EFFECT OF PAPAYA LEAVES (*Carica papaya*) SUSPENSION ON JAPANESE QUAIL (*Coturnix japonica*)

A THESIS

BY

SETARA AKTER SETU

REGISTRATION NO: 1605509

SEMESTER: JANUARY– JUNE 2018

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MASTER OF SCIENCE (MS)

IN

PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

JUNE 2018

DEDICATED

TO MY

BELOVED PARENTS

DEDICATED

TO MY

BELOVED PARENTS

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ABSTRACT

This work was conducted for a period of two months from October to November 2017 at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh to evaluate the effect of papaya (*Carica papaya*) leaves suspension (PLS) on Japanese quail. One hundred and twenty (120) female Japanese quails (*Coturnix japonica*) ten days old were randomly assigned into four groups with three replications (each containing ten birds) per treatment to investigate the comparative efficacy of papaya leaves suspension (PLS) and doxycycline on bacterial load and blood constituents on *E. coli* infected Japanese quail. For this purpose, birds were assigned into control group (T_0), *E. coli* treated group (T_1), *E. coli* plus PLS treated group (T_2) and *E. coli* plus doxacil-vet powder treated group (T_3). All groups were supplied *E. coli* orally except T_0 group and after 24 hours T_2 & T_3 groups were treated with PLS (0.25%) and doxacil-vet powder suspension (1g/2lit) respectively. EMB agar was used for CFU and microbial load significantly ($P<0.05$) decreased in T_2 & T_3 group where significantly ($P<0.05$) increased in T_0 and T_1 group. TEC, Hb, PCV & ESR in different treatment groups were almost similar and the differences were statistically non-significant ($P>0.05$) except TLC which was statistically significant ($P<0.05$) and the highest TLC was recorded in T_2 group. Furthermore, ten days old sixty (60) female Japanese quails were randomly assigned into two groups named T_0 (control diet) and T_1 (0.25% PLS) with three replications (each containing ten birds) per treatment to investigate the effect of papaya leaves suspension on live body weight, egg production and egg quality of Japanese quail. Egg weight, egg shape index, shell plus membrane weight, shell plus membrane thickness, haugh unit, albumen index and yolk index were performed for the determination of egg quality. The body weight of T_0 and T_1 at 65^{th} day were 164.95 and 182.32 g respectively which was statistically significant ($P<0.05$). The Hen-day-egg production (HDEP) observed for 15 days were 68.29% and 73.10% in T_0 and T_1 group respectively which statistically significant ($P<0.05$). In the treatment groups the external and internal quality of eggs (Egg weight, egg shape index, shell plus membrane weight, shell plus membrane thickness, haugh unit, albumen index and yolk index) were almost similar and the differences were statistically non-significant ($P>0.05$).

Key words: *Coturnix japonica*, *Carica papaya*, *E. coli*, hematology, body weight and egg production.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	**Full meanings**
%	Percentage
° C	Degree celcius
/	Per
@	At the rate of
<	Less than
>	Greater than
±	Plus minus
ANOVA	Analysis of variance
Av.	Average
Ca	Calcium
CFU	Colony forming unit
Cm	Centimeter
Cm^2	Square centimeter
Conc.	Concentration
Contd.	Continued
Cu	Copper
DCP	Di calcium phosphate
DM	Dry matter
E. coli	*Escherichia coli*
e.g.	For example
EMB	Eosine Methylene Blue
ESR	Erythrocyte Sedimentation Rate
et al.	And others
Fig.	Figure
g	Gram
Hb	Hemoglobin
HDEP	Hen day egg production
HSTU	Hajee Mohammad Danesh Science and Technology University
HU	Haugh unit
i.e.	That is
Kcal	Kilo-calorie

LIST OF ABBREVIATIONS AND SYMBOLS (Contd.)

kg	Kilogram
Ltd.	Limited
Max	Maximum
mg	Milligram
mm	Millimeter
ml	Mililitre
mm^3	Cubic millimeter
Mn	Manganese
Na	Sodium
No.	Number
NS	Non-significant
PBS	Phosphate buffer solution
PCV	Packed cell volume
PLS	Papaya leaves suspension
SE	Standard error
SEM	Standard error mean
Sq.	Square
T	Treatment
R	Replication
TEC	Total erythrocyte count
TLC	Total leukocyte count

CHAPTER I

INTRODUCTION

Poultry industry can produce very high quality proteins for human nutrition as well as a source of income for the community in many countries, therefore poultry production plays very important role in economic development of any country (Tarhyel *et al*., 2012). For a long time, the poultry industry in Bangladesh has been dominated largely by broiler and egg layer chicken farming. In the recent past, however, rearing of Japanese quail (*Coturnix japonica*) for meat and egg production has fast-gained prominence in the country. Bangladesh was introduced by it for the first time in 1990 and nowadays become very popular in rearing of quail for meat and egg production. Quails have a shorter generation interval (Odunsi *et al*., 2007), lower feed requirement and a lower production costs (Ojo *et al*., 2011) than chickens. Quails are very small sized avian species. Six to seven quails can be raised in the same place that is required for one chicken. An adult quail weights between 150 to 200 grams and an egg weights around 7 to 15 grams. Female quails start laying eggs within their 6 to 7 weeks of age and continuously lay one egg daily. They lay about 300 eggs in their first year of life. After that they produce about 150 to 175 eggs in second year. Egg production gradually decreases after their first year of laying period. Their food to meat or eggs converting efficiency is satisfactory. They can produce one kg meat or eggs by consuming three kg food. Quails do not incubate their eggs. An incubator or brooder chickens can be used for hatching their eggs. Quail egg is very suitable for human health. It contains 2.47% less fat than chicken egg. Quail eggs are very tasty and nutritious than other poultry eggs. Because quail eggs contain comparatively more protein, phosphorus, iron, vitamin A, B_1 and B_2. The egg has more beneficial effect. It cures cancer, high blood pressure, HIV AIDS, ageing, allergy, bronchitis, diabetes, digestive disorder, gallstone etc.

Japanese quails are prone to almost all diseases that are seen in chickens and other poultry breeds. It is observed that most of the diseases seen in quails occur during the first two weeks of brooding. Quail are susceptible to a variety of bacterial, fungal and parasitic diseases. Some of the common diseases seen in quails are colibacillosis**,** staphylococcal infection, proteus syndrome, quail disease, mycoplasmosis, infectious bronchitis, newcastle disease, hemorrhagic enteritis, aspergillosis, candidiasis, aflatoxicosis etc. Among these diseases avian colibacillosis and salmonellosis are considered to be the major bacterial diseases in the poultry industry world-wide. Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages. It is caused by a bacterium called *Escherichia coli* (*E. coli*) which is a gram negative, rod-shaped bacterium found normally in the intestine of the bird. It is a part of the common microbial flora of the intestine of poultry. *E. coli* infection is very common in poultry as well as in quail. *E. coli* infection includes colibacillosis, hjarre's disease, coligranuloma, peritonitis, salpingitis, synovitis, omphalitis, air sac disease etc. Colibacillosis occurs as an acute fatal septicemia or subacute pericarditis and air sacculitis. Difficulty to walk, loss of appetite and epilepsy are the symptoms of this disease. Systemic infection occurs when large numbers of avian pathogenic *Escherichia coli* (APEC) gain access to the bloodstream from the respiratory tract or intestine. Bacteremia progresses to septicemia and death or the infection extends to serosal surfaces, pericardium, joints and other organs. Initial exposure to APEC may occur in the hatchery from infected or contaminated eggs. Large numbers of *E. coli* are maintained in the poultry house environment through fecal contamination. This disease has an important economic impact on quail production. The majority of economic losses results from mortality and decrease in productivity of the affected birds. It is a common systemic disease of economic importance in poultry and is seen worldwide. Chloramphenicol, doxycycline hydrochloride etc. antibiotics can be administered for the treatment of this disease.

Food safety has become one of the most important public health issues in Bangladesh. Nowadays people become more health conscious. Common pathogenic bacteria that associated with food borne diseases include *E. coli* (Clarence *et al*., 2009). Recently quail eggs and meat are gradually gaining popularity in Bangladesh. *E. coli* infection is considered to be the major bacterial diseases in quail. It can be controlled with antibiotic therapy but a significant increase in drug-resistant strains of *E. coli* has complicated the problem in the poultry industry. Also use of antibiotics as feed additive have some negative consequences. For example, Sengul *et al*. (2008) reported that flavomycin consumption in quail's nutrition resulted in DNA damage and increased oxidative stress. Also antibiotics as feed additive can increase abdominal fat in poultry and increase risk of heart disease in consumers (Murwani and Bayuardhi, 2007). Treatment of colibacillosis relies on antimicrobial therapy but with isolates of *E. coli* becoming increasingly resistant to frequently used antibiotics, this treatment may fail. In other hand use of antibiotics in poultry can increase risk of antibiotic resistance in human society.

So, we need to quickly replace of antibiotics with other options. These new options should be inexpensive and available in everywhere as well as healthy for human society. It is thought that medicinal plants are a good alternative for this purpose. Since ancient times, medicinal plants play important role in health management of traditional poultry production (Eevuri and Putturu, 2013). In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.*, 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin *et al.*, 1985). Also, in contrast to antibiotics, most active components of medicinal plants are readily absorbed in intestine and have short half life. Therefore risk of tissue accumulation of these components is probably minimal (kohlert *et al*., 2000). For example, Dalkilic and Guler (2009) showed that clove (*Syzygium aromaticum*) extract reduced coliform counts in small intestine of broiler significantly.

Papaya (*Carica papaya*) belongs to the family of Caricaceae. Papaya is not a tree but herbaceous succulent plants that possess self-supporting stems (Dick, 2003). The papaya fruit as well as all other parts of the plant contain a milky juice in which an active principle known as papain is present. Papaya leaves have potential to supply dietary proteins required by the birds. This because they have a high crude protein content (~30%) with low (~5%) crude fiber levels (Onyimonyi *et al.*, 2009). The phytochemical components of the papaya leaves were amino acids, α- amylase, ß- amylase, carbohydrate, glutamine, protein, proline and phenolic compounds. The components qualitatively analyzed were alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steroids, triterpenoids and tannins (Suresh *et al*., 2008). The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence (Elizabeth, 1994). Elisa *et al*. (2011) was reported that the extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*. Antibacterial activity of papaya leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol, ethyl acetate and hexane. Papaya leaf extracts were tested against *Bacillus stearothermophilus*, *Listeria monocytogenes*, *Pseudomonas sp*. and *E. coli* by agar diffusion method. By realizing all sorts of problem we are planning to rear quail by using herbal medication like papaya leaves suspension instead of any synthetic agent to avoid human health hazards as well as economic quail production in Bangladesh.

This experiment was conducted to study the antibacterial activity of papaya leaves suspension against *E. coli* infection in quail in comparison with therapeutic dose of doxycycline hydrochloride. Growth promoting activity and effect on egg production of the herb, "*Carica papaya*", was also studied.

The general objective of this study was to know the effect of papaya leaves suspension in Japanese quail with the following specific objectives:

1. To investigate the antibacterial and hematological effects of papaya leaf on *E. coli* inoculated Japanese quails
2. To observe the effect of papaya leaf on body weight and egg production performances in Japanese quails.

CHAPTER II
REVIEW OF LITERATURE

This chapter presents the review of relevant literatures which consist of the effect of papaya leaves in Japanese quail production.

2.1 Origin and domestication of Japanese quail

The earliest records of domesticated Japanese quail populations are from 12th century in Japan; however, there is evidence that the species is actually domesticated as early as the 11th century (Hubrecht and Kirkwood, 2010). These birds were originally bred as songbirds and it is thought that they were regularly used in song contests (Hubrecht and Kirkwood, 2010; Mills, 1997).

Populations of the Japanese quail are known to mainly inhabit East Asia and Russia including India, Korea, Japan and China (Barilani *et al.*, 2005; Puigcerver *et al.*, 2007). Though several resident populations of this quail have been shown to winter in Japan, most migrate south to the areas such as Vietnam, Cambodia, Laos and southern China (Birdlife International, 2013). This quail has also been found to reside in many parts of Africa including Tanzania, Malawi, Kenya, Namibia, Madagascar and the area of the Nile River Valley extending from Kenya to Egypt (Pappas, 2013).

This quail and its European counterpart are migratory. Japanese quail will migrate to India (Finn, 1911), northern Japan and Korea for the summer (Hoffmann, 1988). Their migration covers 400-1000 km which is remarkable for a bird not known for its flying capability (Hoffmann, 1988). Overall, their migration route follows a north-south pattern (Johnsgard, 1988).

2.2 Physical description and behavior

The Japanese quail is similar in appearance to the European Common Quail, *Coturnix coturnix*. Overall, they are dark brown with buff mottling above and lighter brown underneath. They have a whitish stripe above the eye on the side of the head. Legs are orange-gray to pinkish-gray as is the beak (Hoffmann, 1988). In contrast to the males and usually females lack the rufous coloring on the breast and black flecking on the throat (Johnsgard, 1988). There are variations in plumage color. Some birds are whitish to buff with rufous to chestnut mottling above. Others have a very dark brown appearance with little or no mottling. In addition, there have been golden-brown varieties bred in captive condition (Hoffmann, 1988). Wing sizes in males and females are similar ranging from 92 to 101 mm. Both male and female have similar sized tails ranging from 35-49 mm in length (Johnsgard, 1988).

In their natural habitat, Japanese quail live amongst grasses and bushes, flying over short distances. The Japanese quail are seen in grassy fields, on river banks or in rice fields (Takatsukasa, 1941). They are primarily a ground-living species that tends to stay within areas of dense vegetation in order to take cover and evade predation (Buchwalder and Wechsler, 1997). It has also been reported to prefer open habitats such as steppes, meadows and mountain slopes near a water source.

These quail eat many kinds of grass seeds including pannicum and white millet. Their diet consists of a higher degree of protein. In the summer, they will especially seek and eat a variety of insects and small invertebrates (Johnsgard, 1988). In addition, they eat grit especially egg-laying females (Lambert, 1970).

This quail may breed in parts of Europe, Turkey and central Asia to parts of China (Alderton, 1992). From studies of captive-bred Japanese quail, seven distinct displays and calls have been recognized in males. Three of the calls were also observed to be uttered by females (Johnsgard, 1988). The call of this quail consists of deep hollow sounds with several times repeated in quick succession (Finn, 1911). The male's call is typically three notes. The female will utter a long call which alerts the male to her receptivity to copulate (Johnsgard, 1988). In addition, these quail engage in courtship-feeding. The male will hold a small worm in his beak and uttering a soft croaking call. The female approaches the male and takes the small worm to eat. The male then attempts to copulate with the female (Lambert, 1970).

2.3 Reproduction

As with other quail, eggs were laid at a rate of one per day (Lambert, 1970) with 7-14 eggs per clutch (Hoffmann, 1988). An egg averages 29.8 by 21.5 mm in size and weighs 7.6 g (Johnsgard, 1988). Incubation time is 19-20 days (Lambert, 1970), although clutch sizes have been associated with latitude and length of photoperiod. In Japan, clutch size is 5-8 eggs while in Russia clutch size is 5-9 eggs (Johnsgard, 1988). The chicks are considered to be mature and able to mate after four weeks old (Hoffmann, 1988). The breeding season varies with location. In Russia, the season starts in late April and continues to early August. In

Japan, nesting occurs from late in May and usually ends in August. On the rare occasion, eggs may be found in nests in September (Johnsgard, 1988).

The production of eggs with eggshell quality is an important concern of the egg industry. Losses due to low eggshell quality or other reasons may reach 20% before the eggs arrive at retail (Roland, 1988). In the uterus, the organic fraction of the eggshell is synthesized by the glands and calcium its largest component is mobilized from the blood. Eggshell is sensitive to calcium availability and carbonate is influenced by dietary factors that affect acid-base balance. This author also observes that eggshell mineral content is 90% out of which 98% consist of calcium carbonate. It is well known that, during eggshell formation, the transference of calcium from the plasma to the uterus in layers is very fast, of an average of one minute. Eggshell is formed mostly during the night when birds do not eat and this may increase calcium deficiency for egg formation (Etches, 1996). Therefore, calcium is mobilized from the bones. Calcium requirements are generally very low except at the time eggshell is deposited (Leeson *et al.*, 1991). Commercial layers lay more frequently during the morning after a period of fasting during the night when eggshell is formed (Faria *et al.*, 2000). Aiming at improving eggshell quality in commercial layers, feeding calcium-rich feeds in the afternoon, high particle size calcium dietary addition and short lighting period during the night (Joly *et al.*, 2003). However, in order to successfully apply these techniques in quails, feed intake behavior and lay times must be similar between these two bird species.

2.4 Description of papaya

Scientific name is *Carica papaya* belongs to the family of Caricaceae. It is widely cultivated and a tropical fruit tree. It grows rapidly, fruiting within three years and originally from southern Mexico (particularly Chiapas and Veracruz), Central America and northern South America (Morton, 1987), the papaya is now cultivated in most tropical countries.

Nutritive value of Papaya:

Nutritional value per 100 g

Energy	179 kJ (43 kcal)
Carbohydrates	10.82 g
Sugars	7.82 g
Dietary fiber	1.7 g
Fat	0.26 g

Protein	0.47 g
Vitamins	
Vitamin A equivalent	47 μg
Beta-Carotene	274 μg
Lutein zeaxanthin	89 μg
Thiamine (B1)	0.023 mg
Riboflavin (B2)	0.027 mg
Niacin (B3)	0.357 mg
Pantothenic acid (B5)	0.191 mg
Folate (B9)	38 μg
Vitamin C	62 mg
Vitamin E	0.3 mg
Vitamin K	2.6 μg
Minerals	
Calcium	20 mg
Iron	0.25 mg
Magnesium	21 mg
Manganese	0.04 mg
Phosphorus	10 mg
Potassium	182 mg
Sodium	8 mg
Zinc	0.08 mg
Other constituents	
Water	88 g
Lycopene	1828 μg

The papaya fruit as well as all other parts of the plant contain a milky juice in which an active principle known as papain is present. Pawpaw (*Carica papaya*) leaves have potential to supply dietary proteins required by the birds. This because they have a high crude protein (CP) content (~30%) with low (~5%) crude fiber (CF) levels (Onyimonyi *et al.*, 2009).

Papaya skin, pulp and seeds contain a variety of phytochemicals including carotenoids and polyphenols (Rivera *et al.*, 2010). As well as benzyl isothiocyanates and benzyl glucosinates with skin and pulp levels that increase during ripening (Rossetto *et al.*, 2008). Papaya seeds also contain the cyanogenic substance prunasin (Seigler *et al.*, 2002).

The phytochemical components of the *Carica papaya* leaves were amino acids, α-amylase, ß-amylase, carbohydrate, glutamine, protein, proline and phenolic compounds. The components qualitatively analysed were alkaloids, anthroquinone, catachol, flavonoids, phenols, saponins, steriods, triterpenoids and tannins (Suresh *et al*., 2008). They showed the antibacterial activity of the leaf extracts of *Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach* and *Psidium guajava* against pathogenic bacteria like gram positive (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria by invitro agar well diffusion method. The plants aqueous leaf extracts showed pronounced inhibition than chloroform leaf extracts. Leaf extracts showed more inhibitory action on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*.

Zulu *et al.* (2014) assessed nutrient digestibility and growth performance of Japanese quails (*Coturnix japonica*) fed diets with 0, 2, 4 and 6% pawpaw leaf meal (PLM) as a protein source. 108 three-weeks-old quails were individually weighed, divided into four groups based on body weights and groups randomly assigned 0, 2, 4 and 6% PLM-diets. They observed Feed intake, weight gain, body weights, feed conversion ratios (FCR) and digestibility of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE). Birds fed 6% PLM-diet were superior ($p<0.05$) in all these parameters, followed by those in 4% PLM group. In this study, optimal results were achieved at 6% PLM replacement level, no significant differences were observed between control & 2% PLM-diet fed birds and partially replacing soybean meal with PLM improved nutrient digestibility.

2.5 Medicinal use of papaya

Studies at the University of Nigeria have revealed that extracts of ripe and unripe papaya fruits and of the seeds are active against gram positive bacteria. Strong doses are effective against gram negative bacteria. The substance has protein like properties. The fresh crushed seeds yield the aglycone of glucotropaeolin benzyl isothiocyanate (BITC) which is bacteriostatic, bactericidal and fungicidal. A single effective does is 4-5 g seeds (25-30 mg BITC). Papain has been employed to treat ulcers, dissolve membranes in diphtheria and reduce swelling, fever and adhesions after surgery. With considerable risk, it has been applied on meat impacted in the gullet. Chemopapain is sometimes injected in cases of slipped spinal discs or pinched nerves.

Elisa *et al.* (2011) was reported that the extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*. Antibacterial activity of *Carica papaya* leaf extracts on pathogenic bacteria was observed in this study. The objectives of this study were to determine extract ability against pathogenic bacteria to observe the influence of pH, NaCl, heat on extracts ability and to observe extract ability against *B. stearothermophilus* spores. The data showed that ethyl acetate extract could inhibit *B. stearothermophilus*, *L. monocytogenes*, *Pseudomonas sp.* and *E. coli.* From this study, it is found that Papaya leaves are potential natural antibacterial substance which might be used in certain kinds of food. Atta (1999) found that fresh, green papaya leaf is an antiseptic whilst the brown, dried papaya leaf is the best as a tonic and blood purifier.

James *et al.* (1993) showed that the meat, seed and pulp of papaya are bacteriostatic against several enteropathogens such as *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa and Klebsiella pneumoniae* by the agar-cup method. This study correlates the bacteriostatic activity of papaya with its scavenging action on superoxide and hydroxyl radicals which could be part of the cellular metabolism of such enteropathogens.

Anibijuwon *et al.* (2009) were investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The bioactive compound of leaf and root extracts of *Carica papaya* was extracted using water and organic solvents. The root extracts demonstrated higher activities against all the gram positive bacteria than the gram negative bacteria tested with the highest activity (14 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa* while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the gram positive bacteria than the gram negative bacteria tested with the highest activity (4.2 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa*. The minimum inhibitory concentration and minimum bactericidal concentration of the root extracts ranged between 50-200 mg/ml.

Ahaotu *et al.* (2018) observed carcass and organ weight indices of feeding different levels of pawpaw leaf meal on finisher broiler birds. The study was conducted with four weeks old sixty Anak broiler birds .The birds were assigned into four treatments with three replicates (containing five birds) per treatment. Broiler finisher rations were formulated in which pawpaw leaf meal was incorporated at levels of 0%, 5%, 10% and 15% in the control T_1, T_2, T_3 and T_4 diets respectively. Results showed that the effect of treatments on gizzard weight (gram per bird per day), liver weight, heart weight and live weight were significant ($p<0.05$). There were improved daily weight gain and daily feed intake as the dietary levels of pawpaw leaf meal increased from T_1 to T_4 in all the parameters evaluated. The incorporation of pawpaw leaf meal into finisher broiler diets had nutritional benefits which led to general improved performance in body weight changes, feed conversion ratio, feed cost/kg gain, carcass and organ weights. It was concluded that 15% pawpaw leaf meal can be included in the diets of broiler finisher birds without any adverse effect on performance.

2.6 Effect on blood parameters

Salihu *et al.* (2012) were studied in 40 Isa Brown commercial layers infected naturally with nematodes to observe the anthelmintic efficacy of the aqueous and crude extract of *Carica papaya* seeds. The results of this study showed that the powdered and aqueous extract of *Carica papaya* after its administration, produced a significant increase ($P<0.001$) in packed cell volume, red blood cells, haemoglobin concentration, lymphocyte counts and significant decrease in eosinophil counts. In this study, before treatment, the PCV mean values for animals in Groups A (untreated), B (piperazine 322 mg/kg body weight/day), C (powdery *Carica papaya*, 300 mg/day/bird) and D (aqueous extracts of *Carica papaya*, 1:10 ml water required/day) were 20.00 ± 0.50, 24.00 ± 0.6, 23.00 ± 0.4 and 23.00 ± 0.2. After treatment, this parameter showed a significant increase. Pre-treatment values of hemoglobin in groups A, B, C and D were 6.30 ± 0.4, 7.5 ± 0.5, 7.4 ± 0.3 and 7.4 ± 0.5 respectively which significantly increased after treatment.

2.7 Infection and therapeutic

Escherichia coli is a gram negative, rod shaped, non acid fast, uniform staining, non sporulating bacillus and many strains are motile and have peritrichous flagella. Air sacculitis is a respiratory disease of poultry, frequently caused by *Escherichia coli* (*E.coli*) and characterized by thickening and inflaming of air sacs with fibrinous exudates, pericarditis and perihepatitis as sequel to colisepticemia (Stebbins *et al.*, 1992).

Several serotypes of *E. coli* have been associated with disease conditions in poultry, the most common manifestation being colisepticemia (Sojka, 1965). Although most serotypes are nonpathogenic, a limited number produce extraintestinal infections. Avian pathogenic *E. coli* (APEC) strains are commonly of the O_1, O_2 and O_{78} serogroups but many others have also been associated with cellulitis and colibacillosis. There is considerable diversity of serogroups among clinical isolates with a high percentage of APEC isolates being untypeable. Therefore, no single *E. coli* serogroup used as a bacterin can provide full protection against all of the serogroups that cause infections. Virulence factors include possession of large virulence plasmids and the abilities to resist phagocytosis and serum killing, acquire iron in low iron conditions and adhere to host structures. APEC is generally nontoxigenic and poorly invasive.

Colibacillosis causes typical pathology which can be seen during post mortems of affected birds. Isolation of a pure culture of *E. coli* from the heart, liver or other lesions confirms the diagnosis.

Atheer and Nawal (2009) showed that the broiler chicks developed signs of airsacculitis second day which were inoculated at 22 days of age in the caudal thoracic air sac with 0.5 ml normal saline containing 105 colony forming unit of pathogenic field isolate of *E. coli*. For this study, G_1 kept as negative control and G_2 as positive control. G_3 and G_4 were fed 1% black seed (*Nigila sativa*) and 1% garlic powder (*Allium sativum*) from day 1 to slaughter at 42 days of age respectively. Mean body weights at slaughter were 2540, 2504, 2718, 2607 and 2452 grams for the groups respectively. Group BS (Black Seed) was heavier (P= 0.05) than all other groups and group GP (Garlic Powder) was heavier than positive control and Enrofloxacin. There were insignificant differences in mortalities between the groups. Veterinary drugs, such as antimicrobial compounds are widely used in poultry and may lead to the presence of residues in matrices of animal origin such as muscle and liver tissue.

Battaa *et al.* (2015) conducted an experiment to assess the response of 132 $Dokki_4$ hens and 12 $Dokki_4$ cocks at the age of 32 weeks to diet containing papaya latex (PL) ground powder at levels 0.01% (PL_1), 0.03% (PL_2) and 0.05% (PL_3). From that experiment they found that the final body weight, body weight gain, egg production, egg weight and egg mass for the layers fed diet supplemented with PL were increased significantly than those fed control diet. A feed conversion ratio was significantly improved with PL whereas daily feed intake significantly ($P \leq 0.05$) decreased with PL inclusion. They also found the digestibility coefficient values were significantly improved for hens fed diet supplemented with PL compared to those fed control diet. Diets supplemented with PL had significantly increased shell thickness, egg shape index and haugh unit. However, no effect on shell weight, yolk percentages and albumen were found. Semen quality, fertility and hatchability were significantly ($P \leq 0.05$) improved by PL supplementation. Feeding PL at different levels led to significant ($P<0.05$) decrease of total lipids and cholesterol on plasma and yolk. Also add PL led to improved immune response. These results suggest that supplementation of papaya latex diets of laying hens improved egg production, feed conversion ratio, nutrients digestibility, immunity, semen quality, fertility, hatchability and economical efficiency especially with the addition of 0.05% papaya latex and also good economic efficiencies were observed.

CHAPTER III

INVESTIGATION OF ANTIBACTERIAL AND HEMATOLOGICAL EFFECTS OF PAPAYA LEAVES (*Carica papaya*) ON *E. coli* INOCULATED JAPANESE QUAIL (*Coturnix japonica*)

3.1 INTRODUCTION

Escherichia coli infection is an economically important disease in poultry industry which is prevalent throughout the world (Margie and Lawrence, 1999). *Escherichia coli* causes a variety of lesions in poultry including yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma and colibacillosis (Gross, 1994). In a commercial hatchery of Japanese quail, hatchability, dead-in-shell embryos and one-day-old Japanese quail chick mortality were 70, 2, and 6%, respectively. Japanese quail were also raised on a farm adjacent to the commercial hatchery. Sporadic mortality in one to five weeks old Japanese quail flocks of 30,000 birds for a period of 10 d ranged from 0.01% to 0.5%. An investigation was undertaken to find out the cause of increased mortality at the affected farm and the decrease in hatchability. Colibacillosis can be controlled with antibiotic therapy but a significant increase in drug resistance of *E. coli* has complicated the problem in the poultry industry. The risk of the presence of antibiotic residues and their harmful effects on human health also may consider as a great threat. Medicinal plants have been used for centuries and the oldest friend of mankind. Papaya plant (*Carica papaya*) is such a plant like many other medicinal plants and widely found in Bangladesh. Almost all parts of the plant can be utilized by human for food and also widely used for medicinal purposes. Papaya leaf extracts have phenolic compounds such as protocatechuic acid, p-coumaric acid, 5-7 dimethoxycoumarin, caffeic acid, kaempferol, quercetin and chlorogenic acid. These compounds have antimicrobial activity (Srivastava *et al.*, 2010). The present experiment describes the use of herbal medication like papaya leaves instead of any synthetic agent in the treatment of *E. coli* infection in Japanese quail. This experiment was conducted to study the antibacterial activity of papaya leaf suspension against *E. coli* infection in Japanese quail in comparison with therapeutic dose of doxycycline hydrochloride with following specific objectives:

1. To investigate the antibacterial and hematological effect of papaya leaves suspension on *E. coli* inoculated Japanese quail
2. To differentiate the antibacterial effect of papaya leaves from synthetic drug (Doxycycline hydrochloride)

3.2 MATERIALS AND METHODS

This study was conducted in collaboration with the Department of Physiology and Pharmacology and Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur to evaluate the antibacterial effect of papaya leaves suspension on *E. coli* infected Japanese quail. The following procedures were followed during study period.

3.2.1 Research location and duration

The experiment was conducted at the experimental shed under the Department of Physiology and Pharmacology and microbial test was performed at microbiology laboratory of the Department of Microbiology, HSTU, Dinajpur. The experiment was performed for a period of 25 days from 3rd October to 28th October, 2017 to investigate the effects of papaya leaf plus doxacil-vet powder suspension on *E. coli* infected Japanese quail.

3.2.2 Preparation of experimental house

At first the room as well as the wire cages were washed by sweeping with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant and allowed to dry leaving the room unused with the electric fan and the bulb switched on overnight. The room was properly ventilated. All the utensils required for the experiment such as feeder, water pot, beakers, pestle and mortar, syringe, needle etc. were collected and the experimental shed was properly designed.

3.2.3 Experimental birds

One hundred and twenty (120) female Japanese quail at the age of 10 days were purchased from Bahadur bazar, Dinajpur. All the birds were kept in the wire cages of the experimental shed. Proper ventilation and lighting was maintained inside the shed throughout the experimental period.

3.2.4 Collection of feed

Quail mash commercial feed were collected from Power Feed Ltd., Gazipur, Bangladesh, which was available at Griholokkhi Poultry Feed, Kalitola, Dinajpur, a reputed quail feed exporter. Feed and water were provided *ad-libitum* during the whole experimental period. The ingredients that were used for the composition of mash feed are described in (Table 3.1).

Table 3.1 Composition of the commercial quail feed

Ingredient	Percentage (%)	Chemical composition	Percentage (%)
Maize	51.2	Crude protein	22.29
Rice polish	6	Crude fibre	3.64
Soybean meal	22	Ether extract	5.11
Protein concentrate	8	Calcium	3.76
Meat and bone meal	3.5	Phosphorus	0.78
DCP	0.800	Lysine	1.38
Limestone	8	Methionine	0.36
Salt	0.500	Metabolizable Energy (KCal/kg)	2704.12
Vitamin-mineral premix	0.250		
Lysine	0.060		
Methionine	0.170		
Toxin binder	0.060		

Fig 3.1: Quail commercial feed

3.2.5 Acclimatization of quails

Immediately after reaching the destination the quails were shifted to wire cages. They were fed with feed and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for first three days to overcome the transportation stress. Quails were allowed to acclimatize in their new environment for 6 days before the commencement of the experiment.

3.2.6 Lighting

During the whole experimental period, all quails were exposed to a 16 hours continuous photoperiod (natural light plus artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

3.2.7 Routine management

Quails were provided to similar care and management in all groups throughout the experimental period. Adequate hygiene and sanitation were maintained properly.

3.2.8 Experimental design

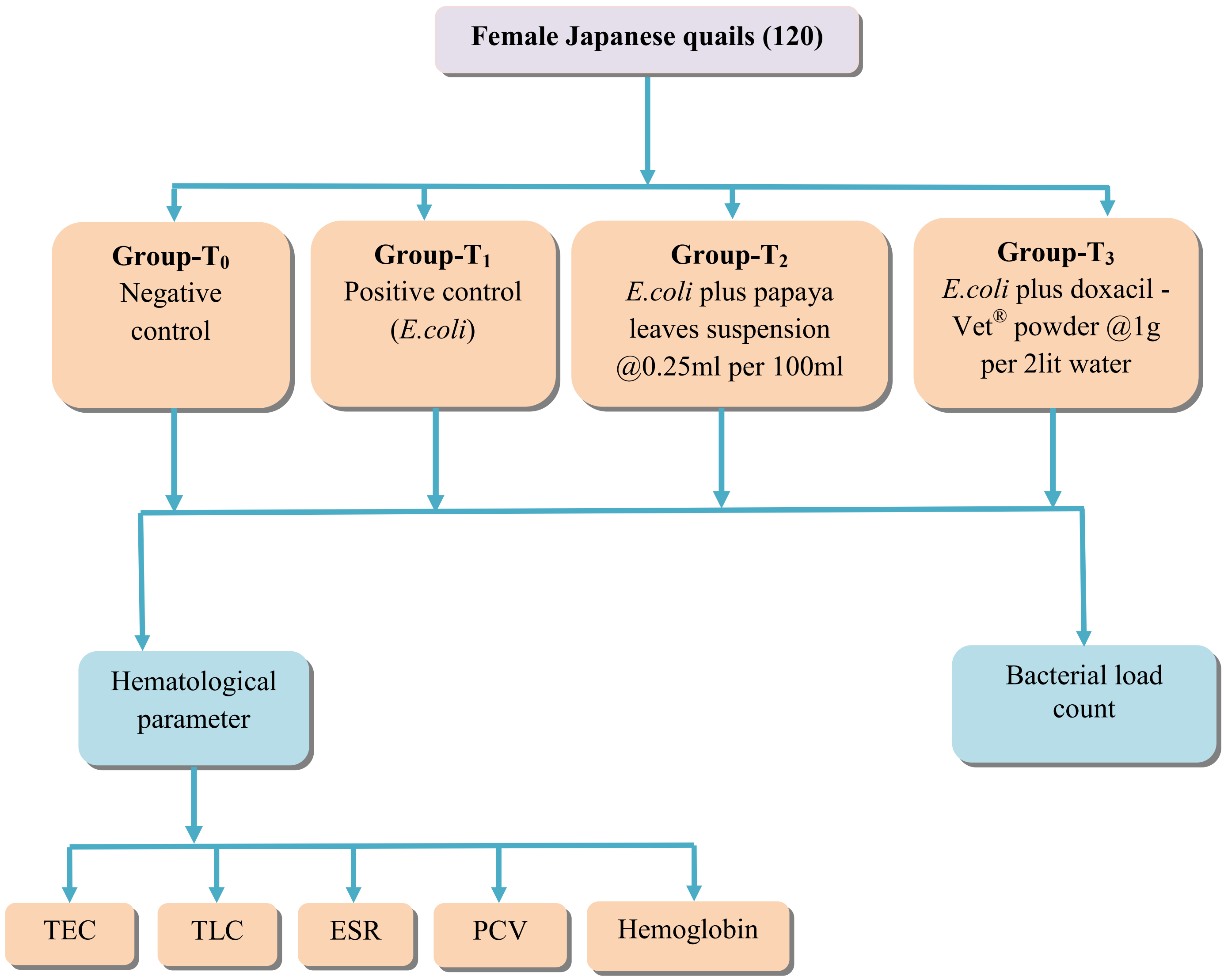

Fig 3.2: Layout of the experiment

3.2.9 Experimental birds grouping

One hundred and twenty Japanese quails were used to evaluate the effect of papaya leaves suspension on *E. coli* load and hematological parameters. The quails were assigned into four treatments with three replications (each containing 10 quails) per treatment. Ten quails kept in each replication were considered as an experimental unit. Quails were randomly distributed in every replication. The groups were designated and maintained as follows:

Group T_0: The quails were fed normal diet and given water *ad-libitum* and their blood parameters and bacterial load were measured at five days intervals. This group was served as "**Negative control"** group.

Group T_1: The quails were supplied with *E. coli* bacteria after acclimatization to induce *E. coli* infection in this group. No antibacterial treatment was done against *E. coli* in T_1 group. Adequate feed and drinking water was given. This group served as "**Positive control"** group.

Group T_2: Quails were supplied with *E. coli* bacteria after acclimatization to induce *E. coli* infection as like as T_1 group. This group left as about 24 hours for establishment of *E. coli* infection. After 24 hours, this group was treated with papaya leaf suspension as an herbal antibacterial substance at a dose rate of 0.25 ml per 100 ml of drinking water. This group served as **"Papaya leaf"** group to find out the effect of formulation as antibacterial drug and effect on blood profile.

Group T_3: After acclimatization to induce *E. coli* infection, quails of this group were supplied with *E. coli* bacteria as like as T_1 & T_2 group. This group left as about 24 hours for establishment of *E. coli* infection. After 24 hours, this group was treated with antibiotic (Doxacil-Vet powder @ 1 g per 2 liter water). This group served as "**Doxycycline"** group to compare the antibacterial effect with papaya leaves suspension.

Table: 3.2 Table showing the distribution of quails

Replication (R)	Treatment (T)			
	T_0	T_1	T_2	T_3
R_1	10	10	10	10
R_2	10	10	10	10
R_3	10	10	10	10

T_0 = Negative control group (Basal diet)

T_1 = Positive control group (Basal diet + *E. coli*)

T_2 = Papaya leaf group (Basal diet + *E. coli* + 0.25% Papaya leaves suspension)

T_3= Doxycycline group (Basal diet + *E. coli* + Doxacil-Vet powder @ 1 g per 2 litre water)

3.2.10 Collection and preparation of test organism

The test organism (*E. coli*) was collected from the laboratory under the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Nutrient broth was used to grow the organisms from the collected samples before feeding the quails orally.

3.2.11 Preparation of nutrient broth and administration of organism

Nutrient broth was prepared by dissolving 13.0 grams of bacto-nutrient broth (Difco) into 1000 ml of distilled water and heated up to boiling to dissolve it completely. The solution was then dispensed into tubes (10 ml per tube), closed with cotton plugs and sterilized in the autoclave machine at l21º C and 15 lb pressure for 15 minutes. After preparing the Nutrient broth, the organism (*E. coli*) was inoculated into nutrient broth media by metallic loop and was incubated for overnight in incubator at 37º C temperature.

After growing of *E. coli* in the nutrient broth, the culture media was shacked properly and the quails of T_1, T_2 & T_3 group with each replication were supplied orally with 2-3 drops of the inoculum.

Fig 3.3: Administration of *E. coli*

3.2.12 Collection and processing of plant materials

Papaya leaves were collected from the HSTU, Dinajpur. Young papaya leaves were collected and washed with fresh water. Before chopping it into small pieces, it was soaked with cotton for removing the adhesive water. Then the leaf was chopped into small pieces and was mashed with the help of pestle and mortar. Leaf juice was collected by squeezing mashed leaf. Then it had been produced 0.25% of suspension of grinded papaya leaf with distilled water.

Fig 3.4: Papaya leaf

Fig 3.5: Processing of papaya leaf

3.2.13 Collection, preservation and administration of Doxacil - Vet® powder

Doxacil-vet powder was collected from Square Pharmaceuticals Ltd. It was preserved in a dry place at room temperature and was administered at a dose rate of 1 gram per 2 liter of drinking water.

Composition: Each gram powder contains Doxycycline HCl BP equivalent to 100 mg doxycycline.

Fig 3.6: Doxacil-Vet® powder

3.2.14 Sample collection for bacteriological analysis

The fecal samples were collected to estimate the bacterial load. Samples were collected from each replication and immediately transferred from the collection site to microbiology laboratory, HSTU for analysis. The first fecal sample was collected after 24 hours of *E. coli* organism induction and just before the commencement of other treatments. Then sample was collected every 5 days interval up to the last of experimental period.

3.2.15 Estimation of bacterial loads

Eosine Methylene Blue (EMB) agar was used as a selective media for the culture of *E. coli* organisms.

3.2.16 Preparation of Eosine Methylene Blue (EMB) agar media

EMB agar about 37.46 g (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving at 121º C, 15 lbs pressure for 15 minutes; 20 ml of the medium was poured in to each sterile glass petridish. To accomplish the surface be quite dry, the medium was allowed to cool at 45º C to 50º C and allowed to solidify for about 2 hours with the covers of petridishes partially removed. The sterility of the medium was judged and used or stored at 4ºC in refrigerator for future use (Carter, 1979).

3.2.17 Preparation of serial tenfold dilution of sample

The numbers of total twelve feces samples were weighted 1 g individually on digital weighting balance and diluted into 100 ml phosphate buffer solution (PBS) as stock solution for serial ten fold dilution. A series of test tube, each containing 9 ml of diluents (PBS) were taken. Serial ten fold dilutions of each of the fecal samples in a series of dilution tubes were prepared. From the original sample, 1 ml was transferred in the 1st test tube and mixed thoroughly in order to make 10^{-1} dilution. Then 1ml solution transferred from 1st test tube to 2nd test tube, then from 2nd test tube to 3rd test tube and this way dilution was made up to 10th test tube. Finally 1ml discarded from 10th test tube and in every step mixing was done properly.

Fig 3.7: Preparation of serial tenfold dilution

3.2.18 Enumeration of bacterial colony

For the determination of total viable bacterial count, 10^{-9} dilution was considered for each sample. For each 10^{-9} dilution, three petridishes were taken containing EMB agar. 1 ml of each 10^{-9} dilution was transferred and spread on previously prepared EMB agar using a fresh pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a cotton bud. Individual cotton bud was used for individual numbering of plate. The plates were then kept in the incubator at 37º C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted by the digital colony counter machine. Only plates (or replicate plates from the same dilution) with 30-300 colonies are counted. Plates with fewer than 30 colonies give statistically unreliable results while plates with more than 300 colonies are too crowded to allow all the bacteria to form distinct colonies. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The results of the total bacterial count were expressed as the number of organism or colony forming units per ml (CFU/ml) of feces sample.

Fig 3.8: Green metallic sheen on EMB agar

Fig 3.9: Colony counting at colony counter

3.2.19 Colony Forming Unit (CFU)

Colony forming unit (CFU) is a measure of viable bacterial cells. For convenience the results were given as CFU/ml (colony forming units per milliliter) for liquids and CFU/g (colony-forming units per gram) for solids.

CFU/ml = (no. of colonies × dilution factor) / volume of culture plate

3.2.20 Collection and examination of blood

0.5 ml blood from each group was collected from wing vein with the help of syringe (1 ml) and needle. The 1st blood sample was collected after the commencement of treatments and then every 5 days of interval up to the end of the experiment. The collected blood was sent to the Taufique Agro Lab, Rangpur, Bangladesh for the estimation of different blood parameters such as TLC (Total Leucocytes Count), TEC (Total Erythrocyte count), Hemoglobin (Hb) percentage, PCV (Packed Cell Volume) and ESR (Erythrocyte Sedimentation Rate). The blood parameters were determined by semi automatic hematological analyzer machine (Cure inc. U.S.A.).

Fig 3.10: Collection of blood from wing vein

3.2.21 Statistical analysis

The collected data were recorded and analyzed by one way ANOVA following Duncan post hoc test using SPSS program version 20 software to find out the difference among the treatments.

3.3 RESULTS AND DISCUSSION

To perform the experiment, one hundred and twenty female Japanese quails were randomly divided into four groups with three replications each containing ten birds. They were fed with *E. coli* organism for induction of infection. Group T_0 birds were kept as control (non infected) without giving *E. coli* and any other treatment whereas T_1 treated with *E. coli* and next two groups (T_2 &T_3) were treated with 0.25% papaya leaves suspension and doxacil-vet powder suspension at a dose of 1 g/2 lit water respectively. In this experiment, the efficacy of papaya leaves suspension on bacterial load and blood constituents of Japanese quail infected with *E. coli* were investigated. The results of this study are discussed under following subheadings.

3.3.1 Antimicrobial effect of papaya leaves suspension and doxycycline

Microbial load in feces of *E. coli* infected quail was presented on Table 3.3. This study showed that, microbial load in feces was significantly differed among the treated groups. Microbial load significantly (P<0.05) increased in T_0 (negative control) and T_1 (positive control) group. Microbial load significantly (P<0.05) decreased in T_2 group supplied with 0.25% papaya leaves suspension and doxacil-vet powder suspension. Elisa *et al.* (2011) showed that papaya leaves have antibacterial activity. Papaya leaf extracts were tested against *Bacillus stearothermophilus, Listeria monocytogenes, Pseudomonas sp.* and *Escherichia coli* by agar diffusion method in that study.

Table: 3.3 Effect of papaya leaves suspension and doxycycline on bacterial load in feces of *E. coli* inoculated Japanese quail

Age (Days)	T_0 (Mean ± SEM)	T_1 (Mean ± SEM)	T_2 (Mean ± SEM)	T_3 (Mean ± SEM)	Level of significance
17	$10.95 \pm .01^{a}$	$11.26 \pm .01^{b}$	$11.27 \pm .01^{b}$	$11.27 \pm .01^{b}$	*
22	$10.98 \pm .01^{a}$	$11.27 \pm .01^{b}$	$11.28 \pm .01^{c}$	$11.24 \pm .01^{b}$	*
27	$11.01 \pm .01^{a}$	$11.33 \pm .01^{c}$	$11.25 \pm .01^{b}$	$11.05 \pm .01^{a}$	*
32	$11.13 \pm .01^{b}$	$11.39 \pm .01^{c}$	$11.17 \pm .05^{b}$	$10.80 \pm .01^{a}$	*

Values are expressed as mean ± standard error of means. a, b, c Means within and between column with different superscripts are statistically different (P <0.05). * = Significant at 5% level of significance

3.3.2 Hematological parameter

The effect of papaya leaves suspension and doxycycline on different blood parameters (TEC, TLC, PCV, Hb and ESR) of *E. coli* infected Japanese quail were presented on Table 3.4. The values of TEC, PCV, Hb and ESR in all treated groups and control group were more or less similar and within the normal range at 22^{nd}, 27^{th} and 32^{nd} day of age. These values showed a little fluctuation but were not statistically significant (P>0.05). The values of TLC of T_0 (Basal diet) and T_3 (*E. coli* plus doxacil vet powder) group are more or less similar followed by same superscripts in the same column. But in T_1 (Basal diet plus *E. coli*) and T_2 (*E. coli* plus papaya leaves suspension) group, the values are differed followed by different superscripts in the same column. The values are also differed between columns among the treated groups. The lowest TLC value (5851±58.65) was recorded from T_2 group at initial stage of experimental period and the highest TLC value was also recorded from T_2 group at last stage of experimental period. The TLC value increased in the group (T_2) fed with 0.25% papaya leaves suspension which agrees with the findings of Battaa *et al.* (2015). They showed that WBCs were significantly (P<0.05) increased with addition of papaya latex than those fed control diet experimented on hens and cocks of $Dokki_4$ strain. The increase in WBCs in treated groups might also be concluded leukocytes count. This can be attributed mainly to the antibacterial, antifungal and antioxidant functions of papaya latex. Miyamoto *et al.* (2004) showed that papaya enzyme increase immune system function. The white blood corpuscles a good indicator of increasing the immunity efficiency (Wieslaw *et al*., 2006)

Table 3.4: Effect of treatment with papaya leaves suspension and doxycycline on the different blood parameters (TEC, TLC, PCV, Hb and ESR) of quails inoculated with *E. coli*

Age (Days)	T_0 (Mean ± SEM)	T_1 (Mean ± SEM)	T_2 (Mean ± SEM)	T_3 (Mean ± SEM)	Level of significance
TEC(million/mm^3)					
22	2.00±0.15	2.06±0.28	2.10±0.10	1.57±0.28	**NS**
27	2.06±0.28	2.40±0.15	2.20±0.25	1.80±0.02	**NS**
32	2.58±0.03	2.27±0.03	2.32±0.02	2.28±0.28	**NS**
TLC					
22	6500±208.16^b	5799±284.05^a	5851±58.65^a	6354±86.23^b	*
27	6412.33±64.3^b	6155±57.55^{ab}	6533±72.64^b	6649±63.54^b	*
32	6216.66±116.67^b	6583.33±79.57^b	6675±44.81^b	6260±100.29^b	*
PCV (%)					
22	31.21±0.01	30.85±0.58	30.76±0.56	29.87±0.57	**NS**
27	32.14±0.57	31.29±0.57	31.87±0.58	31.66±0.58	**NS**
32	35.71±0.58	34.63±0.57	35.67±0.76	35.65±0.62	**NS**
Hb (mg/dl)					
22	7.69±0.03	7.72±0.02	8.10±0.26	7.59±0.26	**NS**
27	7.79±0.04	7.72±0.14	8.04±0.07	7.87±0.14	**NS**
32	8.09±0.17	7.79±0.32	8.30±0.25	7.88±0.13	**NS**
ESR (mm/1st hour)					
22	10.33±0.57	10.49±0.54	11.15±0.58	11.86±0.69	**NS**
27	10.46±0.03	10.52±0.56	10.97±0.07	11.55±0.32	**NS**
32	11.77±0.38	10.99±0.32	10.91±0.35	12.16±0.31	**NS**

Values are expressed as mean ± standard error of means. a, b, c Means within and between column with different superscripts are statistically different ($P < 0.05$). * = Significant at 5% level of significance. NS=Not significant

CHAPTER IV

EFFECT OF PAPAYA LEAVES (*Carica papaya*) ON BODY WEIGHT AND EGG PRODUCTION PERFORMANCES IN JAPANESE QUAIL (*Coturnix japonica*)

4.1 INTRODUCTION

Poultry production remains the fast going means to provide animal protein. Poultry meat and egg are still widely consumed with little or no religious and social constraints. In the recent past, Japanese quail (*Coturnix japonica*) rearing for meat and egg has fast-gained prominence in Bangladesh. Quails have a shorter generation interval, lower feed requirement and a lower production costs than chickens. Quail eggs are very nutritious than other poultry eggs because of containing comparatively more protein, phosphorus, iron, vitamin A, B_1 and B_2. The structure of humans and animal is built on protein. Man relies on animal and vegetable protein for the supply of amino acids. Egg is a good source of low cost high quality protein providing 6.3 grams of protein (13% of the daily value for protein) in one egg for a caloric cost of only 68 calories (Oluyemi and Robert, 2007). Papaya (*Carica papaya*) leaves have potential to supply dietary proteins required by the birds because of having a high crude protein content (~30%) with low (~5%) crude fiber levels (Onyimonyi *et al*., 2009). They also contain high levels of base metals (potassium, sodium, calcium and magnesium) and appreciable levels of iron (Maisarah *et al*. 2014). Papaya leaf meals in quail diets would not only serve as a protein source but also provide some vitamins, minerals and oxycarotenoids which may improve egg and carcass quality of the birds as observed in chickens by D'Mello *et al*. (1987) and Opara (1996). Papaya leaves contain papain (5.3%), a natural proteolytic enzyme that helps in the digestion of proteins (Singh *et al*., 2011) and enhances the digestibility of ingested feed in the tract, thereby accelerates bird's growth.

Therefore, the aim of this study was to determine the effect on body weight and egg production performance of papaya leaves suspension in Japanese quails with following specific objectives:

1. To determine the effect of papaya leaves suspension on growth performances of Japanese quails
2. To measure the number of egg
3. To investigate the internal and external quality of eggs

4.2 MATERIALS AND METHODS

This study was conducted in collaboration with the Department of Physiology and Pharmacology and Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur to investigate the effect of papaya leaves suspension on body weight and egg production of Japanese quail. The following procedures were followed for conducting this study.

4.2.1 Research location and duration

The experiment was conducted at the experimental shed under the Department of Physiology and Pharmacology and the egg quality determination was performed at Dairy and Poultry Science laboratory under the Department of Dairy and Poultry Science, HSTU, Dinajpur. The experiment was performed for a period of 55 days from 3rd October to 29th November, 2017 to observe the effects of papaya leaves suspension on body weight and egg production of Japanese quail.

4.2.2 Preparation of experimental house

At first the room as well as the wire cages were washed by sweeping with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant and allowed to dry leaving the room unused with the electric fan and the bulb switched on overnight. The room was properly ventilated. All the utensils required for the experiment such as feeder, water pot, beakers, pestle and mortar, syringe, needle etc. were collected and the experimental shed was properly designed.

4.2.3 Experimental birds

Sixty (60) female Japanese quail at the age of 10 days were purchased from Bahadur bazar, Dinajpur. All the birds were kept in the wire cages of the experimental shed. Proper ventilation and lighting was maintained inside the shed throughout the experimental period.

4.2.4 Collection of feed

Quail mash commercial feed were collected from Power Feed Ltd., Gazipur, Bangladesh. Feed and water were provided *ad-libitum* during the whole experimental period.

Fig 4.1: Quail commercial feed

4.2.5 Acclimatization of birds

Immediately after reaching the destination the quails were shifted to wire cages. They were fed with feed and drinking water *ad-libitum*. Glucose and vitamin C were supplied with drinking water for first three days to overcome the transportation stress. Quails were allowed to acclimatize in their new environment for 10 days before the commencement of the treatment.

4.2.6 Lighting

During the whole experimental period, all quails were exposed to a 16 hours continuous photoperiod (natural light plus artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

4.2.7 Routine management

Quails were provided to similar care and management in all groups throughout the experimental period. Adequate hygiene and sanitation were maintained properly.

4.2.8 Experimental design

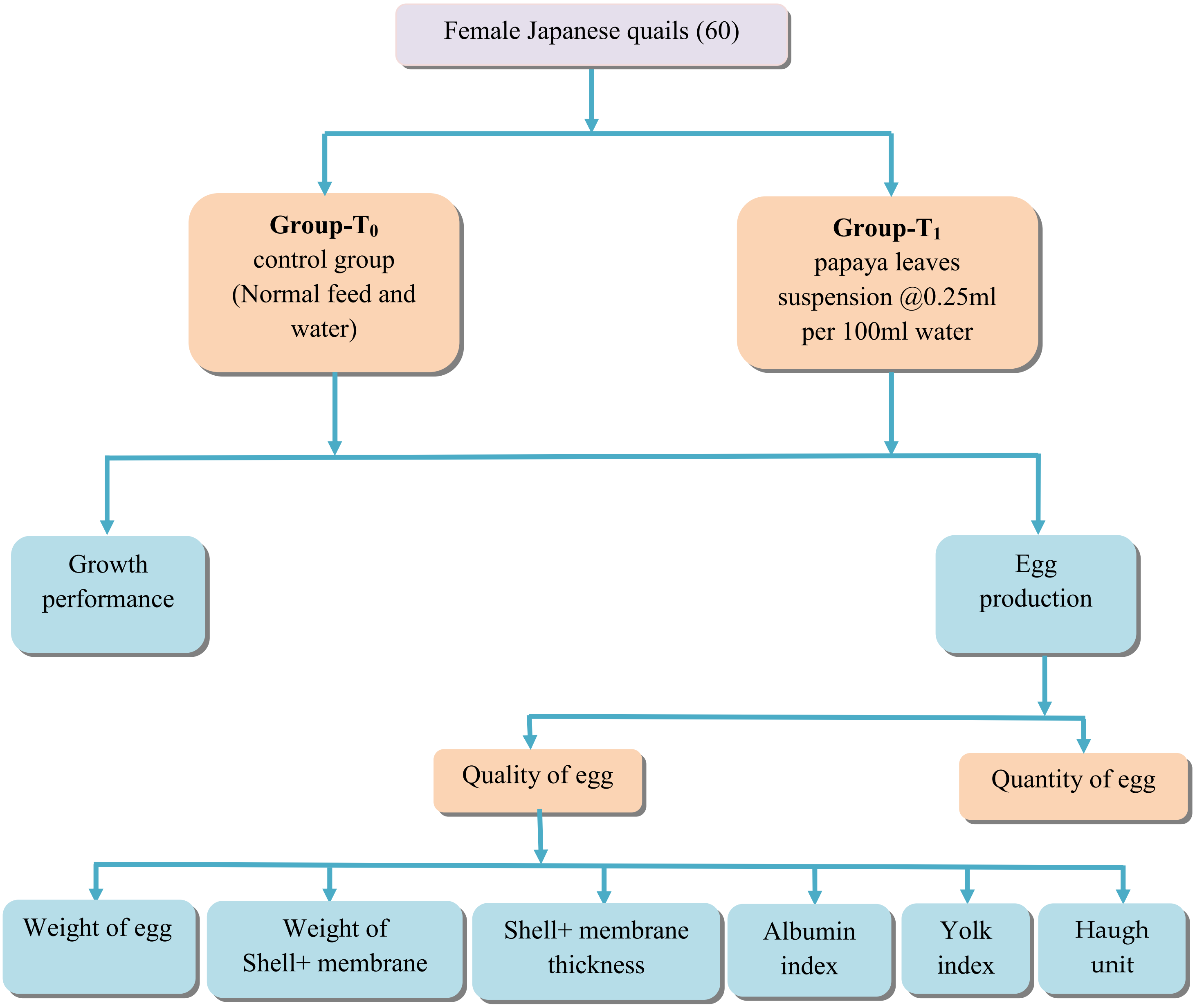

Fig 4.2: Layout of the experiment

4.2.9 Experimental birds grouping

Sixty female Japanese quails were used to evaluate the effect of papaya leaves suspension on growth performance and egg production. The quails were assigned into two groups with three replications containing 10 quails in each replication. Ten quails kept in each replication were considered as an experimental unit (Replication). Quails were randomly distributed in every replication. The groups were designated and maintained as follows:

Group T_0: The quails were fed normal diet and given water *ad-libitum* and their body weight was recorded at every 15 days interval. Total egg production and egg quality were also measured at first laying (50^{th} day of age) and at the end (65^{th} day of age) of the experiment. This group was served as "**Control**" group.

Group T_1: Quails were fed normal diet and water supplied with papaya leaves suspension as an herbal treatment at a dose rate of 0.25 ml per 100 ml of drinking water. This group served as **"Papaya leaf"** group to find out the effect of formulation as a growth promoter, effect on egg production and egg quality.

Table: 4.1 Table showing the distribution of quails

Replication (R)	**Treatment (T)**	
	T_0	T_1
R_1	10	10
R_2	10	10
R_3	10	10

T_0 = Control group (Basal diet)

T_1 = Papaya leaf group (Basal diet plus 0.25% Papaya leaves suspension)

4.2.10 Collection and processing of plant materials

Papaya leaves were collected from the HSTU, Dinajpur. Young papaya leaves were collected and washed with fresh water. Before chopping it into small pieces, it was soaked with cotton for removing the adhesive water. Then the leaf was chopped into small pieces and was mashed with the help of pestle and mortar. Leaf juice was collected by squeezing mashed leaf. Then it had been produced 0.25% of suspension of grinded papaya leaf with distilled water.

Fig 4.3: Papaya leaf **Fig 4.4: Processing of papaya leaf**

4.2.11 Recording of body weight

The quails were weighed just before to the commencement of treatment at the 20th day of age and then every 15 days interval body weight was recorded. Last body weight was recorded at the end of the experimental period (65th day of age) with the help of digital balance.

Fig 4.5: Recording of body weight

4.2.12 Hen day egg production

Eggs were collected from each replication everyday from the beginning to fifteen days of laying and the number of eggs were recorded.

Egg production percent was determined replication wise by the following formula.

Hen day egg production (HDEP) (%) = $\frac{\text{No.of eggs laid}}{\text{Total no.of days}} \times 100$

4.2.13 Determination of egg quality

Two eggs from each replication of two treatments were considered randomly during the first day of laying (50th day of age) and last day (65th day of age) of experimental period to determine the egg quality characteristics. Each egg for quality determination was cleaned by wet cloth and then numbered by permanent marker pen.

Fig 4.6: Collection and marking of quail eggs

4.2.14 Egg weight

Weight of each egg was recorded before quality determination by using a digital balance.

Fig 4.7: Weighing of egg

4.2.15 Egg shape index

Measurements of egg length and width were taken with a calliper to the nearest 0.01 mm. The egg shape index was determined from these measurements according to Reddy *et al.* (1979) and Anderson *et al.* (2004) as given with the following formula.

$$\text{Egg shape index} = \frac{\text{Egg width}}{\text{Egg length}} \times 100(\%)$$

Fig 4.8: Egg length determination

Fig 4.9: Egg width determination

4.2.16 Albumen index

The albumen index was determined according to the formula developed by Heiman and Carven (1936).

$$\text{Albumen index} = \frac{\text{Average height of thick albumen}}{\text{Average diameter of thick albumen}} \times 100(\%)$$

Average height of thick albumen was determined as the mean of three measurements taken by a spherometer in three different locations of the albumen avoiding the location of chalazae. Average diameter of the thick albumen was recorded as the mean value of three measurements taken by slide calipers.

Fig 4.10: Recording of thick albumin height

Fig 4.11: Recording of diameter of albumin

4.2.17 Yolk index

The yolk index was determined by the formula developed by Wesley and Stadelman (1959).

$$\text{Yolk index} = \frac{\text{Average height of yolk}}{\text{Average diameter of yolk}} \times 100(\%)$$

The height of the yolk was measured by a spherometer and the diameter by slide calipers. In each parameter, three measurements were taken and the mean value was taken for final calculation.

Fig 4.12: Recording of yolk height

Fig 4.13: Recording of yolk diameter

4.2.18 Haugh unit

Haugh unit (HU) of egg was calculated according to Haugh (1937),

$$HU=100\log (H+7.57 -1.7W^{0.17})$$

Where, HU=Haugh unit

H= Height of thick albumen

W= Egg weight (g)

Average height of thick albumen was determined as the mean of three measurements taken by a spherometer in three different locations of the albumen avoiding the location of chalazae. Egg weight was determined by digital electrical balance.

4.2.19 Shell plus membrane weight and thickness

Immediately after breaking the eggs, the egg shell was soaked with cotton to remove adhesive albumin and egg shell plus membrane weight was recorded with the help of a digital balance. Egg shell plus membrane thickness (mm) was measured by screw gauze. Three measurements were taken from three different locations of each shell; two reading from the waist region and one reading from each end of egg.

Fig 4.14: Weighing of egg shell plus membrane **Fig 4.15: Egg shell plus membrane thickness**

4.2.20 Statistical analysis

Data were analyzed by independent-samples t-test. All analyses were performed by SPSS program version 20. The level of significance was set when the P value is <0.05.

4.3 RESULTS AND DISCUSSION

To perform the experiment, sixty female Japanese quails were randomly assigned into two groups with three replications each containing ten birds. Group T_0 birds were kept as control whereas T_1 treated with 0.25% papaya leaf suspension. In this experiment, the efficacy of papaya leaf suspension on body weight, egg production and egg quality of Japanese quail were investigated. The results of this experiment were discussed under following subheadings.

4.3.1 Body weight

Body weights of two treatment groups were presented in Table 4.2. Results (Table 4.2) indicated that there were a significant (P<0.05) increase in body weight in T_1 (papaya leaf suspension) group than the T_0 (Basal diet) group. Mean body weights at 20^{th} day of age were 38.85 and 37.79 grams where 164.95 and 182.32 grams mean body weights were found at 65^{th} day of age for T_0 and T_1 groups respectively. Birds treated with papaya leaf suspension (T_1 group) had the highest final body weight compared to the birds fed control diet (T_0). This weight gain due to papaya leaf containing papain enzyme. Papain is a protease enzyme that hydrolyzes proteins to short peptides which are the key factors to increase protein digestibility, fast absorption and helps to increase growth factors (Wong *et al*., 1996). Battaa *et al*. (2015) also showed significant (P<0.05) body weight gain for $Dokki_4$ laying hens with different levels of papaya latex. These findings were also confirmed upon examination of papaya latex on rabbits (El Kholy *et al.*, 2008, Zeedan *et al.*, 2009 and El Neney *et al.*, 2013).

Table: 4.2 Effect of papaya leaf suspension on body weight (g) of Japanese quail.

Age (Days)	**T_0** (Mean ± SEM)	**T_1** (Mean ± SEM)	**Level of significance**
20	38.85±0.76	37.79±0.20	**NS**
35	79.63±4.89	98.44±0.94	*
50	151.55±0.20	161.13±2.31	*
65	164.95±0.62	182.32±3.17	*

Values are expressed as mean ± standard error of means. Means between columns are statistically different (P<0.05), NS=Not significant, * = Significant at 5% level of significance

4.3.2 Egg production

The Hen-day-egg production (HDEP) observed for 15 days in two dietary treatments were statistically significant ($P<0.05$). Result indicated (Table 4.3) that the feeding of papaya leaf suspension in the drinking water of laying quail has significant effect on egg production. Quails treated with papaya leaf suspension (T_1 group) showed HDEP (%) 73.10 where birds of control diet (T_0 group) showed 68.29 HDEP (%).

Table: 4.3 Effect of papaya leaf suspension on egg production (%) of laying quails

Parameter	**T_0** (Mean ± SEM)	**T_1** (Mean ± SEM)	**Level of significance**
HDEP (%)	68.29±1.92	73.10±0.82	*

Note: Values are expressed as mean ± standard error of means. Means between column are statistically significant ($P<0.05$). * = Significant at 5% level of significance

4.3.3 External and internal quality of egg

Egg weight, egg shape index, shell plus membrane weight, shell plus membrane thickness, haugh unit, albumen index and yolk index were presented in table 4.4. It was observed that egg weight, egg shape index, shell plus membrane weight, shell plus membrane thickness, haugh unit, albumen index and yolk index of the eggs laid by quails supplied papaya leaf suspension and control diet were almost similar during experimental period and differences were non-significant. These results indicated that the experimental birds supplied with papaya leaf suspension had no effect on external and internal qualities of egg. However, egg weight and haugh unit slightly improved in the group (T_1) supplied with papaya leaf suspension than the group (T_0) supplied with normal diet. These findings showed dissimilarities with Battaa *et al.* (2015). They found that diets supplemented with papaya latex had significantly increased shell thickness, egg shape index and haugh unit in eggs of $Dokki_4$ hens. These results agree with them in findings of no effect on shell weight, yolk index and albumen index.

Table: 4.4 Effect of papaya leaf suspension on external and internal quality of egg

Parameter	Age (Days)	T_0 (Mean ± SEM)	T_1 (Mean ± SEM)	Level of significance
Egg weight (g)	**50**	9.20±0.01	10.11±0.32	NS
	65	9.49±0.21	10.30±0.22	NS
Egg shape index (%)	**50**	77.35±0.356	77.23±0.569	NS
	65	76.98±0.587	76.78±0.876	NS
Shell + Membrane weight (g)	**50**	1.67±0.027	1.66±0.005	NS
	65	1.69±0.04	1.68±0.03	NS
Shell + Membrane thickness (mm)	**50**	0.22±0.004	0.23±0.004	NS
	65	0.21±0.004	0.22±0.003	NS
Haugh unit	**50**	81.44±0.96	83.80±0.34	NS
	65	78.16±0.48	80.52±0.87	NS
Albumen index (%)	**50**	9.26±0.04	9.37±0.035	NS
	65	8.86±0.072	9.00±0.15	NS
Yolk index (%)	**50**	43.12±1.43	45.12±2.39	NS
	65	42.46±1.16	44.82±0.76	NS

Note: Values are expressed as mean ± standard error of means. NS=Not significant.

CHAPTER V
CONCLUSIONS

Herbal medicines are being progressively used all over the world. Nevertheless, herbal remedies are not without hazards and several cases of adverse reactions have been described. From the current experimental data, it may concluded that papaya leaf (*Carica papaya*) suspension have antimicrobial effect and effect on blood constituents, growth performance and egg production of Japanese quail. This experiment supports the traditional use of papaya leaf (*Carica papaya*) for the control of *E. coli* infection. It may be stated that the papaya leaf (*Carica papaya*) may provide a new therapeutic avenue against *E. coli* because of its availability in our country and effectiveness. Papaya leaf can also be considered as leukocyte enhancer, efficient growth promoter and remarkable role on egg production in Japanese quail. To draw a definite conclusion in this regard it demands elaborate study specially mechanism of action, side effect and contraindication of this ancient herbal medicine.

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Name of Exam	Name of Institution	Board/University	Year of Passing
SSC	Dinajpur Govt. Girls' High School, Dinajpur	Rajshahi	2007
HSC	Dinajpur Govt. College, Dinajpur	Dinajpur	2009
DVM	Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200	HSTU, Dinajpur	2015
MS (Physiology)	Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200	HSTU, Dinajpur	2018