IMPACT OF VITAMINS AND SELENIUM ON PRODUCTION PERFORMANCE AND IMMUNITY IN GOLA PIGEON

JESMIN MAHMOOD

DEPARTMENT OF POULTRY SCIENCE
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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BY

JESMIN MAHMOOD

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APPROVED BY:

Prof. Dr. Md. Anwarul Haque Beg

Supervisor

Department of Poultry Science

Sher-e-Bangla Agricultural University,

Dhaka-1207

Dr. Maksuda Begum

Co-Supervisor

Assistant Professor

Department of Poultry Science

Sher-e-Bangla Agricultural University,

Dhaka-1207

Dr. Md. Aftabuzzaman

Associate Professor and Chairman

Department of Poultry Science

Sher-E-Bangla Agricultural University,

Dhaka-1207

Dr. Md. Anwarul Haque Beg
Professor
Department of Poultry Science
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh
Mobile No.: +8801718-026200
Email: mahbegsau@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled, "IMPACT OF VITAMINS AND SELENIUM ON PRODUCTION PERFORMANCE AND IMMUNITY IN GOLA PIGEON" Submitted to the Department of Poultry Science, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE (MS) IN POULTRY SCIENCE, embodies the result of a piece of bona fide research work carried out by JESMIN MAHMOOD, REGISTRATION NO. 15-06481 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during this investigation has duly been acknowledged.

Dated: December 2022

Dhaka, Bangladesh

Prof. Dr. Md. Anwarul Haque Beg
Supervisor
Department of Poultry Science
Sher-e-Bangla Agricultural University,
Dhaka-1207

THIS PAPER IS DEDICATED

TO

MY BELOVED PARENTS

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LIST OF ACRONYMS AND ABBREVIATIONS

ABBREVIATION	FULL MEANING
ANOVA	Analysis of Variance
BLRI	Bangladesh Livestock Research Institute
BWG	Body Weight Gain
cm^2	Square Centimeter
CONTD.	Continued
CP	Crude Protein
DLS	Department of Livestock Services
DMRT	Duncan's Multiple Range Test
e.g.	For Example
et al.	And Others/Associates
FC	Feed Consumption
FCR	Feed Conversion Ratio
gm	Gram
HI	Hemagglutination Inhibition
i.e.	That is
Kcal	Kilocalorie
Kg	Kilogram
LRI	Livestock Research Institute
LW	Live Weight
MS	Master of Science
ml	Milliliter
mm	Millimeter
No.	Number
Nos	Numbers
NS	Non-significant
SAU	Sher-e-Bangla Agricultural University
SE	Standard Error
SPSS	Statistical Package for Social Sciences
viz.	Such as
WHO	World Health Organization

LIST OF SYMBOLS

SYMBOLS	FULL MEANING
@	at the rate of
&	and
%	percentage
<	less than
>	greater than
≤	less than equal
≥	greater than equal
/	per
+	plus
=	equal
±	plus-minus
℃	degree celsius

IMPACT OF VITAMINS AND SELENIUM ON PRODUCTION PERFORMANCE AND IMMUNITY IN GOLA PIGEON

ABSTRACT

In the growing poultry industry, pigeon farming is an important agribusiness in Bangladesh. According to most pigeon owners, common health issues in pigeons include pigeon pox, Newcastle disease, salmonellosis, and the common cold. An experiment was conducted to estimate the impact of different combinations of vitamins and Selenium (Se) on the production performance and immune response against the Newcastle disease vaccine. A total of 16 pair of Gola pigeon (3 months old) were randomly assigned to 4 treatment groups namely; T0 (Control), T1 (Basal diet + Vitamin A, D, E & Se), T2 (Basal diet + Vitamin B-complex), and T3 (Basal diet + Vitamin A, D, E, B-complex & Se) having 4 replications. The treatment groups (T1, T2 and T3) were vaccinated with the Ranikhet disease vaccine and the control group was kept unvaccinated. Performance indices indicated non-significant ($P>0.05$) differences in final live weight, live weight gain and feed consumption. Statistical analysis revealed non-significant ($P>0.05$) differences in egg weight values. However, significant ($P<0.05$) differences were found for egg production, shape index and hatchability in the T1 (23.75), T3 (76.29) and T2 + T3 (87.50) groups respectively. The hemagglutination inhibition titers of the experimental birds were significantly ($P<0.05$) different and higher individual titer was found in the vitamin A, D, E and Se supplemented T1 group (272). The thymus length and width were the best in group T1, 12.75 and 4.25 respectively with a significant ($P<0.05$) difference. From the study, it can be concluded that supplementation of vitamin A, D, E and Se combination gave a better humoral immune response against Newcastle disease vaccine, egg production, thymus length and width; supplementation of vitamin B-complex separate supplementation gave only a better response to egg hatchability, and supplementation of vitamin A, D, E, B-complex, and Se combination gave better performances in egg shape index and hatchability.

CHAPTER 1

INTRODUCTION

In the growing poultry industry, pigeon farming is an important agribusiness in Bangladesh. Approximately 10 million pigeons can be found in Bangladesh (Jalal, 2020). According to most pigeon owners, pigeon pox, Newcastle disease, salmonellosis, and the common cold are among the most frequent health issues affecting pigeons, particularly in the first few days of winter. Approximately 80% of pigeon owners attribute mortality to Newcastle disease (Al-Garib et al., 2003). The initial documentation of the presence of Newcastle disease in pigeons dates back to 1971. NDV is of high economic significance in domestic pigeons, and the global existence of the subject has been recorded, which encompasses India as well (Kataria et al., 2004; Naveen et al., 2009). Newcastle disease (ND) is caused by a virus called the (NDV), also referred to as avian Paramyxovirus (APMV-1) which is made up of single-stranded RNA that belongs to the Paramyxoviridae family. The disease is characterized by gastrointestinal and respiratory symptoms, frequently the presence of nervous disorders, and elevated mortality rates (reaching up to 100%) are often observed.

In the realm of literature, there have been numerous documented instances of outbreaks that have occurred in flocks that were previously vaccinated against Newcastle Disease (ND). These outbreaks were mainly due to improper immune response as well as vaccine failures (Alders *et al.*, 2001). Many outbreaks were additionally ascribed to feedstuffs that were infected with pigeon paramyxovirus (PPMV), as pigeons conveniently encounter free-range birds, and as a result, can disperse NDV among diverse avian species (Toro *et al.*, 2005; Ezema *et al.*, 2009). Cumulatively, the irrational utilization of antibiotics and the substandard quality of compound poultry feeds have rendered the avian species susceptible to a multitude of infectious ailments (Beard, 1984). Immunosuppression may additionally result in the ineffectiveness of vaccines and the prevalence of diseases, as flocks with weakened immune systems frequently encounter a higher occurrence of infections that take advantage of their vulnerability and exhibit insufficient response towards the regular administration of vaccines. Consequently, ensuring appropriate vaccination procedures and closely

monitoring the immune response following vaccination play a pivotal role in effectively managing Newcastle disease (Adene, 1990).

Poultry reared within intensive production systems exhibit a marked vulnerability toward the occurrence of vitamin insufficiencies as a result of various factors. Firstly, they don't gain significant advantages from producing vitamins by microbes in their digestive systems. Secondly, modern poultry operations are characterized by a high stocking density of birds, which imposes significant stress on them and consequently leads to an elevation in their vitamin needs. Thirdly, Poultry requires a substantial number of vitamins and minerals to meet their nutritional needs. The vitamin requirements of avian creatures are contingent upon their physiological composition, age, well-being, nutritional condition, and operational duties (Dudley-Cash, 1994).

The concept of immuno-stimulation encompasses both prophylactic and therapeutic measures aimed at stimulating both nonspecific and specific immune responses (Spradbrow, 1997). Successful administration of immune-stimulating substances in poultry has included Levamisole, vitamin E, and Selenium (Alexander, 2000; Orajaka *et al*., 1999; Dudley-Cash, 1994), as well as Ascorbic acid and vitamin D. These findings suggest that achieving optimal immune response to ND vaccinations, it may be necessary to supplement poultry feeds with vitamins and minerals such as vitamins A, C, and E, as well as Selenium while ensuring optimal vitamin and mineral allowances (McDowell, 2000).

Multivitamin-mineral premixes have been utilized in avian feed to enhance the growth of birds and optimize feed consumption, thus leading to improved production and economic outcomes (Rahman *et al.,* 2012). These vitamins positively impact growth performance by enhancing the utilization of feed and metabolism and functionality of the immune system, and mitigating various stressors (Sahin *et al*., 2003). In addition to vitamins, the absence of minerals like selenium, copper, zinc, and iron has been shown to have an impact on the immune system of birds (Dardenne *et al*., 1985; Suttle and Jones, 1989; Macpherson, 1994).

Vitamin A is a molecule that is soluble in fat. Studies conducted on both animals and humans have demonstrated that adequate levels of vitamin A are essential for immune function. A lack of vitamin A hinders innate immunity by slowing down the recovery of mucosal barriers damaged by infection (Stephensen, 2001). Research has

demonstrated a correlation between insufficiency of vitamin A and a heightened susceptibility to diarrhea, as well as an elevated susceptibility to the progression toward the acquisition of the syndrome known as acquired immune deficiency (Kennedy *et al*., 2000; Rich *et al*., 2000).

Vitamin B-complex has been found to have effects on the immunity of pigeons (Spinas *et al.*, 2015). Inadequate levels of vitamin B_1 can lead to neuroinflammation and T-cell infiltration. Vitamin B-complex has also been reported to enhance feed conversion rate, promote growth, and offer economic benefits.

Vitamin D, a highly advantageous nutrient for avian species, is a lipid-soluble vitamin that regulates the concentration of calcium and phosphorus in the organism (Shojadoost *et al.*, 2015).

Furthermore, it has been noticed that vitamin E boosts both the quantity and effectiveness of immune system cells and stimulates the production of antibodies in response to chicken vaccine administration (Adolfsson *et al*., 2001; Khan *et al*., 2014

Selenium (Se), a micromineral deemed necessary, plays a crucial role in various physiological functions, such as reproduction, immunity, growth, and development of farm animals, including poultry (Surai & Dvorska, 2002). Furthermore, Se possesses anti-carcinogenic and antiviral characteristics, and it is likely to have a significant role in the immune system's operation (Surai, 2002). Reports suggest that the parental administration of selenium enhances the humoral immune response (Droke and Loerch, 1989).

Extensive research has provided a clear understanding of how vitamins and minerals play important roles in the normal functioning of the immune system. Insufficient vitamin levels can also result in dysfunction of the immune system (Koutsos *et al*., 2006). Among all the vitamins, vitamins A, B, D and E have been found to have the most notable impacts on immune system function through various mechanisms. This study aimed to assess how supplementing pigeons with vitamins and minerals, either separately or in combination via drinking water, affected their immune response to the ND vaccine and their overall production. The performance of the native Gola pigeon was assessed through Feed consumption, body weight gain, egg production, egg weight, hatchability, shape index, gross characteristics of lymphoid organs, and antibody titers.

Objectives:

i. To study the impacts of different combinations of vitamin A, D, E, B-complex and Selenium (Se) supplementation on the production performance and immunity in pigeon

ii. To evaluate antibody titers using hemagglutination inhibition (HI) test against the Newcastle disease vaccine

iii. To compare the immune organs with the control group

CHAPTER 2

REVIEW OF LITERATURE

Performing any form of survey or experiment literature review holds significant importance for the proposed study because it helps make the research process easier and more effective. The literature analyzed in this particular framework has been limited to works that are considered compatible and applicable to the aims of the current investigation. Since there is a scarcity of studies conducted on pigeons, this review of the literature was focused on the research conducted on other poultry species with similar objectives. Around 115 sources of literature were reviewed to understand the background, limitations, and future possibilities of our research, and also looked at past findings and the current state of research in this field. Out of the aforementioned sources, 30 consisted of full articles, 50 were abstracts, 20 were comprised solely of titles, and the remaining constituted miscellaneous materials.

Sources of Literature

1. Books and Journals of Sher-e-Bangla Agricultural University (SAU) Library, Dhaka
2. Internet browsing- Google Scholar, PubMed, ResearchGate, Academia, Scopus, etc.

2.1 General Information on the Immune System

The immune system is a vital part of any living organism, protecting the host from infections in the surrounding environment including viruses, bacteria, parasites, and other non-infectious foreign substances, such as proteins and polysaccharides (Abbas *et al.*, 2001; Calder and Kew, 2002). The immune system relies on essential parts like the bone marrow, lymph nodes, spleen, thymus, and bursa of Fabricius. In vertebrates, including chickens, there are two types of immune responses to microorganisms, namely innate (or natural) immunity, and adaptive (specific, acquired) immunity (Abbas *et al*., 2001).

2.1.1 Innate Immunity

The first line of defense against infections is called the innate immune system. This type of immunity comprises anatomic, physiologic, and phagocytic/endocytic barriers,

as well as chemical protection such as gastric acid (Medzhitov and Janeway, 2000). The first line of defense against invaders includes physical barriers like the skin and mucous membranes. Physiological barriers, such as pH, temperature, and oxygen tension, limit microbial growth. Phagocytic cells, such as neutrophils (heterophils in chickens), monocytes (in the blood), and phagocytic macrophages (in the tissues), play a vital role in protecting the body against harmful pathogens. Macrophages are crucial phagocytic cells that participate in non-specific and specific immunity. These cells can destroy infected cells and microorganisms that they ingest, as well as assist other immune system cells in initiating an immune response (Abbas *et al*., 2001).

2.1.2 Adaptive Immunity

When the innate immune system proves inadequate in eliminating encountered pathogens, adaptive immunity serves as the subsequent line of defense. Acquired immunity, known for its specificity and immunologic memory, allows the body to remember and recognize features of pathogens it has encountered before. The humoral and cell-mediated immune responses encompass adaptive immunity (Abbas *et al*., 2001).

2.1.2.1 Humoral (B cell-mediated) Immunity

Humoral immunity fights specific infections by using circulating antibodies, specifically immunoglobulin (Ig) (Devereux, 2002). These antibodies are produced when the immune system encounters a pathogen and are retained within the immune system. Immunoglobulin molecules act as cell surface receptors on B-lymphocytes that originate from the bursa of Fabricius in chickens. In birds, antibodies are primarily categorized into three types: IgM, IgG (also known as IgY), and IgA (Abbas *et al*., 2001). These antibodies are not very effective against viruses and certain types of bacteria that infect cells, but they are highly skilled at eliminating pathogens that are outside of cells.

2.1.2.2 Cell-mediated (T cell-mediated) Immunity

Cell-mediated immune responses are activated when the humoral immune responses prove insufficient in eliminating the antigen, as documented by Erf (2004). T-lymphocytes play a crucial role in the cell-mediated immune system by effectively dealing with and reducing the risk of intracellular pathogens (Chen *et al*., 1991;

Devereux, 2002). T-cells identify antigens using the T-cell receptor (TCR) and other additional adhesion molecules. These cells produce cytokines that are crucial for activating T- and B-cells (Chan *et al.*, 1988; Janeway *et al*., 2001), which then activate elements of non-specific immunity and boost the overall functioning of the immune system.

2.2 Factors Influencing the Avian Immune System

The avian immune system is influenced by both intrinsic and extrinsic factors. Intrinsic factors include an individual's age and sex, while extrinsic factors encompass environmental conditions, social interactions, diet, and exposure to toxins (Koutsos and Klasing, 2014). Birds with a weakened immune system are more susceptible to diseases and infections, and their ability to mount an effective immune response against external threats is significantly reduced (Huff *et al*., 2005).

2.3 Immunomodulation in Poultry

Immunomodulation in poultry is important for improving disease resistance and overall health. Immunomodulators are a class of agents that have the specific ability to regulate the immune system, thereby enhancing immunity and resistance to diseases and infections as well as enhancing vaccine efficacy. In the context of poultry, the health and immunity of chickens and other poultry species are of paramount importance as they directly affect growth, disease resistance, FCR, body weight gain, and production output. Prebiotics, probiotics, vitamins, adjuvants, polysaccharides, herbs, and other similar agents are the various types of immunomodulators available (Das *et al.*, 2020).

2.4 History of Newcastle Disease

The initial recognition and designation of Newcastle disease (ND) were observed in poultry in 1926, in Java, Indonesia (Kraneveld, 1926; Ashraf and Shah, 2014), as well as in Newcastle-upon-Tyne, England (Doyle, 1927). However, before the year 1926, there had been earlier accounts of comparable outbreaks of the disease in Central Europe (Halasz, 1912). Specifically, Macpherson (Macpherson, 1956) attributed the loss of all chickens in the Western Isles of Scotland in 1896 to Newcastle disease. To avoid any confusion with other ailments, Doyle coined the term "Newcastle disease" (naming it after the initial outbreaks in Great Britain) as a provisional measure (Doyle, 1935). Despite this, the name has stuck, although the term ‘Avian Paramyxovirus type

1 (APMV-1)' is now commonly used when discussing the Newcastle disease virus (NDV).

2.4.1 Etiology

Newcastle disease (ND), is a member of the Paramyxoviridae family, belonging to the genus *Avulavirus*. The avian paramyxoviruses have been classified into ten serotypes, namely APMV-1 to APMV-10. Among these, the Newcastle disease virus (NDV) has been classified as Avian Paramyxovirus type 1 (APMV-1). NDV strains are categorized as highly virulent (velogenic), moderately virulent (mesogenic), or nonvirulent (lentogenic) following their pathogenicity (Beard, 1984). The clinical manifestations of Newcastle disease virus infection in chickens have led to its categorization into five pathotypes, namely viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic or respiratory, and asymptomatic (Alexander *et al.*, 2004).

Table 1. Characteristics of Newcastle Disease Virus

Temperature	Inactivated by 56 °C for 3 hours or 60°C for 30 minutes
p^H	Inactivated by acid pH ≤ 2
Chemicals / Disinfectants	Ether sensitive; inactivated by formalin, phenolics, and oxidizing agents; chlorhexidine, sodium hypochlorite (6%)
Survival	Survives for long periods at ambient temperature, particularly in feces

Source: (Alexander *et al.*, 2004)

2.4.2 Epidemiology

Newcastle disease virus (NDV) is indeed a major pathogen in poultry, but there are variants of the virus that can also impact other species, such as pigeons. While NDV is widespread in Bangladesh, there is limited knowledge about the viruses affecting pigeons. It is important to note that the virulence of NDV strains varies depending on

the host. Chickens are highly susceptible, while ducks and turkeys typically do not show severe manifestations (Higgins, 1971). The susceptibility to the disease varies among game birds like pheasants, partridges, quail, and guinea fowl, as well as parrots belonging to the Psittaciformes order, however, cockatiels are sensitive. Wild birds and waterfowl, which belong to the Anseriformes order, can carry the Newcastle disease virus without showing obvious clinical symptoms. Young cormorants of the *Phalacrocorax spp.* have shown disease linked to APMV-1, while ostriches of the Struthioniformes order and pigeons of the Columbiformes order are known to be susceptible to the virus. Raptors are typically immune to ND, although cases of acute disease have been documented in the bearded vulture (*Gypaetus barbatus*), white-tailed sea eagle (*Haliaeetus albicilla*), a wild osprey (*Pandion haliaetus*), and some species of falcons. Gulls of the Charadriiformes order, fowls of the Strigiformes order, and pelicans of the Pelecaniformes order are other birds that have been affected by NDV. Passerine birds of the Passeriformes order exhibit variable susceptibility, with some species displaying healthy but excreting NDV, while others may encounter serious illness. Fatalities in crows and ravens of the genus *Corvus* have been noted, and acute ND has been observed in penguins of the Sphenisciformes order. The rates of morbidity and mortality can vary among species and with the virus strain. Humans can also get infected, as manifested by unilateral or bilateral reddening, excessive lachrymation, eyelid edema, conjunctivitis, and sub-conjunctival hemorrhage (Afreen *et al.*, 2012).

2.4.3 Transmission

The transmission of Newcastle disease virus occurs through direct contact with secretions of infected birds, mainly through ingestion (fecal/oral route) and inhalation, as well as through fomites such as feed, water, equipment, surfaces, human clothing, boots, bags, egg trays/crates, and other objects. The presence of feces, including soiled eggshells, prolongs the survival of the agent. Additionally, fleas, rodents, insects, and dogs may mechanically spread the virus from infected feces (Ullah *et al.*, 2004; Alders and Spradbrow, 2001; Yune and Abdela, 2017).

2.4.4 Occurrence

Velogenic Newcastle Disease Virus (NDV) is prevalent in various sites of Mexico, Central, and South America, and is extensively distributed in Asia, the Middle East, and Africa. While lentogenic strains of NDV have a global presence, mesogenic pathotypes,

which have a specific adaptation to pigeons, i.e., pigeon Paramyxovirus, do not seem to readily infect other poultry species, according to research findings (Naveen *et al*., 2013).

2.4.5 Pathogenicity and Clinical Signs

The pathogenicity of the Newcastle strain can be classified into five different pathotypes. These include the asymptomatic enteric strain, which presents as a sub-clinical enteric infection without clear symptoms. The lentogenic strain is characterized by a mild respiratory infection. The mesogenic stain presents with rare nervous and respiratory signs, while the mortality rate is related to the age of the susceptible birds (young birds are more susceptible compared to adults). The viscerotropic velogenic strain causes hemorrhagic intestinal lesions and is highly pathogenic. Finally, the neurotropic velogenic strain causes high mortalities followed by respiratory and nervous signs (Ashraf and Shah, 2014).

Initially, the disease presents with a range of symptoms, including fatigue, loss of appetite, disheveled feathers, swelling, and redness of the eye membranes (conjunctiva). Birds may also experience diarrhea with a greenish or white watery appearance, difficulty breathing (dyspnea), and inflammation in the head and neck region, often accompanied by a bluish discoloration as the disease advances. In the later stages of the disease, neurological symptoms may become apparent, displaying a range of issues such as trembling, spasms characterized by muscle stiffness and convulsions, weakness or paralysis in the wings or legs, abnormal head positioning (torticollis), and erratic circling behavior. Additionally, there might be a significant decline in egg production, with eggs showing irregular shapes and abnormal coloring, as well as having rough or thin shells filled with watery albumin. In some cases, highly virulent strains of the disease can lead to sudden death, often with minimal or no preceding signs. When birds survive severe infections, they may develop neurological problems and may completely stop laying eggs. In the absence of vaccination, unvaccinated chickens can experience extremely high rates of illness and death, approaching 100% (Alexander *et al.*, 2004).

The clinical signs associated with the three most aggressive strains of avian Paramyxovirus (APMV-1) (Linde *et al.*, 2010; Beard, 1984): Lentogenic strains- are commonly linked with subclinical manifestations characterized by mild respiratory

illness, including coughing, gasping, sneezing and rales. Mortality rates are deemed negligible. Mesogenic strains- have been found to potentially induce acute respiratory disease and neurological symptoms in certain species, with the mortality rate typically remaining low, at less than 10%. Velogenic strains are known to induce severe diseases in chickens, usually leading to mortality.

2.4.6 Diagnosis

2.4.6.1 Clinical Sign and Lesions

Common clinical signs include birds appearing lethargic and downcast, with ruffled feathers, as well as showing greenish-white diarrhea and various neurological symptoms like head twisting (torticollis), or paralysis in their legs or wings. The disease also spreads quickly, has a mortality rate exceeding 50% in local bird populations, and an incubation period ranging from 3 to 6 days, occasionally extending to 2 to 15 days (Alexander *et al.*, 2004; Allan and Gough, 1974).

During postmortem examinations, typical findings include mucus in the trachea and bleeding in the intestine, primarily in the proventriculus (Alexander *et al*., 2004).

2.4.6.2 Serological Diagnosis

The hemagglutination inhibition (HI) test and the enzyme-linked immunosorbent assay (ELISA) are the two methods utilized to measure antibody titers. In both cases, obtaining blood samples from the birds is imperative (Getabalew *et al.*, 2019).

2.4.7 Newcastle Disease Vaccine

At the time when the virus seemed to be emerging, vaccination using inactivated virus was being considered as a potential method for controlling Newcastle Disease (ND). However, an attenuated live vaccine, known as strain H, was produced following the 1933 outbreak in England. Later on, the most commonly used veterinary vaccines worldwide became the USA isolates of low virulence, Hitchner B1 (HB1) and La Sota. Over more than half a century since the first use of vaccines to protect village poultry against ND, a diverse range of vaccine types have been developed, many of which have undergone testing on village poultry (Placidi and Santucci, 1952). Different vaccination methods have been employed for Newcastle disease (ND), with three specific vaccine types: live lentogenic, live mesogenic, and inactivated vaccines (Alexander, 2000).

Most live vaccines are derived from enteric or lentogenic strains that don't cause symptoms, although some vaccines from mesogenic strains are still used. Live lentogenic vaccines are typically developed from field viruses that have low pathogenicity in poultry but still trigger a sufficient immune response (Alexander *et al.*, 2004).

2.5 Nutrition and the Immunity

Nutritional immunology has been a recognized field of study since 1810, as indicated by Silverstein in 2009. In the early 20th century, there was growing awareness of the positive impact of nutrients, including vitamins, on the immune system. Bendich in 1996 and Field *et al.* in 2002 provided evidence that the proper intake of nutrients significantly enhances the immune system. Various aspects of the immune system, such as the gastrointestinal tract, thymus, spleen, regional lymph nodes, and circulating immune cells, are influenced by nutrients (Chandra, 1997, Cunningham-Rundles, 2001). In both mammals and birds, a lack of protein, specific amino acids, vitamins A, E, B6, folate, and trace minerals like copper, zinc, selenium, chlorine, sodium, and iron can reduce immunocompetence. It's important to note that excessive amounts of nutrients can also have detrimental effects on the immune system (Flynn, 1985).

2.6 Effects of Vitamin A Supplementation

2.6.1 Effects of Supplementing Vitamin A on Feed Consumption and Body Weight

Lin *et al.* (2002) discovered that providing a significant amount of vitamin A as a supplement had a positive impact on the feed consumption and egg-laying rate of hens experiencing heat stress.

2.6.2 Effects of Supplementing Vitamin A on Egg Production

Elsherif *et al.* (2017) conducted a study that discovered the addition of vitamin A to the diet had a substantial positive impact on the egg production of Lohmann brown laying hens. The results showed that egg production experienced a notable enhancement through the addition of vitamin A. During the period of peak production, the groups that were given 30,000; 40,000, or 50,000 IU of vitamin A per kilogram of diet demonstrated an improvement in egg production compared to the groups that received 10,000 or 20,000 IU of vitamin A per kilogram.

Mahrose *et al.* (2011) found that increasing the amount of vitamin A supplementation to 16,000 IU/kg diet led to the greatest monthly egg count and egg weight in laying hens.

2.6.3 Effects of Vitamin A Supplementation on Immune Organs and Immunity

Bhatti *et al.* (2016) found that vitamin A as a supplement did not significantly impact the weights of the spleen and thymus, but there was a tendency for improvement in the bursa of Fabricius index in broiler chicks.

Supplementing vitamin A in pigeons has been shown to have positive effects on their immune function. One study showed that pigeons fed with a diet supplemented with mannan oligosaccharides (MOSs), which are a type of vitamin A, had higher levels of serum immunoglobulin M (IgM) concentrations when compared to those birds that were fed a basal diet (Muir *et al.*, 2002).

Another study found that vitamin A and related retinoids have a notable impact on immunity, including the production of antibodies and the function of various immune cells (Swain *et al.*, 2000). The result indicated that the chicks fed with a diet supplemented with 12500 IU/kg vitamin A resulted in the best growth, most efficient feed utilization, and the highest cellular response. The humoral immune response was obtained on a diet supplemented with vitamin A level of 12500 IU/kg. Both hemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) titers reached their highest levels on the 10th day after immunization.

2.7 Effects of Vitamin D Supplementation

2.7.1 Effects of Supplementing Vitamin D on Feed Consumption and Body Weight

Vazquez *et al.* (2018) found that in broiler chickens, the addition of 25-hydroxycholecalciferol [25(OH)D_3] to the diet has been found to improve growth performance and cellular immune response.

Safamehr *et al.* (2013) showed that egg weight, egg production, egg mass, feed intake, feed conversion ratio, and egg quality traits remained unaffected by different levels of vitamin D_3.

2.7.2 Effects of Supplementing Vitamin D on Egg Production

Safamehr *et al.* (2013) showed that the weight of the eggs, the production of eggs, the mass of eggs, the intake of feed, the ratio of feed conversion, and the traits of egg quality were not influenced by the sources of different levels of vitamin D_3.

Mattila *et al.* (2004) discovered that the administration of vitamin D supplements did not result in any observable changes in the production parameters of birds when compared to the control diet.

2.7.3 Effects of Vitamin D Supplementation on Immune Organs and Immunity

Higher doses of vitamin D supplementation have been shown to enhance the innate immune response in broiler chickens (Misiorowski, 2020).

In broiler chickens, incorporating 25-hydroxycholecalciferol [$25(OH)D_3$] into the diet has been found to improve growth performance, and cellular immune response (Vazquez *et al.*, 2018).

Zhang and Wangy (2010) found that increasing levels of vitamin D in broilers enhanced immune function, as evidenced by increased thymus and spleen indices.

2.8 Effects of Vitamin E Supplementation

2.8.1 Effects of Supplementing Vitamin E on Feed Consumption and Body Weight

Pompeu *et al.* (2018) studied the effect of vitamin E supplementation on the growth performance, meat quality, and immune response of male broiler chickens using a meta-analysis of 51 scientific papers. The results showed that dietary vitamin E supplementation did not influence growth performance.

Adebiyi (2011) investigated the effect of tocopherol (vitamin E) supplementation on the performance characteristics and serum enzymes of broiler chicks at different stocking densities. The results showed that there were no significant changes in the weight gain and final weight of the birds across the various dietary treatments. However, the birds on the negative control diet (T2) did consume significantly more feed compared to those that received vitamin E supplementation.

Lin and Chang (2006) conducted a study to examine how the immune responses in breeder chickens during the maturing period are affected by dietary vitamin E

supplementation. The researchers found that providing additional vitamin E improved the body weight gain of laying pullets during the peak-laying period. However, they observed no significant effect on the growth performance of cockerels.

Lohakare *et al.* (2004) conducted a study to assess the impact of vitamin E supplementation in both feed and water on the growth performance and meat quality of broilers. The findings indicated that vitamin E supplementation led to better growth performance, improved nutrient digestibility, increased dressing percentage, enhanced bone strength, and reduced meat oxidation levels.

2.8.2 Effects of Supplementing Vitamin E on Egg Production

Nguyễn *et al.* (2021) found that adding either 75 mg or 100 mg of vitamin E to the feed improved the egg performance of Japanese quails. The groups E100, control, and E75 had notably higher egg-laying rates and heavier eggs compared to the E125 group.

Research conducted by Torki *et al.* in 2018 revealed that dietary supplementation of vitamin E improved egg production and egg quality in laying hens, even under heat-stress conditions.

Another study by Gjorgovska *et al.* (2012) showed that vitamin E supplementation in the diet resulted in higher egg production and egg weight in laying hens.

Additionally, Scheideler *et al.* (2010) found that vitamin E supplementation improved egg production and vitelline membrane strength in laying hens, especially when combined with selenium supplementation.

Lin *et al.* (2004) found that supplementing with 80 mg/kg of vitamin E improved egg production in Taiwan Native Breeder pullets.

2.8.3 Effects of Vitamin E Supplementation on Immune Organs and Immunity

Amevor *et al*. (2021) found that vitamin E, separately or in combination, significantly improved egg production, egg quality, and immune function in aging hens.

Rostami *et al.* (2018) assessed the effects of vitamin E treatments in birds and found that neither antibody titers against viruses nor the weight of lymphoid tissues were influenced by vitamin E ($P>0.05$).

Pompeu *et al.* (2018) showed that dietary vitamin E supplementation did not affect growth performance, but it raised the vitamin E content in the muscle, decreased lipid peroxidation, and improved the immune response in birds.

Darabighane *et al.* (2017) showed that vitamin E can improve the humoral and cellular immune reactions of broilers. Moreover, the highest level of antibodies against Newcastle disease virus was achieved on days 25 and 35 among the broilers that were given vitamin E.

Rehman *et al.* (2017) reported that supplementing vitamin E at a rate of 250 mg/kg enhanced the antioxidant status and immune response in two broiler strains. Birds that received vitamin E supplementation exhibited significantly higher body weight and feed conversion ratio (FCR).

One study found that vitamin E supplementation at different levels did not affect the weight of lymphoid tissues, including the thymus, spleen, and bursa (Bhatti *et al.*, 2016).

Faluyi *et al.* (2014) showed that birds that were fed a diet containing 300mg/kg of vitamin E consumed the highest amount of feed. The antibody titer values, after the second ND vaccination, were highest in birds that were fed a diet supplemented with 100mg/kg of vitamin E, with a titer of log210.

Silva *et al.* (2011) reported that birds were given a dosage of 65mg/kg of vitamin E, and this dosage showed an improvement in the cell immune response of the birds.

2.9 Effects of Vitamin B-complex Supplementation

2.9.1 Effects of Supplementing Vitamin B-complex on Feed Consumption and Body Weight

Halle and Ebrahem (2012) investigated how vitamin B_{12} and Cobalt affected the performance of laying hens. The findings indicated that adding 5 µg of vitamin B_{12} per kg of hen feed was enough to offset deficiencies in feed intake and laying performance. Furthermore, supplementing the diet with 5 to 20 µg of B_{12} led to a notable increase in the final body weight of the hens.

Halle *et al.* (2011) investigated the effects of dietary vitamin B_{12} and cobalt supplementation on growth performance, feed intake, feed-to-gain ratio, carcass

composition, and nutrient content of breast meat of broiler chickens and Pekin ducks. The results showed that increasing the B_{12} concentration from 0 to 40 µg/kg in the feed improved the daily feed intake, daily weight gain, and final body weight of broiler chickens.

2.9.2 Effects of Supplementing Vitamin B Complex on Egg Production

Halle and Ebrahem (2012) studied the effect of vitamin B_{12} and cobalt on the performance of laying hens. The results showed that supplementation of 5 µg vitamin B_{12} per kg laying hen feed was sufficient to compensate for deficiencies in feed intake and laying performance. Egg weight and daily egg mass production were significantly increased with supplementation of vitamin B_{12} and $B_{12} \times Co$.

Krishnan Rajalekshmy (2010) found that folic acid at 2 ppm increased egg production in laying hens.

Day and Dilworth (1966) conducted two tests to determine the need for certain supplemental B vitamins in laying rations for commercial White Leghorn layers. The first test used multiple supplements containing riboflavin, pantothenic acid, niacin, and choline, while the second test used single and combination additions of riboflavin, pantothenic acid, and niacin. The findings revealed that all levels of vitamin supplementation led to a significant boost in egg production and egg size, with even the lowest level of vitamin supplementation being equally effective as higher levels. Additionally, riboflavin supplementation notably increased egg production in the second test.

2.9.3 Effects of Vitamin B Complex Supplementation on Immune Organs and Immunity

Vitamin B_1, B_2, and B_6 are important components of the optimized complex vitamin composition for poultry, which can improve the immune system and enhance responses to vaccines (Shojadoost *et al*., 2021).

Furthermore, the vitamin B complex, along with other vitamins such as A, D, E and C can modulate cell-mediated and antibody-mediated responses, regulate the immune system, and have anti-inflammatory effects in chickens (Sanda, 2015).

Overall, supplementing vitamin B complex in poultry diets can contribute to the improvement of immune function and enhance the ability of chickens to combat microbial pathogens (Swain *et al.*, 2000).

2.10 Effects of Selenium (Se) Supplementation

2.10.1 Effects of Supplementing Selenium on Feed Consumption and Body Weight

In one study, dietary sodium selenite (SS) supplementation did not have a significant effect on the final body weight of pigeon squabs (Wang *et al.*, 2019).

According to Khashaba *et al.*, (2009), a study comparing organic and inorganic sources of selenium found that increasing dietary selenium levels increased the number of weaned squabs, body weight, and weight gain in local Baladi squabs and pigeons under Egyptian conditions.

2.10.2 Effects of Supplementing Selenium on Egg Production

Supplementing selenium in pigeon diets has been shown to have positive impacts on egg production. Wang *et al.* (2017) found that dietary supplementation of sodium selenite (SS) at a concentration of 1.0 mg/kg resulted in higher egg production compared to the control group.

Additionally, Liu, H. *et al.* (2020) showed that adding a high level of selenium to the diet of laying hens significantly increased egg production.

Furthermore, Liu *et al.* (2020) demonstrated that selenium yeast supplementation improved the laying rate in older laying hens.

However, Lin *et al.* (2020) found that nano-selenium supplementation decreased egg production and increased laying hens' feed conversion rate and eggshell thickness.

2.10.3 Effects of Selenium (Se) Supplementation on Immune Organs and Immunity

Sun *et al.* (2020) found that providing laying hens with selenium-enriched earthworm powder (SEP) in their diet enhanced their antioxidant capabilities and immune system.

Korzeniowska *et al.* (2019) found that both organic and inorganic forms of selenium had no impact on the relative weight of immune organs like the bursa of Fabricius, thymus, and spleen.

Bakhshalinejad *et al.* (2018) showed that higher levels of selenium supplementation were found to have a positive effect on the immune parameters of the chickens.

Marković *et al.* (2017) discussed that adding selenium to animal diets enhances their overall performance, health, and immune system.

Habibian *et al.* (2015) demonstrated that the addition of selenium to the diet can increase the antioxidant levels and immune function of broiler chicks, laying hens, and quails when raised in hot conditions.

Bao (2009) studied the effects of two different sources of selenium, sodium selenite, and nano selenium, on the growth performance of laying chicks and the development of their immune organs. The results showed that the addition of nano selenium in the laying chicks' feed could promote their growth and strengthen the function of their immunity organs.

Singh *et al.* (2006) examined how dietary supplements of selenium, vitamin E, or a combination of both affected the antibody responses in broiler chickens. Their findings indicated that chicks given supplements containing 200 mg of vitamin E per kilogram and 0.2 mg of selenium per kilogram exhibited significantly higher antibody titers for hemagglutination inhibition (HI). Furthermore, these chicks also had significantly larger spleens and bursa. This suggests that vitamin E and selenium together have a positive and synergistic impact on the immune system's responses.

2.11 Effects of Different Combinations of Vitamin A, D, E, B-complex, and Selenium on Production and Immunity

Nemati *et al.* (2020) showed that there was no substantial effect of vitamin E and Se on feed consumption, FCR, and egg yield rate.

Dalia *et al.* (2018) found that supplementation of vitamin E and selenium in broiler chicken feed improved immune response and increased beneficial bacteria in the gut.

Sanda (2015) showed that vitamin-mineral supplementation (VMS) had a substantial impact on the immune response of broiler chicks to Newcastle disease vaccination. The group that received VMS before and after vaccination had a significantly higher mean NDV antibody titer.

Naik *et al.* (2015) found that broilers receiving 0.1 ppm organic selenium with 300 mg/kg vitamin E had higher feed intake and body weight.

Safarizadeh and Zakeri (2013) found that the addition of vitamin A and the complex of vitamin E and selenium to the diets of broiler chickens improved their humoral immune system, but had no significant effect on their growth factors.

Salahuddin *et al.* (2012) studied the effects of extra protein and vitamin ADE supplementation on the growth and blood parameters of broiler chicks. The chicks were divided into four groups: one serving as the control and the other three receiving supplements of protein, vitamin ADE, or a combination of both. The research revealed that when protein and vitamin ADE were given together, it led to improved body weight and growth of various organs.

Giri *et al.* (2012) investigated the impact of dietary vitamin E and selenium supplementation on egg production, fertility, and hatchability in native ducks. The group that underwent treatment with a basal diet containing 0.5g of vitamin E and 50 ppm of selenium per 100 kg of feed exhibited noteworthy enhancements in egg production, fertility, and hatchability when compared to the control group, which received only the basic diet.

Yamuna and Thangavel (2011) suggested that selenium and vitamin E supplementation can improve the immune status of broiler chickens.

Mahrose *et al.* (2011) investigated a study to examine the impact of additional dietary supplements of vitamins A, E, and Selenium on the performance of laying hens during the summer season. The results showed that for most of the parameters related to living body weight and its change over time, there were no significant differences due to the supplements of vitamins A, E, and Se, or their combinations, across all age groups studied. Increasing the supplementation levels was associated with a reduction in feed intake while improving feed conversion. The effects of these supplementations and their interactions were highly significant ($P \leq 0.01$) for feed intake and feed conversion. Moreover, increasing the level of vitamin A up to 16000 IU/kg in the diet resulted in the highest ($P \leq 0.05$) monthly egg production and egg mass. However, a higher level of dietary vitamin E supplementation led to a significant ($P \leq 0.05$) decrease in shell thickness and Haugh units, affecting egg quality. Additionally, most of the egg quality traits fluctuated significantly ($P \leq 0.05$ and 0.01) with the higher level of selenium

supplementation. Notably, the interactions among the different supplementations did not have a significant impact on egg quality traits.

Kanchana and Jeyanthi (2010) showed that the body weight of chicks receiving both vitamin E and selenium (basal diet with 100 mg/kg vitamin E plus 0.2 mg/kg selenium, and basal diet with 200 mg/kg vitamin E plus 0.4 mg/kg selenium) was significantly increased ($P<0.05$). Chicks receiving supplements of 100 mg vitamin E/kg and 0.2 mg selenium/kg exhibited significantly higher HI titer against NDV ($P<0.01$).

Yalçinkaya and colleagues (2010) examined how diets with organic selenium and vitamin E impacted the growth, feed intake, organ sizes, and blood measurements of broiler chicks. Their research revealed that there were no notable variations in live weight, feed consumption, feed efficiency, and relative organ weight between the different groups.

Na et al. (2007) carried out a study to investigate how dietary organic selenium and vitamin E affected weight gain, feed intake, feed conversion, and selenium content in the meat of broiler chickens. In each growth phase, they added supplements to the basic diet, including 0 (control), 150 IU/kg of vitamin E, and combinations of 1.2 ppm selenium from selenium yeast (SY) and varying levels of vitamin E (100, 150, 200, and 300 IU/kg). The research revealed that during the first 21 days, weight gain was significantly higher in the control group and the group with 150 IU of vitamin E compared to the group receiving the combination of 1.2 ppm SY and 150 IU of vitamin E.

Shaik *et al.* (2005) studied the effect of supplementation of vitamin E, selenium, and their combinations on the growth and immune response in broiler chickens. The results showed that the experimental diets did not influence body weight, feed intake, or weights of the bursa and spleen.

Although there is no specific mention of the effects of vitamins and Se supplementation on production and immunity in pigeons in the provided abstracts, the effects of vitamins and Se observed in other avian species suggest that it may have similar effects in pigeons as well.

CHAPTER 3

MATERIALS AND METHODS

3.1 Site of the Experiment:

The research work was conducted at the Poultry Farm of Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experimental Period:

The experiment was held for a period of 38 weeks from May 2022 to February 2023.

3.3 Experimental Pigeons:

A total of 16 pair of around 3-month-old Gola pigeon were used in the experiment. They were randomly distributed in four treatments following a Complete Randomize Design (CRD) consisting of four (4) replicates, one pair in each replicate. The distribution and arrangement of the pigeons are given in the following table.

Table 2. The Layout of the Experiment

Treatment	No. of Replication				Total
	R1	R2	R3	R4	
T0	2	2	2	2	8
T1	2	2	2	2	8
T2	2	2	2	2	8
T3	2	2	2	2	8
Total	8	8	8	8	32

3.4 Experimental Treatments:

Treatments Description

T0: Basal diet (Control group without vitamins and Selenium)

T1: Basal diet + Vitamin A, D, E & Selenium (0.5ml/liter drinking water)

T2: Basal diet + Vitamin B-complex (0.5ml/liter drinking water)

T3: Basal diet + Vitamin A, D, E, B-complex & Selenium (0.5ml/ liter drinking water)

3.5 Experimental Diets:

Pigeons were given farm-made protein-based ration. The feed ingredients were purchased from the local market to formulate pigeon ration. The energy and protein level of the ratio was maintained as 2900 ME Kcal/Kg and 17.6% CP. The pigeons were fed as above-mentioned vitamin A, D, E, B-complex and Selenium (Se). The whole grain feeding system was followed. Cereal grain- maize and wheat; pulse seed- black gram and pea; oil seed- mustard and soybean, and grit mixture- broken bricks, oyster shell, and DCP (Di-calcium phosphate) were used in the ration.

Table 3. Formulated Ration for Pigeon (Protein Based)

Ingredients	Amount (Kg)	Supplied CP% in ration
Maize	30	2.7
Wheat	23	2.2
Soybean	15	5.4
Pea	20	4.4
Mustard	05	1.7
Black Gram	05	1.2
Mineral Mixture	02	-
Total	100	17.6

3.6 Preparation of the Experimental House:

Pigeons were reared in an intensive (cage) system. A large house was used for keeping the cages. Required feeders and drinkers were supplied inside the cage. The experimental house was thoroughly cleaned and washed using fresh tap water. The house was meticulously cleaned and disinfected using a mixture of detergent and diluted Virocid® disinfectant solution (6 ml per 1 liter of water). A foot bath containing potassium permanganate ($KMnO_4$) was employed at the entrance of the farm gate to prevent the potential transmission of pathogens. The curtain was used to avoid wind and rainwater entering the house. Entry of wild birds and rodents was strictly prohibited. A comprehensive hygiene and sanitation program was implemented on the

farm and its surroundings. Regular cleaning of the farm and disinfection of the feeders and waterers were maintained.

3.7 Biosecurity Measures:

During the experimental period, the biosecurity measures were adequately upheld. The entrance of wild birds and animals was strictly forbidden, and a footbath was placed immediately after the farm's entrance to reduce the chances of pathogen transmission. The footbath contained a potassium permanganate ($KMnO_4$) solution. Furthermore, a comprehensive hygienic and sanitation program was implemented encompassing both the farm and its surrounding areas. Stringent sanitary protocols were enforced throughout the entire experimental duration, including the disinfection of both the bird cages and the housing using Virocid® solution.

3.8 Medications:

Metronidazole tablet at the dose of 1 Tab./4 L water, was used to prevent coccidiosis among the birds. Albendazole (1 Tab./4 L water) and Ivermectin pour-on (1 drop/pigeon) were used for the parasitic treatment at 4 monthly intervals.

3.9 Collection of Vitamins and Selenium:

The supplemented vitamins and minerals were collected from a veterinary medicine shop. For vitamins A, D and E, Renasol AD_3E vet (Renata Company); For the vitamin B-complex, B-Com-Vit Liquid (Square Company) and for the Selenium, E-Sel Solution (Square Company) was used. These supplementations were given orally in drinking water for 5 consecutive days twice a month.

3.10 Newcastle Disease (ND) Vaccine:

In this experiment, the Ranikhet disease live lentogenic vaccine (RDV) was acquired from the Livestock Research Institute (LRI) in Mohakhali, Dhaka.

3.11 Immunization with ND Vaccine:

Treatment groups T1, T2, and T3 were primarily vaccinated with RDV through an intramuscular (IM) route of 0.5 ml per bird at 12 week of age followed by booster vaccination at 38 week of age and 0.5 ml per bird. The T0 group was kept as unvaccinated control.

Table 4. Vaccination Programs in the Study Groups

Age at ND vaccination	T0	T1	T2	T3
12 weeks	-	RDV Live (IM)	RDV Live (IM)	RDV Live (IM)
38 weeks	-	RDV Live (IM)	RDV Live (IM)	RDV Live (IM)

IM = Intramuscular

3.12 Feed Consumption:

The daily feed intake in all the groups was monitored. A measured amount of feed per pair was distributed in their feed troughs and the birds allowed feed for 24 hours. The difference between the quantity given and the remainder was calculated as the weekly feed intake. The average feed intake per bird per day was calculated for each group.

3.13 Body Weight:

The initial live weight at 12 weeks of age and the final live weight at 38 weeks of age were recorded per bird.

3.14 Body Weight Gain:

The change in body weight per bird was determined by subtracting the initial weight from the final weight.

3.15 Egg Production and Egg Weight:

Regular records were maintained for egg production and the total weight of eggs produced. Birds were mature sexually at 5^{th} week of age. By the sixth week of age, the birds had reached peak production. Almost 7 days after mating, females laid 1 to 3 (usually 2) white eggs which hatched after 17 to 18 days.

3.16 Blood Collection:

Blood samples were obtained from the birds following the booster vaccination. Four pigeons were randomly chosen from each group, and blood was collected from the jugular vein after their slaughter. Blood was collected in the apparatus tube without

adding any anticoagulant and the tubes were then kept in a slanting position for 8-12 hours to separate serum and stored in a refrigerator.

3.17 Serum from Blood Samples:

The serum was gathered in a test tube through micro pipetting. Subsequently, it was centrifuged at 1330 rpm for 2 minutes and 20 seconds to separate the clear serum. This clear serum was then transferred into small vials, appropriately labeled, and stored at a temperature of -20°C until it was needed.

3.17.1 Serological Test:

The hemagglutination inhibition (HI) test and the enzyme-linked immunosorbent assay (ELISA) are the two methods utilized to measure antibody titers. In both cases, obtaining blood samples from the birds is imperative (Getabalew *et al.*, 2019). Here hemagglutination inhibition (HI) test was used to evaluate the antibody titers against ND.

3.17.2 Hemagglutination Inhibition (HI) Test:

The sera were utilized to assess the pigeons' immune response to vaccination using the hemagglutination inhibition (HI) test, following the procedure outlined in OIE (2000). The test involved employing a constant 4HA unit antigen and a serum dilution method (β-procedure) as described in Anon (1971). Initially, the hemagglutination titer of the HA antigen was determined through the HA test. Then, the antigen was diluted in PBS to achieve 4HA units per 50µl of suspension. A series of two-fold serum dilutions were prepared for conducting the HI test. Antibody titers against NDV in the sera were determined via the HI assay at the ACI Animal Health Diagnostic Laboratory in Dhaka.

3.18 Collection of Samples:

Samples were collected after the slaughtering of birds. The pigeons were weighed and slaughtered at 38 weeks of age. The thymus, spleen, and cecal tonsil were promptly extracted, with the utmost care given to the removal of adhering connective tissues, and subsequently weighed individually. The relative weights of these organs were determined by computing the ratio of their respective weights to that of the live body. The data about body weight (BW), feed consumption, feed conversion, as well as the weights of lymphoid organs were aggregated for statistical analysis.

3.19 Calculations:

3.19.1 Feed Intake:

The calculation for feed intake per pigeon involved dividing the total feed consumption in a replication by the number of birds present in that replication.

Feed intake (g/bird) = Total Feed intake in a replication / No. of birds in a replication

3.19.2 Body Weight Gain:

The average body weight gain of each replication was calculated by deducting the initial body weight of the birds from their final body weight.

Body weight gain = Final body weight – Initial body weight

3.19.3 Egg Shape Index:

The egg shape index is a crucial parameter for assessing egg quality, defined as the ratio of the egg's width to its length. It can be calculated using the following equation:

Shape index (%) = Egg width (mm) / Egg length (mm) × 100

3.19.4 Egg Hatchability:

Hatchability refers to the percentage of eggs that successfully reach the end of the incubation period and result in the hatching of chicks (Khalid *et al.*, 2015).

Hatchability (%) = (No. of squabs hatched / No. of fertile eggs incubated) × 100

3.20 Analysis of Data:

The study objectives were followed in compiling, tabulating, and analyzing the total dataset. For preliminary data calculation, the utilization of the Excel Program was employed. The data obtained for different parameters were subjected to one-way ANOVA using the principles of the completely randomized design (CRD) procedure provided by the SPSS software (Version 26.0). To analyze the treatment means, Duncan's Multiple Range Test (DMRT) was employed in an analysis of variance (ANOVA). Statistical differences were considered significant at a threshold of $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production Performances of Gola Pigeon

4.1.1 Final Body Weight

Data presented in Table 5 showed that the impact of vitamins and Se on final body weight (gram per pigeon) was not significant (P>0.05). The relative final live weight (gm) of pigeons in the supplementary groups T0, T1, T2, and T3 were 347.25, 334.75, 340, and 327.50 respectively. The highest result was found in T0 (Control) (347.25) and the lowest result was in the T3 (Vitamin A, D, E, B-complex and Se) (327.50) group.

Although their final body weight was increased from the initial live weight, the control group's final live weight was higher compared to the other treatment groups. It may be due to the treatment groups' pigeons did not intake vitamin-mineral supplementation properly and there were different feeding choices among the pigeons. So, the impact of the treatments was not observed on the final live weight.

These results are in agreement with those obtained by Mahrose *et al.*, (2011); Yalçinkaya *et al.*, (2010); Shaik *et al.*, (2005), and Wang *et al*. in 2019, the provision of vitamins and Se as a dietary supplement did not manifest a statistically significant influence on the ultimate body mass.

In addition, these results are contradictory with those of previous researchers (Salahuddin *et al.*, 2012; Naik *et al.*, 2015; Kanchana and Jeyanthi, 2010) showed that the effects of additional supplementation significantly (P<0.05) improved live body weight of birds compared with the control groups.

4.1.2 Average Body Weight Gain

Data presented in Table 5 showed that the effect of treatments on total body weight gain (gm/pigeon) at the end of 38 weeks was non-significant (P>0.05). The relative total body weight gain (gm) of pigeons in the dietary groups T0, T1, T2, and T3 were 58.50, 50.25, 50.75, and 72 respectively. The highest result was found in T3 (Vitamin A, D, E, B-complex and Se) (72) and the lowest result was in the T1 (Vitamin A, D, E, and Se) (50.25) group. However, the live weight gain of pigeons supplemented with vitamin A, D, E, B-complex and Se increased but that was insignificant (P>0.05) compared to that

of the control and other treatment groups. So, it can be said that there was no significant impact of vitamins and Se on the body weight gain in Gola pigeon.

These results are in agreement with the previous findings of (Yalçinkaya *et al.*, 2010; Day and Dilworth, 1966; Adebiyi, 2011). The results showed that there were no significant changes in weight gain of the birds fed different dietary treatments.

Moreover, the outcomes presented are in opposition to the findings of prior scholars. Na *et al.*, (2007), and Lin and Chang, (2006) were conducted to examine the effects of vitamins and Se supplementation on body weight gain in chickens. The results found improved body weight gain significantly ($P<0.05$).

4.1.3 Average Feed Consumption (FC)

Data presented in Figure 1 showed that the effect of treatments on total feed consumption (gram per pigeon) at the end of 38 weeks was statistically non-significant ($P>0.05$). However, the feed consumption was higher in the T2 (Vitamin B-complex) group (8183) compared to the T0 (6898.25), T1 (7321.25), and T3 (6930) groups respectively. Although the highest feed consumption was recorded in the T2 group, the body weight gain was not up to the mark. So, there was no impact of vitamin B-complex in body weight gain with feed intake.

These findings are in concurrence with the outcomes achieved through prior investigations. Day and Dilworth, (1966) conducted two tests to determine the need for certain supplemental B vitamins in laying rations for commercial White Leghorn layers. The tested results showed that feed consumption was not significantly affected by B-vitamin fortification.

On the contrary, Halle *et al.*, (2011) and Halle and Ebrahem, (2012) reported that supplementation of vitamin B-complex significantly ($P<0.05$) improved feed consumption (FC) of chickens and ducks.

Table 5. Impact of Supplementing Vitamins and Selenium on the Production Performances of Gola Pigeon

Characteristics	Treatment				Mean ± SE	Level of significance
	T0	T1	T2	T3		
Final Body Weight (gm)	347.25	334.75	340	327.50	337.38 ± 11.84	NS
Body Weight Gain (gm)	58.50	50.25	50.75	72	57.88 ± 7.40	NS

Here, T0 = (Control), T1 = (Vitamin A, D, E, and Se), T2 = (Vitamin B-complex), T3 = (Vitamin A, D, E, B complex, and Se). Values are Mean ± SE (n=16) One-way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ SE= Standard Error
- ✓ NS = Non-significant

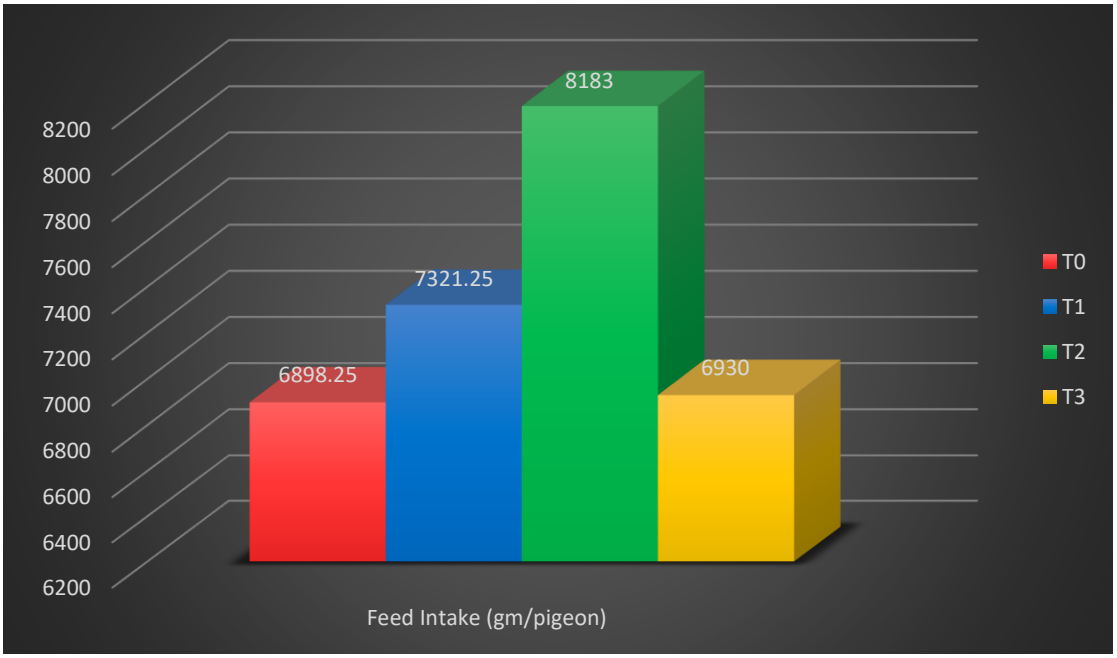

Figure 1. Impact of Supplementing Vitamins & Se on the Total Feed Consumption (gm/pigeon) from 12 to 38 weeks of Age

4.2 Egg Production Performances of Gola Pigeon

4.2.1 Egg Number

Egg production by number was significantly influenced ($P<0.05$) due to different supplementation treatments for Gola pigeon (Figure 2). Results indicated that the treatment T1 (Vitamin A, D, E and Se) (23.75) showed the overall best performance on egg production in number compared to other vitamin-mineral supplementation treatments whereas the treatment T2 (Vitamin B-complex) (18.00) showed the least performance. So, it can be said there was no impact of vitamin B-complex on egg production in Gola pigeon.

A similar result was also observed by Giri *et al.*, (2012) exhibited noteworthy enhancements in egg production.

4.2.2 Egg Weight (gm)

Different treatment groups of vitamins and Se supplementation in the Gola pigeons showed non-significant variation ($P>0.05$) (Table 6). The relative egg weight (gm) of pigeons in the supplementary groups T0, T1, T2, and T3 were 16.26, 17.68, 17.30, and 16.75 respectively. The numerically highest value was found in the T1 (Vitamin A, D, E and Se) group (17.68) and the lowest was in the T0 (Control) (16.26) group. However, the egg weight of pigeons supplemented with the combination of vitamins A, D, E and Se increased but that was insignificant ($P>0.05$) compared to that of the control and other treatment groups. So, there was no substantial impact of vitamins and Se on egg weight in Gola pigeon.

These results are in agreement with the previous findings of Mahrose *et al.*, (2011); who showed that egg quality traits were not significantly affected by extra dietary supplementations of vitamins A, E and Se. However, these results are contradictory to the findings of Giri *et al.*, (2012).

4.2.3 Egg Shape Index (%)

The egg shape index differed ($P<0.05$) significantly due to different feed supplementation treatments (Table 6). Results showed that the treatment T3 (Vitamin A, D, E, B-complex and Se) showed the highest shape index (76.29) followed by T1 (Vitamin A, D, E and Se) (74.67) and T2 (Vitamin B-complex) (73.11) treatments. On

the other hand, the lowest shape index (71.40) was recorded from T0 (Control). So, there was a significant impact of vitamins and Se on egg shape index in Gola pigeon.

Giri *et al.*, (2012) also found similar results and observed that vitamin E and selenium-supplemented feed had a significant effect on egg quality parameters in poultry.

4.2.4 Egg Hatchability (%)

Different treatment groups supplementing with vitamins and Se in the Gola pigeon showed significant variation ($P<0.05$) (Table 6). The highest result was found in both the T2 and T3 (87.50) groups and the lowest result was in the T1 (55) group. It can be said that there was a significant impact of vitamins and Se on hatchability in Gola pigeon.

These results are in harmony with the previous researcher Giri *et al.,* (2012).

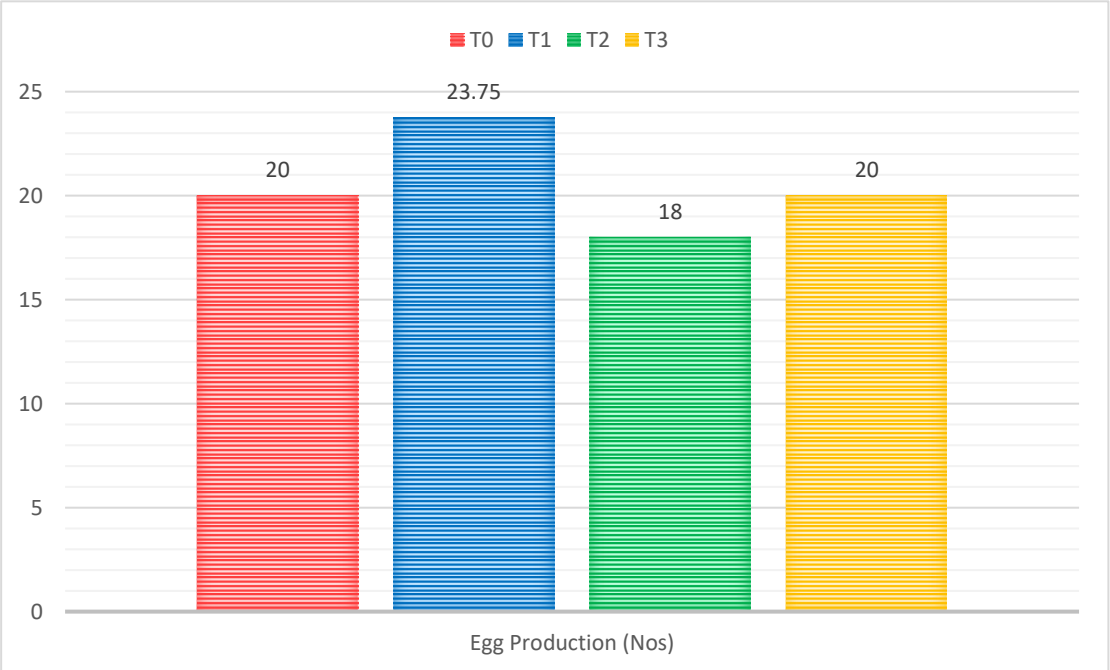

Figure 2. Impact of Vitamins & Se on the Egg Production (Nos) of Gola Pigeon

Table 6. Egg Production Performances of Gola Pigeon with the Supplementation of Vitamins and Selenium

Characteristic	Treatment					Level of Significance
	T0	T1	T2	T3	Mean ± SE	
Egg Weight (gm/egg)	16.26	17.68	17.30	16.75	17 ± 0.26	NS
Egg Shape Index (%)	71.40^{c}	74.67^{ab}	73.11^{bc}	76.29^{a}	73.87 ± 0.55	*
Hatchability (%)	66.67^{ab}	55^{b}	87.50^{a}	87.50^{a}	74.17 ± 4.82	*

Here, T0 = (Control), T1 = (Vitamin A, D, E, and Se), T2 = (Vitamin B-complex), and T3 = (Vitamin A, D, E, B-complex, and Se). Values are Mean ± SE (n=16) One-way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within the same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ NS = Non-significant
- ✓ *Means significant at a 5% level of significance (P<0.05)

4.3 Immune Organs Characteristics of Gola Pigeon

4.3.1 Thymus Weight

The effects of treatments on the thymus weight (gm) of Gola pigeons were demonstrated by the data in Table 7. A significant difference (P<0.05) was found among the groups. The highest thymus weight was seen in the T0 (Control) group (0.13 ± 0.01) following the T1 (0.07), T3 (0.06) and T2 (0.04) groups.

4.3.2 Thymus Length

The effects of treatments on the thymus length (mm) of Gola pigeons were demonstrated by the data in Figure 3. Significant difference (P<0.05) was found among the different treatment groups. The highest thymus length was seen in T1 (Vitamin A, D, E and Se) (12.75) following T0 (9.00), T3 (7.75) and T2 (7.25) groups.

4.3.3 Thymus Width

The effects of treatments on the thymus width (mm) of native Gola pigeons were demonstrated by the data in Figure 4. A significant difference ($P<0.05$) was found among the different treatment groups. The highest thymus width was seen in T1 (Vitamin A, D, E and Se) (4.25) following T0 (4.00), T3 (3.75) and T2 (3.13) groups.

4.3.4 Spleen Weight

The effects of treatments on the spleen weight (gm) of Gola pigeons were demonstrated by the data in Table 7. Non-significant difference ($P>0.05$) was found among the different treatment groups. The highest spleen weight was seen in the T1 (Vitamin A, D, E and Se) group (0.29) following T2 (0.23), T0 (0.19) and T3 (0.18) groups.

4.3.5 Spleen Length

The effects of treatments on the spleen length (mm) of Gola pigeons were demonstrated by the data in Figure 3. A significant difference ($P<0.05$) was found among the groups. The highest spleen length was seen in T0 (Control) (14.75) following T1 (11.25), T2 (11.25) and T3 (10.50) groups.

4.3.6 Spleen Width

The effects of treatments on the spleen width (mm) of Gola pigeons were demonstrated by the data in Figure 4. A significant difference ($P<0.05$) was found among the groups. The highest spleen width was seen in T0 (Control) (4.13) following T2 (3.88), T3 (3.63) and T1 (3.00) groups.

4.3.7 Cecal Weight

The effects of treatments on the cecal weight (gm) of Gola pigeons were demonstrated by the data in Table 7. A significant difference ($P<0.05$) was found among the groups. The highest cecal weight was seen in the T0 (Control) group (0.05) following T1 (0.03), T2 (0.03) and T3 (0.03) groups.

4.3.8 Cecal Length

The effects of treatments on the cecal length (mm) of Gola pigeons were demonstrated by the data in Figure 3. A significant difference ($P<0.05$) was found among the groups.

The highest cecal length was seen in T0 (Control) (6.50) following T3 (4.88), T2 (4.38) and T1 (3.50) groups.

4.3.9 Cecal Width

The effects of treatments on the cecal width (mm) of Gola pigeons were demonstrated by the data in Figure 4. A significant difference ($P<0.05$) was found among the groups. The highest cecal width was seen in T0 (Control) (2.75) following T3 (2.00), T1 (1.63) and T2 (1.63) groups.

The Bursa of Fabricius, spleen, and thymus are the main lymphoid organs in birds and their weights are directly related to immune systems and protection. The thymus is a primary lymphoid organ and it becomes atrophied with age. The spleen is considered the secondary lymphoid organ and the cecal tonsil consists of mucosa-associated lymphoid tissue. The avian immune system is influenced by both intrinsic and extrinsic factors. Intrinsic factors include the age and sex of the individual, while extrinsic factors include environmental conditions, social interactions, type of diet, and exposure to toxicants (Koutsos and Klasing, 2014). The gastrointestinal tract, thymus, spleen, regional lymph nodes, and circulating immune cells, as detailed by Chandra (1997) and Cunningham-Rundles (2001), are all influenced by nutrients. Immunocompetence is reduced by the lack of protein, certain amino acids, vitamins A, E, B_6, folate, and trace minerals, such as copper, zinc, selenium, chlorine, sodium, and iron, in mammals and birds. Excessive amounts of nutrients, however, may also have a negative impact on the immune system (Flynn, 1985).

In this study, the results showed the control group was better in almost all the performance parameters measured except for thymus length and width. The values obtained may indicate that the vitamin-mineral supplementation did not contribute to immune organ characteristics. This observation supports the previous findings of (Shaik *et al.*, 2005; Bhatti *et al.*, 2016; and Rostami *et al.*, 2018) that vitamins A, D, E, B complex and Se supplementation did not have a beneficial effect on the immune organs.

But the presented outcomes do not support Zhang and Wangy, (2010) and Bao, (2009).

Table 7. Impact of Supplementing Vitamins and Selenium on Gross Characteristics of Immune Organs of Native Gola Pigeon

Characteristics	Treatment					Level of significance
	T0	T1	T2	T3	Mean ± SE	
Thymus Weight (gm)	0.13^{a}	0.07^{b}	0.04^{b}	0.06^{b}	0.07 ± 0.01	*
Spleen Weight (gm)	0.19	0.29	0.23	0.18	0.22 ± 0.03	NS
Cecal Weight (gm)	0.05^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.03 ± 0.00	*

Here, T0 = (Control), T1 = (Vitamin A, D, E, and Se), T2 = (Vitamin B-complex), T3 = (Vitamin A, D, E, B-complex, and Se). Values are Mean ± SE (n=16) One-way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different ($P<0.05$)
- ✓ Mean within the same superscripts don't differ ($P>0.05$) significantly
- ✓ SE= Standard Error
- ✓ NS = Non-significant
- ✓ *Means significant at a 5% level of significance ($P<0.05$)

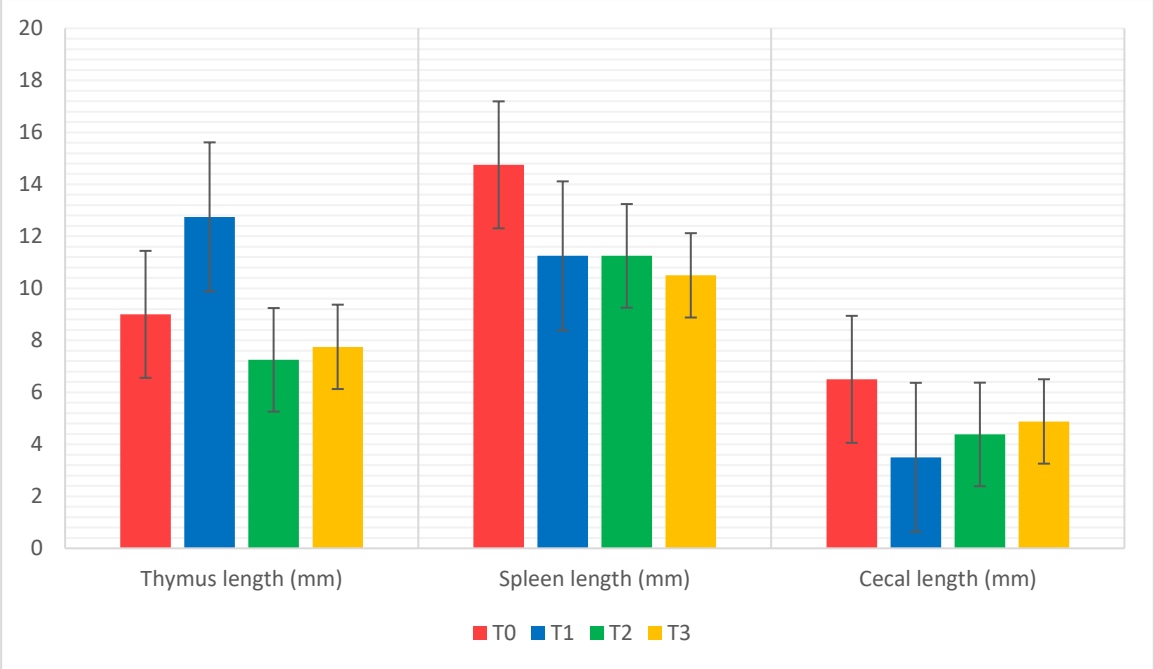

Figure 3. Impact of Supplementing Vitamins and Se on the Lymphoid Organs Length

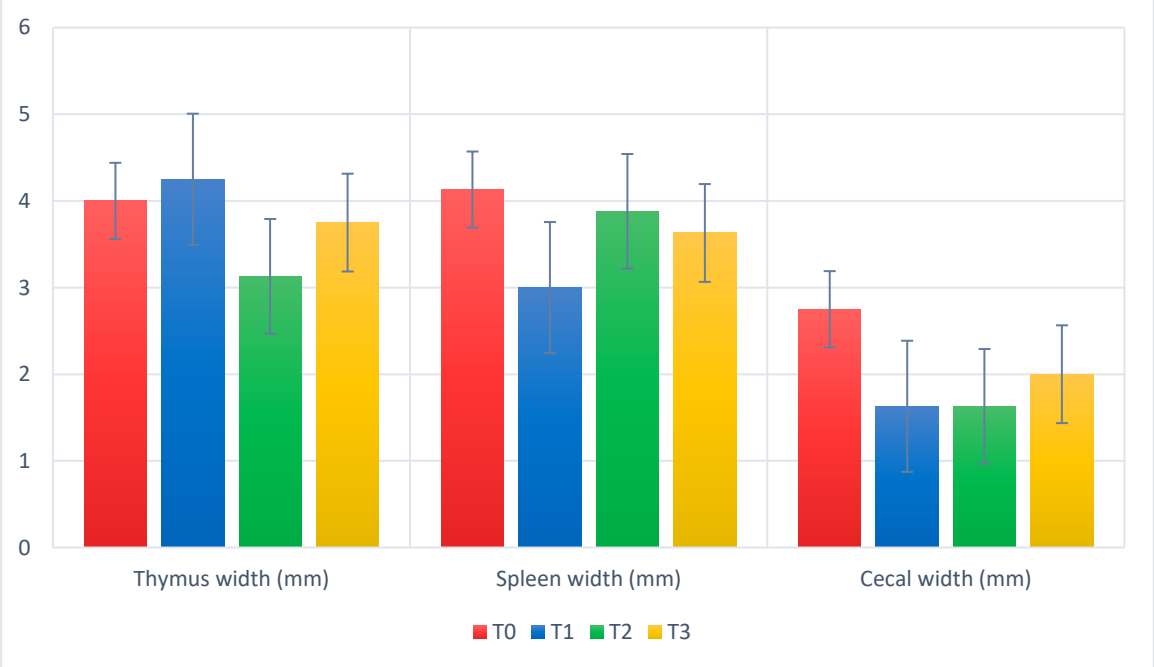

Figure 4. Impact of Supplementing Vitamins and Se on the Lymphoid Organs Width

4.4 Hemagglutination Inhibition (HI) Antibody Titers of Native Gola Pigeon Vaccinated Against Newcastle Disease

The study was carried out to observe the effects of supplementation of vitamin A, D, E, B-complex and Se in different combinations. The results revealed that both vitamins and Se have beneficial effects on the immune response when used separately or in combination (Table 8). The raised HI antibody titers were observed in the treatment groups more than in the control group. The data revealed that pigeons fortified with vitamin A, D, E and Selenium had significantly higher ($P<0.05$) immunity than all the other groups. The supplementation of a combination of vitamin A, D, E and Se in group T1 (272) showed better results in improving humoral immune response against NDV than individual supplementation of vitamin B-complex in group T2 (144) and a combination of vitamin A, D, E, B-complex and Se in group T3 (128). So, there was a noteworthy impact of vitamins and Se on immune response against NDV in Gola pigeon.

The immune system is significantly enhanced by the proper intake of nutrients, as demonstrated by Bendich (1996) and Field *et al.* (2002).

The observed values showed that birds on diets supplemented with vitamins and Se had consistently higher HI titers than the control birds. The high immunity to NDV in birds on vitamin-mineral supplemented diets agrees with the findings of Kanchana and Jeyanthi, (2010); Yamuna and Thangavel, (2011); Dalia *et al.*, (2018); and Sanda, (2015) who observed a positive correlation between antibody titer and vitamins and Se supplementation.

Table 8. Impact of Vitamins and Selenium Supplementation on the HI Antibody Titers against Newcastle Disease Vaccine of Gola Pigeon

Characteristics	Treatment					Level of Significance
	T0	T1	T2	T3	Mean ± SE	
Individual Titer	80^{b}	272^{a}	144^{b}	128^{b}	156 ± 93.39	*

Here, T0 = (Control), T1 = (Vitamin A, D, E, and Se), T2 = (Vitamin B-complex), T3 = (Vitamin A, D, E, B-complex, and Se). Values are Mean ± SE (n=16) One-way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different (P<0.05)
- ✓ Mean within the same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ NS = Non-significant
- ✓ *Means significant at a 5% level of significance (P<0.05)

CHAPTER 5

SUMMARY AND CONCLUSION

A supplementation trial was conducted on 3-month-old Gola pigeon for 38 weeks in the poultry farm of Sher-e-Bangla Agricultural University, Dhaka. The pigeons were assigned to 4 treatment groups namely T0 (Control), T1 (Basal diet + Vitamin A, D, E, and Se), T2 (Basal diet + Vitamin B-complex), and T3 (Basal diet + Vitamin A, D, E, B-complex, and Se) respectively having 4 replications. Treatment groups were vaccinated with Ranikhet disease vaccine and the control group was kept unvaccinated. At 38 weeks of age, randomly selected 16 pigeons from each group were sacrificed to evaluate the efficacy of different combinations of vitamin A, D, E, B-complex, and Selenium (Se) supplementation on production and immunity. The production performance indices *viz.* final body weight, body weight gain, feed consumption, egg production, egg weight, shape index and hatchability; immunity parameters *viz.* immune organs weight, length, and width and antibody titers against Newcastle disease vaccine of pigeons on different replication of different treatments were recorded and compared.

There was no significant difference ($P>0.05$) in the final body weight, body weight gain, and feed consumption of pigeons among different treatment groups. However, numerically ($P>0.05$) improved average body weight gain and feed intake were found in the vitamin A, D, E, B-complex and Se treated T3 group and vitamin B-complex treated T2 group respectively compared to other groups. This indicated that, although the highest feed consumption was observed in group T2, the body weight gain did not satisfactorily increase. In the case of egg parameters, no significant difference ($P>0.05$) was found in egg weight values. Significantly ($P<0.05$) higher levels of egg production, shape index, and hatchability were found in the T1, T3, and T2 + T3 groups respectively than in the control group.

The average weight, length, and width of the thymus, spleen, and cecal tonsil were measured. All the parameters showed significant differences ($P<0.05$) except spleen weight, where numerically improved average weight was found in the T1 (Vitamin A, D, E and Se) treated group. The largest values were found in the T1 treatment group for thymus length and width with significant ($P<0.05$) difference. Different treatment groups showed a significant ($P<0.05$) effect on the serum antibody titers against the

Newcastle disease vaccine. Significantly ($P<0.05$) higher individual titer was found in Vitamin A, D, E and Se supplemented T1 group than in T2 (Vitamin B-complex), T3 (Vitamin A, D, E, B-complex and Se), and T0 (control) group.

Based on the examination of the research results provided above, it can be concluded that different combinations of vitamins and Selenium supplementation had a very effective impact on the production performances and immune responses against the ND vaccine of Gola pigeon. There were no significant effects of vitamins and Se on growth performances. Vitamins and Se supplementation of pigeon diets did not improve thymus weight, spleen length and width, and cecal length, width, and weight, however, supplementation of vitamin A, D, E and Se combination gave a better humoral immune response against NDV, egg production, thymus length and width; supplementation of vitamin A, D, E, B-complex and Se in combination gave better performances in egg shape index, and hatchability and supplementation of vitamin B-complex in separate supplementation gave only a better response to egg hatchability. Pigeons fed combinations of vitamin A, D, E and Selenium supplemented diet achieved superior results.

However, the available abstracts do not provide any specific information regarding the impact of different combinations of vitamin A, D, E, B-complex and Se supplementation on production and immunity in pigeons. Therefore, it is necessary to conduct specific research on the effects of vitamin-mineral supplementation on pigeon immunity and production to determine its potential benefits or drawbacks.

CHAPTER 6

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CHAPTER 7

APPENDICES

Appendix 1. Initial Body Weight (gm/pigeon), Final Body Weight (gm/pigeon), Body Weight Gain (gm/pigeon), and Feed Intake (gm/pigeon) under Different Treatment Groups

Treatment	Replication	Initial body weight	Final body weight	Body weight gain	Feed intake
T0	R1 (M)	286	323	37	5910
	R1 (F)	288	339	51	5909
	R4 (M)	338	426	88	7887
	R4 (F)	243	301	58	7887
T1	R1 (M)	288	352	64	6476
	R1 (F)	310	400	90	6475
	R4 (M)	324	363	39	8167
	R4 (F)	216	224	8	8167
T2	R2 (M)	308	323	15	9035
	R2 (F)	263	355	92	9034
	R4 (M)	276	354	78	7332
	R4 (F)	310	328	18	7331
T3	R3 (M)	265	303	38	7325
	R3 (F)	270	340	70	7324
	R4 (M)	302	381	79	6536
	R4 (F)	185	286	101	6535

Here, M = male pigeon; F = female pigeon

Appendix 2. Egg Production (Nos), Egg Weight, Egg Shape Index, and Hatchability under Different Treatment Groups

Treatment	Replication	No. of Eggs	Egg Weight (gm/egg)	Shape Index (%)	Hatchability (%)
T0	R1 (M)	12	17.46	72.67	50
	R1 (F)				
	R4 (M)	8	15.06	70.13	83.33
	R4 (F)				
T1	R1 (M)	12	18.83	75.67	50
	R1 (F)				
	R4 (M)	12	16.52	73.67	60
	R4 (F)				
T2	R2 (M)	8	17.25	72.97	100
	R2 (F)				
	R4 (M)	10	17.35	73.25	75
	R4 (F)				
T3	R3 (M)	10	16.31	74.77	100
	R3 (F)				
	R4 (M)	10	17.18	77.80	75
	R4 (F)				

Here, M = male pigeon; F = female pigeon

Appendix 3. Measurement of the Immune Organs (Thymus, Spleen, and Cecal tonsil) under Different Treatment Groups

Treatment	Replication	Thymus			Spleen			Cecal tonsil		
		Weight (gm)	Length (mm)	Width (mm)	Weight (gm)	Length (mm)	Width (mm)	Weight (gm)	Length (mm)	Width (mm)
T0	R1 (M)	0.15	11	5	0.13	12	4.5	0.04	8	3
	R1 (F)	0.11	10	4	0.2	17	4	0.03	5	2
	R4 (M)	0.13	7	3	0.24	16	4	0.06	7	3
	R4 (F)	0.13	8	4	0.18	14	4	0.05	6	3
T1	R1 (M)	0.14	11	5	0.11	10	3	0.02	4	2
	R1 (F)	0.06	15	4	0.52	16	4	0.04	3	1.5
	R4 (M)	0.04	12	4	0.4	9	2	0.02	4	1.5
	R4 (F)	0.05	13	4	0.12	10	3	0.03	3	1.5
T2	R2 (M)	0.05	9	3	0.3	14	5	0.03	5	2
	R2 (F)	0.04	8	3	0.19	10	4	0.02	5	1.5
	R4 (M)	0.02	5	3	0.21	10	3.5	0.04	3.5	1.5
	R4 (F)	0.04	7	3.5	0.23	11	3	0.03	4	1.5
T3	R3 (M)	0.12	10	4	0.15	12	4	0.02	5	2
	R3 (F)	0.02	5.5	3	0.18	9	3	0.02	5	2
	R4 (M)	0.05	8	4	0.19	11	4	0.03	5	2
	R4 (F)	0.04	7.5	4	0.2	10	3.5	0.03	4.5	2

Here, M = male pigeon; F = female pigeon

Appendix 4. Hemagglutination Inhibition (HI) Antibody Titers against Newcastle Disease Vaccine under Different Treatment Groups

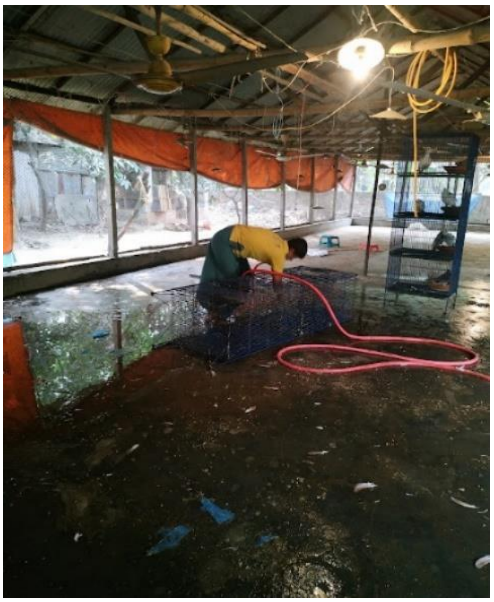
Treatment	Replication	Individual Titer
T0	R1 (M)	64
	R1 (F)	128
	R4 (M)	64
	R4 (F)	64
T1	R1 (M)	320
	R1 (F)	256
	R4 (M)	256
	R4 (F)	256
T2	R2 (M)	256
	R2 (F)	128
	R4 (M)	64
	R4 (F)	128
T3	R3 (M)	64
	R3 (F)	256
	R4 (M)	64
	R4 (F)	128

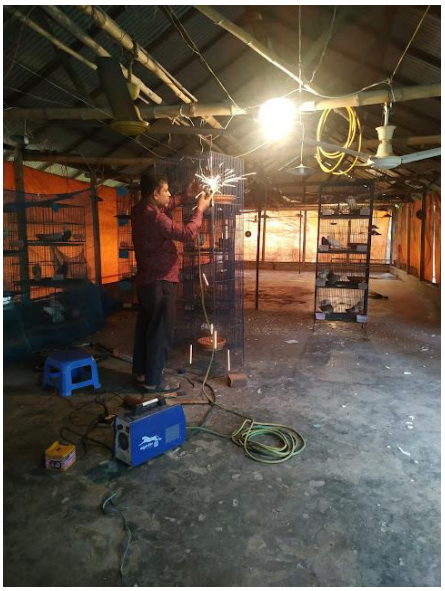
Here, M = male pigeon; F = female pigeon

PLATES

Plate 1. Experimental Site and House Preparation for Pigeons

In front of the SAU Poultry Farm





Preparation of the House

Plate 2. Labeling and Distribution of Pigeons

Labeling and Tagging

Distribution of pigeons in their cages

Plate 3. Feed Collection and Ration Formulation

Collection of the feed ingredients for ration formulation

Feed mixing

Ready to supply feed

Plate 4. Collection and Supplementation of Vitamins and Selenium

Collection of directed Vitamins and Selenium

Giving treatments through water

Feed and water intake by pigeons

Plate 5. Vaccine Collection and Vaccination to the Pigeons


Vaccine collection maintaining cooling chain

Preparation before vaccination

Primary vaccination at 12 weeks of age

Booster dose at 38 weeks of age

Plate 6. Weighing and Data Collection

Weighing of pigeon

Weighing of egg

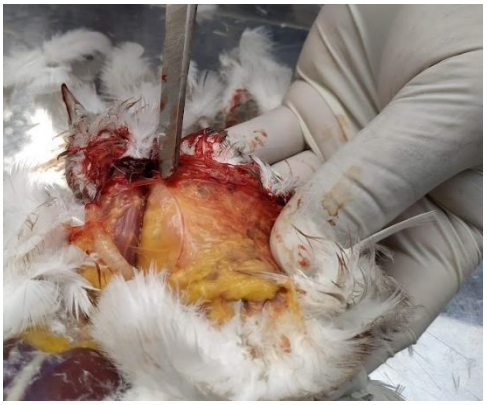
Weighing of feed

Measuring of feed wastage

Data collection

Plate 7. Slaughtering and Sample Collection

Slaughtering of pigeon



Sample collection with the help of our respected teacher

Sample: Spleen

Sample: Cecal tonsil

Sample: Thymus

Plate 8. Collection of Serum from Blood Samples for Serological Test

Blood sample

Centrifuging of blood

Serum collection

Supervision of research work by respected supervisor