Jensen et al. (1963)*** noted that dietary calcium levels of 2.5 to 3.5 % depressed the hatchability of fertile eggs in comparison to levels of 1.0 to 1.75 percent. While ***Jensen et al. (1964)*** noted that hatchability were not depressed by the use of high calcium levels in turkey breeders.

***Balloun and Miller (1964)*** obtained the best hatchability with 2.0 and 2.5 % calcium in the diet.

***National Research Council (1971)*** suggested that the calcium requirement for turkey breeder hens is 2.25 percent for best hatchability.

***Waldroup et al. (1974)*** found that Nicolas turkey breeders needed 2.25 percent calcium for the best hatchability .

***Potter et al. (1974)*** reported that increasing the dietary calcium level from 0.99 to 1.77% resulted in significant improvements in the hatchability of the turkey eggs. While ***Mehring and Johnson (1965)*** found that high dietary calcium levels have not been affected hatchability of chicken egg.

**c) Thiamine and hatchability**

The vitamin was qualitatively shown to be necessary for embryo viability, ***Polin et al. (1962a&b)*** added 0.1% of amprolium as a week antimetabolite of thiamin, to a commercial breeder ration to produce a thiamin deficiency in egg yolks. Associated with low yolk thiamin value there were a peak embryo mortality during the late stage of incubation and numbers of weak and dead chicks in the hatching trays.

***Polin et al. (1963)*** found that 0.68 p.p.m thiamin in the diet is the minimum level calculated to yield a yolk value of 0.63 p.p.m which is the thiamin yolk concentration required for optimum hatchability.

***d) Time of lay and hatchability .***

***Funk (1934)*** reported that there is a significant increase in hatchability for eggs laid in the afternoon, which was confirmed by the work of ***McNally and Byerly(1963)***. However, ***Hays (1937)*** reported that the hatchability of fertile eggs was not affected by the hour of laying.

***McConachif et al. (1959)*** stated that the time at which the eggs were laid exerted no significant effect on hatchability.

***e) Hatchability and Chicks breed differences:***

***Singth (1981)*** reported that genetic factors play a definite role in hatchability of eggs .Some investigators, stated that fertility and hatchability differ significantly from one breed to another, while others found no significant difference.

***McConachif et al. (1959)*** reported that the breed differences significantly affect hatchability. ***Ghany (1960)*** showed that Rhode Island red exceeded Fayoumi in hatchability.

***El-Gammal and Hassan (1977)*** stated that the Rhode Island red was better in hatchability than Fayomi and Docki-4 chickens .

# Material & Methods

Five hundred fertile eggs about 60-64 gm were collected from a local breeder flock 52 weeks old at Mansoura city-Egypt .Eggs were obtained from Saso breeders hen that were naturally met by Saso breeder strain males .All eggs had been candled to discard the abnormal eggs .The average hatchability % among the egg of that flock was 75 %.

### *Egg incubation*

The eggs were incubated in forced incubator by using a dry bulb temperature at 37.5 °C and 60% relative humidity .

The incubator was adjusted to turn the eggs once/hour. At day 7$^{th}$ and day 17$^{th}$ of incubation , all eggs were candled and the infertile eggs and eggs with dead embryos were removed .At the day 18$^{th}$ of incubation , eggs were transferred to compartmentalized hatching baskets to maintain the egg identity and identity of chicks that hatched from them .Dry bulb temperature was then reduced to 36.9 °C and humidity was increased to about 80% at day 20$^{th}$ by increasing the water level and the amount of forced air .

Three hundred twenty eggs were classified into sixteen equal groups and eggs were dipped for five seconds at 15 –18°C ***(Wilson and Glick , 1966),*** in 100 ml of one of the following solutions

***1- First group:***

The eggs in this group were immersed in 100 ml distilled water (1$^{st}$ control group ).

***2-Second group :***

The eggs in this group were immersed in 100 ml ethyl alcohol (95%) only ($2^{nd}$ control group) .

***3- Third group :***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 0.04 mg calcitonin.

***4- Fourth group :***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 0.08 mg of calcitonin.

***5- Fifth group :***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 0.16 mg calcitonin .

***6- Sixth group :***

The eggs in this group were immersed in 100 ml of ethyl alcohol containing 200 I.U of vitamin D3 .

***7- Seventh group :***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 400 I.U of vitamin D3 .

***8- Eighth group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 800 I.U of vitamin D3 .

***9- Ninth group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 0.5 mg verapamil

***10-Tenth group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 1.0 mg verapamil.

***11-Eleventh group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 5.0 mg verapamil

***12- Twelvth group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 20.0 mg verapamil

***13-Thirteen group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 0.5 mg verapamil

***14-Fourteen group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 1.0 mg verapamil.

***15-Fifteen group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 5.0 mg verapamil

***16- Sixteen group***

The eggs in this group were immersed in 100 ml ethyl alcohol containing 20.0 mg verapamil.

***Egg dipping***

The eggs were dipped in the 2nd day of incubation on the first twelve groups (1-12) and on the twelve day of incubation for the last four groups (13-16). About 3.5 cm of the pointed end of the egg was immersed in 95% ethyl alcohol (El-Nasr Pharmaceutical Chemicals Company, Egypt) containing cibacalcin[R] (synthetic calcitonin, Novartis pharma , Switzerland) vitamin D3[R] (Natural Pharmaceuticals Co., Cairo –Egypt ) and Isoptin[R] (Verapamil hydrochloride, the Arab Drug Company, Cairo ,Egypt ).

All eggs were re-incubated again after dipping procedure until hatching .

## ***Sampling:***

The chicks were sacrificed, the legs and the blood samples were collected from each group. Sera were separated in dry clean vials, then kept in deep freeze (at-20°C) until determination of calcium and phosphorus level.

The legs were proceeded for determination of $Ca^{++}$ and $P^{++}$ as will discuss later .

The egg shell samples were prepared according to the procedure adapted by ***Christensen and Edens (1985)*** as the following steps:

The egg shells of each group were boiled in 0.5 percent sodium hydroxide (NaOH) for 10-15 minutes (to remove the shell membranes), rinsed three times in distilled water and dried in hot air oven at 105°C for 3 hours and weighed to the nearest 0.1mg.

***Extraction of calcium and phosphorus content of the shell***

Dried egg shells were grounded, 5g of the ground was weighed in clean dried crucible and transferred to muffle furnace (Kulfirini, NF- 120, 06287. Ankra, Turkiye) for complete ashing at 550 °C until 3 constant weights were obtained (about 12hrs).The ash was weighed and then transferred to a clean dry beaker and moistened with distilled water to which 5ml conc.HCl (El-Nasr Pharmaceutical Chemicals, Company, Egypt) were added, the mixture was heated electrically till boiling for 5 minutes ,5ml analar nitric acid (El-Nasr Pharmaceutical Chemicals, company, Egypt ) were then added with continuous boiling till the mixture was reduced to half the original volume .The mixture was left to cool then filtrated in a 50 ml volumetric flask .The filter paper was washed with distilled water to dilute the acid concentration till 50ml. Now , the filtrate is ready for determination of minerals e.g calcium and phosphorus ***(Gindler and King, 1972)*** .

**Extraction of calcium and phosphorus content of the bone:**

***1- Preparation of bones***

The legs of chicks were prepared according to the procedure described by ***Cantor et al. (1980)*** as the following steps:

The legs of sacrificed chicks of each group were immersed in boiling water for 3 minutes (to coagulate the flesh to be easily peeled from the bones) then the legs were left at room temperature to cool .The legs were defleshed manually to obtain tibia and femur .

The bones (tibia & femur) were dried at 100 °C in hot air oven for 10 hours and weighed .These bones were ashed in muffle furnace (Kulfirini, NF- 120, 06287. Ankra, Turkiye), at 750 °C for 22 hours in porcelain crucibles for complete ashing, until 3 constant weights were obtained , then complete as the previously mentioned in extraction of calcium and phosphorus content of the egg shell.

***D) Determination of free calcium in blood , egg shell &bones***

Calcium content of the bone was determined using the same procedure used for determination of calcium content of the shell according to ***Gindler and King (1972).***

Diamond Diagnostic Kits (Modern Laboratory Chemical-Egypt ), were used for determination of ionized calcium.

Ionized calcium was determined by, O-cresolphthalein complexon, without deproteinization .$Ca^{++}$ forms a violet complex with O- cresolphthalein complexon in alkaline medium and the absorbance of the standards and the samples was measured against the reagent blank using a spectrophotometer***( Shimadz -UV-1601, Tokyo,Japan)*** at 570 nm .

***Calculation :***

$$\textbf{Calcium (mg/dl)} = \frac{\text{A Sample}}{\text{A Standard}} \times 10$$

-A Sample = Absorbance of the sample

-A standard = Absorbance of the standard

***Determination of phosphorus in blood :***

Phosphorus % was determined according to the procedure of ***Kuttner and Lichtenstein (1930).***

1- 0.2 ml serum sample was mixed well with 4.8 ml of 10 percent, trichloracetic acid in a test tube then the mixture was filtrated.

2- 2ml of the filtrate were add to 3.7 ml of distilled water , and 0.2ml sulphuric acid–molybdate reagent, and 0.1 ml of dilute stannous chloride solution.

3- The contents of the test tube were mixed well .

4- 2 ml of the phosphate standard were treated similarly.

5- Compare in the a spectrophotometer at 680 n.m .

***Calculation :***

$$\text{Phosphorus (mg/100ml)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 5$$

## ***Determination of phosphorus in egg shell and bone :***

The above procedures used for determination of phosphorus in the blood was used for determination of phosphorus in egg shell and bone samples but without filtration since the samples are protein free.

***Statistical analysis***

All data were subjected to statistical analysis according to ***Snedecor and Cochran (1980)*** .

## Summary for egg treatments and time of dipping

| *Group* | No. of eggs | *Treatment (Dipping solution) | **Time of dipping | N.B |
|---|---|---|---|---|
| *Control I* | 20 | 100 ml Dist water | 2 days | |
| *Control II* | 20 | 100 ml ethyl alcohol | 2 days | |
| *Third* | 20 | Ethyl alcoh. Containing 0.04 mg cibacalcin | 2 days | |
| *Fourth* | 20 | Ethyl alcoh. Containing 0.08 mg cibacalcin | 2 days | |
| *Fifth* | 20 | Ethyl alcoh. Containing 0.16 mg cibacalcin | 2 days | |
| *Sixth* | 20 | Ethyl alcoh. Containing 200 I.U Vit D3 | 2 days | |
| *Seventh* | 20 | Ethyl alcoh. Containing 400 I.U Vit D3 | 2 days | |
| *Eighth .G* | 20 | Ethyl alcoh. Containing 800 I.U Vit D3 | 2 days | |
| *Ninth* | 20 | Ethyl alcoh. Containing 0.5 mg Isoptin | 2 days | |
| *Tenth* | 20 | Ethyl alcoh. Containing 1.0 mg Isoptin | 2 days | |
| *Eleventh* | 20 | Ethyl alcoh. Containing 5.0 mg Isoptin | 2 days | |
| *Twelfth* | 20 | Ethyl alcoh. Containing 20.0 mg Isoptin | 2 days | |
| *Thirteen* | 20 | Ethyl alcoh. Containing 0.5 mg Isoptin | 12 days | |
| *Fourteen* | 20 | Ethyl alcoh. Containing 1.0 mg Isoptin | 12 days | |
| *Fifteen* | 20 | Ethyl alcoh. Containing 5.0 mg Isoptin | 12 days | |
| *Sixteen* | 20 | Ethyl alcoh. Containing 20.0 mg Isoptin | 12 days | |

**Eggs at 37.5 C were dipped in every solution at 15-18 C for 5 seconds.*

*** Days after incubation*

# RESULTS

## 1) The effect of dipping in calcitonin hormone, vitamin $D_3$ and verapamil on hatching percentage.

***a)Effect of dipping in distilled water and ethyl alcohol .***

From table. (1) and fig (1) it can be observed that dipping of eggs in distilled water or ethyl alcohol at the second day of incubation did not affect the hatching percentage , among eggs as compared to that of eggs not exposed to treatment (according to data of parent stock breed , where the average hatching % among the eggs of the flock was 75 %) .

***b) Effect of dipping in calcitonin hormone :-***

The results present in table (1) and fig (1) showed that dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin increased the egg hatching percentage and reduced the number of embryos dead before pipping as compared to that of eggs dipped in distilled water or ethyl alcohol.

It was also observed that the pipping started earlier in eggs dipped in 0.16 mg calcitonin as compared to that of eggs of other groups .

***(c) Effect of dipping in vitamin $D_3$ :-***

As shown in table(1) and fig (1) it can be observed that dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ improved egg hatching percentage and reduced the number of embryos dead before pipping as compared to that of eggs dipped in distilled water , ethyl alcohol or ethyl alcohol containing 200 I.U vitamin $D_3$.

***(d) Effect of dipping in verapamil on hatching percentage***

**a) Eggs dipped at second day of incubation:**

From table (1) and fig (1) it can be noted that dipping of the eggs in ethyl alcohol containing 5 and 20 mg verapamil reduced the hatching percentage as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.5 and 0.1 mg of verapamil or even eggs without dipping. The number of dead embryos before pipping was increased following dipping in verapamil as compared to those dead after pipping. While the pipping time started more later in eggs dipped in ethyl alcohol containing 20 mg verapamil as compared to that of eggs in other groups .

**b) Eggs dipped at 12$^{th}$ day :-**

The results presented in table (1) revealed that the growing chick embryos were all dead within two days from egg dipping in ethyl alcohol containing 0.5, 1.0, 5 or 20 mg verapamil at the 12$^{th}$ day of incubation.

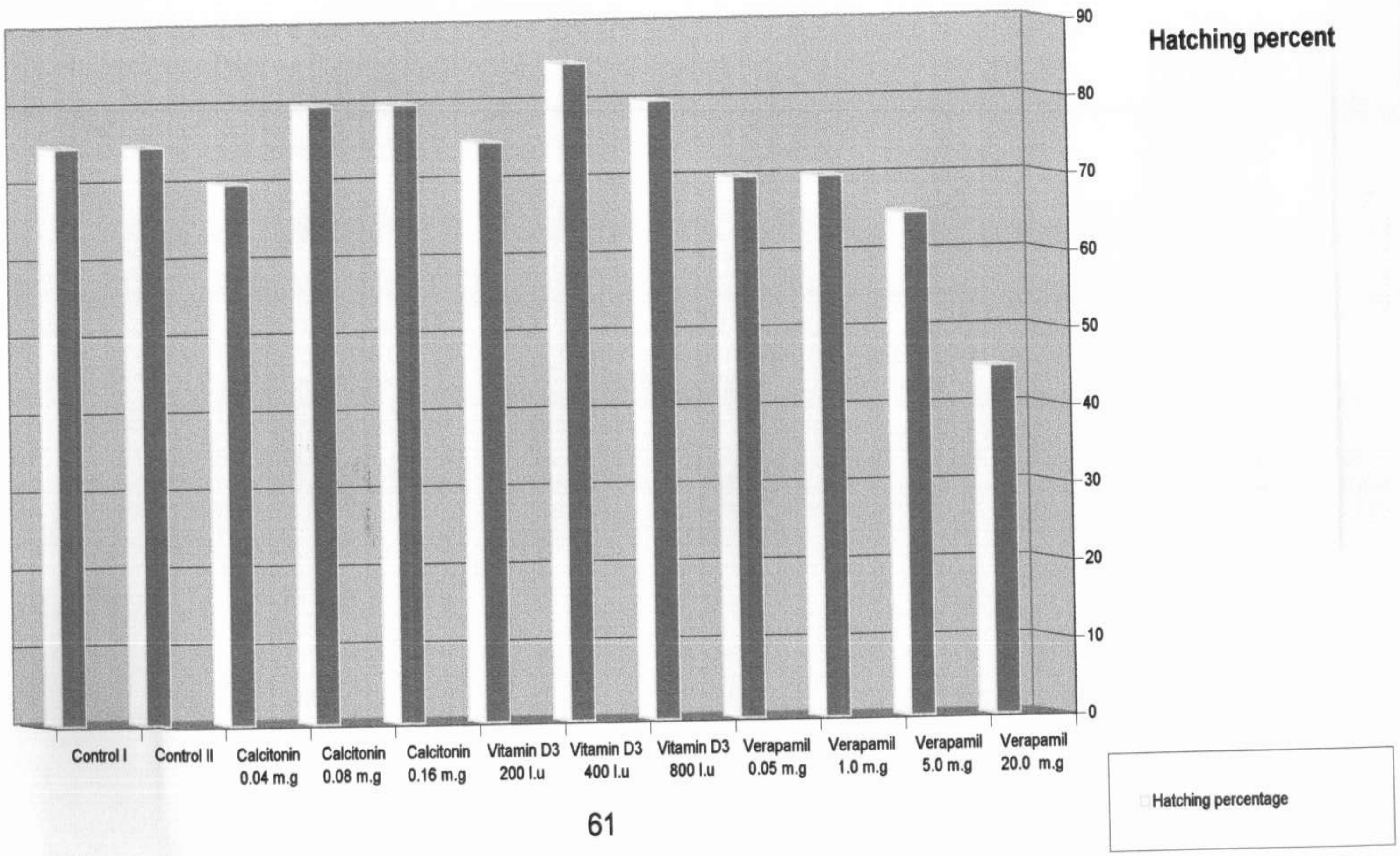
**Table(1) The effect of dipping in calcitonin hormone, vitamin $D_3$ and verapamil on hatching percentage .**

| Group . | | Total number of eggs | No. of hatched eggs | No . of unhatched eggs | Dead embryos | | Hatching % |
|---|---|---|---|---|---|---|---|
| | | | | | *Before pipping* | *After pipping* | |
| Control I * | | 20 | 15 | 5 | 3 | 2 | 75% |
| Control II** | | 20 | 15 | 5 | 2 | 3 | 75% |
| Calcitonin hormone | 0.04mg | 20 | 14 | 6 | 3 | 3 | 70% |
| | 0.08 mg | 20 | 16 | 4 | 1 | 3 | 80% |
| | 0.16 mg | 20 | 16 | 4 | 0 | 4 | 80% |
| Vitamin $D_3$ | 200.I.U | 20 | 15 | 5 | 2 | 2 | 75% |
| | 400 I.U | 20 | 17 | 3 | 0 | 3 | 85% |
| | 800 I.U | 20 | 16 | 4 | 1 | 3 | 80% |
| Verapamil Dipping at the 2nd day | 0.5 mg | 20 | 14 | 6 | 3 | 3 | 70% |
| | 1 mg | 20 | 14 | 6 | 4 | 2 | 70 % |
| | 5 mg | 20 | 13 | 7 | 5 | 2 | 65 % |
| | 20 mg | 20 | 9 | 11 | 7 | 4 | 45 % |
| Verapamil Dipping at the 12th day | 0.5 mg | 20 | 0 | 20 | 20 | 0 | 0% |
| | 1mg | 20 | 0 | 20 | 20 | 0 | 0% |
| | 5mg | 20 | 0 | 20 | 20 | 0 | 0% |
| | 20mg | 20 | 0 | 20 | 20 | 0 | 0% |

** Means eggs were dipped in distilled water for 5 second .*

*** Means eggs were dipped in ethyl alcohol for 5 second.*

Fig (1) The effect of egg dipping in calcitonin, vitamin D3 and Verapamil on the hatching percentage



## (2) The effect of egg dipping in calcitonin hormone on calcium content in the serum, egg shell and bone of newly hatched chicks .

### *(a) Effect on serum calcium :-*

As shown in table (2) and fig (2) dipping of eggs in distilled water , ethyl alcohol containing 0.04 or 0.08 mg calcitonin did not significantly reduce the calcium content of serum in newly hatched chicks . Whereas, dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin significantly reduced ($P<0.05$) the serum calcium content as compared to that of eggs dipped in ethyl alcohol (second control group).

### *(b) Effect on egg shell calcium :-*

As observed in table (2) and fig (2) dipping of eggs in ethyl alcohol containing 0.08 mg calcitonin significantly reduced ($P<0.01$) the calcium content of egg shell as compared that of to eggs dipped in, distilled water, ethyl alcohol or ethyl alcohol containing 0.04 mg calcitonin. While Eggs dipped in ethyl alcohol containing 0.16 mg calcitonin had reduced ($P<0.01$) calcium content of egg shell as compared to that of eggs dipped in distilled water, ethyl alcohol and ethyl alcohol containing 0.04 and 0.08 mg calcitonin.

### *(c) Effect on bone calcium :-*

As shown in table (2) and fig (2) dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin significantly increased ($P<0.01$) the calcium content of bones in newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.04, 0.08 mg calcitonin.

**Table(2) The effect of egg dipping in calcitonin hormone on calcium content in the serum, egg shell and bone of newly hatched chicks *** .**

| Group | | Calcium (mg/100ml) in serum | Calcium % in egg shell | Calcium % in the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | 9.29 ± 0.11 | 44.44 ±0.33 [ag] | 39.29 ± 0.34[a] |
| **Control II | | 9.34 ± 0.13 [A] | 44.56 ±0.17 [bf] | 39.43 ± 0.24[b] |
| Calcitonin hormone | 0.04 mg | 9.18 ± 0.20 | 44.68 ±0.3 [ce] | 39.68 ± 0.20[c] |
| | 0.08 mg | 8.97 ± 0.18 | 43.22 ± 0.16 [abcd] | 40.20 ± 0.50[d] |
| | 0.16 mg | 8.61 ±0.18 [A] | 41.43 ± 0.16 [defg] | 41.17 ± 0.34[abcd] |

**Means eggs were dipped in distilled water for 5 second .*

*** Means eggs were dipped in ethyl alcohol for 5 second .*

****Means ± S.E .*

*Means in the same column having the same superscript small letter are significantly different at (P<0.01) .*

*Means in the same column having the same superscript capital letter are significantly different at (P<0.05) .*

## (3) The effect of egg dipping in vitamin $D_3$ on calcium content in serum, egg shell and bone of newly hatched chicks

***(a) Effect on serum calcium :.***

As shown in table(3) and fig (2) dipping of eggs in ethyl alcohol containing 800 I.U vitamin $D_3$ did not significantly reduce the calcium content of serum in newly hatched chicks. Whereas, dipping of eggs in ethyl alcohol containing 400 I.U vitamin $D_3$ significantly reduced ($P<0.01$) the calcium content of serum in newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 I.U of vitamin $D_3$ .

***(b) Effect on egg shell calcium :.***

The results presented in table(3) and fig (2) revealed that dipping of eggs in ethyl alcohol containing 200 and 800 I.U of vitamin $D_3$ did not significantly reduce the calcium content of egg shell, whereas the dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ significantly reduced ($P<0.01$) calcium content of egg shell as compared to that of eggs dipped in distilled water or ethyl alcohol .

***(c) Effect on bone calcium :.***

As shown in table(3) and fig (2) dipping of eggs in ethyl alcohol containing 200 and 800 I.U of vitamin $D_3$ did not significantly increase the calcium content of bones in newly hatched chicks . Whereas, the dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ significantly ($P<0.01$) increased calcium content of the bone as compared to that of eggs dipped in distilled water and ethyl alcohol .

**Table(3) The effect of egg dipping in vitamin $D_3$ on calcium content in serum, egg shell and bone of newly hatched chicks***.**

| Group | | Calcium(mg/100 ml) in serum | Calcium % in egg shell | Calcium % in the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | 9.29 ±0.11$^{a}$ | 44.44 ±0.33$^{a}$ | 39.29 ± 0.34$^{a}$ |
| **Control II | | 9.34 ± 0.34$^{b}$ | 44.56 ±0.17$^{b}$ | 39.43 ± 0.24$^{b}$ |
| Vitamin $D_3$ | 200I.U | 9.12 ± 0.14$^{c}$ | 44.28 ±0.27 | 39.71 ± 0.34 |
| | 400I.U | 8.37 ± 0.15$^{abc}$ | 43.1 ± 0.22$^{ab}$ | 41.23 ± 0.51$^{ab}$ |
| | 800I.U | 8.75 ±0.17 | 43.3 ± 0.23 | 40.30 ± 0.30 |

** Means eggs were dipped in distilled water for 5 second.*

*** Means eggs were dipped in ethyl alcohol for 5 second.*

****Means ± S.E.*

*Means in the same column having the same superscript letter are significantly different at (P<0.01)*

## 4) The effect of egg dipping in verapamil on calcium content in the egg shell and bone of newly hatched chicks :

### *(a) Effect on egg shell calcium :.*

The results presented in table(4) and fig (2) revealed that dipping of eggs in ethyl alcohol containing 20 mg verapamil significantly increased ($P<0.01$) calcium content of the egg shell as compared to that of eggs dipped in distilled water , ethyl alcohol or ethyl alcohol containing 0.5 and 1.0 mg verapamil.

### *(b) Effect on bone calcium :.*

As observed in table(4) and fig(2) the eggs dipped in ethyl alcohol containing 20.0 mg verapamil significantly reduced ($P<0.01$) bone calcium content of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol or ethyl alcohol containing 0.5 , 1.0 and 5 mg verapamil.

**Table (4) The effect of egg dipping in verapamil on calcium content in the egg shell and bone of newly hatched chicks*****

| Group | | Calcium (mg/100ml) in serum | Calcium % in egg shell | Calcium % In the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | Blood samples were not collected where hatched chicks were dead after hatching | 44.44±0.33[a] | 39.29 ± 0.37[a] |
| **Control II | | | 44.59 ±0.16[b] | 39.43 ± 0.26 [b] |
| Verapamil | 0.5 mg | | 44.68 ±0.29[c] | 39.09 ± 0.34[c] |
| | 1mg | | 44.92 ± 0.32 [d] | 38.85 ± 0.36[d] |
| | 5mg | | 45.40 ± 0.29 | 37.06± 0.39[e] |
| | 20mg | | 46.65 ± 0.27[abcd] | 34.08 ± 0.36[abcde] |

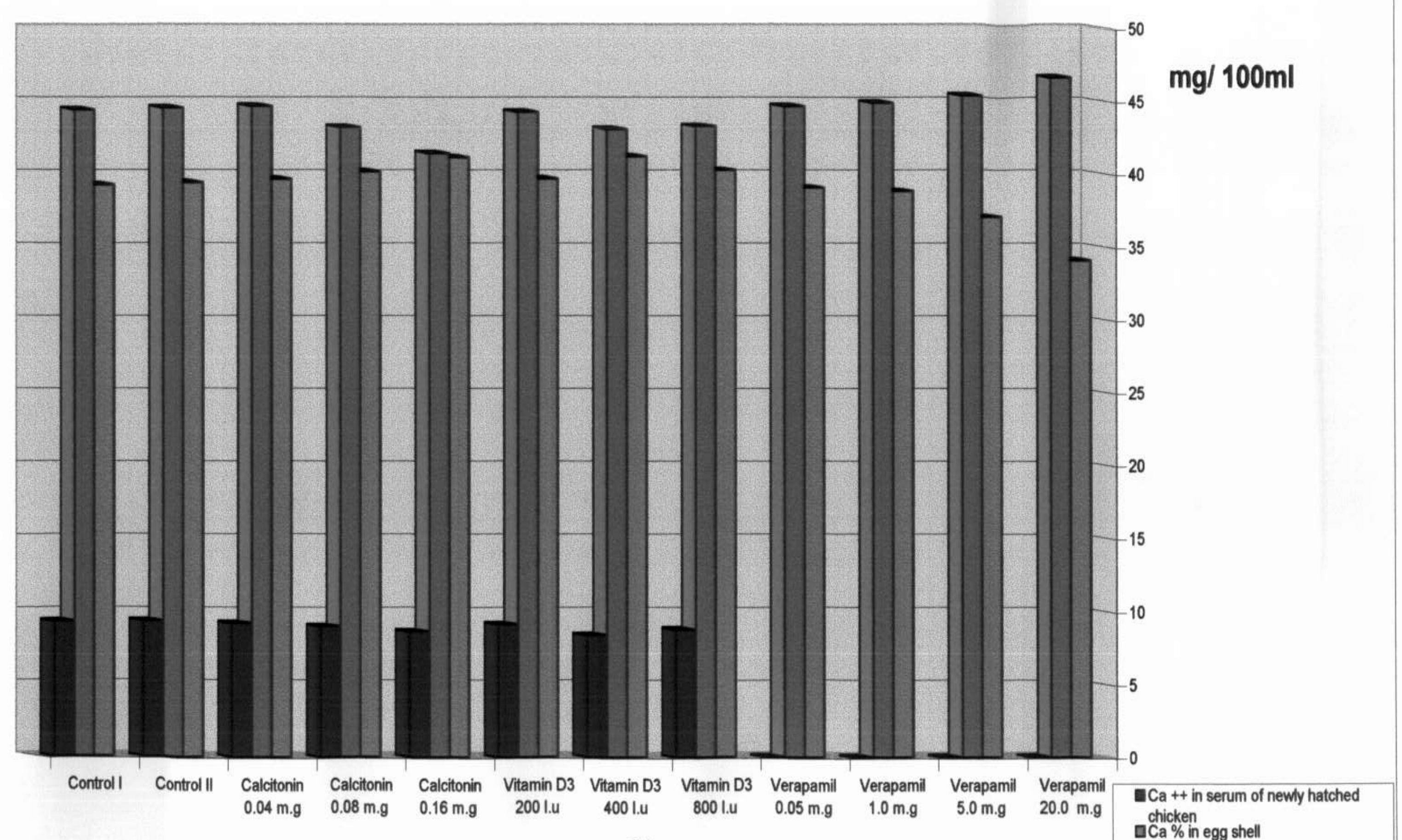
** Means eggs were dipped in distilled water for 5 second .*

*** Means eggs were dipped in ethyl alcohol for 5 second .*

****Means ± S.E .*

*Means in the same column having the same superscript letter are significantly different at (P<0.01) .*

Fig (2) The effect of egg dipping in calcitonin, vitamin D3 and Verapamil on the calcium content in , serum,egg shell and bone of newly hatched chicks



## (5) The effect of egg dipping in calcitonin hormone, vitamin $D_3$ and verapamil on the egg shell ash percentage .

***(a) Effect of calcitonin***

The results showed in table(5) and fig (3) revealed that dipping of eggs in ethyl alcohol containing 0.04 and 0.08 mg calcitonin hormone did not significantly reduced the egg shell ash percentage . Whereas, the dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin significantly reduced ($P<0.01$) egg shell ash percent as compared to that of eggs dipped in distilled water, ethyl alcohol and ethyl alcohol containing 0.04 mg calcitonin.

***(b) Effect of vitamin $D_3$ :.***

As shown in table (5) and fig (3) dipping of eggs in ethyl alcohol containing 200 and 800 I.U of vitamin $D_3$ did not significantly reduce the egg shell ash percent. Whereas, dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ significantly reduced ($P<0.05$) egg shell ash percent as compared to that of egg dipped in distilled water and ethyl alcohol .

***(c) Effect of verapamil :.***

As shown in table(5) and fig(3) the eggs dipped in ethyl alcohol containing 20.0 mg verapamil significantly increased ($P<0.01$) egg shell ash percentage as compared to that of eggs dipped in distilled water or ethyl alcohol .

**Table (5) The effect of egg dipping in calcitonin hormone, vitamin D3 and verapamil on the egg shell ash percentage*****

| Group | | Egg shell ash % |
|---|---|---|
| *Control I | | 54.02 ± 0.66 [Acf] |
| **Control II | | 54.00 ± 0.65 [Bdg] |
| Calcitonin hormone | 0.04 mg | 53.37 ± 0.57 [e] |
| | 0.08 mg | 54.09 ± 0.34 |
| | 0.16 mg | 50.80 ± 0.56 [cde] |
| Vitamin D3 | 200I.U | 52.66 ± 0.46 |
| | 400I.U | 51.08 ± 0.71 [AB] |
| | 800 I.U | 52.58 ± 0.44 |
| Verapamil | 0.5 mg | 54.66 ± 0.41 |
| | 1 mg | 54.97 ± 0.43 |
| | 5.0 mg | 56.16 ± 0.40 |
| | 20.0 mg | 57.05 ± 0.73 [fg] |

** Means eggs were dipped in distilled water for 5 second.*

*** Means eggs were dipped in ethyl alcohol for 5 second .*

****Means ± S.E .*

*Means in the same column having the same superscript small letter are significantly different at (P<0.01).*

*Means in the same column having the same superscript capital letter are significantly different at (P<0.05).*

## (6) The effect of egg dipping in clcitonin hormone vitamin $D_3$ and verapamil on bone ash percentage of newly hatched chicks

***(a) Effect of calcitonin :.***

The results showed in table(6) and fig (3) revealed that eggs dipped in ethyl alcohol containing 0.16 mg calcitonin significantly increased ($P<0.01$) bone ash percentage of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.04 and 0.08 mg of calcitonin hormone.

***(b) Effect of vitamin $D_3$ :.***

As shown in table(6) and fig(3) dipped of eggs in ethyl alcohol containing 400 I.U vitamin $D_3$ significantly increased ($P<0.01$) bone ash percentage of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 and 800 I.U of vitamin $D_3$ .

***(c) Effect of verapamil :.***

The results presented in table(6), fig(3) revealed that dipping of eggs in ethyl alcohol containing 20.0 mg verapamil HCL decreased significantly ($P<0.01$) bone ash percentage of newly hatched chicks as compared to that of eggs dipped in distilled water ethyl alcohol or ethyl alcohol containing 0.5 and 1.0 mg verapamil. Whereas, dipping of eggs in ethyl alcohol containing 5.0 mg verapamil also decreased significantly ($P<0.01$) bone ash % as compared to that of the two control groups only (eggs dipped in distilled water and ethyl alcohol).

**Table(6) The effect of egg dipping in calcitonin hormone, vitamin $D_3$ and verapamil on bone ash percentage of newly hatched chicks*****

| Group | | Egg shell ash % |
|---|---|---|
| *Control I | | $37.45 \pm 0.26^{agko}$ |
| **Control II | | $37.41 \pm 0.25^{bhlp}$ |
| Calcitonin hormone | 0.04 mg | $37.58 \pm 0.33^{c}$ |
| | 0.08 mg | $38.53 \pm 0.22^{d}$ |
| | 0.16 mg | $40.13 \pm 0.48^{abcd}$ |
| Vitamin D3 | 200I.U | $37.77 \pm 0.20^{f}$ |
| | 400I.U | $40.39 \pm 0.57^{efgh}$ |
| | 800 I.U | $38.45 \pm 0.26^{e}$ |
| Verapamil | 0.5 mg | $37.15 \pm 0.30^{m}$ |
| | 1 mg | $36.08 \pm 0.70^{n}$ |
| | 5.0 mg | $35.17 \pm 0.32^{op}$ |
| | 20.0 mg | $33.32 \pm 0.35^{klmn}$ |

** Means eggs were dipped in distilled water for 5 second.*

*** Means eggs were dipped in ethyl alcohol for 5 second.*

****Means ± S.E.*

*Means having the same superscript letter are significantly different at (P<0.01)*

Fig (3)The effect of egg dipping in calcitonin, vitamin D3 and Verapamil on the ash % on egg shell and bone of newly hatched chicks

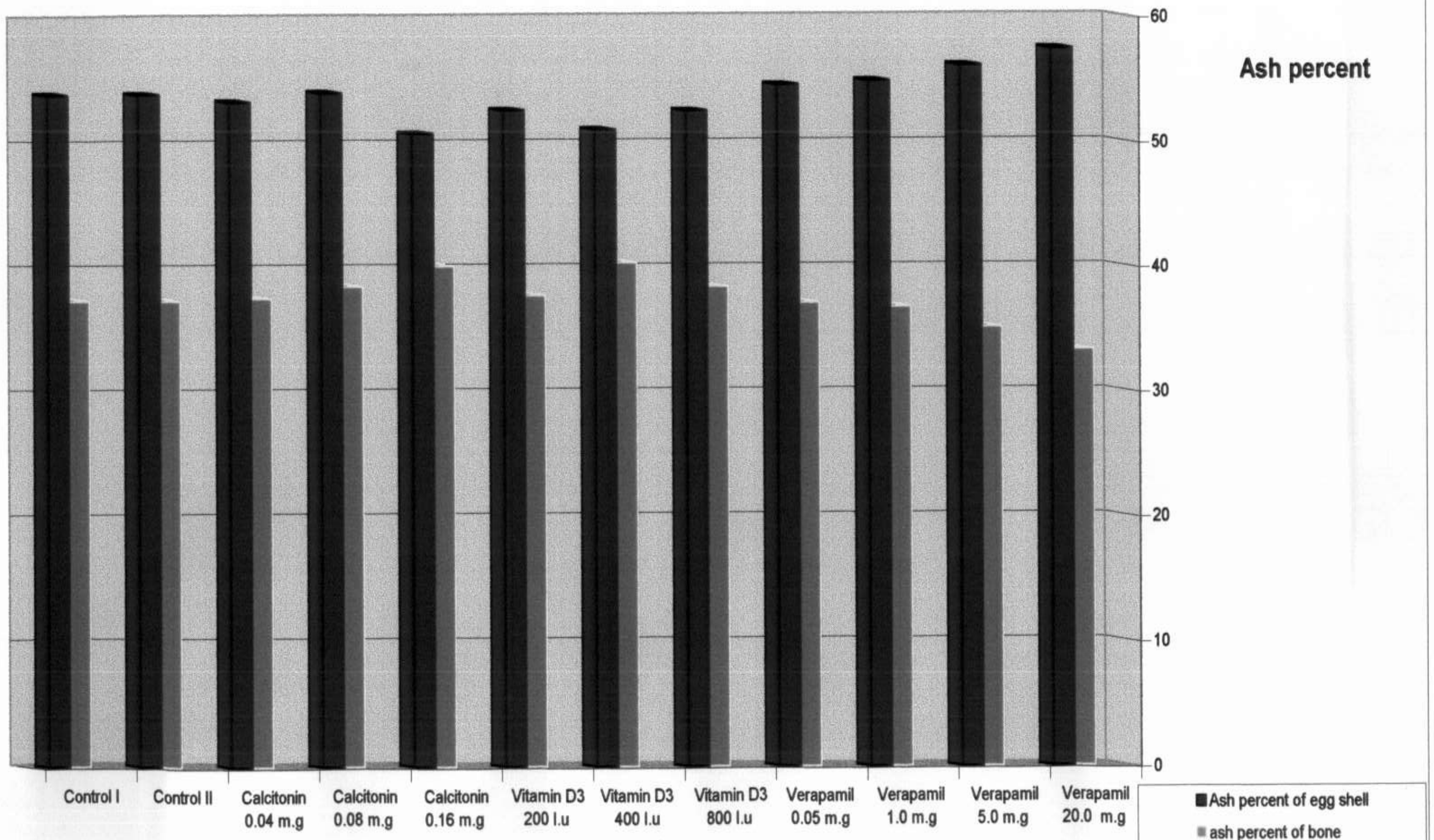


**Table (7) The effect of egg dipping in calcionin hormone on phosphorus content in the serum, egg shell and bone of newly hatched chicks .**

***(a) Effect on serum phosphorus :.***

The results showed in table( 7) and fig (4) revealed that dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin significantly reduced ($P<0.05$) phosphorus content of serum in newly hatched chicks as compared to that of eggs dipped in ethyl alcohol .

***(b) Effect on egg shell phosphorus :.***

The results presented in table(7) and fig (4) revealed that dipping of eggs in distilled water, ethyl alcohol or ethyl alcohol containing 0.04, 0.08 and 0.16 mg calcitonin did not significantly decrease phosphorus content in egg shell .

***(c) Effect on bone phosphorus :.***

As observed in table(7) and fig (4) the results revealed that dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin significantly increased ($P<0.01$) phosphorus content in bones of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.04 and 0.08 mg of calcitonin hormone. Whereas, dipping of eggs in ethyl alcohol containing 0.08 mg calcitonin significantly increased ($P< 0.01$) phosphorus content in bones as compared to that of the eggs on the two control groups.

**Table (7) The effect of egg dipping in calcionin hormone on phosphorus content in the serum, egg shell and bone of newly hatched chicks***.**

| Group | | Phosphorus (mg/100ml) in serum | Phosphorus % in egg shell | Phosphorus % in the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | 4.58 ± 0.06 | 0.21 ±0.015 | 19.65 ± 0.17[cd] |
| **Control II | | 4.68 ± 0.06 [A] | 0.22 ±0.016 | 19.62± 0.16 [ab] |
| Calcitonin hormone | 0.04 mg | 4.60 ± 0.1 | 0.21 ±0.017 | 19.75 ± 0.14[e] |
| | 0.08 mg | 4.49 ± 0.09 | 0.19± 0.015 | 20.1 ± 0.3[acf] |
| | 0.16 mg | 4.27 ±0.1 [A] | 0.17 ± 0.013 | 20.59 ± 0.17[bdef] |

** Means eggs were dipped in distilled water for 5 second.*

*** Means eggs were dipped in ethyl alcohol for 5 second.*

****Means ± S.E.*

*Means in the same column having the same superscript small letter are significantly different at (P<0.01).*

*Means in the same column having the same superscript capital letter are significantly different at (P<0.05).*

**Table (8) The effect of egg dipping in vitamin $D_3$ on the phosphorus content in the serum, egg shell and bone of newly hatched chicks .**

***(a) Effect on serum phosphorus :.***

As shown in table(8) and fig (4) dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ significantly reduced ($P<0.01$) phosphorus content of serum as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 I.U of vitamin $D_3$ .

***(b) Effect on egg shell phosphorus :.***

The results showed in table(8) and fig (4) revealed that dipping of eggs in ethyl alcohol containing 200,400,800 I.U of vitamin $D_3$ did not significantly reduced phosphorus content in egg shell as compared to that of eggs dipped in distilled water or ethyl alcohol .

***(c) Effect on bone phosphorus :.***

Dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ significantly increased ($P<0.01$) bone phosphorus content of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 I.U vitamin $D_3$ (table (8) and fig (4).

**Table(8) The effect of egg dipping in vitamin $D_3$ on the phosphorus content in the serum, egg shell and bone of newly hatched chicks***.**

| Group | | Phosphorus (mg/100ml) in serum | Phosphorus % in egg shell | Phosphorus % in the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | 4.58± 0.06[a] | 0.21 ±0.015 | 19.65 ± 0.17[b] |
| **Control II | | 4.68 ± 0.06 [b] | 0.22 ±0.016 | 19.62± 0.16 [a] |
| Vitamin D3 | 200I.u | 4.6 ± 0.6[c] | 0.195 ±0.11 | 19.85 ± 0.17[c] |
| | 400 I.u | 4.1 ± 0.7[abc] | 0.16± 0.011 | 20.37 ± 0.32[abc] |
| | 800 I.u | 4.3 ±0.1 | 0.18 ± 0.12 | 20.61 ± 0.15 |

** Means eggs were dipped in distilled water for 5 second.*

*** Means eggs were dipped in ethyl alcohol for 5 second.*

****Means ±S.E .*

*Means in the same column having the same superscript letter are significantly different at (P<0.01)*

## Table(9) The effect of egg dipping in verapamil on phosphorus content in the egg shell and bone of newly hatched chicks .

***(a) Effect on egg shell phosphorus content :.***

As shown in table (9) and fig (4) results revealed that phosphorus content of the egg shell did not significantly increased in eggs dipped in ethyl alcohol containing 0.5, 1.0 ,5.0 and 20.0 mg verapamil as compared to that of other groups in which eggs were dipped in distilled water or ethyl alcohol.

***(c) Effect on bone phosphorus content :.***

As shown in table (9) and fig.(4) the eggs dipped in ethyl alcohol containing 20.0 mg verapamil reduced significantly ($P<0.01$) the phosphorus content of the bone of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol and ethyl alcohol containing 0.5 and 1.0 mg verapamil. Whereas dipping eggs in ethyl alcohol containing 5.0 verapamil decreased significantly ($P<0.01$) bone phosphorus content as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.5 mg verapamil.

**Table(9). The effect of egg dipping in verapamil hydrochloride on phosphorus percentage in the egg shell and bone of newly hatched chicks*** .**

| Group | | Phosphorus (mg/100ml) in serum | Phosphorus % in egg shell | Phosphorus % in the bone of newly hatched chick |
|---|---|---|---|---|
| *Control I | | Blood samples were not collected where hatched chicks dead just hatched | $0.21 \pm 0.015$ | $19.65 \pm 0.18^{ae}$ |
| **Control II | | | $0.22 \pm 0.016$ | $19.62 \pm 0.18^{bf}$ |
| Verapamil | 0.5 mg | | $0.24 \pm 0.015$ | $19.53 \pm 0.17^{cg}$ |
| | 1 mg | | $0.22 \pm 0.11$ | $19.43 \pm 0.18^{d}$ |
| | 5 mg | | $0.27 \pm 0.017$ | $18.53 \pm 0.20^{efg}$ |
| | 20 mg | | $0.28 \pm 0.024$ | $17.39 \pm 0.17^{abcd}$ |

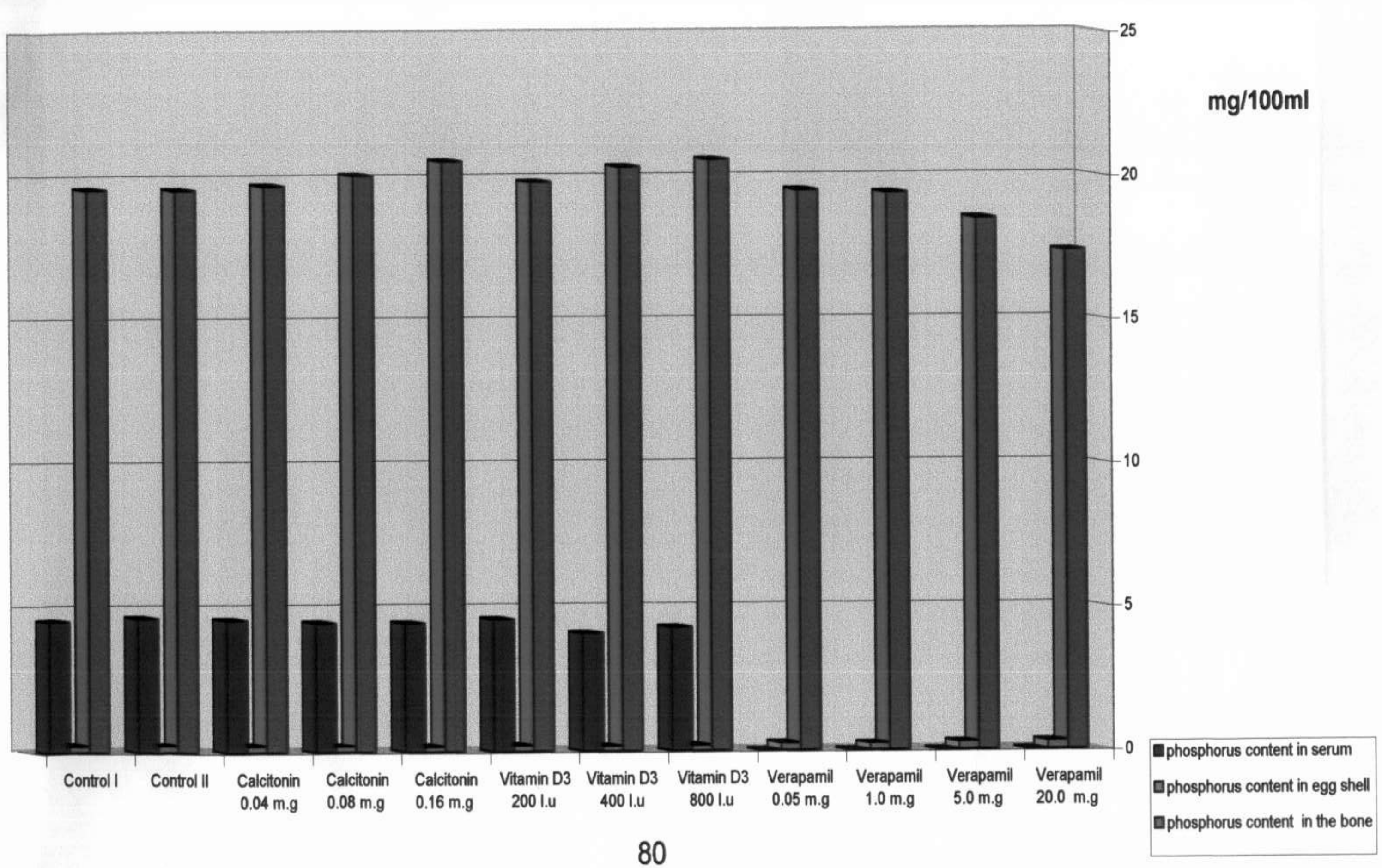
** Means eggs were dipped in distilled water for 5 second .*

*** Means eggs were dipped in ethyl alcohol for 5 second .*

****Means $\pm$ S.E .*

*Means having the same superscript letter are significantly different at (P<0.01)*

Fig (4)The effect of egg dipping in calcitonin, vitamin D3 and Verapamil on the phosphorus content in , serum,egg shell and bone of newly hatched chicks



## Discussion

Egg shell is considered a rich source of calcium for newly hatched chick embryo during incubation time. About 75-80% of total calcium in chick embryo is derived from the egg shell at the late period of incubation ***(Richards, 1982 and Ono and Wakasugi, 1984)***.

Calcium ions are responsible for activation of pipping muscles (Musculus Complexus), which play an important role in liberation of chick embryo from egg and consequently improve egg hatching percentage ***(Christensen and Biellier, 1982).***

The role of calcium in muscular activity which is a vital process in hatching was declared by ***Narbaitz et al.(1980)*** who reported that the transfer of avian embryo from the dark to the light incubator increased the synthesis of 1,25-$(OH)_2$ $D_3$ and resorption of calcium from the shell, and the increased calcium concentration would be expected to increase embryonic muscular activity during pipping and hatchability.

The fluctuations in blood calcium levels during egg incubation was previously studied by ***Tuan and Scott (1977)*** who found that , the onset of calcium transfer by the chorioallantoic membrane occurs about days 10-12 of embryonic development. Maximal calcium transport activity occurs at day 19 and then decline. While ***Christensen and Biller (1982)*** recorded that, the plasma calcium in chick embryos increased significantly until day 19 and then decline. On day 21 of incubation the plasma calcium level was the lowest observed among the experiment.

In the present study both egg shell and serum calcium content was significantly reduced in newly hatched chicks from eggs dipped in 400 I.U. vitamin $D_3$ this is could be due to vitamin $D_3$ is increase within the egg could be responsible for the calcium withdrawal from the egg shell and blood and subsequently deposited in chicks skeleton and increased skeletal calcification of chicks ***(Kubota et al., 1981) Jensen et al. (1963). Balloun and Miller (1964)*** found that, the high calcium levels decreased the hatchability. However, ***Christensen and Edens (1985)*** reported that calcium injection in late period of incubation increased hatchability .

The present finding that egg dipping in vitamin D3 improved egg hatchability coincides with the previous reports of ***Sund et al. (1978)*** who found that , chick embryo can effectively use 1,25-(OH)2 D3, and eggs injected with 1,25-(OH)2 D3, had greater percentage of hatchability, and ***Ammenuddin et al. (1982)*** who found that, 1,25-(OH)2 D3, and 25-(OH) D3 support normal hatchability .

***Kubota et al. (1981)*** found that 25-(OH) $D_3$, is converted to 1,25-$(OH)_2$ $D_3$, after the $8^{th}$ day of incubation, where the embryonic chick renal 25-hydroxy, 1-α- hydroxylase doesn't become active until the $8^{th}$ day of incubation.

In the present study it was found that egg dipping in calcitonin, resulted in significant decrease in calcium content in sera and egg shell, while the bone calcium content in newly hatched chicks was significantly increased . The significant decrease in egg shell calcium may be due to increased transfer of calcium ions from the egg shell to the embryo during incubation period

***(Johnston and Comar, 1955; Simkiss, 1961; Rommanoff, 1967; Crooke and Simkiss , 1974 and Tuan, 1983).***

The hypocalcemic effect of calcitonin is due to its effects on calcium transefer from the blood to the bone ***(Capen., 1975 and Georgievskii., 1981)*** and its inhibiting effect on bone resorption process ***(Freeman., 1984 and Heerche., 1992)***. In addition calcitonin was reported to increase osteoblastic population of the bone and inhibit the bone resorption through blockage of osteoclastic osteolysis (***Chambers and Moor., 1983)***.

The ash content of hatched egg shells was significantly reduced after dipping in calcitonin or vitamin D3, while bone ash content was significantly increased. Meanwhile, the dipping of eggs in verapamil significantly increased calcium content of egg shell and decreased bone calcium content of newly hatched chicks as compared to that of other groups and this coincides with the present results on calcium content in both hatching egg shells and bones of newly hatched chicks. The results were confirmed by ***Boris et al. (1977)*** who found that , all of the metabolites and analogies of cholecalciferol increased tibia ash weight . In addition ***Vohra et al. (1979)*** showed that, the dietary deficiencies in calcium or vitamin D3 resulted in reduced tibia ash of quail and Leghorn hens. Similar results were obtained by ***Edward (1989)*** who found that 1,25-$(OH)_2$ $D_3$ significantly increased bone ash levels in broiler chicks .

It may be suggested that withdrawal of calcium from the egg shell and consequently decrease in its thickness would facilitate the pipping and hatching process .

The results of the present work indicated that , the egg dipping in verapamil resulted in a significant increase of egg shell calcium as compared to control groups. While it decreased bone calcium content of newly hatched chicks.

Verapamil is a calcium channel blockers acting as slow channel blocking agent ***(Johnson et al., 1991 and Levorse et al., 1991)***, which would suppress the transferee of calcium from egg shell through the chorioallantoic circulation to the bones of newly hatched chicks resulting in reduced hatchability percentage .

Calcium entry blockers have a wide spectrum of pharmacological activities as calcium is involved in several vital cellular processes including contractile, secretory and neural activities ***(Godfraind et al., 1986).***

Transport of calcium through the cellular membrane plays an important role in the stimulus, contraction, coupling process ***(Rasmussen., 1970 and Schwartz and Triggle., 1984).*** Thus it would be suggested that verapamil treatment of eggs resulted in embryonic death due to cardiac and respiratory impairment . Moreover the impairment of muscle contraction during pipping and hatching could result in embryonic death.

It could be concluded that dipping of eggs in other solution containing 400 I.U. Vitamin $D_3$ or calcitonin solution at concentration of 0.16 mg could improve the hatchability. This could be due to improvement of calcium transefer from the egg shell and embryo blood to the chicks muscle and bone.

# Summary

The present investigation aimed to study the effect of vitamin D3, calcitonin hormone in addition to calcium channel blocker verapamil on the transfer of calcium and phosphorus from the egg shell to the blood and bones of newly hatched chick, during the period of incubation, and also their effect on egg hatchability.

Five hundred fertile eggs were collected from 52 weeks old Saso breeder hens that were naturally met by Saso breeders males . Apparently normal eggs were incubated in a forced draft incubator.

Three hundred twenty eggs were classified into sixteen equal groups. Eggs were dipped for 5 seconds on the 2nd day of incubation for the first twelve group, while eggs of the last four groups (13-16) were dipped on the twelve day of incubation. All eggs were dipped in 100 ml at 15-18 C of one the following solution:-

***Group 1***: Distilled water.
***Group 2***: Ethyl alcohol (95 %) .
***Group 3***: Ethyl alcohol containing 0.04 mg calcitonin
***Group 4***: Ethyl alcohol containing 0.08 mg calcitonin
***Group 5***: Ethyl alcohol containing 0.16 mg calcitonin
***Group 6***: Ethyl alcohol containing 200 I.U Vit $D_3$.
***Group 7***: Ethyl alcohol containing 400 I.U Vit $D_3$.

*Group 8*: Ethyl alcohol containing 800 I.U Vit $D_3$.
*Group 9*: Ethyl alcohol containing 0.5 mg verapamil .
*Group 10*: Ethyl alcohol containing 1.0 mg verapamil
*Group 11*: Ethyl alcohol containing 5.0 mg verapamil
*Group 12*: Ethyl alcohol containing 20 mg verapamil
*Group 13*: Ethyl alcohol containing 0.5 mg verapamil
*Group 14*: Ethyl alcohol containing 1.0 mg verapamil
*Group 15*: Ethyl alcohol containing 5.0 mg verapamil
*Group16*:Ethyl alcohol containing 20.0 mg verapamil

All eggs were re-incubated again after the dipping procedures. Serum and bones (femur and tibia) were obtained from newly hatched chicks, and also the egg shells of hatched eggs were collected . Egg shells, and bones were ashed and the serum was prepared.

**The following parameters were determined :-**

1-The egg hatchability percentage .
2-Serum calcium and phosphorus content.
3-Egg shell calcium and phosphorus content .
4-The egg hatchability percentage .
5-The ash % of the egg shell.
6-The ash % of the bone of newly hatched chicks.

Statistical analysis of the data was performed and the results revealed the following:-

***A) Effect of calcitonin :-***

Dipping of eggs in ethyl alcohol containing 0.16 mg calcitonin results in :-

1) Significant reduction in the ionized serum calcium and phosphorus as compared to the 2nd control group (eggs dipped in ethyl alcohol).

2) Significant decrease in egg shell calcium content as compared to that of the 1st and 2nd control group (eggs dipped in distilled water or ethyl alcohol), or ethyl alcohol containing 0.04 mg calcitonin and insignificant reduction in egg shell phosphorus content as compared to that of other groups.

3) Significant increase in calcium and phosphorus content of the bones in newly hatched chicks as compared to eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.04,0.08 mg calcitonin .

4) Significant reduction in egg shell ash percentage of hatched eggs.

5) Significant increase for the bone ash percentage of the newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 0.04 and 0.08 mg calcitonin .

6) Improve of the hatching percentage in chicken eggs as compared to that of eggs dipped in distilled water or ethyl alcohol or even eggs without dipping .

***B) Effect of Vitamin $D_3$***

Dipping of eggs in ethyl alcohol containing 400 I.U vitamin D3 produce the following changes :

1) Significant reduction of egg shell calcium content and insignificant reduction of egg shell phosphorus content as compared to that of other groups.

2) Increase in bone calcium and phosphorus content as compared to that of eggs dipped in distilled water or ethyl alcohol.

3) Decrease in ionized serum calcium and phosphorus as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 I.U of vitamin $D_3$.

4) Significant reduction of egg shell ash % as compared to that of eggs dipped in distilled water or ethyl alcohol :-

5) Significant increase in bone Ash % of newly hatched chicks as compared to that of eggs dipped in distilled water, ethyl alcohol or ethyl alcohol containing 200 and 800 I.U of vitamin $D_3$ .

6) Dipping of eggs in ethyl alcohol containing 400 I.U of vitamin $D_3$ improved the hatchability percentage in chicken compared to that of eggs dipped in distilled water, ethyl alcohol or even without dipping eggs .

***C) Effect of verapamil :***

1) Dipping of eggs in verapamil (20mg) on the 2nd day of incubation reduced the hatchability percent to 45 as compared to that of control value of 75%. Eggs dipped at the 12th day of incubation in 0.5, 1.0, 5 and 20ml verapamil did not hatch ( zero hatchability %).

2) Verapamil treatment (20mg) significantly reduced the transfer of calcium from egg shell to the embryo as compared to that of control eggs .

3) Bone calcium and phosphorus content were significantly reduced in 20mg verapamil group as compared to that of control eggs.

4) The eggs dipped in ethyl alcohol containing 20 mg verapamil significantly increased egg shell ash percent and decreased bone ash percent as compared to those of control eggs respectively.

# Conclusion

It can be concluded that addition of 0.16 mg calcitonin or 400 I.U vitamin D3 per 100 ml in the dipping solution at the second day of incubation for five second at 15-18 °C would improve the egg hatchability which may be recommended in poultry industry.

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بسم الله الرحمن الرحيم

# الملخص العربي

استهدفت فى هذه الدراسة القاء الضوء علـــى تـأثير كـل مـن فيتـامين د ٣ وهرمــون الكالسيتونين بالاضافة الى تاثير الفيراباميل على انتقال الكالسيوم والفوسفور من قشرة البيــض فــى اثناء فترة التحضين الى كل من دم وعظام الكتاكيت الفاقسة وكذلك ايضا تاثير هـــذه المـواد علـى النسبة المئوية للفقس .

وقد تم استخدام عدد خمسمائة بيضة مخصبة وصالحة للتفريخ من قطيع امهات عمـــره ٥٢ اسبوعا من سلالة الساسو النقى وتم تحضين البيض فى ماكينة تفريخ وتم تقسيم عـــدد ٣٢٠ بيضـــة الى ست عشر مجموعة متساوية بكل منها ٢٠ بيضة حيث تم غمس كل المجموعات لمدة ٥ ثوانــــى عند درجة حرارة من (٥ –١٨) فى ١٠٠سم من احد السوائل التالية :

- مجموعة رقم (١) تم غمس البيض فى ماء مقطر فقط.
- مجموعة رقم (٢) كحول ايثيلى فقط تركيزه ٩٥%
- مجموعة رقم (٣) كحول ايثيلى يحتوى على ٠,٠٤ ملليجرام من هرمون الكالسيتونين .
- مجموعة رقم (٤) كحول ايثيلى يحتوى على ٠,٠٨ ملليجرام من هرمون الكالسيتونين .
- مجموعة رقم (٥) كحول ايثيلى يحتوى على ٠،١٦ ملليجرام من هرمون الكالسيتونين .
- مجموعة رقم (٦) كحول ايثيلى يحتوى على ٢٠٠ وحدة دولية من فيتامين د٣
- مجموعة رقم (٧) كحول ايثيلى يحتوى على ٤٠٠ وحدة دولية من فيتامين د٣
- مجموعة رقم (٨) كحول ايثيلى يحتوى على ٨٠٠ وحدة دولية من فيتامين د٣
- مجموعة رقم (٩) كحول ايثيلى يحتوى على ٠,٥ ملليجرام من الفيراباميل
- مجموعة رقم (١٠) كحول ايثيلى يحتوى على ١ ملليجرام من الفيراباميل
- مجموعة رقم (١١) كحول ايثيلى يحتوى على ٥ ملليجرام من الفيراباميل
- مجموعة رقم (١٢) كحول ايثيلى يحتوى على ٢٠ ملليجرام من الفيراباميل
- مجموعة رقم (١٣) كحول ايثيلى يحتوى على ٠,٥ ملليجرام من الفيراباميل
- مجموعة رقم (١٤) كحول ايثيلى يحتوى على ١ ملليجرام من الفيراباميل
- مجموعة رقم (١٥) كحول ايثيلى يحتوى على ٥ ملليجرام من الفيراباميل
- مجموعة رقم (١٦) كحول ايثيلى يحتوى على ٢٠ ملليجرام من الفيراباميل

وتم غمس البيض فى اليوم الثانى من التحضين فى المجموعات الاثنتا عشرة الاولـــى (١-١٢) بينما فى المجموعات الاربع الاخيرة (١٣-١٦) تم غمس البيض فى اليوم الثـــانى عشـــر مـــن

التحضين ثم اعيد البيض الى ماكينة التفريخ بعد الغمس مباشرة . وبعد فقس الكتاكيت تم تجميع الـــدم من كل كتكوت على حده للحصول على المصل وكذلك العظام (عظمة الفخذ والساق) اضافـــة الــى قشر البيض الذى فقس منها الكتاكيت وتم تجهيز كل من العظام والقشر للحصول على الرماد وبعـــد ذلك تم اجراء القياسات التالية :-

١- تقدير مستوى الكالسيوم والفوسفور فى مصل دم الكتاكيت .
٢- تقدير مستوى الكالسيوم والفوسفور فى قشر البيض الفاقس .
٣- تقدير مستوى الكالسيوم والفوسفور فى عظام الكتاكيت الفاقسة .
٤- تقدير النسبة المئوية للرماد فى قشر البيض الفاقس .
٥- تقدير النسبة المئوية للرماد فى عظام الكتاكيت الفاقسة .
٦- تقدير النسبة المئوية لفقس الكتاكيت .

وبتحليل البيانات احصائيا .... اوضحت النتائج ما يلى :

**اولا :- تاثير هرمون الكالسيتونين**

عند غمس البيض فى الكحول الايثيلى المحتوى على ٠،١٦ ملليجرام مـــن هرمـــون الكالســيتونين كانت النتائج التالية :

١- انخفض مستوى الكالسيوم المتاين والفوسفور فى مصل الـــدم انخفاضـــا معنويـــا بمقارنتـــه بالمجموعة الضابطة الثانية .
٢- انخفض مستوى الكالسيوم فى قشر البيض انخفاضا معنويا بينما كان انخفـــاض الفوســفور غير معنوى فى قشر البيض وذلك بمقارنتهما باقى المجموعات .
٣- اظهر مستوى الكالسيوم والفوسفور فى عظام الكتـــاكيت الفاقســـة ارتفاعـــا معنويـــا وذلـــك بمقارنته بباقى المجموعات .
٤- اظهرت مستويات الرماد فى قشر البيض انخفاضا معنويا فى نسبتها المئوية بمقارنتها ببـــاقى المجموعات .
٥- ارتفعت النسبة المئوية لرماد عظام الكتاكيت الفاقسة ارتفاعا معنويا وذلك بمقارنتـــها ببـــاقى المجموعات .
٦- ارتفعت النسبة المئوية لفقس الكتاكيت من البيض المعالج بالجرعة المذكورة وذلك بمقارنتـــها بالمجموعتين الضابطتين وكذلك نسبة الفقس المئوية فى القطيع المنتج لبيض العينة .

**ثانيا تأثير فيتامين د٣**

ترتب على غمس البيض فى الكحول الايثيلى المحتوى على ٤٠٠ وحدة دولية من فيتامين د٣ النتائج التالية :-

١- انخفض مستوى الكالسيوم المتاين والفوسفور فى مصل الدم انخفاضا معنويا بالمقارنة بباقى المجموعات .

٢- اظهر مستوى الكالسيوم فى قشر البيض انخفاضا معنويا بينما كان انخفاض الفوسفور غير معنوى .

٣- اظهرت مستويات الكالسيوم والفوسفور فى عظام الكتاكيت ارتفاعا معنويا وذلك بمقارنتها بالمجموعتين الضابطتين .

٤- انخفضت النسبة المئوية للرماد فى قشر البيض انخفاضا معنويا .

٥- اظهرت مستويات الرماد فى عظام الكتاكيت ارتفاعا معنويا فى نسبتها المئوية مقارنة بباقى المجموعات .

٦- اظهرت نتائج الفقس ارتفاعا ملحوظا فى نسبتها المئوية مقارنة بالمجموعتين الضابطين وكذلك نسبة الفقس بالقطيع المنتج لبيض العينة .

**ثالثا تاثير الفيراباميل :-**

اظهرت نتائج غمس البيض فى الكحول الايثيلى المحتوى على ٢٠ ملليجرام من الفيراباميل فى اليوم الثانى من التحضين انخفاضا ملحوظا فى النسبة المئوية للفقس حيث انخفضت من ٧٥ % فى المجموعة الضابطة الى ٤٥ % فى المجموعة المعالجة بينما نفقت كل الاجنة فى المجموعات الاربع الاخيرة التى تم غمس البيض فيها فى اليوم الثانى عشر من التحضير .

كما اظهرت نتائج غمس البيض فى الكحول الايثيلى المحتوى على ٢٠ مج من الفيراباميل النتائج التالية ايضا .

١- اظهرت نتائج مستوى الكالسيوم فى قشر البيض الفاقس ارتفاعا معنويا بينما كان ارتفاع الفوسفور غير معنوى بالمقارنة بالمجموعتين الضابطتين .

٢- انخفض مستوى الكالسيوم والفوسفور فى عظام الكتاكيت (عظمة الفخذ والساق) انخفاضا معنويا بمقارنتها بالمجموعتين الضابطتين .

٣- اظهرت مستويات الرماد فى قشر البيض ارتفاعا معنويا بمقارنتها بباقى المجموعات .

٤- انخفضت مستويات الرماد فى عظام الكتاكيت بصورة معنوية بمقارنتها بالمجموعتين الضابطتين.

نستنتج من هذه الدراسة انه يمكن الاستفادة من نتيجتها فى مجال صناعة الدواجن وذلك بغمس البيض فى اليوم التالى من التحضين لمدة ٥ ثوانى عند درجة حرارة من ١٥-١٨ درجة مئوية فى محلول الكحول الايثيلى المحتوى على الكاسيتونين او فيتامين د٣ بمعدل ٠,١٦ ملليجرام او ٤٠٠ وحدة دولية لكل ١٠٠ سم من المحلول حيث يؤدى ذلك الى ارتفاع نسبة فقس الكتاكيت وكذلك انخفاض نسبة النفوق فى المراحل المتاخرة من التحضين

# تحـــت إشـــــــــــراف

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# دراسات فسيولوجية على مستوى الكالسيوم فى دم وبيض الدواجن

رسالة مقدمة من


**ط.ب/ سمير احمد ابو العيون**

بكالوريوس العلوم الطبية البيطرية

جامعة القاهرة

١٩٨٢


للحصول على

درجة الماجستير فى العلوم الطبية البيطرية

**(فسيولوجيا الحيوان)**

مقدمة الى

**كليـــة الـطب البيـطرى جامعة الاسكندرية**

٢٠٠٠