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Effects of UVB Light on Laying Hens and Eggshell Quality

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Abstract

The effects of UVB lights on laying hens and eggshell quality were investigated in this study. UVB light treatment was provided by Philips TL 20W/12 RS SLV/25 tube light. The lights were on 2x30 min per day and were turned on during feeding. It was placed at the front centre of each cage such that the feet were exposed to the light. The daily exposure of UVB light on hens will be 1147 mJ/cm2. Both the baseline value and room without UVB light served as the control to assess the effect of light. In additional to baseline sampling, the sample were collected after 4weeks and 14 weeks.

The current study demonstrated that exposure of UVB light on laying hens had no effects on the eggshell quality of eggs. Additional treatment with UVB radiation could not further improve the quality of eggs including weight, length, diameter, eggshell thickness, eggshell breaking strength and percentage of the shell. Eggshell formation and stability totally depends upon vitamin D status. There was no any impact of UVB radiation on laying hens' performance, egg weight, eggshell quality and thickness. The weight and size of the eggs increases with production time. The eggshell breaking strength is related to eggshell thickness and eggshell weight.

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1. INTRODUCTION

Chicken eggs and eggs products are nutrient-rich food which plays an important role in human diet and nutrition. Eggs contain highly digestible protein, lipids, minerals and vitamins (Fisinin et. al, 2008). The global production of eggs has increased rapidly due to high nutritional value and reasonable price. Higher demand for poultry products had led to genetic improvement in production. But the bone development of chicken has not been satisfactory which resulted in locomotion problem. Vitamin D plays a significant role in calcium and phosphorus metabolism, bone mineralization and mobilization. Nutritional factor plays an important role in bone development.

Exposure of UVB to hens assists to synthesise Vitamin D3 from 7-dehydroxycholesterol in the skin of feet and legs. It helps to minimize the occurrence of tibial dyschondroplasia and prevents rickets in chickens with a cholecalciferol deficiency. Chicken feed with vitamin D3 deficiency diet had normal growth and bone ash with exposure to UVB light (Lewis et. al, 2009). Supplementation of vitamin D decreases the incidence of the bone disorder. Vitamin D also affects the growth performance and meat quality of hens (Han et. al, 2012).

The most important concern in the poultry industry is the quality of eggshell. Profitability of the egg production is governed by the quality of eggshell. Eggshell quality decreases due to increase in the egg weight without an increase in calcium carbonate deposit in the shell. Absorption and mobilization of calcium during shell synthesis is essential which is managed by vitamin D3. The adequate amount of vitamin D3 is required for proper calcium and phosphorus utilization. Ca-binding protein is synthesized for calcium transportation in the gut and uterus. Deficiency of calcium leads to the higher requirement of vitamin D3 (Bar et. al, 2008).

Supplementing vitamin D3 or its metabolites in the diets of laying hens has shown an increase in eggshell quality (Tsang et. al, 1993). Exposure of UVB light on laying hens without vitamin D3 in the diet has shown improvement in eggshell quality, laying performance and bone stability (Schutkowski et. al, 2013). Additional UVB radiation on laying hens feeding with 3,000 IU vitamin D3/kg feed has also shown an effective increment of vitamin D content on eggs and meat (Schutkowski et. al, 2013).

The aim of the study was to investigate the effect of UVB light on laying hen's performance and eggshell quality.

2. LITERATURE REVIEW

2.1 Egg

Eggs have been used as a food sources from decades. Chicken eggs are one of most common foods used throughout the world. Eggs are the good source of all essential nutrients like protein, fat, vitamins and minerals with exception of vitamin C. The chicken egg consists of yolk (30-33%), albumen egg white (60%) and the protective eggshell (9-12%) within various thin membranes (Roberts et. al, 2004). The eggs produced commercially for eating are not fertilized by rooster. Hence, it cannot be developed into embryo for production of chicken. Minerals, antioxidants and vitamins can possibly be enhanced in eggs through adding those components in chicken feeds.

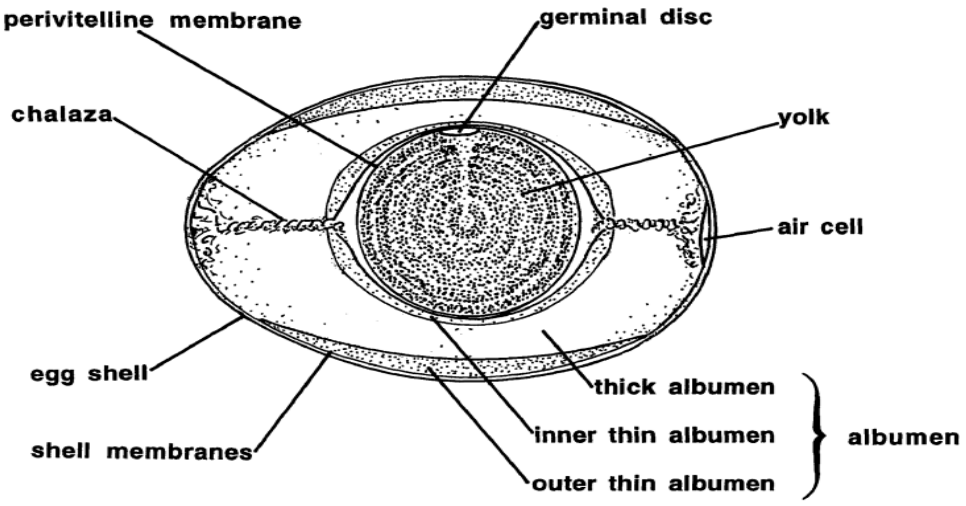

Figure 1. Diagram of egg (Roberts et. al, 2004)

2.2 Composition of Egg

Albumen and folk of eggs are used for consumption. More than half of the egg's total protein is comprised in albumen egg white. Four alternating layers of thick and thin consistencies are included in albumen. The main function of albumen is to keep yolk away from microorganisms and provide water, minerals and protein to yolk. The innermost layer attached to the yolk is known as chalaziferous layer (2.7%) followed by inner thin layer (16%), middle thick layer (50%) and outer thin layer (25%) (Poultryhub.org).

Yolk consists of higher amount of lipid, 17% protein and small amounts of vitamins, minerals and carbohydrates. All of the vitamins A, D, E and fats are in the yolk of eggs. Egg yolk is one of the natural food containing Vitamin D. Yolk provides lipid and protein for embryonic growth.

The composition of eggshells is calcium carbonate, magnesium and phosphorous. It is composed of protein fibers, calcium carbonate crystals and cuticles. The cuticles are a foamy layer of protein which covers the shells (Romanoff et. al, 1949).

Table 1. Nutritional composition of an egg (Engormix.com)

Nutrient(Unit)	**Whole Egg**
Weight	60g
Water (percentage)	65-68.5
Calories (kcal)	70
Protein (g)	6.3
Carbohydrates(g)	0.36
Total fat (g)	4.8
Polyunsaturated fat (g)	1
Monounsaturated fat (g)	1.8
Saturated fat(g)	1.6
Cholesterol (mg)	185
Choline (mg)	126
Vitamin A(IU)	270
Vitamin D (IU)	41
Vitamin E (mg)	0.5

2.3 Vitamin D

Vitamin D is very essential nutrition for human beings as well as laying hens to maintain good health. Vitamin D is required to maintain serum calcium concentration in the body within the physiological homeostatic range (Browning et. al, 2014). Vitamin D plays a vital role in bone formation. There is chronic Vitamin D deficiency in human body which leads to rickets in children and bone disease osteomalacia in adults (Holick et. al, 2004). The transfer of calcium and phosphorous within the gastrointestinal wall are regulated by vitamin D and also consequent mineralization of bone tissue (Borle, 1974). It also maintains the immune system and helps to maintain healthy skin and strength the muscle (DeLuca, 1998).

Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the two-fat soluble prohormes which refer vitamin D. Both vitamins are produced by the exposure to ultraviolet (UV) radiation. Vitamin D2 is produced by invertebrates whereas vitamin D3 is produced by vertebrates in their skin (O'Mahony et. al, 2011). To fulfil the requirement of vitamin D in human body we totally depend upon the sun exposure. Solar ultraviolet B photons are absorbed by human body to convert it into Vitamin D3. 7-dehydrocholesterol in the skin absorbs the solar ultraviolet B photons and transforms to previtamin D3 and converts it to Vitamin D3. It is then absorbed in the liver to form 25-hydroxyvitamin D3 which is then transformed in its biologically active form, 1,25-dihydroxyvitamin D3 in kidney (Holick et. al, 2004).

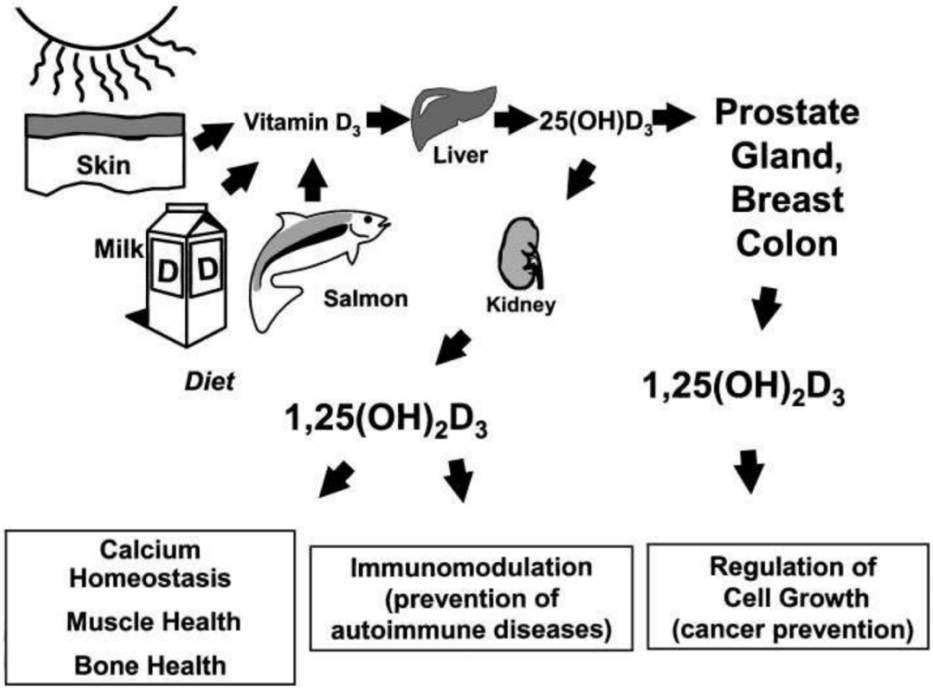

Figure 2. Production, metabolism and biologic functions of vitamin D3 (Holick et. al, 2004).

Very few natural foods are rich in vitamin D contain and only few foods are fortified with vitamin D. Due to less exposure to sunlight and few natural and fortified Vitamin D food, there has been wide spread of vitamin D deficiency in all age groups of people in Europe and United States. Deficiency of vitamin D is associated with different diseases such as cardiovascular, rheumatoid arthritis, multiple sclerosis, type 1 diabetes and deadly cancers. It is very important to maintain blood concentration of 25-hydroxyvitaminD above 80 nmol/L (30 ng/mL). It maximizes intestinal

calcium absorption as well as provides extrarenal 1-α hydroxylase which is required to produce 1,25-dihydroxyvitaminD3 (Holick et. al, 2004).

Insufficiency of vitamin D during winter in Europe is a common problem. The production of vitamin D can be ease through production of vitamin D-fortified eggs and meats. Vitamin D plays an important role in mineralization of bones and regulation of calcium and phosphorous in the human body. Vitamin D deficiency is a global health problem. 80-90% of vitamin D_3 is synthesis in the skin by exposure to sunlight and only 10-20% of vitamin D_3 supply is contributed by nutrition. Vitamin D_3 recommendation in European countries range between 5 to 20 µg daily for adult. But the recommended amount of Vitamin D_3 are not meet in most of the European countries by intake of dietary sources. Therefore, it is necessary to develop food based strategies to improve vitamin D_3 status.

2.4 Vitamin D Physiology and Importance in Laying Hens

Vitamin D is an important nutrient for growing chicks and laying hens. Deficiency of vitamin D causes rickets, which causes leg and beak deformities in young chicks. Deficiency of vitamin D in hens adversely affects the egg production and also causes calcium deficiency. Adequate levels of vitamin D3 is required for proper absorption of calcium (Ca) and phosphorus (P). Vitamin D3 are obtained in the body of chicken through feed and exposure to sunlight.

The major function of Vitamin D is to promotes plasma calcium and phosphorus to normal mineralization of bone and skeletal growth. Vitamin D also plays an important role in chicken and hens in regulation of parathyroid gland in immune system. Its helps in metabolism of foreign chemicals and cancer prevention in skin and cellular development and differentiation (Bouillon et. al,2014). The vitamin D (1,25-(OH)2D) regulates in immune cell function and plays regulatory role in reproduction in both male and female (DeLuca, 2008).

The calcium and phosphorus are actively transported across the intestinal epithelium through Vitamin D stimulation. The vitamin D (1,25-(OH)2D) are transferred to the nucleus of intestinal cell and interacts with chromatin materials. The specific proteins are translated by ribosomes which leads to enhancement of calcium and phosphorus absorption. The magnesium (Mg)

absorption are also influenced by vitamin D as well as calcium and phosphorous balance (Miller et. al, 1965).

The minerals are deposited in protein matrix during bone formation. The bones are elongated by rise in the trabecular bone which are accompanied by invasion of blood vessels. The organic matrix fails to mineralize due to vitamin D deficiency which causes rickets in young and osteomalacia in adults. The mineralization of bone matrix is actively metabolized by vitamin D (1,25-(OH)2D). Another functions of vitamin D in bone is to mobilize calcium from bone to extracellular fluid compartment. Biochemical changes occur in intestine, bone and kidney due to vitamin D for mineralization and skeletal growth (Reinhardt et. al,1987).

Vitamin D also plays an important role in embryonic development of chick. Yolk calcium mobilization are stimulated by vitamin D treatment. Calbindin, vitamin D-dependent calcium-binding proteins in the intestine and kidney are present in yolk sac. 1,25-(OH)2D is also essential to transport eggshell calcium to embryo (Elaroussi et al., 1994).

There are very few natural food sources that are rich in vitamin D. Fish liver oil, oily fish, egg yolk and wild mushrooms are the richest sources of vitamin D. Cod liver oil is important for bone health, as it is a key source of vitamin D3. Egg yolk contains both vitamin D3 (D3) and 25-hydroxyvitamin D3 (25(OH)D3. In compare to other animal based food, eggs contain higher level of 25-hydroxyvitamin D3 (25(OH)D3 (Mattila et. al, 1995). In human nutrition, 25-Hydroxyvitamin D3 is very essential as it offers five times the relative biological activity of vitamin D (Browning et. al, 2014).

2.5 Vitamin D Fortification

Fortified foods are those food in which one or more essential nutrients are added. Those nutrients are added in food to remedy the deficiency or preserve the food. It may or mayn't be normally contained in those food. Enriched foods are those foods which nutrients were restored which were lost during processing. It is considered interchangeably with fortified foods. The level of vitamin D in the animal products can be enhance through feeding animals with vitamin D supplemented feed. Fortified foods and enriched vitamin D foods are considered as Vitamin D-enhanced foods. Very few research has been conducted to produced vitamin D fortified food. Research are

conducted on milk, orange juices, some breads and cereals to produce fortified foods (Tangpricha et. al, 2003).

Vitamin D fortification of food in Europe is highly regulated and discussed. Bio-addition is a novel approach to enrich foods with specific nutrients. Specific nutrients are added to animal feed during livestock farming production for production of fortified food. Egg is an attractive target for vitamin D_3 fortification since, it is widely and regularly used. The Council of the European Communities (Council Directive 70/524/EEC) has specified maximum amount of supplemented cholecalciferol 3000 IU/kg feed for laying hens. Animal feed cannot be fortified with vitamin D beyond maximum limit. UVB exposure of laying hens might become a favourable option to improve the vitamin D content in eggs and meats.

2.6 UVB Light

In commercial layer production, artificial lightning is one of the most important management tools. It improves the egg production and quality, allows anticipating or delaying the beginning of lay and optimizes the feed efficiency.

In recent studies, exposure to artificial UVB and sunlight has shown improvement of vitamin D_3 content in eggs (Schutkowski et. al 2013). Whereas, the chickens whose upper part of body was exposed to UVB did not produced vitamin D enriched eggs (Lietzow et al. 2012). The unfeathers skin of chicken legs contains most of the 7-dehydrocholesterol (7-DHC), the pre-cursor and limiting factors for vitamin D_3 synthesis. The Chicken exposed to UV light especially in unfeathers legs, are capable of vitamin D_3 synthesis compared to other skin parts. Vitamin D3 is not synthesized in the upper leg skin which is covered by skin. These feathers block most of the UV rays. UVB light were exposed to chicks for three hours per day (Schutkowski et. al, 2013). The eggs produced contained 4-to 5-fold higher vitamin D than eggs that were produced without expose to UVB light. The vitamin D_3 is incorporated into the egg yolk as non-hydroxylated vitamin D which is formed in exposed skin by UVB light.

Exposure of UVB light for 300 minutes per day has shown 95% of maximum attainable vitamin D content in eggs. Whereas, 60 minutes per day UVB exposure has shown 50% of achievable increment (Kühn et al. 2015). The maximum of vitamin D3 in eggs in response to the respective

UVB exposure time was only achieved after 3 weeks of daily UVB treatment. Eggs collected after two weeks had on average 20% less vitamin D3 compared to those collected after 3 and 4 weeks. One egg from chicks that were exposed to UVB light for 300 min per day provides on average 2.21 µg vitamin D3 and 0.33 µg 25(OH)D3 (Kühn et al. 2015).

New technology has emerged to enhance the vitamin D contain in the food. The concentration of vitamin D has significantly increased without affecting the quality of mushroom by use of UVB light (Ko et. al, 2008). The rate of increment depends upon the irradiation dose and temperature. Vitamin D enhancements has also been success in animal products. Increase in the dietary vitamin D3 content in pig feed has shown significant increase in the vitamin D3 level in meat and liver of pig (Wilborn et. al, 2004). Similarly, Vitamin D contain in fish was improved through feeding vitamin D3 rich feed (Greff et. al, 2002). Feeding vitamin D3-rich diets has also shown increase in vitamin D content on eggs. Supplemented diet with vitamin D3 increased vitamin D in egg yolk more effectively than with vitamin D2 as well as vitamin D3 strength the bone (Mattila et. al. 2004).

The main source of vitamin D for the human beings is through exposure to sunlight (Holick et. al, 2004). Exposure to UVB radiation has also shown significant effects on blood concentration of vitamin D. Research conducted in Great Britain in nursing home suggested that use of UVB lamps were effective to maintain blood concentration of 25(OH)D. Since the bone density is directly related to 25(OH)D concentrations, it has higher benefit for bone health (Chuck et. al, 2001).

Exposure of UVB has shown significant enhancement in hatching in panther chameleon. Increased dose of UVB enhanced vitamin D3 content in egg. There was higher hatching success rate with eggs containing higher vitamin D3 of panther chameleon compared to lower vitamin D3. 25(OH) vitamin D3 plays a vital role in embryonic development. Vitamin D3 are generated in skin through UVB light as well as from feed. 25(OH) vitamin D3 are transferred to eggs and is responsible for successful hatching of eggs (Ferguson et. al, 2005).

2.7 Factors Affecting Eggshell Quality

Production of eggs having good shells is the major problems in production of high quality eggs. The common methods to measure the shell quality are thickness, smoothness, porosity of eggshell. Percentage of egg shell, breaking strength are as measured to analysis the quality of eggshell. The

breaking strength of the eggs are affected due to seasonal fluctuations and changes. The egg shell thickness is also affected under controlled air temperature. High air temperature during production of egg leads for more fragile eggs (Bennion et. al, 1933). The egg shell quality is inherited and white shelled eggs have lower breaking strength compared to brown shelled eggs (Taylor et. al, 1938).

Contamination of feeds, production system, diseases, general and heat stress also affects the egg shell quality. Age and strain of hen, storage, induced moult, diseases and nutrition also may affect the internal quality of eggs (Roberts et. al, 2004).

2.8 Effects of Vitamin D3 and Calcium on Eggshell Quality

Eggshell quality of laying hens is also governed by nutritional factor. Calcium and vitamin D3 are the most important nutritional factors. The eggshell is primarily composed of calcium carbonate. 38% of eggshell are made of calcium and plays vital role in eggshell formation and maintaining the quality (Plaimast, et. al, 2015). Calcium is closely associated with vitamin D3. Vitamin D3 is required for calcium metabolism and is essential for intestinal uptake in layers. The synthesis of Ca-binding protein is promoted by vitamin D3 which transports calcium in the guts. If the supply of calcium to the layers is not at optimum level then the requirement of vitamin D3 increases (Bar et. al, 2008).

The amount and ratio of dietary Ca and P, their availability, species and physiological factors also effect the Vitamin D3 requirement. The symptoms of vitamin D3 in laying hens occurs after 1 to 2 months when then are deprived of vitamin D3. The eggshell become thin and the egg production consequently decreases (Panda et. al,2006). Eggshell quality gradually reduced in aged hen's due to disorder in calcium and vitamin D3. In old laying hens, the problem of egg breakage due to poor shell quality is high (Bar et. al, 2002).

Supplementing calcium and vitamin D3 might be good approach to maintain eggshell quality in later stage of laying hens. More than 3.5% Ca is required in the diet of laying hens to maintain good eggshell quality (Safaa et. al, 2008). Eggshell quality improved with addition of vitamin D3 in the diet of laying hens. The eggshell thickness was significantly influenced by dietary vitamin D3 (Schutkowski et. al, 2013). Increasing dietary calcium level has shown linear increment in

eggshell percentage and thickness and egg specific gravity. Increase of calcium in dietary level contributes better eggshell synthesis since, calcium plays an important role in eggshell formation.

3. EXPERIMENTS

3.1 Introduction

The experiment was conducted at Ole Egges farm in Kroer, Ås and Laboratory of Norwegian University of Life Sciences (NMBU). The eggs were produced and provided by Ole Egges farm and then stored and analysed at NMBU. Felleskjøpet conducted this experiment on top of their own feed test. New chickens which arrived around July 11nd at 15 weeks of age were used for experiment. The experiment started in week 41 (October 9th) with baseline of same day.

3.2 Material and Methods

3.2.1 Experimental Setup

The facility consists of three rooms. Each room had two floors with separate feeding on each floor. Each floor consists of 22 cages with 9 hens per cage. Lower floor of room 2 were used to study the effect of UV light whereas upper floor of room 1 were used as the control. Same feed was used for control as well as with UV light treatment. Philips TL 20W/12 RS SLV/25 tube light were used which was 60 cm long. It was placed at the front centre of each cage such that the feet were exposed to light. The lights were on 2x30 min per day, turned on when feeding started in the afternoon, at 14.05 and 16.05. With 60 minutes of UV exposure, the daily exposure will be 1147 mJ/cm^2.

The eggs from hens were sampled from lower floor on room 2 whereas upper floor on room 1. The sample were collected from the same cages throughout the experiment. 4 eggs from 8 cages were collected from lower floor of room 2 as treatment whereas 4 eggs from 4 cages were collected as control at baseline and at later times. Eggs from cages number 3,5,7 and 9 counted from the door on each side were used to collect sample from room 2. Whereas eggs from cage number 3 and 7 were used from room 1. In this way, both the baseline value and room without lighting served as control to assess the effect of light. In addition to baseline sampling, the sample were collected after 4 weeks on November 6th and after 14 weeks January 17th. The last collection of eggs was planned to be after 12 weeks on January 2nd but due to some technique error in lighting. To allow the effect of light the collection of sample was delayed by 2 weeks.

Table 2. The total number of samples for egg quality

Date	Number of Eggs
Baseline October 9th	
Control: 4 cages*4 eggs	16
Treatment: 8 cages*4 eggs	32
November 6th	
Control: 4 cages*4 eggs	16
Treatment: 8 cages*4 eggs	32
January 17th	
Control: 4 cages*4 eggs	16
Treatment: 8 cages*4 eggs	32
Total	**144**

3.2.2 Egg Analysis

The sample of eggs were collected from the farm for quality analysis and are stored in refrigerator at 4°C for 1 weeks. 16 eggs from control and 32 eggs from treatment were collected and were individually labelled. The eggs were used for quality analysis after 1 weeks of storage.

3.2.2.1 Egg Weight

The initial weights of the eggs were recorded immediately after collection from the farm. The egg weights were determined to the nearest 0.01g using a digital scale (Sartorius AX2202). Then the eggs were stored in refrigerator for 1 weeks. Then the eggs samples were again weighted to analyse the weight loss in 1 weeks during storage. After that they were used for further quality analysis.

Figure 3. Digital scale to measure weight of egg

3.2.2.2 Eggshell Breaking Strength

Length and width of each egg were measured individually using electronic digital caliber. Tinius Olsen Texture Analyzer HK5T were used to determining the egg shell breaking strength values. This texture analysis instrument is capable of measuring ultimate force (N) and break distance. The eggs were placed horizontally between a stainless-steel plate. Cylindrical Feed Pellet Compression Test 100N target Methods were used to determine the eggshell breaking strength. Breaking force (N) was defined as the compression force required to fracture an eggshell at a constant compression speed.

Figure 4. Digital caliber and Tinius Olsen Texture Analyzer HK5T

3.2.2.3 Eggshell Thickness

The eggshell thicknesses were measured with shell membrane intact. After the eggshell breaking strength analysis were performed, the contents of the eggs were emptied and the shell was thoroughly washed in running water. Eggshell thickness with membranes were measured with a 0.01millimeter accuracy using thickness measurer Digital micro meter MS25LP. Each 2 repeated measurements were taken at the broad and the narrow poles and at the equator of each shell (Peebles et. al., 2004 and Snapir et al.,1969). Shell thicknesses were designated as the arithmetic average of the six measurements. In order to eliminate errors due to the natural curvature of the shell, pieces off 2-3 mm^2 were measured.

Figure 5. Digital micro meter MS25LP

3.2.2.4 Eggshell Weight

After emptying all the egg's contents, the entire eggshells were retained. Fragments belonging to common shell were kept together and labelled. The external cuticle and internal shell membranes were retained. The eggshells were then weighted on electronic digital scale and recorded. Then the eggshells were dried at 105 degree overnight with the shell membranes intact (Snapir et al.,1969). Then the dried eggshells were placed in the desiccator for half an hour. Then the dried eggshells were weighed on an analytical scale to the nearest 0.0001-gram digital scale.

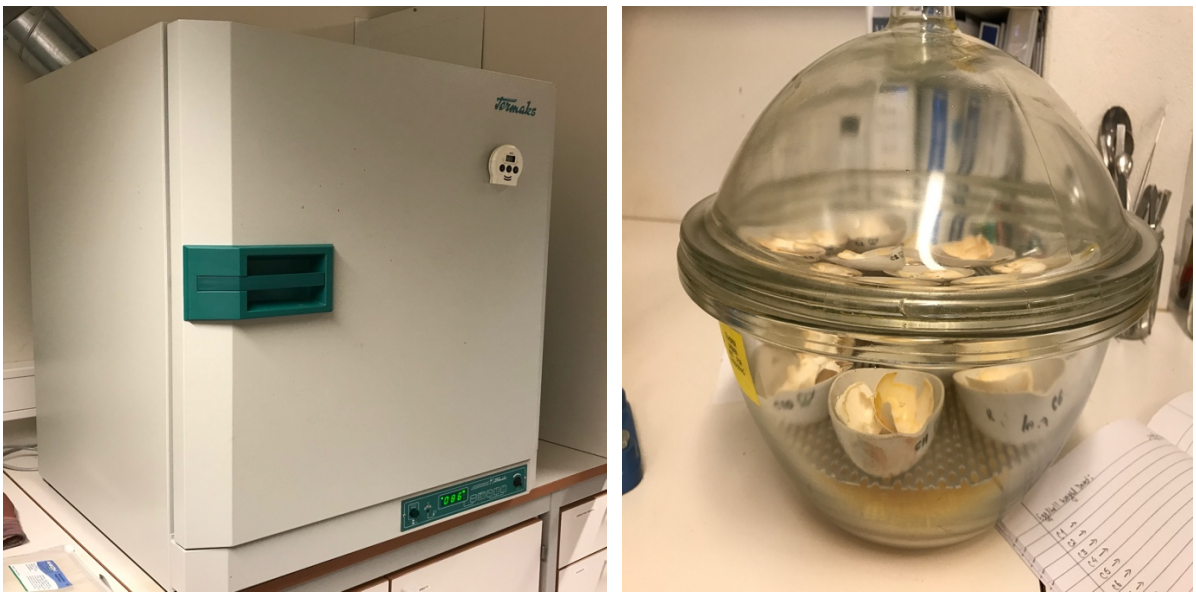

Figure 6. Oven drier and Desiccator

3.2.2.5 Percentage of Eggshell

After measuring the dried eggshells weight the percentage of eggshell were calculated as dried eggshell weight X 100/whole egg weight (Wilhelm, L.A., 1940).

3.2.3 Data Analysis

To compare the quality of eggs an independent sample t-test was carried out by using SPSS statistics. Mean comparisons were performed at p=0.05 For calculation of mean and graphical presentation Microsoft Excel was used.

3.3 Results

3.3.1 Disaggregated Descriptive Statistics

This section deals with the analysis of disaggregated data on the quality of eggs including weight, length, diameter, thickness, eggshell breaking strength, percentage of shell, among others. The descriptive statistics of the eggs quality between control and treatment groups are presented below.

3.3.1.1 Eggs Fresh Weight

The fresh weight of eggs in control as well as UVB light treatment showed slightly higher in weight in third reading compared to first and second reading. The average fresh weight of eggs in control in first reading was 60.718 gram whereas in UVB light treatment it was 61.275 gram. In third reading, there was small increment in weight of eggs. The average weight of eggs in control was 66.512 gram whereas in UVB light treatment it was 65.694 gram.

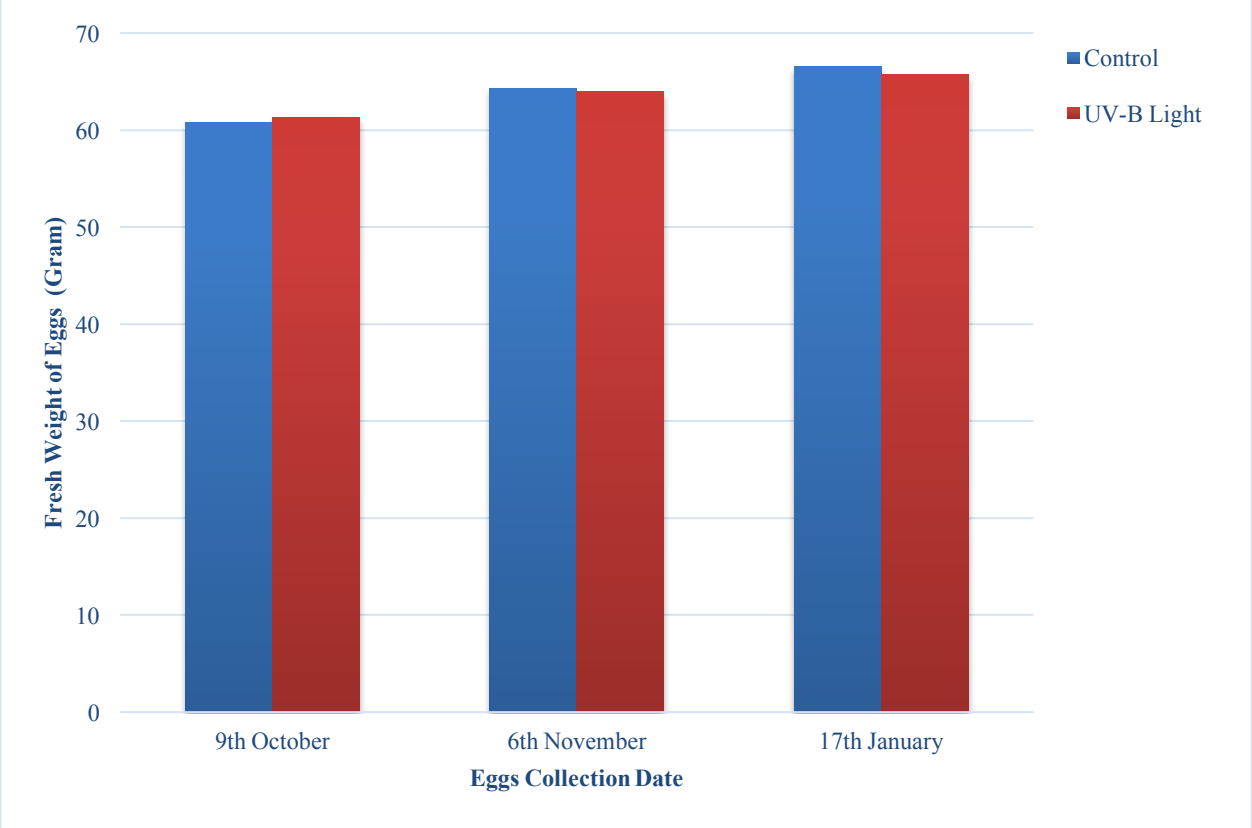

Figure 7. Mean Eggs Fresh Weight of Control and UVB light treatment.

3.3.1.2 Weight Loss

The loss of weight after one week of storage in the UVB light treatrment was slightly higher compared to the controlled group. The average mean percentage of weight loss after one week for the control group was found to be 0.397% while the same was found to be slightly higher at 0.416% after 1 week for the treatment group. The weight loss was slightly higher in first reading compared to second and third reading in both control and UVB light treatment.

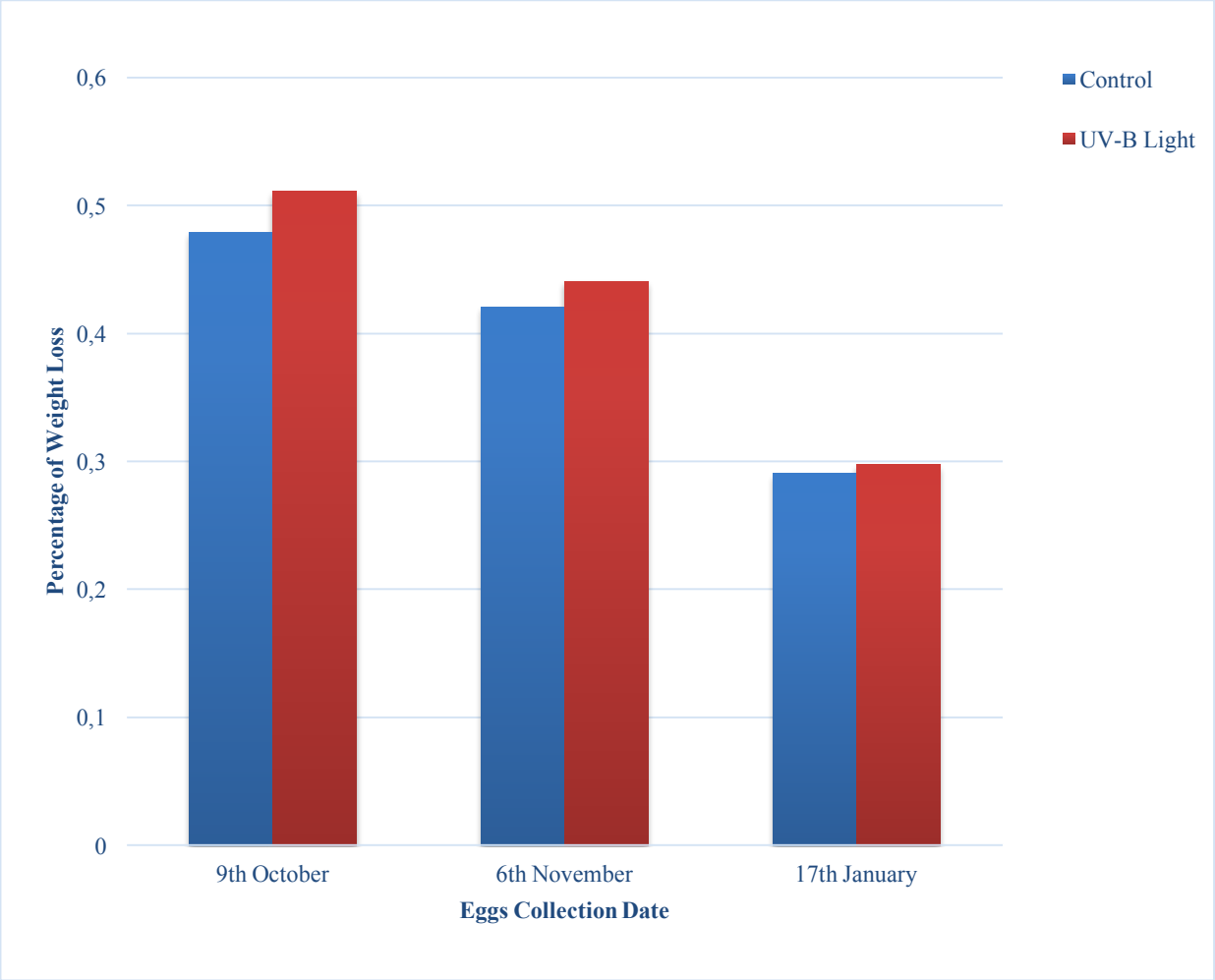

Figure 8. Mean % Weight loss comparison between Control and UVB light treatment.

3.3.1.3 Eggs Length

There were not much differences in the aggregate mean of the length of eggs in compared to control and UVB light treatments. The aggregate mean of the length of control eggs were 57.6625 mm whereas with UVB light treatments were 57.6350 mm. The lengths of the eggs were slightly lower during first collection and slightly increased during second and third collection. The average length of eggs of control in first collection were 56.288 mm which slightly increased to 57.569 mm in second collection and reached to 59.128 mm. Similarly, length of eggs increased in UVB light treatment during different collection. The average length in first collection were 56.539 mm which increased to 57.668 mm and reached 58.696 mm in third collection.

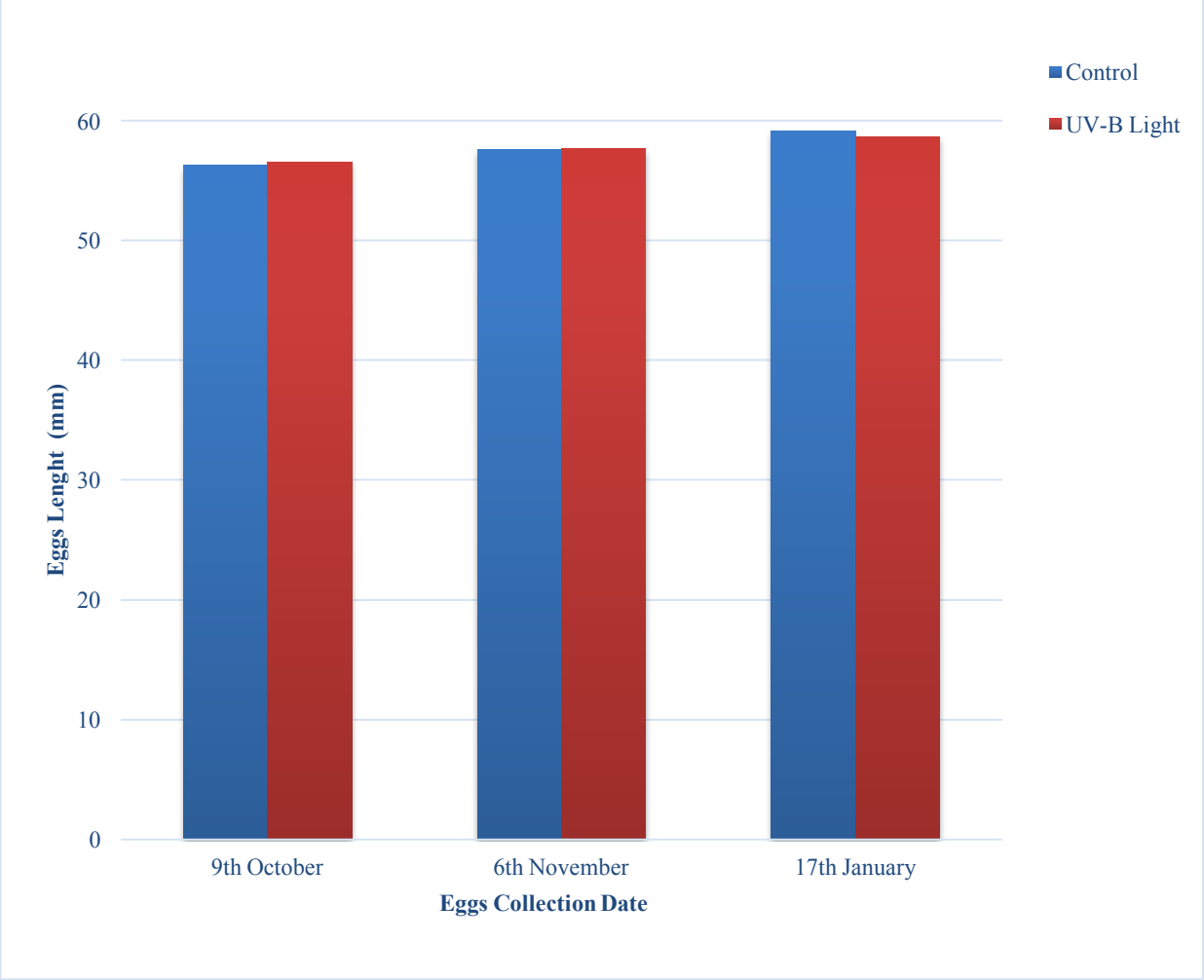

Figure 9. Mean Eggs Length of Control and UVB light treatment.

3.3.1.4 Eggs Diameter

There were not much differences as well in the aggregate mean of diameter of eggs in compared to control and UVB light treatments. The aggregate mean of the diameter of control eggs were 44.1613 mm whereas with UVB light treatments were 44.0167 mm. The diameters of the eggs were slightly lower during first reading and slightly increased during second and third reading in both control and treatments. The average diameter of eggs of control in first collection were 43.337 mm which slightly increased to 44.493 mm in second collection and reached to 44.651 mm. Similarly, diameter of eggs increased in UVB light treatment during different collection. The average diameter in first collection were 43.623 mm which increased to 43.833 mm and reached 44.359 mm in third collection.

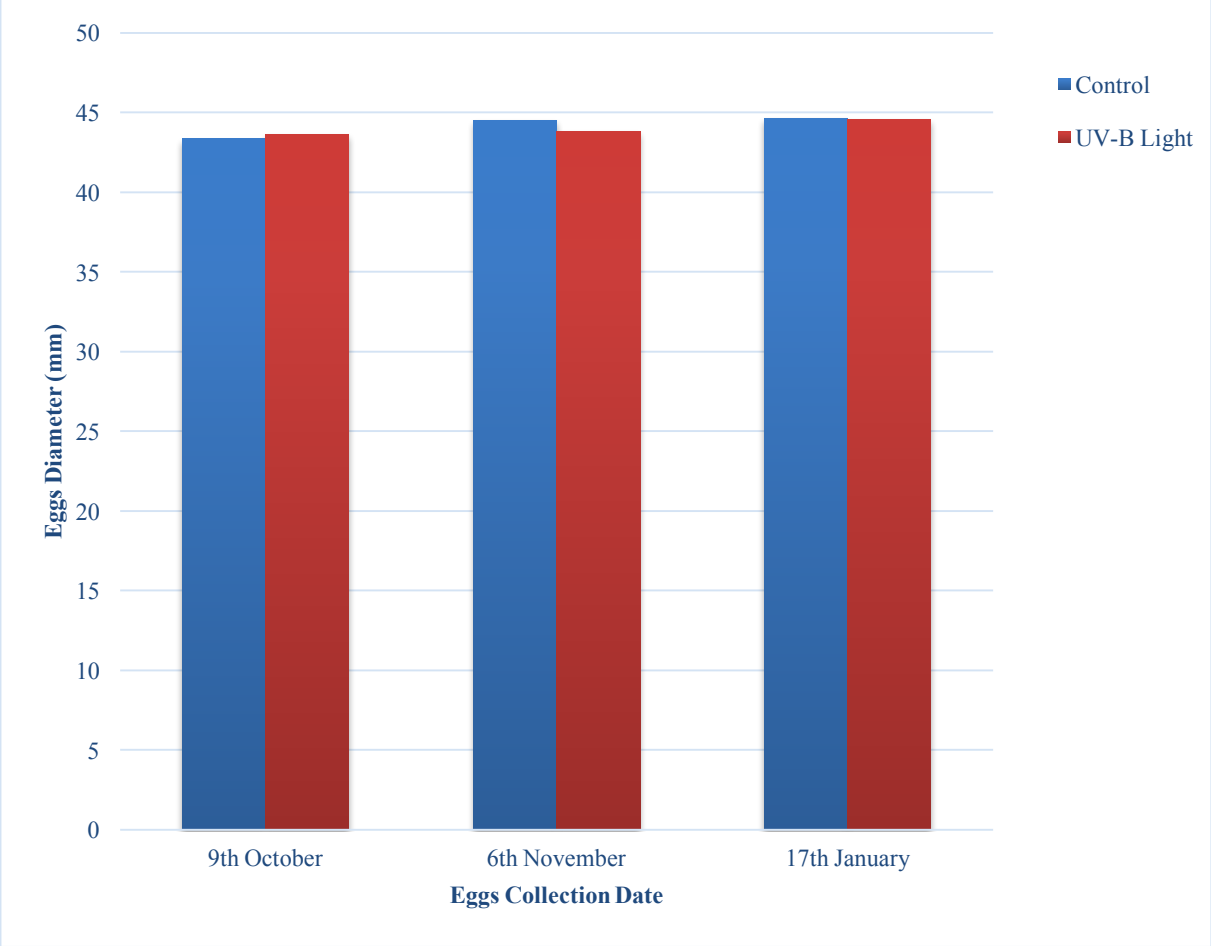

Figure 10. Mean Eggs Diameter of Control and UVB light treatment.

3.3.1.4 Eggshell Thickness

There were no differences in the aggregate mean of eggshell thickness in compared to control and UVB light treatments. The aggregate mean of the eggshell thickness of control eggs were 0.4377 mm whereas with UVB light treatments were 0.4348 mm. The eggshell thickness decreased after every collection. The average eggshells thickness of control in first collection were 0.491mm which slightly decreased to 0.4397 mm in second collection and further decreased to 0.3823 mm in last collection. Similarly, eggshell thickness decreased in UVB light treatment during different collection. The average eggshell thickness in first collection were 0.4822 mm which decreased to 0.4308 mm in second collection and further decreased 0.3914 mm in third collection.

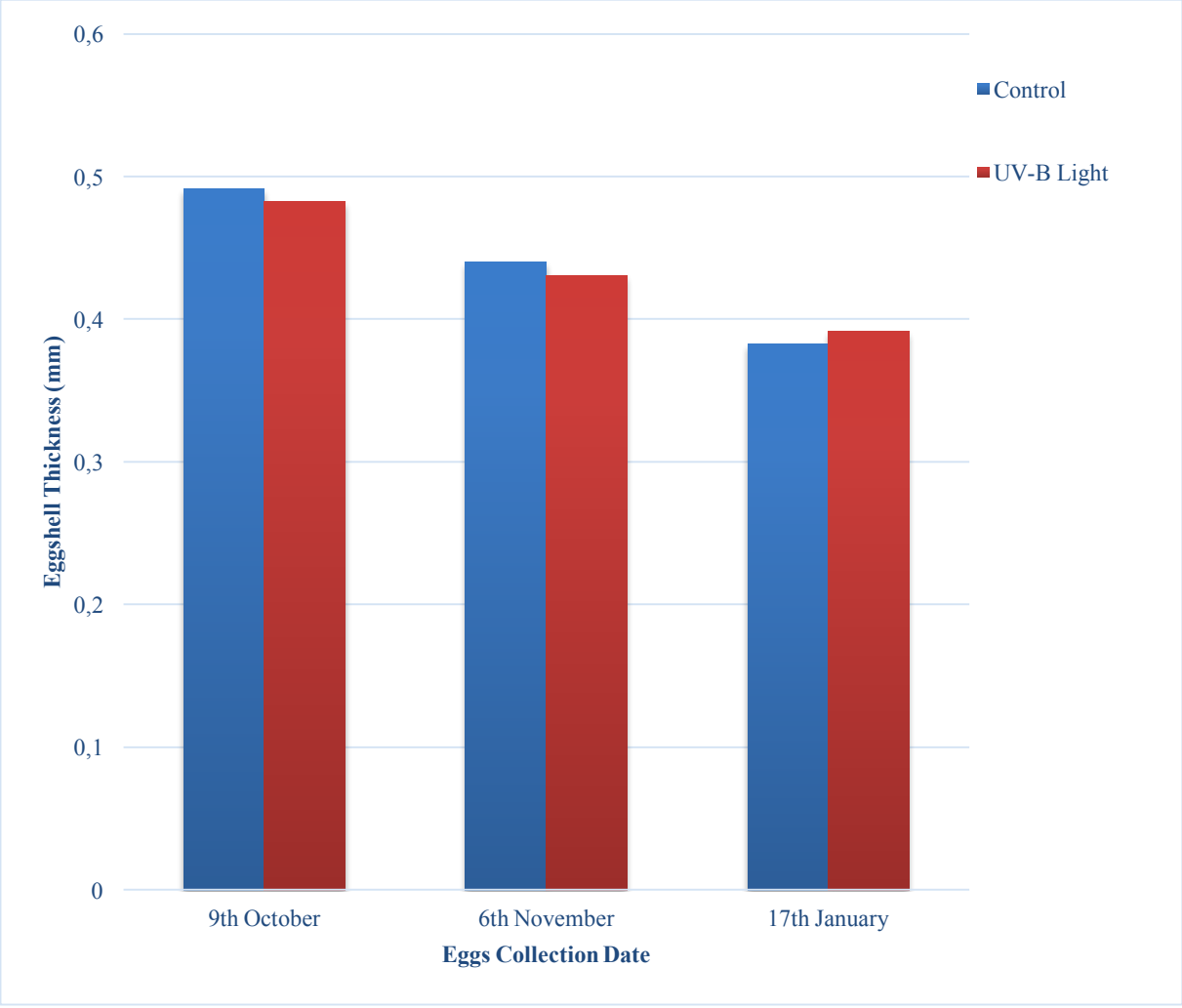

Figure 11. Mean Eggshell Thickness of Control and UVB light treatment.

3.3.1.5 Eggshell Breaking Strength

There were no differences in the eggshell breaking strength between the control and UVB light treatment. The average eggshell breaking strength of the control group were found to be 43.56458 N whereas UVB light treatment were 43.38969 N. The average eggshell breaking strength of the control during first, second and third collection were found to be 44.18 N, 43.94 N and 42.56 N respectively. Whereas, the average eggshell breaking strength of UVB light treatment were found to be 46.60 N, 40.43 N and 43.12 N in first, second and third collection respectively.

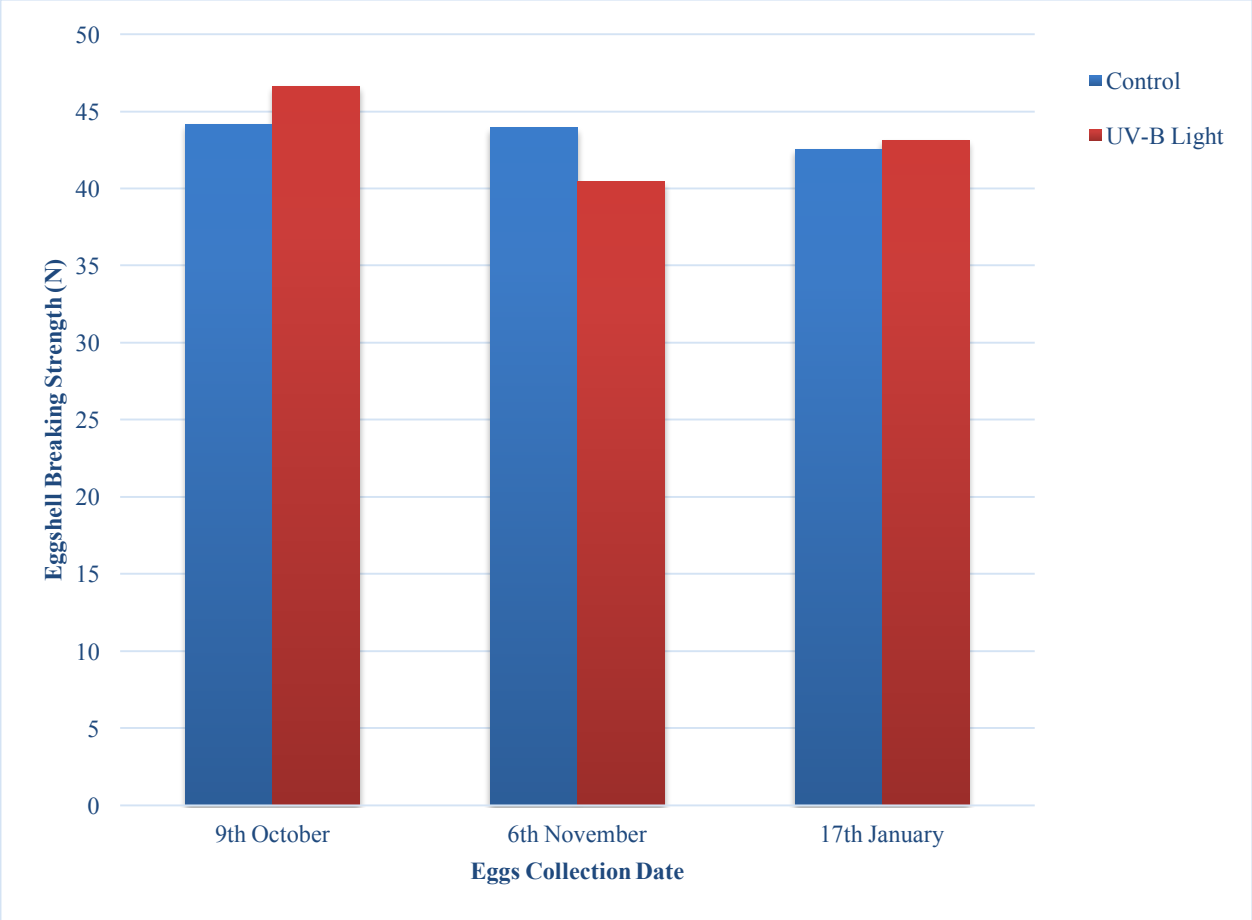

Figure 12. Eggshell Breaking Strength of control and UVB light treatment.

3.3.1.5 Dried Eggshell Weight

The average dried eggshell weight in all three different collections were slightly higher in control compare to UVB light treatments. The average dried eggshells weights of control were 6.542856 gm compare to 6.437006 gm of UVB light treatment.

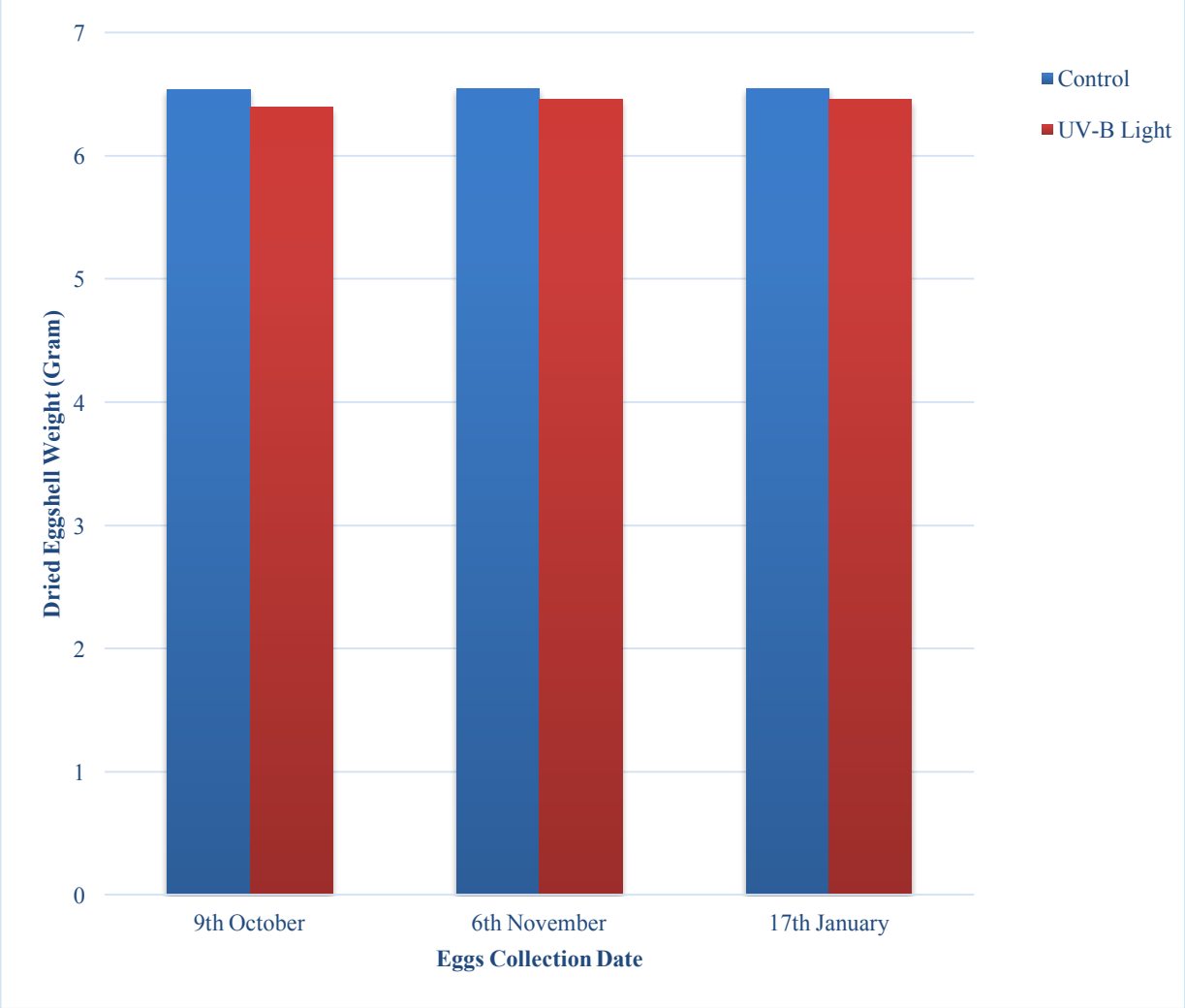

Figure 13. Dried Eggshell Weight of Control and UVB light treatment.

3.3.1.6 Percentage of Eggshell

The percentage of eggshell in the control was slightly higher compare to UVB light treatment. The average percentage of eggshell in control were 10.268 % whereas UVB light treatment were 10.125%. Second and third collection had small decrease in percentage of eggshells compare to first collection.

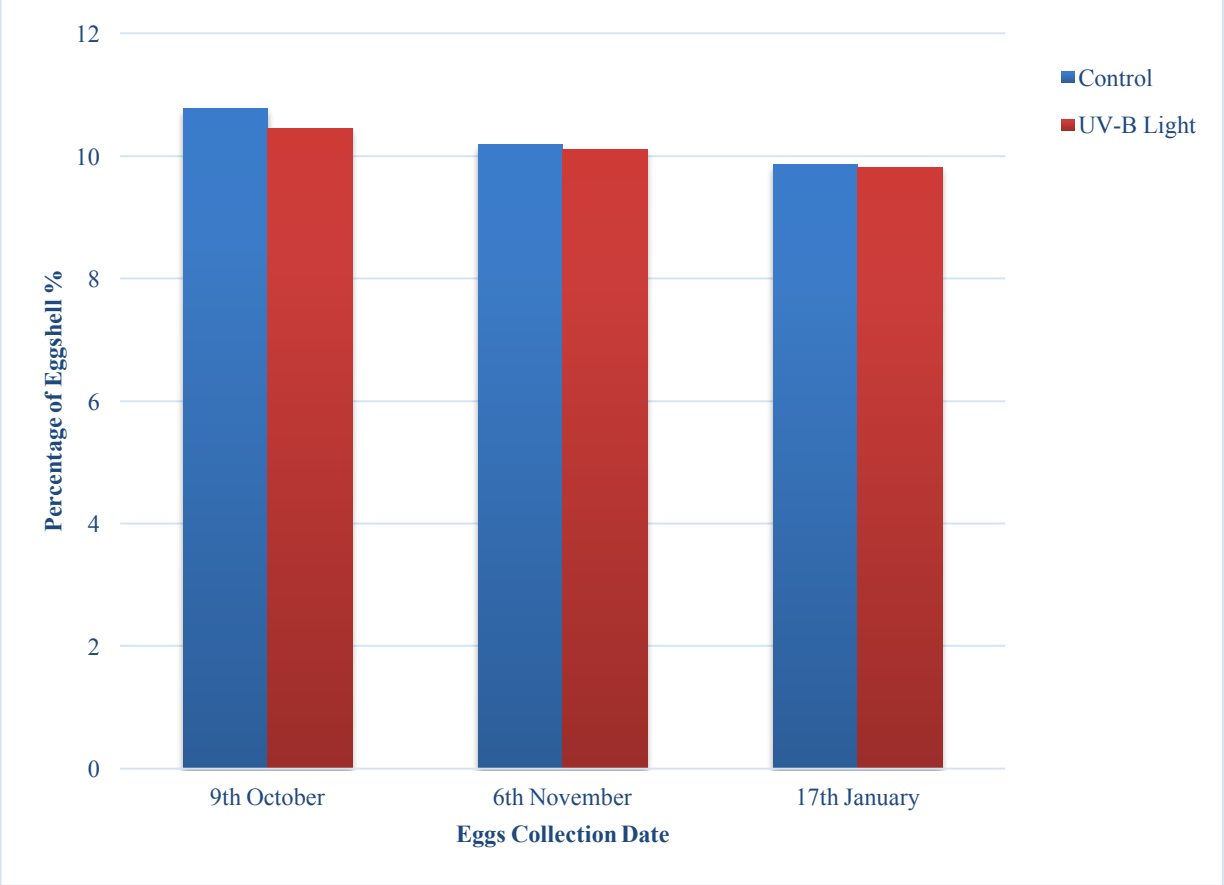

Figure 14. Percentage of Eggshell of Control and UVB light treatment.

Disaggregated comparison between different characteristics of egg quality were compared and it showed a level of differences in the different means of characteristics of eggs.

3.3.2 Independent Sample T-Test

To compare the quality of eggs under control and treatment groups, an independent sample t-test were carried out. The table below shows the statistical data showing the significance at 95% confidence level.

Table 3. Comparison of quality of eggs under control and UVB light treatments in basline date (09th October).

		N	Mean	Std. Deviation	Std. Error Mean	t-statistics	p-value	Significance at 5% level of significance
Initial	Control	16	60,718	3,207	0,802	-,522	,605	Not significant
Weight(Gram)	Treatment	32	61,275	4,000	0,707			
Weight after 1	Control	16	60,238	3,156	0,789	-,499	,621	Not significant
week(Gram)	Treatment	32	60,764	3,958	0,700			
Weight Loss %	Control	16	0,479	0,068	0,017	-,990	,327	Not significant
	Treatment	32	0,511	0,152	0,027			
Length(mm)	Control	16	56,289	1,045	0,261	-,660	,513	Not significant
	Treatment	32	56,539	1,559	0,276			
Diameter(mm)	Control	16	43,338	0,813	0,203	-1,089	,284	Not significant
	Treatment	32	43,625	0,951	0,168			
Thickness(mm)	Control	16	0,491	0,023	0,006	1,183	,245	Not significant
	Treatment	32	0,482	0,026	0,005			
Eggshell	Control	16	44,181	9,266	2,316	-,897	,378	Not significant
Breaking Strength (N)	Treatment	32	46,603	7,836	1,385			
Fresh Eggshell	Control	16	8,270	0,463	0,116	1,626	,113	Not significant
Weight (Gram)	Treatment	32	8,020	0,569	0,101			
Dried Eggshell	Control	16	6,539	0,372	0,093	,110	,219	Not significant
Weight (Gram)	Treatment	32	6,395	0,376	0,067			
Percentage of	Control	16	10,771	0,337	0,084	2,518	,016	Significant
shell	Treatment	32	10,454	0,530	0,094			

There were no significant differences between the means of the control and treatment except percent of eggshell during first collection of eggs.

Table 4. Comparison of quality of eggs under control and UVB light treatments during second collection of eggs (07th November).

		N	Mean	Std. Deviation	Std. Error Mean	t-statistics	p-value	Significance at 5% level of significance
Initial Weight(Gram)	Control	16	64,324	3,118	0,779	,305	,762	Not significant
	Treatment	32	63,959	5,135	0,908			
Weight after 1 week(Gram)	Control	16	63,904	3,124	0,781	,324	,748	Not significant
	Treatment	32	63,519	5,079	0,898			
Weight Loss %	Control	16	0,421	0,109	0,027	-,599	,554	Not significant
	Treatment	32	0,441	0,109	0,019			
Length(mm)	Control	16	57,569	1,190	0,298	-,280	,782	Not significant
	Treatment	32	57,669	1,101	0,195			
Diameter(mm)	Control	16	44,494	0,908	0,227	2,436	,021	Significant
	Treatment	32	43,834	0,837	0,148			
Thickness(mm)	Control	16	0,491	0,023	0,006	1,183	,245	Not significant
	Treatment	32	0,482	0,026	0,005			
Eggshell Breaking Strength (N)	Control	16	43,944	9,266	2,317	1,225	,230	Not significant
	Treatment	32	40,438	9,496	1,679			
Fresh Eggshell Weight (Gram)	Control	16	8,168	0,524	0,131	1,140	,263	Not significant
	Treatment	32	7,984	0,538	0,095			
Dried Eggshell Weight (Gram)	Control	16	6,544	0,315	0,079	,780	,440	Not significant
	Treatment	32	6,462	0,402	0,071			
Percentage of shell	Control	16	10,179	0,333	0,083	0,596	,555	Not significant
	Treatment	32	10,107	0,496	0,088			

There were no significant differences between the means of the control and treatment except diameter of eggs during second collection of eggs.

Table 5. Comparison of quality of eggs under control and UVB light treatments during third collection of eggs (17th January).

		N	Mean	Std. Deviation	Std. Error Mean	t-statistics	p-value	Significance at 5% level of significance
Initial Weight(Gram)	Control	16	66,512	3,499	0,875	,703	,486	Not significant
	Treatment	32	65,694	4,342	0,136			
Weight after 1 week(Gram)	Control	16	66,221	3,467	0,867	,715	,479	Not significant
	Treatment	32	65,397	4,310	0,135			
Weight Loss %	Control	16	0,291	0,068	0,017	-,321	,751	Not significant
	Treatment	32	0,297	0,064	0,002			
Length(mm)	Control	16	59,129	1,497	0,374	,974	,339	Not significant
	Treatment	32	58,697	1,348	0,042			
Diameter(mm)	Control	16	44,651	0,824	0,206	0,211	,834	Not significant
	Treatment	32	44,592	1,100	0,034			
Thickness(mm)	Control	16	0,382	0,022	0,006	-1,490	,150	Not significant
	Treatment	32	0,391	0,015	0,000			
Eggshell Breaking Strength (N)	Control	16	42,569	5,137	1,284	-,338	,737	Not significant
	Treatment	32	43,128	5,905	0,185			
Fresh Eggshell Weight (Gram)	Control	16	8,043	0,486	4,476	,549	,587	Not significant
	Treatment	32	7,960	0,524	0,016			
Dried Eggshell Weight (Gram)	Control	16	6,546	0,300	0,075	,832	,410	Not significant
	Treatment	32	6,454	0,454	0,014			
Percentage of shell	Control	16	9,854	0,453	0,113	0,287	,776	Not significant
	Treatment	32	9,815	0,431	0,013			

There were no any significant differences between the means of the control and the UVB light treatments. Since there were no significant differences between means in second and third collection, it is statistically concluded that there is no significant effect of UVB light in the quality of eggs.

Due to lack of time and data, we were unable to study the performance of hens on UVB light treatment.

4. DISCUSSION

The current study demonstrated that exposure of UVB light on laying hens had no effects on the eggshell quality of eggs. Exposure of UVB on hens is assumed to be an appropriate and highly effective approach to enhance the vitamin D content in hens and eggs. Researchers indicate that exposure of UVB is the more efficient method to increase of vitamin D content in muscle of hens and egg yolk compared to feeding hens containing a high dosage of vitamin D in diets. Ko et. al (2008) established an efficient approach to use of UVB radiation to enhance the vitamin D content in mushroom without affecting the quality of mushroom. Vitamin D3 enriched feeds were used in previous studies to increase the vitamin D3 content in eggs and meats of the hen. The maximum limit of supplemented vitamin D3 in feed in Europe for laying hens is 3,000 IU per kg feed. It is not likely to increase the vitamin D3 content of eggs and hens beyond this restricted limit by feeding diet. Exposure to UVB radiation or natural sunlight is a favourable alternative. Schutkowski et. al (2013) stated that exposure of UVB lights on hens is an effective method to provide consumers vitamin D3 enriched food from animal sources.

Exposure of UVB light on laying hens increases the vitamin D contents in meats and eggs (Schutkowski et. al, 2013). However, additional treatment with UVB radiation could not further improve the quality of eggs including weight, length, diameter, eggshell thickness, eggshell breaking strength and percentage of the shell. According to Carson et. al (1995), there were also no any significant effect of UVB radiation on breeders' hens on egg production, weight, laying performance and eggshell quality.

Stability and eggshell formation totally depends upon vitamin D status. Calcium and phosphorous are essential macro minerals for eggshell formation. Calcium is a significant component of the shell. Whereas, phosphorous plays a vital role in skeletal calcium deposition. 1,25(OH)2D active form of vitamin D plays a most important role in calcium homeostasis. It regulates the concentration of calcium ions in extracellular fluid and plays a vital role in egg shell formation (Jonchère et. al, 2012). Increase in specific gravity, shell weight and shell thickness of egg has been reported due to supplementation of 1,25(OH)2D3 (Chennaiah et. al, 2004). 6-8% of economic losses occur in poultry industry due to crack and damages of eggs. The response of eggshell quality

with response to UVB light treatment were examined. However, there were no any impact of UVB iradiation on eggshell quality and thickness.

The weight of the egg usually depends upon the hen's age and nutritional factors rather than UVB light (Borille et al.,2013). In our study, there were no any effects of UVB treatment on eggs fresh weight. The weight of the eggs increases with the ages of hens, as the age of hens affects the egg solids (Fletcher et al., 1983). The eggs weight gradually increased during the collection time. Shell, albumen and yolk are genetically linked with eggs weight. There is a higher link between egg weight and albumen weight compare to egg weight and shell or yolk weight. The size of the eggs increases as the percentage of albumen increases. The variation in egg weight is mainly determined by albumen weight within a strain (Fletcher et al., 1983).

The weight of albumen decreases during storage which causes the loss in egg weight (Scott et. al, 2000). Temperature, humidity and length of storage are related to the weight losses which occur during storage. Lose of moisture due to long storage time causes the decline in internal quality of eggs (Khan et. al, 2013).

The fresh weight and size (length and diameter) of the eggs increased with the production time. Whereas weight loss during storage were slightly higher in early stage compared to the later stage of production. Minimum egg weight losses were recorded with higher weight and bigger size eggs at the later stage of production. This is in accordance with previous data that showed the minimum weight loss in large egg size (Iqbal et. al, 2016). The amount of water removed is high in small eggs due to higher surface to volume ratio. There is a greater proportion of albumen in larger eggs which contain higher moisture than small eggs. As the size eggs increased, the percentage of egg weight loss was decreased in breeder hen (Ulmer-Franco et. al, 2010).

Most of the report have shown that the eggshell quality i.e., eggshell weight, thickness and eggshell breaking strength is influenced by age of laying hen (Silversides et. al, 2001; Zita et. al, 2009). The eggshell proportion and thickness decreases as hens grow older (Abrahamsson et. al, 1998). The contents of eggs are protected by eggshell from mechanical impact and micro bacterial invasion. The exchange of water and gases during the development of chick embryo is controlled by eggshell through pores (Nys et al., 2004).

This study shows that addition of UVB light on laying hens had no significant effects on eggshell quality. Carson et. al (1955) also stated that there were no influences on eggshell quality and laying performance of breeder's hen supplemented with sufficient amount of vitamin D3 due to UVB light treatment. The eggshell breaking strength is related to eggshell thickness and eggshell weight. The eggshell thickness is correlated with temperature. Percentage of eggshell and dried eggshell weight are also influenced by temperature. The eggshell thickness is significantly correlated with dried eggshell weight and percentage of eggshell and is not independent of egg weight (Wilhelm et. al, 1940). UVB has shown a non-linear increase of vitamin D3 and 25(OH)D3 contents in eggs. UVB light (76 $\mu W/cm^{2)}$ were exposed for 300 minutes to gain 95 % maximum attainable vitamin D content of eggs (Kuhn et. al, 2014). In contrast to vitamin D3, 25(OH)D3 contents in egg gained maximum at an exposure of UVB radiation for 60 minutes. The maximum of vitamin D3 in eggs are obtained only after more than two weeks of treatments.

The limiting factor for vitamin D3 synthesis is 7-DHC (Dehydrocholesterol). Exposure of UVB in hens have shown strong variations in concentration of 7-DHC in different skin areas. Unfeather skin legs of hens play an important role for the synthesis of vitamin D3. It has shown an amazingly high level of 7-DHC compared to other parts of the skin. 7-DHC concentration were found to be 30 times higher in unfeather skin legs compared to body skin (Tian et. al, 1994). Thus, an increment of vitamin D in meats and eggs can only be achieved by exposure of UVB radiation on unfeather feet skin of hens.

There were no any differences in body weight and mortality due to different vitamin D2 and vitamin D3 supplemented feed. The force required to fracture the tibia was lower for chicken fed with lower vitamin D3 compared to higher vitamin D3 infeed. The chicken fed with vitamin D3 have shown higher tibia breaking strength compared to chicken fed with vitamin D2 rich diets (Mattila et. al, 2004). Ash content of the tibia were also reported to be increased in broiler supplemented with vitamin D3 enriched diets (Barker et. al, 1998). Vitamin D3 improves the bone strength compared to vitamin D2 enrich diet (Mattila et. al, 2004).

Exposure of UV light on chicken has shown reduced incidence and severity of tibial dyschondroplasia (Edwards et al 1992). Increased in body weight and bone ash were observed chicken exposed to UV light (Mitchell et. al, 1197). Exposure of UV light on sheep have shown

higher plasma levels of cholecalciferol and 25-(OH)D3 compared to oral cholecalciferol supplemented (Hidiroglou et. al, 1989). Birds exposed to UV light without dietary cholecalciferol have shown similar bone and tissue characteristics compared to birds feed with dietary cholecalciferol (Edwards et al, 1994).

Plasma concentration of 25(OH)D3 have shown more response in hens compared to plasma level of $1,25(OH)_2D$ to UVB radiation and dietary vitamin D3. Treatment of UVB light on laying hens were effective only in increasing the $25(OH)D_3$ plasma levels with vitamin D3-deficient diet. The lowest plasma level of $1,25(OH)_2D$ were observed in hens that were not exposed to UVB light and feed vitamin D3-deficient diet (Schutkowski et. al, 2013). There was no influence on food intake and body weight of chicken due to UVB radiation and dietary vitamin D3.

Egg production rate and egg weight were also not significantly influenced by UVB treatment and dietary vitamin D3. Eggshell thickness and eggshell stability were significantly influenced by dietary vitamin D3 and UVB treatment. Lower eggshell thickness and stability was observed on treatment without UVB light and vitamin D3 deficient diet. Whereas, treatment with UVB light and dietary vitamin D3 has shown significant increase in eggshell thickness and eggshell stability. Non-exposure to UVB that received vitamin D3 deficient diet also showed lower bone stability (Schutkowski et. al, 2013). Laying performance of hen, eggshell quality and bone stability can be enhanced through UVB irradiation that are fed vitamin D3 deficient diet.

5. CONCLUSION

Exposure of UVB light on the chicken is assumed to have a positive effect on production and vitamin D concentration in meat and eggs. There were no any adverse effects of UVB radiation on laying hens, egg and eggshell quality. Since, dietary vitamin D were received by laying hens in sufficient amount through feed there were no any effect of UVB treatment on hen's performance and eggshell quality. Significant effect of UVB light on hens could be observed when hens receive vitamin D3 deficient diet. Thus, we can consider UVB treatment of laying hen as an effective and safe method for enhancing vitamin D concentration in meats and eggs.

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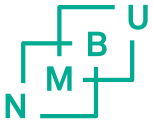

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