Effect of Sea-shell Based Calcium Carbonate Supplement (Cockle) on the Egg Quality of Japanese Quail (*Coturnix coturnix japonica*)

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Roll No: 0120/01

Registration No: 789

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Department of Dairy and Poultry Science

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

December, 2022

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This is to certify that we have examined the above Master's thesis and have found that the thesis is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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The Author

December, 2022

DEDICATED TO MY BELOVED FAMILY

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LIST OF ABBREVIATIONS

Abbreviations	Elaborations
ANOVA	Analysis of Variance
AOAC	Analysis of Official Analytical Chemist
Ca	Calcium
P	Phosphorus
CF	Crude Fiber
CP	Crude Protein
CVASU	Chattogram Veterinary and Animal Sciences University
CRD	Completely Randomized Design
DDPS	Department of Dairy and Poultry Science
DMRT	Duncan’s Multiple Range Test
DOC	Day Old Chick
EP	Egg production
g/b	Gram per bird
HU	Haugh unit score
HHEP	Hen housed egg production
Mg/dL	Milligram Per Deciliter
g/L	Gram Per Litter
DM	Dry Matter
EE	Ether Extract
EW	Egg weight
FCR	Feed Conversion Ratio
LWG	Live weight gain
MS	Master of Science
PRTC	Poultry Research and Training Center
e.g.	Example Given
et al.	And his associates
etc.	Et cetera
ft.	Feet
i.e.	That is

ml	Mili-litre
Sq. ft.	Square Feet
ME	Metabolizable Energy
%	Percentage
<	Less Than
>	Greater Than

Abstract

The study was undertaken to investigate the effects of sea-shell (*Anadara granosa*) supplemented diet on the growth performance, viability, egg production performances and egg quality of layer quail. A total of 60 Japanese quails (*Coturnix coturnix Japonica*) of similar age (50 days old) were distributed randomly into three dietary treatments including T_1 (control), $T_2$and T_3 in a completely randomized design (CRD). Each treatment was replicated four times with 5 birds per replicate. Quails were reared in the battery cages of equal size from d50-71 days (3 weeks). Iso-caloric and iso-nitrogenous formulated diet (mash) was fed the layer quails throughout the trial period. Birds had a free access to test diet mixed with sea-shell powder at the rate of T_1 (0%), T_2 (1.76%), and T_3 (1.88%), and water supplied to the birds *ad-libitum*. Bird was reared under uniform feeding, rearing, lighting and standard management condition throughout the trial period. Data on feed intake (FI), body weight (BW), feed conversion ratio (FCR) and livability (%) were recorded on day71. Besides, egg weight, hen housed egg production (HHEP) %, egg production (EP) number, shell weight, Haugh unit (HU) score, shell weight %, shape index, albumen weight, albumen weight %, albumen index, yolk weight, yolk%, and yolk index were also measured to evaluate the egg quality performance of layer quail during 50 to 71 days of rearing period. The data revealed that dietary treatments did not affect FI, BW, FCR, and viability % of quail ($P>0.05$). The egg weight was unaffected ($P>0.05$) between the dietary treatment, but, EP and HHEP % were differed significantly ($P<0.05$) between treatment from 57 to 71 days. The highest EP (32.25 no/w/b) number and HHEP (92.15%) were observed in the birds fed supplemented diet (T_2) while lowest EP (25.25 no./w/b) and HHEP (72.14%) being in the unsupplemented or control group (T_1) from d65 to 71 days. It is clear from the results that, the most of the egg quality parameters measured in this study was not affected ($P<0.05$) by the dietary treatments except for shell weight % and shape index. Only the shell weight % and shape index were found to be improved ($P<0.05$) in the birds fed supplemented dietary group (T_2, T_3) compared to control or basal diet (T_1) during 2nd (57 to 64 days) and 3rd (65 to 71 days) weeks of ages, respectively. It can be concluded that supplementation of sea-shells (cockle) in layer diet was found to be a more potential natural source of calcium which could enhance the egg production performances (HHEP), egg number and egg quality of quail under commercial farming condition.

Keywords: Growth, viability, cockle shell, egg production performance, egg quality traits.

Chapter-I

INTRODUCTION

Chapter-I

INTRODUCTION

Poultry industry has been a part and parcel of the world food industry, because it provides a very nutritious food items (i.e meat and egg) to the majority of people of the world. For this reason, poultry productions increasing day by day due to meet up the huge protein gap of the world. Egg is a premier quality source of animal protein which contains all sort of essential amino acids in proper ratio and amount necessary for the human health. The egg is available to the consumer world from a variety of poultry species such as chicken, duck, quail, geese and others. Though chicken egg is very dominant in our country, recently quail farming is steadily increasing side by side, and its egg is adding other dimensions to enrich the whole egg industry of the local market in Bangladesh. Because consumer world would like to enjoy variety of food taste appeal coming from the different food sources. Now quail egg is being more conventional and available in the market, and well-known to all the people as a popular food item; because it provides a unique well-balanced source of nutrients for the people of all ages. Availability, moderate cost, popular taste appeal, lower calorie value, ease of preparation, high biological value etc., have given egg a deserved place in human diet in the consumer world. Due to their highly nutritive value, unique functional properties, poultry egg components remain one of the most commonly used ingredients in the food industry.

It goes without saying that, the egg industry is booming steadily across the world, because of its heavy demand to the consumer world for meeting consumer nutrient requirements. This egg demand is giving a heavy pressure on egg producing industry food processing industry, and egg marketing in order to produce, store, sell and market quality egg and egg products to the consumers. However, once egg is derived from the laying flock, then it is inevitable to store and preserve them properly in order to maintain its optimum quality, and then reach them safely for long term consumption by the people. Moreover, the economic success of laying industry depends on number of quality egg produced. So, it is, therefore, important to pay attention to the problems of quality egg production, storing, preservation and marketing of eggs, and to maintain its optimum quality that is acceptable to the consumers (Adeogun & Amole, 2004; Song *et al*., 2000; 18).

For external egg quality characters, physical appearances (e.g cleanliness, freshness, egg weight or size, shell colour) are important for consumer’s preference and acceptance; if the products do not meet perceived expectations, consumer confidence reduces (Hosseini Siyar et al., 2007). Similarly, internal quality factors namely yolk index, yolk colour, Haugh Unit, albumen quality, pH and nutrient composition are also equally important in egg product industry as the demand for liquid egg, frozen egg, egg powder, egg food items, and yolk oil are now on increasing trend (Scott & Silversides, 2001). Albumen quality is not only an important indicator for egg freshness, but it is also important for the egg breaking industry because albumen and yolk have different markets (Ahn et al., 1997; Akbar et al., 1983; Proudfoot, 1962; Lapao et al., 1999).

As we know that egg quality can be deteriorated in different ways or by different factors, for example, genetic factors, storage time, temperature, humidity, bird age, bird size, flock health, nutrition, protein sources, micro-nutrients (Ca, P, Mn, Zn, Vit A, D), dietary fibre, disease incidence and so on. Besides these, interaction effect between storage time and temperature may also affect egg quality (e.g. egg weight loss, albumen height, air cell, pH) significantly reported by Samli et al., 2005). Internal quality of eggs might deteriorate due to time, temperature and storage condition, and this relies on the shell and internal contents of the egg (Adeogun & Amole, 2004; Kul & Seeker, 2004). After production, faulty handling, or transportation, poor storage conditions might result in deterioration of egg quality, and consequently loss and wastage of eggs affecting producers and environment. Moisture loss of egg through the shell pores, retarding of microbial attack, and lower temperature are the salient points of preventing egg quality degradation. Since storage environmental factors could also influence the quality of eggs, methods like lower temperature and modified atmosphere packaging such as refrigeration have been recommended (Chang and Chen., 2000).Poultry egg producers, poultry integrators or egg dealers are very anxious about the egg quality particularly, external protective covering known as egg shell quality, because it can be a main reason for the economic losses (Joshi et al.,2019; Gül et al.,a2022). The functions of eggshell are- it protects the eggs from breaking or physical breakage and pathogens coming from the outsider environment, and provide necessary nutrients for embryonic development, especially calcium. Therefore, it is very important to maintain the optimum shell quality of laying hens along with others components of egg, and it appears a great challenge or threat or burning issue for the poultry scientist or researchers across the globe (Ketta et al., 2020). Modern commercial layer quail strains have become increasingly productive and demanding in terms of management, health, environment and nutrition.

Studies have been developed to improve not only animal productivity but also the quality of final products. In the layers, their final product is egg for human consumption. The main concern of farms related to egg quality is egg shell quality, egg weight or size, price, palatability, color availability including other quality factors. Kussakawa et al. (1998) mentioned that in order to be marketed, the eggshell of eggs must be strong enough to resist lay, collection, grading and transport until they reach the final consumer. Murata et al. (2009) observed better egg production, feed conversion ratio, egg weight and eggshell strength and thickness as dietary calcium levels increased but the different combinations of fine and coarse limestone did not influence the studied parameters.

Amongst the poultry species, Japanese quails are considered as a valuable laboratory animal for conducting poultry or avian research. Various factors say small size, easy handling , little space needs to raise the birds with feeding a very small amount of feed consumption etc., make this birds more favorable tools for conducting avian research works smoothly (Lukanov, Reference Lukanov, 2019; Lukanov and Pavlova, Reference Lukanov and Pavlova, 2020). It is reported that quail eggs are produced in the East Asia and Brazil in a greater amount, and most quail meat is produced in Europe, the USA and China (Lukanov, Reference Lukanov, 2019). Apart from these, recently quail meat and egg production are steadily increased and being more popular in the other parts of the worlds say south East-Asian countries (India, Bangladesh). It is necessary to give particular attention towards nutrition of the birds for improving and development of quail farming (Amoah et al., 2012). As we know that feed cost is the major cost in poultry farming, this cost may goes up by many factors or reasons say the lack of available information on nutritional requirements, underestimating or overestimating their nutritional needs, resulting in economic losses (Sarcinelli et al., 2020). The minerals are the most important micro-nutrients, which need very little amount and make up 5% of an animal's body. Calcium and phosphorus amongst all other minerals play a great role or contribution to skeleton building (80–85%), and their role in egg shells and muscle development, making them essential to animal functions (Stanquevis et al.,2021).

Dietary calcium is an important and essential mineral for bird development and egg shell quality (Ahmed et al., 2022). It considers as the main component of egg shell, which consists of 95% of calcium carbonate (Miles, 1993). Cockle shell (*Anadara granosa*) powder is a rich and naturally purified source of calcium carbonate (98.7%) available in our country at the coastal areas (Awang-Hazmi et al., 2007). This is a natural resource which can be used as a Ca- supplement for our poultry production. We can prepare calcium carbonate supplement for the layer bird from the cockle shell collected from our neighboring sea. This shell can reduce the dependence of using limestone available in the market and cut our production cost tremendously. No research data is available regarding this so far conducted in our country. Much is known about the calcium requirements of laying hens, but little information available on the calcium requirements for laying quails. There is a close relationship between calcium metabolism and poultry aging (Manangi et al., 2018; Zhao et al., 2020; López et al., 2021; Lee et al., 2021), so determining the optimal levels of calcium in the diet is essential to ensure the economic viability of the quail production. Research focusing on cockle shell might be a potential ingredient for the layers as many quail farms are already established in our country. Considering this view, the present study was conducted to assess the growth performance, survivability, egg production performance, external and internal egg quality of Japanese quail fed diet supplemented with sea-shell.

Objectives of the study:

Layer quail diets supplemented with the sea-shell (cockle) as a natural source of calcium supplement could be very cost-effective or might have potential to enhance the productivity, egg production performance, and egg quality by cutting feed cost for raising quail profitably under farming condition. Considering the above, the present study was undertaken to meet the following objectives:

1. To assess the growth performance in terms of body weight, feed intake, feed efficiency and viability of quail fed cockle shell based diet
2. To determine the egg production performance (hen housed egg production) of quail fed diet supplemented with sea-shells (cockle)
3. To investigate the external and internal egg quality (egg weight, shape index, albumen weight, yolk weight, shell%, yolk %, albumen%, yolk index, Haugh unit score, shell weight,) of quail fed cockle shell based diet

Chapter-II

Review of Literature

Chapter-II

REVIEW OF LITERATURE

2.1 Introduction

Egg is a popular food item; because it provides a unique well-balanced source of nutrients for the people of all ages. Egg has a great demand in the world's food industry to provide quality food products to the consumers. And egg quality is vital, as quality food products can be manufactured only from quality raw materials. So, it is inevitable to maintain egg quality to reach the consumer world safely. Once egg is received from the quail, its quality may be deteriorated by various ways, which incurs a lot of economic loss affecting the producers, consumers and environment. So, it is very important to explore the dietary effects and problems of eggs how to ensure its optimum quality and consumer safety across the globe.

The fertile egg is highly complexed reproductive cell and is a tiny center of life, where initial development of embryo takes place. Most of the commercial eggs are infertile. The yolk is surrounded by albumen, having high water content, elasticity and shock absorbing capacity. This entire mass is surrounded by two membranes and an external covering called egg shell. The shell provides a proper shape to the egg and is meant for conserving the valuable nutrients within the egg. Hen egg contains approximately 76% water, 12% protein, 10% lipids and rest vitamins, minerals and carbohydrates. Egg is a major source of human dietary protein with high biological value and excellent protein efficiency ratio.

Egg quality particularly, external protective covering known as egg shell quality, has been a main problem or rising concern tothe poultry egg producers, poultry integrators or egg dealers, because it can be a main reason for the economic losses (Joshi *et al*., 2019; Gül et al., a2022). The functions of eggshell are- it protects the eggs from breaking or physical breakage and pathogens coming from the outsider environment, and provide necessary nutrients for embryonic development, especially calcium. Therefore, it is very important to maintain the optimum shell quality of laying hens along with others components of egg, and it appears a great challenge or threat or burning issue for the poultry scientist or researchers across the globe (Ketta et al., 2020).

It is reported that many factors involved to influence the eggshell formation, it includes genotype, environment and diet calcium content (Ketta and Tůamová, 2016; Tufarelli et al., 2021). Attaining better quality eggshell is closely associated with the dietary supplementation say calcium, phosphorus and vitamin D_3inclusion at the poultry diet in a proper ratio, amount and proportionate level (Al-Zahrani and Roberts, 2015). The optimum rate of calcium relies on the number of factors involving type of breed used, sex, type of birds, nutrient ratio (calcium/phosphorus ratio), vitamin D and vitamin C contents, age, productive or laying phase, availability of nutrients in the ration (Attia et al., 2020; Zhao et al., 2020; Stanquevis et al.,2021).

Many research has been done on the relationship between layers, age and the calcium content on quality egg (Swiatkiewicz et al 2018; Kakhki et al.,2019; Attia et al.,2020; Wang et al.,2021). Many incidences we see or happen, for example, egg weight increases or decreases, thin egg shells, soft egg shells, crackage or breakage egg, soiled egg, crooked egg, deformed egg, egg without shell, internal quality degraded, layer stops laying egg etc., during the course of the egg production cycle. To overcome the deformities of egg and enhancing quality egg production , we think adequate supply of nutrition through dietary supplementation and efficient metabolism of micro-nutrients (Ca, P) is crucial to ensuring saleable quality eggs (Kakhki et al., 2019). Poultry nutritionists often change or modify dietary calcium or other feed sources or requirement to resist or reduce the decreasing eggshell quality as laying birds grow older. Therefore, the requirement or suggestions for dietary calcium supplements in layer ration are always volatile or changeable , not static or stable, and the results of previous research activities regarding calcium supplementation by feedstuffs in layer ration or are inconclusive or often contradictory (Ketta et al., 2020).

Different calcium sources (such as limestone or oyster shell or others) are used in poultry nutrition by many previous investigators (Manangi et al.,2018; Bagheri et al.,2021; López et al.,2021; Lee et al.,2021). Among that, sea-shell (cockle) is an organic or natural source of calcium carbonate and can be used as a supplement in the diet of laying quail (Bagheri et al.,2021). The sea-shells are a new in supplementation strategy in poultry nutrition, so increasing interest in this supplement in the poultry ration might give a new dimension in poultry industry for enhancing quality egg production. Awang-Hazmi et al.(2007) reported that sea-shells (cockle) are a rich source of calcium carbonate (98.7%) along with other sources of many minerals say P, Mg, Zn, Cu, Na, K, B, Si, and Fe etc., in a little amount, which are considered as a very important minerals for enhancing egg quality characters of layer. These nutrients could improve intestinal absorption, facilitates binding to tissues, blood circulation and metabolism. The main advantages of sea-shells are, organic, naturally available, good bioavailability, high solubility and excellent gastrointestinal tolerance, which could favour its absorption in the intestine (Falguera et al., 2010). Further, it suggests that the inclusion of sea-shells in layer quail might improve certain parameters (such appearance, mechanical resistance and functional properties) of egg and bone (Agblo and Duclos, 2011; Al-Zahrani and Roberts, 2015; Joshi et al., 2019). It is noted that research on its use in laying quails is very scarce. However, the egg quality can be influenced by multiple factors say age, sex, breed, genetics, nutrition, temperature, storage and so on. Amongst these factors, nutritional factors (Ca, P, vitamin D, feed composition, feed sources, dietary sources of calcium, proper ratio and amount of the nutrients) stand out salient factors which could take into account for improving egg quality of layer quail.

2.2 Seashell

Seashell**,** hard exoskeleton of marine mollusks such as snails, bivalves, and chitons that serves to protect and support their bodies. It is composed largely of calcium carbonate secreted by the mantle, a skin-like tissue in the mollusk’s body wall. Seashells are usually made up of several layers of distinct microstructures that have differing mechanical properties. The shell layers are secreted by different parts of the mantle, although incremental growth takes place only at the shell margin. One of the most distinctive microstructures is nacre, or mother-of-pearl, which occurs as an inner layer in the shells of some gastropods and bivalves and in those of the cephalopods *Nautilus* and *Spirula*.

2.2.1 What are the Seashells?

A seashell is a hard, protective exoskeleton formed by invertebrate animals who live in the sea and are often found washed up on beaches throughout the world. The most common animals which produce a seashell are mollusks, crabs, oysters, barnacles, brachiopods, annelid worms, and sea urchins. While most seashells are external, some species (e.g., cephalopods) exhibit internal seashells. Seashells are comprised of calcium carbonate and a small quantity of protein.


Fig: Sea-shell

2.2.2 Seashell Formation

Seashells are typically formed in distinct layers via the extracellular secretion of proteins which are then covered by calcium carbonate. Therefore, the shell grows from the bottom upwards, with the constant secretion of new material at the margin between the animal and the shell. The tissue responsible for shell formation is called the mantle. The mantle resides at the interface between the body of the animal and the shell. As the animal grows, the shell also grows and becomes increasingly strong, to accommodate the larger size of the animal and provide adequate protection. There are three distinct layers of the shell produced by the mantle.

2.2.3 Types of sea shells

There are a wide variety of seashells, each distinguished by the species from which they are derived. By far the most common types of seashell encountered on beaches are those produced by mollusks. In addition to mollusks, various other species produce seashells. They are as following:

a. Mollusk shell b. Arthropods Annelids c. Brachiopods

d. Sea urchins e. Corals f. Coccolithopore

2.2.4 Chemical composition of sea shell

They may look very different, but pretty much every shell you pick up on the beach is made of the same stuff: calcium carbonate ($CaCO_3$), otherwise known as the mineral calcite. It's actually the same mineral that stalactites and stalagmites that are found in caves are made of, although obviously the formation process is very different. Shells can also be made of the mineral aragonite, which has exactly the same chemical composition as calcite ($CaCO_3$)but the atoms within the mineral are packed together in a different way, giving the mineral a different structure. Interestingly, the mineral aragonite only forms geologically under conditions of high temperature and high pressure—it's not geologically stable under normal conditions at Earth's surface; it converts to calcite. Exactly how some shellfish manage to make this usually unstable form of calcium carbonate is still a mystery. The sea-shell cockles (*Anadara granosa*) are a rich sources of calcium carbonate (98.7%), and a little sources of Na-0.9%, Mg-0.05%, P-0.02% and others 0.2 % (Awang-Hazmi et al., 2007).

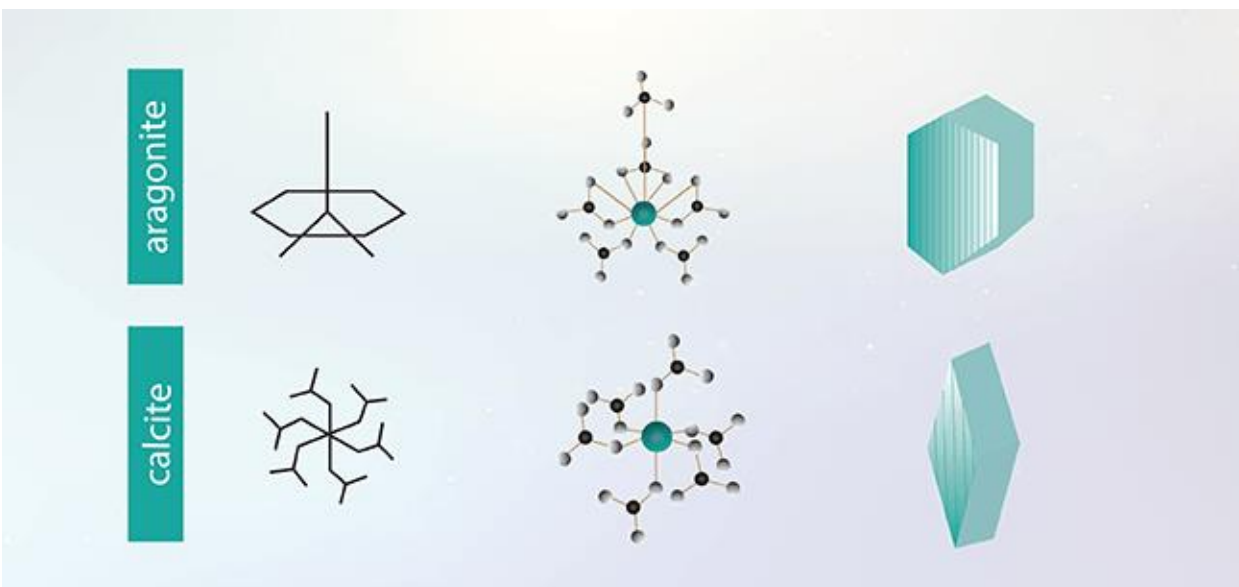

Fig : From left, comparison of form, molecular structure and crystal shape of calcite and aragonite.

2.3 Egg quality assessment

Calcium is an important constituents used for maintenance and egg production of laying chickens (William, et al.,2006; Ahmed et al., 2013). The effect of calcium source on egg weight is in agreement with the results of Lichovnikova (2007); but not with those reported by Pelícia et al (2007). The egg weights (60.8 to 63.9 g) are in the range of 60-65.5 g mostly recorded in the latest laying month of brown eggs layer hens (Nys et al 2008). Using limestone and oyster shell, Khalil and Anwar (2009) did not find a significant effect on eggshell weight and eggshell proportion. The negative effect of snail shell on eggshell quality confirms the effect of calcium source on egg quality reported by Lichovnikova (2007) who observed significant effect of different dietary mineral feedstuffs on eggshell weight, eggshell proportion and eggshell thickness. In high ambient temperature (32-35 °C versus 21-24 °C), hens decrease the synthesis of shell between 6 and 30% (Nys 1995); hence the ambient temperature of 27.9 °C in the poultry house during the experimental period might contribute to the decrease of eggshell thickness. The age of hens is also reported to affect their calcium efficiency (Curtis 2008). In this experiment old hens were used. The quality of eggs could be therefore affected by the age of hens (60 to 72 weeks-old) during this experiment. The similarity of shape index and Haugh units of eggs is in agreement with the results reported by Safaa et al (2008) and Pelícia et al (2007) when they supplied different dietary calcium sources to layer hens. However, the significant depressing effect of the mineral feedstuff on the shell index and on the albumen and yolk percentage is contrary to the findings of Safaa et al (2008) regarding these variables.

Quality is a broad term that captures aspects of physical characteristics, flavour, and odour. A high-quality egg can command a higher price for the producer and will maintain its quality longer during post marketing. Egg quality is determined using external factors such as cleanliness, shell condition, colour, and shape, as well as internal factors such as albumen thickness, yolk condition, air cell size, chemical composition and the presence of defects such as meat or blood spots. Quality is vast term which is the sum total of all characteristics of an individual. Quality refers to the sum of the characteristics of the given food items which influence the acceptability or preference for that food by consumers.

2.3.1 Egg quality factors:

I. **Egg production**
II. **Size of egg**
III. **Haugh Unit**
IV. **Albumen quality**
   - Albumen weight
   - Albumen%
   - Albumen index
V. **Yolk quality**
   - Yolk weight
   - Yolk%
   - Yolk index
VI. **Egg shape**
VII. **Shell quality**
VIII. **Nutritive values**

2.3.2 Egg production

Egg production of laying hen in diets with extruded eggshells was comparable to the diet with limestone or oyster shell (Arvat and Hinners, 1973; Vandepopuliere et al., 1978), (Vandepopuliere et al.,1973; 1975) reported the heavier egg (65.7 g) production of laying hens fed a diet with extruded eggshell compared to the diet with limestone (64.8 g). Extruded eggshell is probably the best and most available unconventional natural source of calcium. In addition, birds highly prefer eggshells even 10-20 times more than limestone or oyster shell. On the other hand, digestion, as well as utilization of extruded eggshell in the body of birds, is much better than limestone or oyster shell (Bee, 2011). Using extruded eggshells in the diet of poultry will also help to save the environment. Considering the above points, the objective of the present study is to assess the effect of extruded eggshell, limestone, or oyster shell on egg production performance and profitability of laying hens for producing a safe and cost-effective egg. Its particle size may play an important role in maintaining eggshell quality and bone mineralization (Blister et al., 1981; Guinotte and Nys, 1991; Keshavarz et al., 1993). Mroz et al., 2007) found a relationship between the mammillae size and hatchability of chicken eggs.

2.3.3 Size of egg and egg quality

Calcium is an important constituents used for maintenance and egg production of laying chickens (William, et al.,2006; Ahmed et al., 2013). The effect of calcium source on egg weight is in agreement with the results of Lichovnikova, 2007; but not with those reported by Pelícia et al., 2007. The egg weights (60.8 to 63.9 g) are in the range of 60-65.5 g mostly recorded in the latest laying month of brown eggs layer hens (Nys et al 2008). Quail eggs are nearly one-fifth of the size of chicken eggs. Generally, egg weight ranges from 6 to 16 g, with an average weight of 10g (Nys et al., 2008.)

2.3.4 Haugh Unit (HU)

Internal quality of egg can be assessed by measuring HU score of the eggs. We can easily detect whether the egg is fresh or old by knowing the HU score of an egg. The higher the HU score the better the quality of egg. Eggs having 60-70 HU score should be selected for hatching purposes. The HU of egg can be measured by the following HU formula: H.U=: 100 log H + 7.37 - 1.37W$^{o.37}$;where, H.U= Haugh unit, H=The height of the albumen in mm; W=Weight of egg in grams. The eggs which possess the HU score below the 55 would not be selected for hatching. The United States Department of Agriculture standard grades(USDA) has been classified the eggs on the basis of the following HU unit score:

Table1: Haugh unit score

Category	H.U score
”AA’’	72
A	60-72
B	31-60
C	<30
Excellent	78
Good	55-75
Off-grade	31-54
Very off grade	<31

Quality has traditionally been measured by albumen height of the broken out egg or Haugh units which include the effect of egg weight to equalise the measurement between eggs of different size (Haugh, 1937). However, the Haugh unit's ability to do that, compared with simple albumen height, has been questioned, although for selection within a genetic line it may be appropriate (Silversides *et al.*, 1993). These measurements were introduced as a way of determining freshness rather than the properties desired by the user or catering processor, for example in custard production (Ericson, 1943)

2.3.5 Albumen quality

Egg albumen is one of the oldest fining agents used for reducing the harshness of red wines. Peynaud (1984) reported that about 12.5% (w/w) protein can be found in fresh egg whites. The principal proteins in egg white are albumen (water soluble) and globular proteins (soluble in neutral dilute salt solutions). To aid solubility of egg white, addition of small amounts of sodium chloride is a common practice.

The egg white, also called albumen, consists of the 60-65% of the total egg weight, and is the egg part with more protein, a key component in egg composition. It is a source or nutrient for the development of the embryo during incubation, so its quality will have a direct effect on the hatching percentage and chick weight and health.

2.3.6 Firmness of the albumen

Funk (1944), Korslund et al. (1956) and Mueller (1958) reported that relative humidity during storage, which controls the rate of evaporation, has little or no effect on albumen and yolk deterioration. Less is known on the relationship between albumen quality breakdown and water movement from albumen to yolk

The higher the firmness of the albumen the better the quality of the eggs. Eggs which are prone to possess more watery albumen do not hatch well. The firmness of albumen may be determined by the albumen index. The standard albumen index of the egg is 0.82—1.0

2.3.7 Yolk quality

Firmness of the yolk:

The yolk index, defined as the ratio of yolk height over yolk diameter, provides indication on the freshness of the egg. According to Ahmed, Dafalla and Malik, eggs with yolk index above 0.38 are considered as extra fresh. Those ranging from 0.28 to 0.38 are fresh and those below 0.28 are considered regular.

Sharp and Powell (1930) reported a procedure for determining a value which they called "Yolk index." The procedure required removal of the yolk from the albumen and then a five-minute waiting period for the yolk to spread prior to measuring its height and width, two ways at right angles. In 1948, Funk proposed a modification which greatly speeded up the procedure. This method involved measuring height and width of yolk without removing it from the albumen and without waiting five minutes. Funk (1948) compared the two methods and suggested that the value determined by his method should be reduced by ten percent for comparison with values determined by the Sharp and Powell (1930) method. In the work herein reported, a similar comparison of the two methods was made. Measurements of yolk index by the Sharp and Powell (1930) and the Funk (1948) method were made on 413 eggs

Heiman and Wilhelm (1937) found no significant correlation between yolk index (ratio of yolk height to yolk width) and percentage firm white or albumen index for eggs kept for the same length of time. Smith (1931) reported that yolks gain approximately 25% less water in an atmosphere of 100% C02 than in air. Needham (1931) observed that in artificial osmotic systems diffusion of water into the yolk is much more rapid than in intact eggs. Smith (1931) concluded from similar results that an unknown factor or factors offer a considerable resistance to the establishment of an osmotic equilibrium between yolk and albumen.

The higher the compactness of the yolk the better the quality of the eggs and contribute the excellent hatching result. The firmness of the yolk is measured by the yolk index. The standard yolk index is 0.40

2.3.8 Egg shape:

Egg shape index is defined as the ratio of width to length of the egg, and it is an important criterion in determining egg quality. Domestic hen eggs that are unusual in shape, such as those that are long and narrow, round, or flat-sided, cannot be placed in grade AA (nearly perfect) or A (slightly worse than AA) since an egg is generally oval in shape (72–76). Round eggs and unusually long eggs have poor appearances and do not fit well in egg cartons; therefore, they are much more likely to be broken during the shipment than the eggs of normal shape (Sarika and Erensayin,2009).Eggs that deviate considerably from the normal do not hatch well due to poor quality. Eggs laving off-shaped, ridged, wrinkled never be selected for hatching. The shape of the egg should be normal or egg shaped. The shape of egg is determined by the shape index by following the under mentioned formula:

Shape index: Width of the eggs/length of the egg

Standard shape Index for eggs: 72---77.

Shape index below 73 should not select for hatching egg.

2.3.9 Egg Shell:

Vandepopuliere et al. (1975) reported that the calcium level in an eggshell is comparable to that of limestone with the benefit of a small amount of protein. The formation of eggshells in the uterus is required to maintain adequate blood Ca+2 levels (Sultana et.al. 2007).The eggshell contains approximately 98.2% calcium carbonate, 0.9% magnesium and 0.9% phosphorus (phosphate) (Romanoff and Romanoff, 1949).

The outer cover of the egg, the shell comprises 10-11% of total egg weight. On an average the eggshell weighs 5-6g, with remarkable mechanical properties of breaking strength (>30N) and is 300-350 micrometer thick. This structure plays a crucial role in protecting the contents of the egg from the microbial and physical environment and in controlling the exchange of water and gases. The calcium content of the eggshell is approximately 1.7-2.5g.

Table 2: Chemical composition of eggshell

Calcium carbonate		:94-97%
Phosphorus		:0.3%
Magnesium		:0.2%
Sodium, Potassium, Manganese, Iron and Copper		:traces
Organic matter		:< 2%

The small amount of organic matter mostly consists of matrix proteins (mixture of proteins and polysaccharides rich in sulphated molecules) and shell pigment. The matrix proteins are critically important in determining the egg shell structure and serves as foundation for the deposition of calcium carbonate. There are about 8000 microscopic pores on the shell. The outer surface of the shell itself consists of a mucous coating (cuticle) which is deposited on the egg just prior to the lay. This proteineous covering helps to protect the interior content of the egg from bacterial penetration through the shell

.2.3.10 Shell Quality

The aesthetic quality of the egg shell relates to the quality factors which one can observe; such as soundness of the shell, shape of the shell and colour of the shell. However, for commercial layer and breeder operations, shell quality means increased shell thickness and shell breaking strength to reduce number of cracked eggs, an increased number of saleable/ hatching eggs and a higher number of viable day old chicks. Scheideler (1998) reported that Ca is available in eggshell which will influences the hatchability and profitability.

2.3.11 Shell weight, shell% and shape index of quail egg fed sea-shell calcium diet:

Using limestone and oyster shell, Khalil and Anwar (2009) did not found a significant effect on eggshell weight and eggshell proportion. The negative effect of snail shell on eggshell quality confirms the effect of calcium source on egg quality reported by Lichovnikova (2007) who observed significant effect of different dietary mineral feedstuffs on eggshell weight, eggshell proportion and eggshell thickness. Besides this Skřivan et al., 2010 stated no influences of large particles limestone upon performance, egg quality, egg shell thickness, egg breaking strength and specific gravity. Cufadar *et al.* (2011) examined the influences of nutritive Ca levels (3.0, 3.6, or 4.2%) and limestone particle sizes (< 2 mm, 2-5 mm, > 5 mm) in moulted hens (76 weeks of age). The achieved findings demonstrated that medium or large limestone particle sizes had a favorable effect on eggshell and tibia bone breaking strength when the diet was low in Ca, but this impact was not found in layers fed a normal or high Ca content. Other findings informed the positive effect of substituting fine with coarse particles of limestone or oyster shell, both of which had longer retention times in the gizzard, and dissolved slowly which provide the hen's organism more evenly with Ca along maintaining sufficient calcium blood concentration during the night, observed by (Koreleski and Świątkiewicz, 2004 ; Lichovnikova, 2007)

2.4 Factors influencing shell quality

Several factors which influence egg shell quality have been identified (Petersen,1965 ; Wolford and Tanaka, 1970). The source and particulate size of calcium used to prepare laying hen diets are two factors tha t have received considerable attention in recent years (Roland, 1978). Meyer et al. (1973) found tha t hens given a diet containing particulate lime stone (LS) produced stronger egg shells than those receiving a diet that contained pulverized LS. Kuhl et al. (1977) also reported tha t particulate LS produced stronger egg shell than pulverized LS in one experiment but not in another. In the former experiment, the strain of hen used was known to produce eggs of good shell quality, whereas, the strain used in the latter experienced shell quality problems especially toward the end of the laying cycle. Both Miller and Sunde (1975) and Muir et al. (1976) found tha t particulate size had little effect on egg shell quality. Roland et al. (1974), Muir et al. (1975), and Roland (1981) concluded tha t particulate size had no effect on shell quality if the diet contained sufficient calcium to allow the hens to consume a minimum of 3.75 g calcium per day for young birds and 4.75 g/day for older hens. Results published by March and Amin (1981) indicate tha t the source of protein and fat in the diet may influence the effect of LS or oyster shell (OS) on shell quality. Scott et al. (1971) demonstrated that shell quality could be improved by incorporating a combination of OS and pulverized LS into the diet of laying hens. Hughes and WoodGush (1971) and Mongin and Sauveur (1974) found that laying hens have a specific appetite for dietary calcium. Thus, some managers of egg-producing flocks routinely add extra calcium, either particulate LS or OS, to diets that contain 3.25 to 4% calcium from pulverized LS (R.M.)

Various researchers have presented a positive effects of coarse particle size on quality of egg shell (Koreleski and Świątkiewicz, 2004; Lichovnikova, 2007; Skřivan et al., 2010), egg specific gravity and also bone strength (Ekmay and Coon, 2011)At high rate of substitution (75 % and 100 %), the snail shell reduces the feed intake. In diet of layer hens, INRA (1989) recommends 6.67 as Ca/P ratio. That explains the efficiency of these diets through the highest laying rate and the lowest feed conversion ratio of hens. In the feeding of Lohmann Brown layer hens, (Safaa et al., 2008) did not found a significant effect in feed intake and feed conversion ratio when evaluating the effect of limestone and oyster shell between 58 to 73 weeks of age. In this study, the results demonstrate that the calcium source and especially snail shell has an effect on feed intake and feed conversion ratio. Thus, a combination of snail shell and oyster shell at an optimum rate 50:50 is more efficient in layer hens feeding than a complete substitution (0:100). The laying rates in SS25 and SS50 (87.6 and 89.1%, respectively) are higher than 79.8% reported with limestone based diets (Saunders-Blades et al 2009). They are also higher than 72.7%, when the limestone and oyster shell were combined at 60:40 in Lohmann Brown hens' diet (Safaa et al., 2008)

Numerous factors affect the functional quality of the egg shell mostly prior to the egg is laid. The thickness of the shell is determined by the amount of time it spends in the shell gland (uterus) and the rate of calcium deposition during shell formation. If the egg spends a short period in the shell gland, the thickness will be less. Also, the time of the day when the egg is laid determines the thickness of the shell. In general, the earlier in the day or light portion of the photoperiod, the thicker the shell will be.

1. Strain
2. Disease
3. Management
4. Moulting
5. Age of bird
6. Drugs
7. Water quality
8. Stress
9. Environmental temperature
10. Nutrition
11. Calcium
12. Phosphorous

2.5 Role of calcium and phosphorous in shell formation

Mineral sources such as bone meal, oyster shell, limestone, calcium, phosphate and gypsum are necessary for bone formation and adequate utilization of the feed (NRC 1994 and Omole et al 2005). They are also important for birds' egg shell formation (Larbier and Leclercq 1992). Calcium is the key feedstuff for shell strength (Nys 1999). Snail shell is a mineral ingredient that contains about 98% of calcium carbonate (Cobbinah et al 2008). It is therefore a biological source of calcium that can be used in animal feeding. Investigations has been done on the use of many sources of calcium such as gypsum, limestone and oyster shell in layers and broilers diets (Omole et al 2005; Safaa et al 2008 and Saunders-Blades et al 2009); but there is a lack of information on the use of snail shell in animal feeding.

There is a complex relationship between calcium, phosphorus, vitamin D^3 and the hormonal system of the layer in calcium metabolism during lay. Calcium and phosphorus balance is critical for proper egg production and eggshell quality. Layer ration should be formulated with correct amount of calcium and phosphorus (usually 3.5 - 4.0% calcium, 0.35-0.40% phosphorus)

Roberts , (2004) and Stringhini ,(2004) reported that Calcium absorption depending on some factors, involving the mineral availability, vitamin D3, parathyroid hormone, gastrointestinal pH, calcium moreover phosphorus serum levels, fiber , fat contents and mineral granulomety of the diet Some of researches.

2.5.1 Calcium

Both excess and deficiency of calcium will negatively affect the shell quality. An egg contains almost 2 grams calcium; hence an average of 4 grams of calcium intake per day is required by a layer to maintain good shell quality since only 50 - 60% of dietary calcium is actually used in shell formation. Calcium requirement of a laying hen is 4 - 6 times that of a non-laying hen. The egg enters the shell-gland region of the oviduct - the uterus - 19 hours prior to oviposition, and the shell does not store calcium ions to attach on protein matrix. During the last 15 hours of shell formation, calcium movement across the shell gland reaches a rate of 100-150 mg/hr. This process draws calcium from two sources: diet and bone. Normal blood calcium level is about 20 - 30 mg/dl with a normal layer ration of

3.56% calcium or higher, while layers on a 2% calcium diet, 30- 40% of the calcium is derived from bone. It is therefore important to have pullets, prior to lay, on a high level of calcium to store it on body. Intestinal absorption of calcium in the diet is about 40% when the shell gland is inactive, but reaches 72% when active. This time closely coincides with late afternoon or the dark hours for the layer. Having higher calcium levels in the gut during this time is important to ensure calcium is being taken from the diet and not bone. Large particle sizes of calcium sources allow calcium to be metered throughout this time. In growers, most importantly, high calcium levels during the growth period will interfere with the proper development of the parathyroid gland by increasing gut pH, which will decrease absorption. The damage to the parathyroid would be permanent and would affect the bird's laying cycle afterwards

2.5.2 Phosphorus

The phosphorus content of the eggshell is small i.e. 20mg, compared with 120mg in the egg contents. There is also uneven distribution of the phosphorus in the inner and outer layers of the shell. Phosphate ions have an inhibitory effect on the $CaCO^3$ and bring the shell formation to an end. High levels of phosphorus in the blood will inhibit the mobilization of calcium from bone.

2.6 Source and form of calcium and phosphorus

Calcium source and particle size plays a role in calcium level in the gut when needed. Phosphorus must be in a form that is available and usable by the layer.

2.7 Intestinal pH

Phosphorus absorption is optimal at pH 5.5-6.0. When the pH is higher than 6.5, absorption of phosphorus markedly decreases. Excess free fatty acids in the diet can cause the pH to decrease and therefore, interfere with calcium and phosphorus absorption.

2.8 Calcium and phosphorus ratio

High calcium or phosphorus levels in the intestine reduce the absorption of both. High calcium increases the pH in the gut and phosphorus absorption is decreased along with zinc and manganese absorption low calcium levels increased phosphorus excretion and low phosphorus levels increased calcium excretion (Abdulrahman *et al.,* 2014) High plasma phosphorus decreases calcium absorption from the gut and calcium mobilization from the bone. Phosphorus is an integral part of the acid-base balance in the body. The proper ratio of calcium to phosphorus (Ca: P ratio) for growing birds is 1.5-2.0 Ca: 1.0P.Lichovnikova (2007) showed that supplementation of eggshell as a Ca source increased Ca and P retention.

2.9 Vitamin D^3

Vitamin D^3 metabolite is essential in absorption of the Calcium. **Vitamin D^3** is vital for absorption and mobilization of calcium during shell synthesis. The importance of adequate vitamin D^3 intake by the hen is obvious and it is essential for proper calcium and phosphorus utilization. However, excess vitamin D^3 and its metabolites have not shown to benefit eggshell quality when normal hens are already consuming adequate vitamin D^3 .Vitamin D^3 is the major control element in stimulating calcium absorption from the intestine. This effect is facilitated by the synthesis of calcium-binding protein (CBP). Vitamin D^3 intake must be adequate. The function of vitamin D^3 is related to its metabolite 1,2,5 dihydroxy D^3 that is formed in the bird's liver and kidneys. Any problem that affects the integrity of these organs or the parathyroid gland will have an adverse effect on the action of vitamin D^3 and thereby calcium absorption and metabolism

2.10 Effects of sea-shell (cockle) as a calcium supplement on the growth performance (body weight, feed intake and FCR) of layer quail

Many previous investigators have conducted multiple research works with calcium supplement of various sources on the laying chicken or quail, who have given both positive and negative effect on the growth performance of poultry. The addition of calcium pidolate to the laying quail diet had no effect on FCR, feed intake and body weight change reported by Samiento-Garcia et al. (2022), which was in accordance with other reports with aged layers (An et al.,2016; Ganjigohari et al.,Reference Ganjigohari, Ziaei, Ramzani Ghara and Tasharrofi, 2018; Kakhki et al., 2019; Islam and Nishibori, 2021; Wang et al., 2021. Similar results had been reported by Arczewska-Włosek et al., 2018) who did not find any influence of dietary calcium level (32–37 g/kg in the diet) on the performance of laying hens (21–70 weeks of age). However, these findings are contradictory. In laying ducks, Xia *et al*. (Reference Xia, Chen, Abouelezz, Azzam, Ruan, Wang, Zhang, Luo, Wang and Zheng2019) reported an improvement in FCR during the early- and entire laying periods as dietary calcium level increased from 28 to 44 g/kg, while the feed intake was unaffected by calcium level. Valderrama and Roulleau (Reference Valderrama and Roulleau2013) observed an improvement on daily body weight gain when they administered 300 ppm of calcium pidolate in the diet of 55-week-old hens. According to Oliveira and Almeida (Reference Oliveira and Almeida2004), those differences could be explained because quails are more tolerant to calcium variations, and excrete excess of this mineral more efficiently compared to other species without affecting performance parameters. In addition, Ganjigohari *et al*. (Reference Ganjigohari, Ziaei, Ramzani Ghara and Tasharrofi2018) reported that laying hens will increase feed intake to compensate for calcium deficiency in the diet, resulting in changes in performance if the diet lacks calcium. The results of this study suggested that calcium pidolate supplementation in aged laying quails is adequate for the maintenance of the same performance parameters as control group.

These strains have the production performance of attaining 210g of weight at 28 days of age, a feed conversion efficiency of 2.5 and livability of about 96%.Japanese quail farming is bestowed with farming-friendly factors such as reduced space and feed requirements, early maturity, fairly high egg production rate, high disease resistance and low financial investment, it soon became immensely popular in several regions of India. Japanese quails are marketed at 4-5 weeks of age for meat with a body weight of 200-240g. Females start laying eggs at about 6-7 weeks of age and are generally reared up to 10-12 months of age for breeding. During this period, each female lays about 250-270 eggs, each egg weighing ~12-13g (Asha, 2011)..

Adult quails consume not more than 20 to 30 grams of food daily. But some quail breeds aged six months old may need about 30 to 35 grams of feed per day and 400 grams of feed for the production of 12 eggs (Sultana et al.) Calcium (Ca) and Phosphorus (P), still remains two of minerals attracting nutritionists and producers as well more than any other mineral (Coon et al., 2002). Ca and P are important and required because they are main constituents of skeleton. Their importance is quantitative, since they make up at least 50% of bone ash (Mc Donald et al., 2002; Underwood and Suttle, 1999), and qualitative being so much implicated in physiological activities that is difficult to find a process where Ca and P are not involved playing a direct or an indirect role (Pond et al., 1995; McDowel, 2003; Rama Rao et al., 2006).

The aim of the study was to identify quails which have different body weight for Some Production Traits such as egg production, egg characteristics, daily feed consumption and feed conversion ratio (FCR). The selection was based on body weight in Japanese quail over two generations using 150 quails (120 female and 30 male). These groups consisted of Low Body Weight (LBW), High Body Weight (HBW) and Control. Average body weights for females at the end of five weeks were 182.3±0.5, 159.5±0.34, and 141.7±0.55 for LBW, HBW and control groups respectively Atilla Taskin et al (2016). There were significant differences between females and males in body weight across the generations. In our experiment all quails are female and it is found that the average weight of quail lies between 190 t0 200 gm which matches with Atilla Taskin et al (2016).

2.11 Egg production quality performance (HHEP) of layer quail and sea-shells as calcium supplement

Quality is a broad term, so egg quality does not mean single characters or traits of individual egg. The outer and inner quality of each component of a whole egg determines the quality of egg. For this reason, both external and internal characters such as egg weight, shell weight, albumen weight, yolk weight, hen housed egg production (HHEP)%, percentages of shell weight, albumen weight and yolk weight, shape index, albumen index, yolk index, and Haugh unit (HU) score of quail egg were also measured in this study for assessing egg quality performance of quail. The improvement in egg quality traits of quails fed sea-shell based diets could be a result of higher bioavailability or availability of calcium for egg shell formation. The improvement in egg quality traits of quails fed sea shell suggests an increased calcium availability for eggshell formation which is consistent with the previous studies (Attia et al., 2020, Wang et al., 2021, Sarmiento-Garcia et al., 2022).

Egg production and dietary calcium level in the diet are a controversial issue. Chen et al., 2015 showed that the egg production and the egg mass of laying ducks were reduced to 84 and 47% of control (36 g/kg calcium) for 18 and 3.8 g/kg calcium, respectively. Similarly, Xia et al., 2019), who studied breed ducks, reported that egg production in the early laying period was directly proportional to the increased calcium level, which is consistent with the findings of (Zhao et al., 2020). In contrast, (Bello et al., 2020) and (Ganjigohari et al., 2020) described that egg production, egg weight and egg mass were not affected by calcium levels. According to (Ganjigohari et al., 2018) the most important factor affecting egg weight is not only feed intake but also the level of protein in the diet. In the current experiment, while egg mass and egg weight were not affected by the inclusion of calcium pidolate, changes were observed for egg production. Previously, (Pelicia et al., 2009) reported that the increase in calcium levels could occur in a decrease in egg production due to a reduced feed intake. These findings could justify the results of our study, although it is true that differences in feed intake were observed, it was not significant.

2.12 Shell weight, shell weight % and shape index of quail and sea-shell diets

Much works on calcium supplementation were done in layer chicken, but the data on sea-shells on quail ration is very rare. However, a research done by(Awang-Hazmi et al., 2007) reported that sea-shells (cockle) are a rich source of calcium carbonate (98.7%) along with other sources of many minerals say P, Mg, Zn, Cu, Na, K, B, Si, and Fe etc., in a trace amount, which are considered as a very important minerals for enhancing egg quality characters of layer. These nutrients could improve intestinal absorption, facilitates binding to tissues, blood circulation and metabolism. The main advantages of sea-shells are, organic, naturally available, good bioavailability, high solubility and excellent gastrointestinal tolerance, which could favour its absorption in the intestine (Falguera et al*.,* 2010). Further, it suggests that the inclusion of sea-shells in layer quail might improve certain parameters (such appearance, mechanical resistance and functional properties) of egg and bone (Agblo and Duclos, 2011; Al-Zahrani and Roberts, 2015; Joshi et al., 2019).

Eggshell breaking strength and eggshell thickness were significantly higher in G3 than G4; however, no differences between treatments were found in damaged egg rate (Sarmiento-garcia et al., 2022). Partially agree with these results, previous authors (An et al*.*, 2016; Joshi et al., 2019; Islam and Nishibori, 2021; Wang et al., 2021) have shown an increase in eggshell thickness and a reduce in damaged eggs when calcium levels increased. In their recent study, Swiatkiewicz et al., (2018) showed that egg and eggshell quality parameters in 30 weeks old hen were unaffected by dietary calcium; however, eggs of older layers (43–69 weeks old) fed the diet with lower calcium level had reduced eggshell percentage, eggshell thickness and eggshell breaking strength. The improvement in egg quality traits of quails fed 0.5 g/kg of calcium pidolate suggests an increased calcium availability for eggshell formation which is consistent with the studies of Wang et al. (2021) and Attia et al. (2020). The results showed that 0.5 g/kg of calcium pidolate was adequate for improving egg quality characteristics in aged laying quails and increasing the calcium pidolate level above 0.50 g/kg had no positive effects. The present findings contradicted the findings of Cufadar et al. (2011) because they reported that dietary calcium level did not significantly affect either eggshell breaking strength or eggshell thickness. There are several explanations for the differences, including a higher calcium content in the quail's diet and older age.

Using limestone and oyster shell, Khalil and Anwar (2009) did not find a significant effect on eggshell weight and eggshell proportion. The negative effect of snail shell on eggshell quality confirms the effect of calcium source on egg quality reported by Lichovnikova (2007) who observed significant effect of different dietary mineral feedstuffs on eggshell weight, eggshell proportion and eggshell thickness. Round eggs and unusually long eggs have poor appearances and do not fit well in egg cartons; therefore, they are much more likely to be broken during the shipment than the eggs of normal shape (Sarika and Erensayin, 2009). The shape of the egg should be normal or egg shaped. The shape of egg is determined by the shape index by following the under mentioned formula. Standard shape Index for eggs is 72 to 77. Shape index below 73 should not select for hatching egg.

2.13 Haugh Unit (HU) Score of layer quail fed sea-shell diets

We can easily detect whether the egg is fresh or old by knowing the HU score of an egg. The higher the HU score the better the quality of egg. Eggs having 60-70 HU score should be selected for hatching purposes. The HU which indicates the relationship of the height of the thick white to the weight of the egg, is the most widely used measure of albumen quality. Quality has traditionally been measured by albumen height of the broken out egg or Haugh units which include the effect of egg weight to equalise the measurement between eggs of different size (Haugh, 1937). However, the Haugh unit's ability to do that, compared with simple albumen height, has been questioned, although for selection within a genetic line it may be appropriate (Silversides *et al.*, 1993). These measurements were introduced as a way of determining freshness rather than the properties desired by the user or catering processor, for example in custard production (Ericson, 1943)Internal quality of egg can be assessed by measuring HU score of the eggs.

2.14 Albumen weight, albumen % and albumen Index of layer quail fed sea-shell diets

Kul and Seker (2009) showed a positive correlation between egg weight and yolk or albumen weight in quails. This study aimed to build models to predict the yolk and albumen weight using egg weight of quails (Coturnix coturnix japonica) supplemented with seashell based calcium carbonate. Though there is positive correlation between yolk and albumen weight, there is no established evidence of dietary effect on albumen weight. Albumen weight of egg clearly depends on egg weight of quail.

The results of the previous study demonstrated that increasing calcium pidolate from 0.25 to 1 g/kg in the diets (G2 to G4) of aged laying quails decreased the albumen index (Sarmiento-garcia et al., 2022). This fact confirms a lower tolerance of quails to levels above 0.50 g/kg of calcium pidolate. These findings are in accordance with Joshi et al. (2019) who reported that the addition of calcium pidolate (0.50 g/kg) had negative effect on the egg albumen index. Also, Attia et al. (2020) observed that there is a linear reduction in albumen index when dietary calcium exceeds 35 g/kg. On the contrary, Al-Zahrani and Roberts (2015) stated that the addition of calcium pidolate (0.30 and 0.60 g/kg) to the diet increased the albumen index in laying hens.

Peynaud (1984) reported that about 12.5% (w/w) protein can be found in fresh egg whites. The principal proteins in egg white are albumen (water soluble) and globular proteins (soluble in neutral dilute salt solutions). The egg white, also called albumen, consists of the 60-65% of the total egg weight, and is the egg part with more protein, a key component in egg composition. Our experiment showed that about 50 to 54% of total egg weight is composed of albumen which is slightly dissimilar with Peynaud (1984)

Funk (1944), Korslund et al. (1956) and Mueller (1958) reported that relative humidity during storage, which controls the rate of evaporation, has little or no effect on albumen and yolk deterioration. Less is known on the relationship between albumen quality breakdown and water movement from albumen to yolk. The higher the firmness of the albumen the better the quality of the eggs. Eggs which are prone to possess more watery albumen do not hatch well. The firmness of albumen may be determined by the albumen index. The standard albumen index of the egg is 0.82—1.0. Our experiment resulted that the albumen index at 1st week varied from 0.70 to 0.75, at 2nd week from 0.73 to 0.76 and at 3rd week from 0.71 to 0.74 which are quite similar to standard albumen index.

2.15 Yolk weight, yolk% and yolk index of layer quail fed sea-shell diets

Fletcher et al (1983) egg weight increases with the age of breeders and that can also affect the egg components. The ratio of yolk to white varies widely with the size of eggs (Marion et al 1964). The proportion of yolk is reported to be less in small eggs than in larger ones (Kaminska and Skraba, 1991. There is no established experiment that showed dietary calcium has any significant egg on egg yolk of quail, there is a positive correlation between yolk and albumen percentage.

A study by Sarmiento-garcia et al., (2022) showed that the yolk index decreased linearly as calcium pidolate levels increased. These findings are consistent with those reported by Islam and Nishibori (2021). They reported that diets with 80 g/kg calcium sources performed better than diets with 40 g/kg calcium sources for yolk index. In another study, Attia et al., (2020) stated that above 35 g/kg calcium in the diet of laying hens negatively affected the yolk index. Those authors describe that the negative impact of calcium on egg quality is more pronounced when dietary calcium levels are inadequate. On the other hand, Joshi et al. (2019) observed that supplementation of calcium pidolate had no negative impact on yolk index of eggs. It is likely that the differences between the parameters of previous studies and ours may be due to variations in the level of calcium used, and therefore, in the assimilation of calcium by the quails.

The condition of the yolk may be evaluated by determining the yolk index, which represents the height of the yolk divided by its width when the yolk is resting on a flat surface (Sauter et al., 1951). As with Haugh units, deterioration of the egg causes a decrease in the yolk index. According to Ahmed, Dafalla and Malik, eggs with yolk index above 0.38 are considered as extra fresh. Those ranging from 0.28 to 0.38 are fresh and those below 0.28 are considered regular. Heiman and Wilhelm (1937) found no significant correlation between yolk index (ratio of yolk height to yolk width) and percentage firm white or albumen index for eggs kept for the same length of time. Smith (1931) reported that yolks gain approximately 25% less water in an atmosphere of 100% C02 than in air. Needham (1931) observed that in artificial osmotic systems diffusion of water into the yolk is much more rapid than in intact eggs. Smith (1931) concluded from similar results that an unknown factor or factors offer a considerable resistance to the establishment of an osmotic equilibrium between yolk and albumen.

2.16 Egg weight of quail and sea-shell

The Japanese quail (*Coturnix coturnix japonica*) is emerging as a promising poultry to the resource-poor rural farmers of India for minimal capital and managemental requirements in rearing. It is the smallest farmed avian species and becoming popular in commercial poultry sector for meat and egg production. Raising quail provides a resource for poor families with meat and eggs. The females are very prolific because they begin laying eggs on average at 6 weeks and can lay between 250 and 300 eggs a year. Furthermore, the quail is an efficient converter of feed with each egg a female deposits an edible package of 8% of her own body weight as compared to 3% in case of chicken.Quail eggs are nearly one-fifth of the size of chicken eggs. Generally, egg weight ranges from 6 to 16 g, with an average weight of 10 g.

2.17 Conclusions

Although the importance of calcium in feed for improving egg quality of quail has been known for over a century, we are still learning the role of calcium in the aspects of both nutrition and production performance. Calcium is critical to maintain overall egg quality, shell quality, reproductive efficiency, FCR and a strong immune system as an essential element of more than 200 metalloenzymes. Unfortunately, there has been only limited research related with the effect of seashell based calcium carbonate in improving egg's internal and shell quality of quail. In addition, as an anticoagulant and its role in blood clotting, calcium may play a significant role in prevention of external and internal bleeding. Better eggs will increase hatchability and will produce healthy chicks. Therefore, further study is needed to improve quail egg quality and better productive and reproductive performance fed seashell based calcium.

Chapter-III

Materials and Methods

Chapter-III

Materials and Methods

3.1 Statement of the experiment

The experiment was carried out at the Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University (CVASU) to ascertain the effect of seashell based calcium carbonate on the egg quality of quail provided seashell as a source of calcium. Feeding trial in quail was performed at the Poultry Research Shed of Research and Farm Based Campus of CVASU (Hathazari), from October to November, 2022. Laboratory analyses were performed in poultry Nutrition Laboratory, Poultry Science Lab, Poultry Research and Training Centre (PRTC) and Biochemistry Laboratory of CVASU, Khushi, Chattogram.

3.2 Preparation of the experimental shed

Firstly, the experimental poultry shed was prepared by swiping and removing of dust dirt by broom. The cage was also washed and cleaned by whisk. Both shed and cages was then washed and cleaned properly with tap water containing detergent. The shed and cages were left for air drying for 3 days. After that, ceiling, wall and floor along with battery cages were treated with disinfectant with FAM 30R (5ml/1L water) via sprayer and again left for drying for 1 week. The cage divided into 12 pens of equal size to accommodate quails. Before allowing the entrance of quails, the individual tube feeder, drinker and each pen were marked properly by sticker (bearing cage no. and treatment). An electric bulb (60 watt) was used to light the room and set 2 bulb at the roof of room by hanging condition. The floor space provided for each bird was 0.6 sq. ft in the cage. The floor of each pan was covered with medium thick paper to reduce leg injury and to maintain warm temperature within each pen. All equipment was cleaned and disinfected accordingly outside the shed.

3.3 Collection of quail and experimental design:

A total of 60layer quails (Japanese) of similar ages (50 days old) was collected from the local hatchery to carry out the experiment for 21 days. The birds were weighed initially, and then randomly distributed into three treatment groups (T_1, T_2 and T_3),as shown in Table 3. Each treatment replicated four times with five birds per replicates in a completely randomized design (CRD). All birds were allotted evenly into 12 battery cages (5 birds per cage) under similar housing lighting, and management condition with *adlibitum* feed (mash) and water throughout the trial period. The layout of the experimental trial was demonstrated below in Table 3.

Table3: Experimental design

Treatments	Number of quail				No. of birds/ treatment
	R_1	R_2	R_3	R_4	
T_1	5	5	5	5	20
T_2	5	5	5	5	20
T_3	5	5	5	5	20
Total	15	15	15	15	Grand Total =60

[T_1refers to control or basal diet, while T_2 and T_3 refers to test diets or treatments which are supplemented with 1.76% and 1.88%, seashell, respectively, R_1, R_2, R_3 and R_4 refer to replicates 1, 2,3, and 4, respectively]

3.4 Collection of the experimental feed and feedstuffs

The macro-feed ingredients (maize, soybean meal, fish meal, palm oil, and limestone) required for the feed formulation were procured by purchasing from the local market of Pahartali and Rajakhali Bazar, Chattogram. Each macro-ingredient was purchased based on thorough selection by visual observation like organoleptic test (color, odor, moisture etc.). The micro-nutrients were procured from another local market (Hazari lane, Terry Bazar, Chattogram). Particularly, test ingredient (seashell) was collected from the different markets of the Cox's-Bazar. The collected seashell was processed by cleaning, washing and drying initially, after that it was ground by coffee grinder machine manually and made it powder form by smashing again and again with the help of metal morter and pestle in the lab. The seashell powder was used as a calcium supplement to formulate the ration or test diet for feeding the quail (Table 4).Samples were taken from the procured and handmade diets prior to supplying the quails in trial pen and sent to the lab for proximate analyses.

Table 4: Nutrient compositions of the test ingredient (Seashell)

Name of the ID	Calcium (mg/g)	Phosphorous (mg/g)
Seashell	28.4	0.2

3.5 Formulation of test diet

Three different test diets (T_1, T_2 and T_3) were formulated with the locally available feed ingredients to meet or exceed the requirements of NRC (1994), as shown below in Table 5.All diets were iso-caloric and iso-nitrogenous. All feedstuffs were used to formulate control or basal diet except for seashell, whereas T_2 and T_3test diets were prepared with the supplementation of seashell, at the rate of 1.76 % and 1.88 % **,** respectively. After that, formulated diets (mash) were allowed to feed the birds from d50- 71 days. All the birds had a free access to the diets, and *ad libitum* fresh, clean and cool drinking water entire the trial period. The composition and nutritive values (calculated and analyzed in the lab) of the formulated or test diets (layer) were shown below in the Table 5.

3.6 Feed grinding, mixing and preparing the diet

First of all, the macro ingredients collected from local market in ground form having a desirable particle size, weighed and mixed. Then micro-ingredients were also weighed by electric balance one by one and then put in a small bucket for each diet and mixed properly by turning layer by layer. After that, the weighed macro-ingredients were spread on the wide plastic paper kept on floor of house and mixed thoroughly by the help of shovel. After that, the micro-nutrients were mixed on feed mixture equally. Vegetable oil (Palm) was added at half of the required amount by sprinkling over the feed mixture and then mixed thoroughly with hand as well as shovel. Remaining half amount of vegetable oil was finally sprinkled over feed mixture and again mixed thoroughly by both hand and shovel. A thorough mixing was done manually with shovel after weighing all ingredients as per the requirement of individual diet. Finally, the mixed diets were stored in the bags with marking, and later used for feeding the bird as mash feed. Same procedures were followed for the preparation of all diets.

Table 5: Nutrient and ingredient composition of layer diet

Ingredient (%)	Treatment		
	T_1	T_2	T_3
Maize	59.00	59.00	59.00
Vegetable oil	2.00	1.96	1.96
Soybean meal	27.40	27.7	27.70
Fish meal	2.10	2.00	2.00
Limestone/Seashell	4.75	1.76	1.88
DCP	3.20	3.26	2.80
Common salt	0.20	.45	0.45
Lysine	0.20	0.4	0.46
Methionine	0.22	0.4	0.45
Choline chloride	0.03	0.03	0.03
Toxin binder	0.17	0.45	0.4
Sand	0.53	0.4	0.45
Vitamin mineral premix	0.20	2.19	2.42
Nutrients (%)-calculated value			
ME(Kcal/kg)	2827.00	2827.00	2827.00
CP	19.00	19.00	19.00
CF	3.42	3.40	3.39
EE	3.48	3.50	3.48
Ca	2.76	2.76	2.76
P	1.00	1.00	1.00
Nutrients –analyzed values (%)			
DM	88.50	88.00	87.30
CP	18.15	18.85	186.63
CF	5.29	6.44	5.32
EE	2.68	2.91	3.17
Ash	9.19	9.17	9.61
Ca	2.8	2.75	2.77
P	1.30	1.27	1.25

[Except for diet T_1, the rest of the diet was supplemented with 1.76, and1.88 % seashell powderin T_2 and T_3, respectively]

3.7 Management

Throughout the entire experimental period, the following management practices were used in an effort to maintain uniformity (similar feeding, lighting, environmental condition) in the management practices as much as possible.

3.7.1 Brooding

We need not to do brooding because we purchased mature quails which age is 50 days of old. Flat square shaped feeder and a bottle drinker were provided for each pen. Electric bulb was used to provide light to get optimum egg production from quail. Two 60watt electric bulbs were hanged at a height of 60 cm in the upper middle of whole cage and a 60 watt bulb was hanged on roof in order to maintain optimum temperature. For the total period experiment, light was provided about 14 to 16 hoursin a day

3.7.2 Floor space

Birds were reared in battery cage of 12 equal size pens. Each pen (0.3 sq. ft.) was marked out for 65birds. Therefore, each bird had 0.6 square feet of floor space.

3.7.3 Feeder and drinker space

One drinker and one feeder were kept in each pen. One square shaped flat feeder and one small sized bottle drinker with a capacity of 250ml were provided for each pen. The flat feeder and drinker were kept beside to make it easy for the birds to intake food and water. Drinkers are cleaned and dried by detergent water 3-5 days interval. Birds were allowed to mash diet from flat feeder from d 50-72 days.

3.7.4 Feeding and watering

Feed and drinking water were supplied *ad-libitum* to the birds throughout the experimental period. Feed was supplied to birds from day 50 to day 72 two times daily, where once in the morning at 6 AM and another in the afternoon at 6 PM. Fresh, clean and cool drinking water was supplied the birds three times a day i.e., at 6 AM, 12 AM, and 6PM.

3.7.5 Lighting

The birds were exposed to 15 to 16 hours of lighting by adjusting the photoperiod of the day.

3.7.6 Immunization of birds

Quails do not need any vaccines to produce immunity against different infectious disease because of their natural immunity, that’s why we did not perform vaccination

3.7.7 Medication

The quails were given glucose and vitamin-C as soon as they were removed from theto minimize any stress that might have occurred due to extra humid and warm weather. During the course of experimental period, electrolytes and vitamin-C were added with the drinking water to combat stress due to high environmental temperature (33 to 37 degree C). Salt and mineral drug Ferovet were provided with drinking water to prevent cannibalism

3.7.8 Sanitation

Adequate and proper hygiene and sanitary measures were adopted and followed throughout the experimental period. Proper cleaning and disinfection of all equipment were done prior to the beginning of the trial. Potassium permanganate ($KMnO4$) solution (1.5 %) was prepared and kept into a plastic bottle fitted with a sprayer at its opening mouth. It was kept at the entry point of poultry shed and used as disinfectant before entry into poultry shed. Hands and feet were also properly disinfected with 70% alcohol before entry into the shed.

3.7.9 Data and sample collection

Feed and test diets samples were collected prior to supplying birds for the assessment of the nutritive values (DM, CP, CF, EE, Ca, P) of each diet. Body weight, feed intake and remaining feeds were recorded in record sheet in weekly basis to calculate body weight gain and feed conversion ratio (FCR). Mortality was recorded as and when it occurred.For egg quality measurement, egg sample was collected from each replicate cage weekly, and marked it properly with the marker in order to assess the egg quality. Data on egg quality parameters (both external and internal characters) say egg weight, shell weight, albumen weight, yolk weight, hen housed egg production (HHEP)%, percentages of shell weight, albumen weight and yolk weight, shape index, albumen index, yolk index, and Haugh unit (HU) score were measured herein this study for assessing the egg quality.

3.8 Record keeping

Throughout the entire experimental period, the following parameters were recorded.

3.8.1 Mortality

Mortality record was kept when it happened.

3.8.2 Body weight

Live weight of quail was taken replication wise for each treatment weekly. Average live weight of the quail was also recorded at the beginning of the experiment and at the end of each weekend.

3.8.3 Feed intake

By calculating the left over from the total amount of feed given to birds on each weekend, the quantity of feed consumed was determined.

3.9 Calculation of data

3.9.1 Weight gain

Weight gain was calculated by deducting initial body weight from the final body weight of the birds.

3.9.2 Feed conversion ratio (FCR)

The amount of feed needed for per unit of production is called feed conversion ratio. The efficiency of converting feed into egg called feed efficiency. It was calculated by using the following formula:

FCR = Feed intake/Total weight of egg laid by birds

3.9.3 Mortality and livability

Mortality of birds was calculated on the basis of number of dead birds throughout the experimental period divided by the total number of birds housed at the start of experiment. Livability was calculated from mortality of birds per replicate cage. Using this formula, the mortality percentage was calculated.

Mortality (%) = Number of quail died/Total number of quail housed × 100

3.10 Feed sample processing and analyses

Feed samples were taken replicate wise from the formulated ration to test the diets prior to feeding the birds. The samples were prepared by using a mortar and pestle to grind them, and they were then thoroughly mixed for laboratory analyses. About 250 gm of each diet of diet were taken and sent to the Poultry Research and Training Center (PRTC) Lab for proximate analysis. The samples were tested for proximate analysis of dry matter (DM %), moisture %, crude protein (CP %), crude fiber (CF %), ether extract (EE %) and ash using standard laboratory procedures (AOAC, 2007). Dry matter estimation was done by oven dry method. Crude protein estimation was accomplished by Kjeldahl Method. Ether Extract estimation was done by Soxhlet apparatus. Ash was measured by igniting the pre-asking sample on a Muffle furnace at a temperature of 600 °C for four to six hours. The percentages of Ca % and P % were determined using by atomic absorption and spectrophotometry (AOAC, 2007)

3.10.1 Chemical analyses of sea shells and egg shells

The powder form of egg samples and seashell samples were sent to the Department of Animal Science and Nutrition to make it dehydrated by using hot air oven at 105 degree centigrade so that it can be easily converted into powder form. The samples were thoroughly dried before being ground. The powder form of egg sample and seashell sample were taken and then used to analyze the bone mineral concentration (Calcium and Phosphorus only) by atomic absorption and spectrophotometry, respectively.

3.11. Measurement of Egg quality parameters

The external quality parameters of collected egg measured were:

i. Egg and shell weights (in grams) were taken weekly by using a weight balance.

ii. Shell percentage (%) was estimated from the expression or formula:

$$\text{Shell \%} = \frac{\text{Shell weight}}{\text{Egg weight}} \times 100$$

iii) Shape index was measured by the following formula:

$$\text{Shape index} = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

iv) Hen housed egg production (HHEP) % : It was measured by the following way :

$$\text{HHEP} = \frac{\text{No. of egg production}}{\text{No. of birds housed}} \times 100$$

The internal quality parameters measured for all the egg sin each replicate of the observation were:

i. Yolk height, yolk width and albumen height (in cm) using a slide callipers.

ii. Yolk index was estimated from ratio of yolk height to yolk width:.

$$\text{Yolk index} = \frac{\text{The height of yolk}}{\text{The width of yolk}}$$

iii. Haugh unit (HU) scores were determined from albumen height and egg weight using the expression below: HU= 100log (H + 7.51 - 1.7EW0.37)2 (9); where, H= Albumen height (cm), W= the weight of egg when tested (g)

iv. Albumen weight and yolk weight (in grams) were taken weekly by using a weight balance

v. Albumen % was estimated from the expression or formula as follows:

$$\text{Albumen \%} = \frac{\text{Albumen weight}}{\text{Egg weight}} \times 100$$

vi) Albumen index was measured by the following formula:

$$\text{Albumen index} = \frac{\text{The height of thick albumen}}{\text{The width of Egg albumen}}$$

vii) Yolk % was estimated from the expression or formula as follows:

$$\text{Yolk \%} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times 100$$

3.12 Statistical analyses

All collected data were subjected to analysis by one way ANOVA procedure using Minitab software (Minitab, Minitab Version, 16, 2000). The significance of differences between means was tested using the Duncan‘s multiple range tests (DMRT). Statistical significance was considered at $P \leq 0.05$.

Pictorial Presentation of Activities During Experiment

Fig: Broken Sea-shell

Fig: Preparation of feed

Fig: Weighing of feed

Fig: Feed separated treatment wise

Fig: Providing feed

Fig: Providing water

Fig: Washing feces tray and drinker

Fig: Weighing of bird

Fig: Housing Of quail on the basis of treatment

Fig: Housing of quail on the basis of treatment

Fig: Egg of quail **Fig: Packing of egg**

Fig: Treatment wise egg collection box

Fig: Egg sample


Fig:Taking different egg parametres

Fig: Separating egg yolk from albumen

Fig: Weighing of egg yolk


Fig: Making powder form of egg shell by grinding

Chapter-IV

RESULTS

Chapter-IV

Results

4. Gross performances of layer quail fed sea-shells (cockle) supplemented diets

The results of gross responses of layer quail say live weight, feed intake, feed conversion ratio (FCR) and viability are presented below in a tubular form. Apart from this, data on egg quality parameters of both external and internal characters such as egg weight, shell weight, albumen weight, yolk weight, hen housed egg production (HHEP)%, percentages of shell weight, albumen weight and yolk weight, shape index, albumen index, yolk index, and Haugh unit (HU) score of quail egg were also presented below in this section.

4.1 Growth performance of quail fed on sea-shells supplemented diets at the last day (71th) of the trial

The results of growth performances of quail in respect to body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were not influenced (P>0.05) by the dietary treatment on days 71 (Table 6).

Table 6: Gross performance of quail on the last day of the experiment

Traits	Treatment			SEM	P- value
	T_1	**T_2**	**T_3**		
Body weight (g/b)	194.25	198.00	195.13	1.795	0.683
Feed conversion ratio (FCR)	2.51	2.49	2.40	0.229	0.269
Feed intake (gm/bird/day)	25.57	24.14	23.57	0.404	0.578

[Data refer to mean values of five birds per replicate on 71 days; T_1 refers to control or basal diet with no supplemental feed, whereas T_2, and T_3 diets are supplemented with 1.76 and 1.88 % sea shell, respectively; SEM-standard error mean]

4.2 Egg production performance of quail fed on sea-shells supplemented diets from d50 to 71 days

The results of egg production performances of quail expressed in terms of egg weight (EW), egg production (EP) number and hen housed egg production (HHEP) % were presented in the Table 7. The data of EW of quail did not differ significantly (P>0.05) at all, but EP (P<0.01) in last week only and HHEP % were influenced (P<0.05) by the dietary treatment during 2nd and 3rd weeks only except for 1stweek (50-56). Significantly (P<0.05) the highest HHEP (92.15%) was found in the treated group (T_2) whereas untreated group (T_1) being the lowest in HHEP (72.14%) during 65 to 75 days. Similar result was also observed in the EP performance of quail during 3rd week only in this study. The data of EP number per week per bird showed that the highest EP number (32.25 no/w/b) was found in T_2 dietary group and lowest EP (25.25 no/w/b) number being in the T_1 group.

Table 7: Egg production performance of quail from d50 to d71 days

Trait	Age (days/week)	Treatment			SEM	P -value
		T_1	**T_2**	**T_3**		
EW (g/b)	50—56(1st wk)	9.52	9.86	9.96	0.183	0.602
	57—64(2nd wk)	10.23	10.81	10.78	0.101	0.107
	65—71 (3rd wk))	10.80	10.82	11.18	0.223	0.753
HHEP %	50—56(1st wk)	71.43	79.28	74.28	3.487	0.661
	57—64 (2nd wk)	78.39^c	85.02^a	80.54^b	2.768	0.05
	65—71 (3rd wk))	72.14^c	92.15^a	82.14^b	2.494	0.029
EP (no./w/b)	50—56(1st wk)	26.00	25.00	27.75	1.220	0.661
	57—64 (2nd wk)	26.00	29.75	26.75	1.094	0.375
	65—71 (3rd wk))	25.25^c	32.25^a	27.00^b	0.540	0.01

[Data refer to mean values of five birds per replicate from day 50 to 71 days; T_1 refers to control or basal diet with no supplemental feed, whereas T_2, and T_3 diets are supplemented with 1.76 and 1.88 % sea shell, respectively; Mean bearing different superscripts in a row is significantly different at $^*P<0.05$ and $^{**}P<0.01$]

4.3 Survivability (%) of quail fed on sea-shells supplemented diets from d50 to 71 days

The response of quail in terms of viability fed with **sea-shells supplemented diets** was not influenced (P>0.05) by treatments (Figure 1). The results showed that the no mortality was counted and survivability being cent percentage (100%) found in the birds of all dietary group entire the trial period.

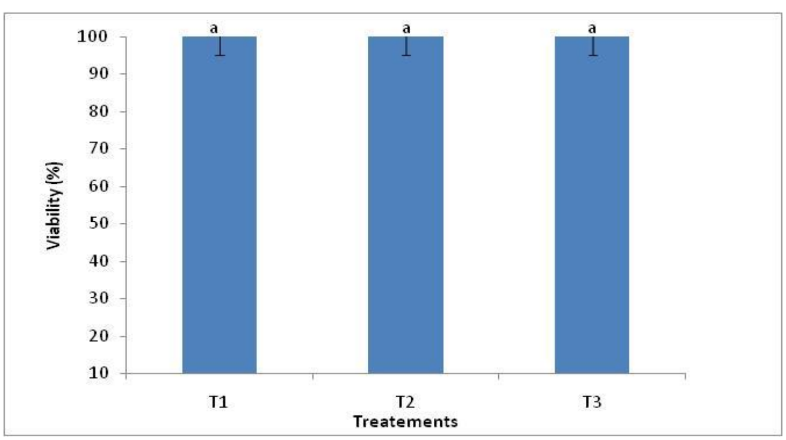

Figure 1: Viability (%) of quail from d50 to 71 days fed sea-shell treated diets; Bar bearing same superscripts has no significant (P>0.05) difference between treatment

4.4 Egg quality traits of quail fed on sea-shells supplemented diets from d50 to 71 days

The results of egg quality characters **(e.g.** Haugh unit (HU) score, shell weight, albumen weight, yolk weight, percentages of shell weight, albumen weight and yolk weight, shape index, albumen index, yolk index) of quail from d50 to 71 days were shown in the three Tables (8, 9, 10), respectively. There was no significant ($P>0.05$) difference was found between treatments in the egg quality parameters of quail during 1st week (50 to 56d) of age. Only shell weight (%) and shape index were influenced significantly ($P<0.05$) between treatments during 2nd and 3rd week of laying periods except for other traits measured in this study. The greater ($P<0.05$) shell weight (%) and shape index were found in the treated group (T_2) than that of untreated or control group.

Table 8: Egg quality traits of quail from 50 to 56 days (1st week):

Traits	Treatment			SEM	P-value
	T_1	T_2	T_3		
Haugh Unit (HU) Score	86.857	86.425	87.027	0.265	0.647
Shell weight (g)	1.3175	1.33	1.44	0.029	0.213
Shellweight%	12.808	12.668	12.61	0.169	0.888
Shape Index	0.7975	0.78	0.7975	0.0077	0.580
Albumen weight	5.39	5.645	6.16	0.116	0.064
Albumen weight %	52.297	53.622	54.078	0.390	0.209
Albumen Index	0.7075	0.7400	0.7525	0.011	0.297
Yolk Weight (g)	3.5925	3.55	3.7975	0.077	0.413
Yolk weight %	34.895	33.710	33.313	0.300	0.135
Yolk Index	0.2575	0.2625	0.2650	0.005	0.845

[Data refer to mean values of four eggs per replicate from day 50 to 56 days; T_1 refers to control or basal diet with no supplemental feed, whereas T_2, and T_3 diets are supplemented with 1.76 and 1.88 % sea shell, respectively; SEM-standard error mean]

Table 9: Egg quality traits of quail from 57 to 64 days (2nd week)

Traits	Treatment			SEM	P-value
	T_1	T_2	T_3		
HU Score	87.063	87.532	86.878	0.229	0.511
Shell weight(g)	1.33	1.4675	1.36	0.033	0.237
Shell weight %	11.14^{c}	13.57^{a}	12.19^{b}	0.245	0.05
Shape Index	0.76^{c}	0.81^{a}	0.79^{b}	0.009	0.050
Albumen weight(g)	5.765	5.5875	6.02	0.146	0.502
Albumen%	53.36	51.55	53.85	0.373	0.075
Albumen Index	0.765	0.745	0.7325	0.012	0.597
Yolk weight (g)	3.7075	3.7675	3.795	.061	0.840
Yolk %	34.335	34.898	33.965	0.284	0.436
Yolk Index	0.28	0.26	0.255	0.004	0.067

[Data refer to mean values of four eggs per replicate from day 57 to 64 days; T_1 refers to control or basal diet with no supplemental feed, whereas T_2, and T_3 diets are supplemented with 1.76 and 1.88 % sea shell, respectively; ^{a,b,c} Means bearing different superscripts in a row differ significantly at *P<0.05; SEM-standard error mean]

Table10: Egg quality traits of quail from 65 to 71 days (3rd week)

Traits	Treatment			SEM	P-value
	T_1	T_2	T_3		
HU score	87.00	88.36	87.66	0.298	0.289
Shell weight (g)	1.14	1.32	1.30	0.035	0.126
Shell weight %	11.81^{b}	13.49^{a}	13.46^{a}	0.286	0.05
Shape Index	0.76^{c}	0.80^{a}	0.78^{b}	0.003	0.05
Albumen weight (g)	4.81	5.11	4.88	0.158	0.735
Albumen%	50.54	52.28	48.88	0.832	0.297
Albumen Index	00.75	0.73	0.72	0.020	0.796
Yolk weight (g)	3.42	3.41	3.70	0.08	0.295
Yolk%	36.02	35.44	37.21	0.905	0.724
Yolk Index	0.31	0.26	0.25	0.016	0.279

[Data refer to mean values of four eggs per replicate from day 65 to 71 days; T_1 refers to control or basal diet with no supplemental feed, whereas T_2, and T_3 diets are supplemented with 1.76 and 1.88 % sea shell, respectively; ^{a,b,c} Means bearing different superscripts in a row differ significantly at *P<0.05; SEM-standard error mean]

Chapter-V

DISCUSSION

Chapter-V

Discussion

5.1 Growth performances of layer quail fed sea-shell (cockle) supplemented diet

Generally, the key performance indicators such as the growth rate, feed consumption and feed efficiency of poultry could play a vital role in measuring gross performance (Rezaei et al, 2004).The purpose of the present study was to determine the effects of **sea-shell (cockle)** and their relationships with growth performance, viability, external and internal quality of egg,. Normally the different supplementation strategies of feed in poultry diet have been known to influence the productivity. Addition of sea-shell (cockle) as calcium supplement in diet can affect egg quality, production and bird development (Ahmed et al., 2022; Sarmiento-Garcia et al., 2022). Sea-shell powder is a new form of calcium supplementation in poultry diets, which could enhance the absorption of this mineral. Many research has been done about the calcium requirements forlaying chickens, but no available data or little work has been worked out on the calcium requirements for laying quails.

In the current study, it was seen that the addition of sea-shells as calcium supplement in layer quail had no significant effect on FCR, feed intake and body weight change. The results of this study indicates that calcium supplementation as sea-shell in laying quail is assumed to be suffice and sufficient for the maintenance of the similar growth performance parameters as control group (Sarmiento-Garcia et al., 2022).Our result is consistent with the findings of previous researchers (Sarmiento-Garcia et al., 2022), who found similar results when layer quail fed diet supplemented with calcium pidolate. The results are also agreed with other reports of many previous investigators worked with layers (An et al., 2016; Ganjigohari et al., 2018; Kakhki et al., 2019; Islam and Nishibori, 2021; Wang et al., 2021). Similar results had been reported by Arczewska-Włosek et al. (2018) who did not find any influence of dietary calcium level (32–37 g/kg in the diet) on the performance of laying hens (21–70 weeks of age).

5.2 Effect of sea-shells on the survivability of layer quail

The data on viability of quail showed that adding sea-shells to diets had no significant effect on the livability or death of birds amongst the treatments. It implies that sea-shells supplementation had no detrimental effect on the birds. Further it can be assumed that sea-shells in the quail diets can be used undoubtedly, as it had no harmful impact on the growth and survivability of the birds. As we know the sea shell is a good sources of calcium along with other minerals particular Zn, Cu, Fe and Na, which could have the immune-booster properties of birds, and thus it could improve the survivability of broiler chicken (Virden et al., 2004; Guo et al. 2002).

5.3 Egg production performance of quail fed diet added with sea-shells as calcium supplement

The egg production performances of quail in terms of egg weight (g), egg production (EP) number and hen housed egg production (HHEP) % were measured in this study. The data revealed that egg weight of quail was not influenced at all, but EP only in last week and HHEP % were significantly affected by the dietary treatment during 2nd and 3rd weeks only except for 1stweek (50-56). The egg weights (10 to 12 g) are in the range of 9-16 g mostly recorded in the latest laying month of eggs of layer quail (Nys et al., 2008). The purpose of our study is to assess the egg quality including egg weight. Through the whole period of this study we have weighed the egg of Japanese quail and got an average weight of around 10gm which is relevant to (Nys et al 2008).

It is obvious from the data that EP and HHEP % were increased in quail fed the supplemented diets ($T_{2,}T_3$) in compare to control group (T_1). The optimum egg production performance of layers in the supplemented diet group might be an outcome of adding sea-shells in quail diets. The result suggests that calcium availability in the supplemented diets appears to be adequate in proper ratio and amount along with other nutrients as required for the layer quail, which could help the birds for contributing optimum egg production performance. Our results are agreed with the previous researchers (Chen et al., 2015, Xia et al. 2019, Zhao et al., 2020). Chen et al. (2015) showed that the egg production and the egg mass of laying ducks were reduced to 84 and 47% of control (36 g/kg calcium) for 18 and 3.8 g/kg calcium, respectively. Similarly, Xia et al. (2019), who studied breed ducks, reported that egg production in the early laying period was directly proportional to the increased calcium level, which is consistent with the findings of Zhao et al. (2020).

Egg production of laying hen in diets with extruded eggshells was comparable to the diet with limestone or oyster shell (Arvat and Hinners, 1973; Vandepopuliere et al., 1978). Vandepopuliere et al. (1973; 1975) reported the heavier egg (65.7 g) production of laying hens fed a diet with extruded eggshell compared to the diet with limestone (64.8 g).Jessy et al. (2016) which respectively show a laying rate between (80 – 90%) and (60 – 99%) at the 4th week of breeding. Our experiment had been conducted over 50 to 72 days of old. The experiment shows that the laying rate at 1st week of our experiment is between (74 to 79%), (78 to 85%) at 2nd week and 72 to 82%) at 3rd week respectively which are very close to Jessy et al (2016.)

5.4 Egg quality performance of quail fed diet added with sea-shells as calcium supplement

Quality is a broad term, so egg quality does not mean single characters or traits of individual egg. The outer and inner quality of each component of a whole egg determines the quality of egg. For this reason, both external and internal characters such as egg weight, shell weight, albumen weight, yolk weight, hen housed egg production (HHEP)%, percentages of shell weight, albumen weight and yolk weight, shape index, albumen index, yolk index, and Haugh unit (HU) score of quail egg were also measured in this study for assessing egg quality performance of quail. It is clear from the data that only shell weight (%) and shape index out of other quality characters measured herein, were influenced significantly between treatments. The greater shell weight (%) and shape index were found in the treated group (T_2) than that of untreated or control group. The improvement in egg quality traits of quails fed sea-shell based diets (1.76; 1.88%) could be a result of higher bioavailability or availability of calcium for egg shell formation. The improvement in egg quality traits of quails fed sea shell suggests an increased calcium availability for eggshell formation which is consistent with the previous studies (Attia et al., 2020, Wang et al., 2021,Sarmiento-Garcia et al., 2022). The results suggests that 1.88% of sea-shell inclusion in quail ration sounds better for improving the egg quality characters.

5.5 Shell weight, shell weight % and shape index of layer quail fed sea-shell diets

In this study, the shell weight % and shape index of quail was found to be improved by inclusion of sea-shell in layer quail diet. The reason for the improvement of egg quality characters might be due to addition of seashells in the diet. Beacuse, Awang-Hazmi et al.(2007) reported that sea-shells (cockle) are a rich source of calcium carbonate (98.7%) along with other sources of many minerals say P, Mg, Zn, Cu, Na, K, B, Si, and Fe etc., in a trace amount, which are considered as a very important minerals for enhancing egg quality characters of layer. These nutrients could improve intestinal absorption, facilitates binding to tissues, blood circulation and metabolism. The main advantages of sea-shells are, organic, naturally available, good bioavailability, high solubility and excellent gastrointestinal tolerance, which could favour its absorption in the intestine (Falguera et al., 2010). Further, it suggests that the inclusion of sea-shells in layer quail might improve certain parameters (such appearance, mechanical resistance and functional properties) of egg and bone (Agblo and Duclos, 2011; Al-Zahrani and Roberts, 2015; Joshi et al., 2019).

Using limestone and oyster shell, Khalil and Anwar (2009) did not find a significant effect on eggshell weight and eggshell proportion. The negative effect of snail shell on eggshell quality confirms the effect of calcium source on egg quality reported by Lichovnikova (2007) who observed significant effect of different dietary mineral feedstuffs on eggshell weight, eggshell proportion and eggshell thickness. Round eggs and unusually long eggs have poor appearances and do not fit well in egg cartons; therefore, they are much more likely to be broken during the shipment than the eggs of normal shape (Sarika and Erensayin, 2009).The shape of the egg should be normal or egg shaped. The shape of egg is determined by the shape index by following the under mentioned formula. Standard shape Index for eggs is 72 to 77. Shape index below 73 should not select for hatching egg.

5.6 Haugh Unit (HU) score of layer quail fed sea-shell diets

The HU score was not influenced by dietary treatment in this study. Though significant variations were not found in HU score, but numerically increased HU score found in the supplemental group. Our experiment found that the HU score is between 86 to 87 over the whole experimental period,it sounds excellent quality according to the United States Department of Agriculture standard grades (USDA) the quality of is excellent. We can easily detect whether the egg is fresh or old by knowing the HU score of an egg. The higher the HU score the better the quality of egg. Eggs having 60-70 HU score should be selected for hatching purposes.

The HU which indicates the relationship of the height of the thick white to the weight of the egg, is the most widely used measure of albumen quality. Quality has traditionally been measured by albumen height of the broken out egg or Haugh units which include the effect of egg weight to equalize the measurement between eggs of different size (Haugh, 1937). However, the Haugh unit's ability to do that, compared with simple albumen height, has been questioned, although for selection within a genetic line it may be appropriate (Silversides et al., 1993). These measurements were introduced as a way of determining freshness rather than the properties desired by the user or catering processor, for example in custard production (Ericson, 1943)Internal quality of egg can be assessed by measuring HU score of the eggs.

5.7 Albumen weight, albumen % and albumen Index of layer quail fed sea-shell diets

Albumen weight (AW), albumen %, and albumen index were unaffected between dietary treatment in this study. Though significant variations were not found in AW, albumen % and albumen index of quail egg, but these parameters were numerically increased in the birds of supplemental group. Kul and Seker (2009) showed a positive correlation between egg weight and yolk or albumen weight in quails. This study aimed to build models to predict the yolk and albumen weight using egg weight of quails (*Coturnix coturnix japonica*) supplemented with seashell based calcium carbonate. Though there is positive correlation between yolk and albumen weight, there is no established evidence of dietary effect on albumen weight. Albumen weight of egg clearly depends on egg weight of quail.

Peynaud (1984) reported that about 12.5% (w/w) protein can be found in fresh egg whites. The principal proteins in egg white are albumen (water soluble) and globular proteins (soluble in neutral dilute salt solutions). The egg white, also called albumen, consists of the 60-65% of the total egg weight, and is the egg part with more protein, a key component in egg composition. Our experiment showed that about 50 to 54% of total egg weight is composed of albumen which is slightly dissimilar with Peynaud (1984)

Funk (1944), Korslund et al. (1956) and Mueller (1958) reported that relative humidity during storage, which controls the rate of evaporation, has little or no effect on albumen and yolk deterioration. Less is known on the relationship between albumen quality breakdown and water movement from albumen to yolk. The higher the firmness of the albumen the better the quality of the eggs. Eggs which are prone to possess more watery albumen do not hatch well. The firmness of albumen may be determined by the albumen index. The standard albumen index of the egg is 0.82-1.0. Our experiment resulted that the albumen index at 1st week varied from 0.70 to 0.75, at 2nd week from 0.73 to 0.76 and at 3rd week from 0.71 to 0.74 which are quite similar to standard albumen index.

5.8 Yolk weight, yolk% and yolk index of layer quail fed sea-shell diets

It is obvious from the data that these characters (yolk weight, yolk% and yolk index) of quail egg were identical between treatment. No significant variation was observed in these quality traits of egg fed quail with the sea-shell diets. Fletcher et al (1983) egg weight increases with the age of breeders and that can also affect the egg components. The ratio of yolk to white varies widely with the size of eggs (Marion et al 1964). The proportion of yolk is reported to be less in small eggs than in larger ones (Kaminska and Skraba 1991. There is no established experiment that showed dietary calcium has any significant egg on egg yolk of quail, there is a positive correlation between yolk and albumen percentage.

The condition of the yolk may be evaluated by determining the yolk index, which represents the height of the yolk divided by its width when the yolk is resting on a flat surface (Sauter et al., 1951). As with Haugh units, deterioration of the egg causes a decrease in the yolk index. According to Ahmed, Dafalla and Malik, eggs with yolk index above 0.38 are considered as extra fresh. Those ranging from 0.28 to 0.38 are fresh and those below 0.28 are considered regular. Heiman and Wilhelm (1937) found no significant correlation between yolk index (ratio of yolk height to yolk width) and percentage firm white or albumen index for eggs kept for the same length of time. Smith (1931) reported that yolks gain approximately 25% less water in an atmosphere of 100% C02 than in air. Needham (1931) observed that in artificial osmotic systems diffusion of water into the yolk is much more rapid than in intact eggs. Smith (1931) concluded from similar results that an unknown factor or factors offer a considerable resistance to the establishment of an osmotic equilibrium between yolk and albumen.

CHAPTER-VI

CONCLUSION AND RECOMMENDATIONS

Chapter VI

Conclusion and Recommendations

From an overview of the results obtained in this study revealed that providing seashell based calcium carbonate diets has no significant effect on the growth performance and viability of quail. But it has a significant effect on egg quality traits like -, Hen Housed Egg production (%), egg production number, shell weight % and shape index of egg without affecting other egg quality traits (egg weight Albumen Weight, Albumen%, Albumen Index, Yolk Weight, Yolk%, Yolk Index) measured in this study. The current findings suggests that seashell based calcium carbonate could play an effective role as an alternative or natural source of calcium for improving egg production performance and egg quality with reduced cost of quail rearing under farming condition. From the result, considerable further research study is required regarding the limitations to the use of seashell based calcium carbonate. Seashell can be a great cheap source of dietary calcium for quail as well as other poultry birds. Though there are some limitations of managing huge demand of seashell, appropriate strategies can be adopted to fulfill the demand of seashell in the poultry industry, More research is needed to make seashell based cheaper diets for profitable layer quail rearing

Suggestions

1. Seashell can be a substitute of dietary calcium source for ration of layer quail.

2. Further research should be conducted on point out the optimum level of seashell based calcium carbonate with in a larger population of Japanese layer quail.

3. Feed mixing should be done mechanically for proper assimilation.

4. Proper sanitization, lighting, ventilation should be ensured for quails in an open sided house is strongly recommended.

5. Cannibalism should be checked which is a major reason of death of quail in layer stage

6. Mineral deficiency should be checked which is proliferative factor of cannibalism

7. More fund should allocate for the continual research studies.

CHAPTER-VII

REFERENCES

Chapter VII

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