EFFECT OF PAPAYA (*Carica papaya*) LEAF JUICE ON PRODUCTION PERFORMANCE AND MICROBIAL LOAD OF SONALI CHICKEN

A THESIS

BY

ZAKARIA SAID ALI
Registration No. 1805718
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MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR – 5200

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE
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Dedicated to
My
Beloved Parents

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The author

ABSTRACT

A study was undertaken to investigate the effect of papaya leaf juice on growth performance and bacterial load in faeces of sonali chicken. A total of one hundred eighty (180) vigorous 28 days sonali chicks were randomly divided into five treatment groups namely T_0 (Control), T_1 (growth promoter (Amino solve) @1ml/1L drinking water), T_2 (10% papaya leaf juice in drinking water), T_3 (20% papaya leaf juice in drinking water) and T_4 (5% dried leaf in feed) having three replication in each treatment group. Data on live weight and feed intake were collected and bacterial load in faeces were recorded and analyzed by ANOVA using the SPSS, version 22.0 software. Total feed intake were non-significant (P>0.05) among the treatment groups but final live weight, live weight gain and FCR were significantly differed among the treatment groups. Total feed intake (gm/bird) and live weight (gm/bird) at 5^{th}, 6^{th}, 7^{th}, 8^{th} week were non-significantly (P>0.05) differed among the treatment groups but live weight (gm/bird) at 9^{th} and 10^{th} week and FCR were significantly (P<0.05) differed among the treatment groups. The final (10^{th} week) average body weight (gm) was significantly (P<0.05) higher T_1 (605.00±7.64) and T_2 (601.88±5.82), followed by T_3 (572.22±8.92), T_4 (569.31±6.38) and T_0 (539.33±14.85), respectively. The best and lowest FCR was found in T_2 (2.63±0.04) and highest FCR was in T_0 (2.98±0.07) whereas in T_1, T_3, and T_4 was 2.63±0.04, 2.79±0.05 and 2.80±0.04 respectively. The load of *Salmonella* sp. and *E. coli* was significantly (P<0.05) lower at increasing concentration of papaya leaf juice compared to control. The present study concludes that supplementation of 10% papaya leaf juice in drinking water can be used as alternative to commercial growth promoter for the production of sonali chicken.

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LIST OF ABBREVIATIONS

AGP	: Antimicrobial growth promoter
CRD	: Completely Randomized Design
DLS	: Department of livestock services
FAO	: Food and Agriculture Organization
FC	: Feed Conversion
FCR	: Feed Conversion Ratio
Kca	: Kilocalorie
MS	: Master of Science
NS	: Not Significant
SD	: Standard Error
W	: Weight
Av	: Average
NRC	: National research council
SPSS	: Statistical package for the social science
SID	: Statistics and informatics Division
%	: Percentage

CHAPTER I

INTRODUCTION

Poultry industry is a promising sector in Bangladesh. Poultry farming offers opportunities for fulltime or part-time employment, particularly for women, children or elderly person in the farm operations. Moreover, from the poultry industry biogas and organic fertilizer can be prepared (Kamal *et al.*, 2015).

Bangladesh is an agriculture oriented country where about 80 percent of the population depends on agriculture. Its economy is particularly dependent on agriculture. Poultry is a part of agricultural farming system in Bangladesh. Poultry production in rural areas is an significant source of livelihood for most of the people living in the rural areas of Bangladesh (Kabir, 2009).

Among the poultry species, indigenous chickens reared in the villages still remains the main genetic resource and plays an important role in rural livelihood, income generation for females and provides important animal protein for large human population in Bangladesh. The national share of commercial strain of chickens and indigenous family poultry in terms of egg production is almost equal 50:50 and that of meat production is 60:40 (Bhuiyan, 2011). About 89% of rural households keep chicken with an average flock size of 5.33 per holding under backyard scavenging system (Bhuiyan *et al*., 2013). Regardless of low output from native chicken in the tropics they can thrive and produce with irregular supply of feed and water and with minimum healthcare. They are part of balanced farming system and have vital roles in the rural households as a source of high quality animal protein and emergency cash income and play a role in the sociocultural life of the rural community. Though local chickens are slow grower and poor layers of small sized eggs they are, however, ideal mothers and good sitters (Tadelle, 2003), excellent foragers, and hardy (Darwish *et al.*, 1990) and possess natural immunity against common diseases (Mtambo, 2000). One of the most important positive characters of native chickens is their hardiness, which is ability to tolerate the harsh environmental condition and poor husbandry practices (climate, handling, watering, and feeding) without much loss in production (Dessie and Ogle, 2011). Identification and characterization of the chicken genetic resources of a country or region generally requires information on their population, adaptation to a specific environment, possession of traits of current or future value and socio cultural importance (Weigend and Romanov, 2001). Native chicken perform a variety of functions, e.g. laying eggs, hatching chicks, brooding and caring of them (Shahjahan *et al.*, 2011). High demand of indigenous (Deshi) cockerel for their tenderness and special taste was observed (Ahmed and Ali, 2007) and indigenous chickens were popular to rural, peri-urban and urban people (Chowdhury, 2012 In the last few decades indigenous chicken has paid great attention by the researchers in all over the world due to its better immunity and adaptability to the existing climatic conditions (Khan, 2015). Indigenous chickens are an excellent genetic resource and their germ-plasm should be preserved and conserved for future genetic improvement program. Horst (1988) reported some useful genes common in tropical indigenous chickens which could be exploited in breeding plans for the development of adaptable breeds suitable in particular to hot-humid climates either for meat or eggs. Indeed, any comprehensive genetic information about such indigenous Bangladeshi chickens other poultry and birds is scarce. In the protracted direction for the evaluation of the genetic compositions of those indigenous chickens, few studies have been carried out. The genetic diversity of native chickens, in Bangladesh when compared with exotic breeds, was found to be greater, and such a phenomenon provides opportunity for poultry breeders to develop a relatively high producing breed of chicken adapted to the rural environment of Bangladesh (Mollah, 2005). In developing countries, native chickens are frequently crossed with exotic stocks to develop crossbreds that may perform better and suitable for scavenging or semi-scavenging systems. The purity of such imported breeds is doubtful and continuous inbreeding may now have caused them to be less productive (Astuti *et al.*, 2001).

Sonali breed is a cross breed, which had been produced from the cross of Road Island Red (RIR) cocks and Fayoumi hens. It has specially been advocated in terms of their higher production rate and better adaptability in rural situation (Ahmed, 1997).

According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for broilers production, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotics as a growth promoter or life savings for chicken. As a result, each and every broiler is a depot of antibiotics. When these broilers are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues (Kamal *et al.,* 2015). Due to the prohibition of most of antimicrobial growth promoters (AGP), plant extracts have gained interest in animal feed strategies (Charis, 2000).

Papaya skin, pulp and seeds also contain a variety of phytochemicals, including lycopene and polyphenols (Kale *et al.,* 2003).

Papaya seeds might contain antibacterial properties against Escherichia coli staphylococcus aureus or *Salmonella* typhi (Kibria *et al.,* 2009). Papaya seed juice may have effects in toxicity-induced kidney failure among various medicinal plant, Nigella sativa is emerging as a miracle herb with a rich historical and religious background since many research revealed its wide spectrum of pharmacology potential (Sarker *et al.,* 2014).

Papaya leave are rich source of the proteolytic enzymes papain and chymopapain (Poulter and Caygill, 1985) which have protein digesting properties and are useful in controlling digestive problems and intestinal worms (Burkhill, 1985). Papaya leaves also contain carotene provitamin a serves as many as 18-50 IU and can be used as a source of natural xanthophyl. Papaya leaves conatain vitamine C Vitamine E, Calcium, phosphorous and iron. Beside that the leaves contain 20.88% crude protein, 099% calcium, 047% phosphorous and 2912kcal / kg gross energy (Mahejabin *et al.,* 2015).

Pawpaw '*Carica papaya*' is a plant native to tropical America. It is known as okwurubekee in Igboland, 'Gonda' in Hausa and Ibepe in Yorube speaking areas of Nigeria. It is popular in the tropics and subtropics because of it easy cultivation, rapid growth quick economic returns and adaptation to diverse soils and climetes (Harkness, 1967; Campbell, 1984) the fruit is high in vitamine (A, B1, B2, C) and minerals (Ca, K, P, Fe) low in sodium, fat and colories and contain practically no starch (Yadava *et al.,* 1990; IIHR, 1979). Papaw latex containe four identified proteolytic enzymes (Yadava *et al.,* 1990) and class 11 chitinaseenzme (Mohamed *et al.,* 1997). Pawpaw plant is the most natural source of papain, an effective natural digestive aid which breaks down protein and cleanses the digestive tract (Poulter and Caygill, 1985). Pawpaw leaf has been used in ethnomedicine application for the treatment of several ailments in Nigeria (Onyimonyi and Ernest, 2009).

Objectives:

- To observe the effect of papaya leaf juice on production performance of sonali chicken
- To determine the effect of papaya leaf juice on bacterial load in faece of sonali.

CHAPTERII

REVIEW OF LITERATURE

The chapter provides a solid background for the research study through review of the literature regarding the effect of papaya juice on production performance and microbial load of sonali chicken. Source of the review literature has been collected from books, publications, journals, reports and other information from the different websites.

2.1 History of sonali chicken

The Sonali is a cross-breed of Rhode Island Red (RIR) cocks and Fayoumi hens and has a similar phenotypic appearance to that of local chickens; it was introduced in 1996–2000 in northern parts of Bangladesh, through SLDP and PLDP. Sonali birds are well adapted to the country's environmental conditions so require less care and attention than other breeds, making them easier for women and children to rear (Saleque and Saha, 2013).and was accepted by all agencies. Between 1992 and 2001, several donors funded projects in Bangladesh, including the Smallholder Livestock Development Project (SLDP-1 and SLDP-2) and the Participatory Livestock Development Project (PLDP-1 and SLDP-2) (Dolberg *et al.* 2002), which involved nearly 1 million women beneficiaries. These projects emphasized the rearing of cross-bred Sonali birds and encouraged other small-scale farmers in rural areas to become involved in the poultry sector.

2.2 Benefits of papaya leaf

Akhila *et al.,* (2015) reported that while plant parts, fruits, roots, bark, peel, seeds and pulp of *Carica papaya* are known to have medicinal properties. It has been used for treatment of numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel thread worms and stimulate reproductive organs. Ayurvedic literature reveals that papaya leaf extract has haemostatic properties and recent studies revealed its ability on platelet augmentation in cyclophosphamide induced thrombocytopenia rat model. Pilot studies done in dengue patients with leaf juice revealed the effect of leaf juice on elevating white blood cells, platelet count and recovery without hospital admission. Hence, in the current study, an effort was taken to study the phytochemical profile of papaya leaf extract using

Liquid Chromatography-Mass Spectroscopy (LCMS). Aqueous extract of young leaves were taken and subjected to LCMS analysis for phytochemical profiling using Water and acetonitrile as mobile phase. On LCMS analysis followed by integrated library search, 21 constituents were identified and it included pharmacologically active phyto compounds, alkaloids, phenolics, flavonoids and also, amino acids. Further studies can be done on these constituents to identify and isolate the most active bio constituent attributing platelet augmentation, anticancer property, anti acne activity, easing menstrual pain and relieving nausea.

2.2.1 Effect of papaya leaves on growth performance

Rumokoy *et al*. (2016) conducted a study to evaluate the effects of papain crude extract addition in mash and pellet feed forms on production performance of broiler chickens in order to obtain the best level of extract papain in mash or pellet form. This natural protease enzyme was extracted from unripe papaya. A complete random design was applied in this study and it was arranged with factorial 4*2 and three replications. The treatments were 4 levels of papain (0, 0.03, 0.05 and 0.07 %) and two physical forms of feed (mash and pellet). Broilers production parameters measured were: feed intake, body weight, feed conversion ratio (FCR) and carcass percentage. The results of analysis of variance showed that the interaction was highly significant ($P<0.01$) for feed intake, body weight, carcass percentage respectively while feed conversions have significant interaction ($P<0.05$). The significant differences in the feed consumption described the role of papain enzyme through treatment of CEP and the physical form of feed. The results indicate that the all treatment of papain crude extract level both in mash and pellet feed form were able to improve feed intake, body weight, FCR and carcass percentage of broiler chickens, whereas the best performance was obtained in the treatment of 0.05% papain crude extract in mash form of diets.

Sudjatinah *et al*. (2009) reported the use of papaya leaf extract to be mixed into the feed of poultry until a concentration of 25ml per liter of drinking water has not shown any significant influence on performance of broiler production. Widjastuti (2009) used the papaya leaf meal up to 10% in layer rations did not provide a significant effect on the quality of the eggs production.

Sorwar *et al.* (2016) determined the effect of papaya leaf (*Carica papaya*) and kalojeera (Nigella sativa) seeds powdered supplementation in drinking water as a growth promoter in broiler chickens. A total of 20 Cobb-500 broiler chicks (day-old) were purchased from local hatchery (Nourish Poultry and Hatchery Ltd.) and after seven days of acclimatization chicks were randomly divided into two groups, A (n=10) and B (n=10). The group A was kept as a control and not treated. The group B was supplemented with papaya leaf and kalojeera powder with feed and water. Weekly observations were recorded for live body weight gain up to 5th weeks and hematological tests were performed at 35th day's age of broiler to search for hematological changes between control (A) and treatment (B) groups. The initial body weight of groups A and B on 1st were 41.00±0.56 and 41.50±0.35, respectively and after 35th day of experiment final body weight were 1470±57.35 gm and 1720±58.56 gm, respectively and economics of production were analyzed and found that net profit per broiler was Tk. 8.91 and Tk. 20.69, respectively. The treatment group B was recorded statistically significant (at 1% level) increased (17.00%) for live body weight than that of control group A. The hematological parameters total erythrocyte count (TEC), erythrocyte sedimentation rate (ESR) and hemoglobin (Hb) estimation value of treatment group shows significant difference, while hemoglobin estimation does not show significant difference from control group. The results suggest that better growth performance could be achieved in broilers supplemented with papaya leaf and kalojeera seeds.

Mahejabin *et al.* (2015) determined the efficacy of mixture of neem, turmeric and papaya leaf extract on growth performances of broilers. A total of 40 days-old broiler chicks, after 7 days acclimatization, were randomly divided into two equal groups. Group A (n=20) was reared as control group with normal feed and water, while group B (n=20) was supplemented with 2% neem, turmeric and papaya leaf extract @ 1 ml per liter of drinking water. No vaccination schedule was practiced and no antibiotics were added in rations. Weekly observations were recorded for live body weight, weekly gain in weight, weekly feed consumption, feed efficiency and blood parameters of birds for five weeks. Body weight in the treatment group was significantly increased ($P<0.05$). However, there was no significant difference in the blood parameters (TEC, Hb, PCV, ESR) between treatment and control groups. Supplementation of neem, turmeric and papaya leaf extract in the treatment group caused improvement in the feed efficiency as compared to that of control group. Treated birds had higher body weight, weekly gain in weight, feed consumption and feed efficiency. These results may be due to antimicrobial and anti-protozoal properties of neem, turmeric and papaya leaf extract which help to reduce the microbial load of birds and improved the feed consumption and feed efficiency of the birds. The study suggests that these medicinal plants may be used as an alternative to antibiotic growth promoters.

Kamal *et al.* (2015) evaluated the efficacy of Neem (*Azadirachta indica*), Nishyinda (*Vitex nogundo*) and Papaya (*Carica papaya*) leaves powdered supplementation in drinking water as a growth promoter in broiler chicks. A total of 40 day-old Cobb 500 broiler chicks were purchased from local hatchery (Nourish Poultry & Hatchery Ltd.) and after seven days of acclimatization chicks were randomly divided into two groups, A and B. The group A was kept as a control and not treated. The group B was supplemented with Neem, Nishyinda and Papaya dried leaves powder with feed and water. Weekly observations were recorded for live body weight gain up to 6th weeks and hematological tests were performed at 17th and 35th day's age of broiler to observe hematological changes between control (A) and treatment (B) groups. The initial body weight of groups A and B on 7th day of this experiment were 140±3.56 gm and 140±4.35 gm, respectively and after 35th day of experiment final body weight were 1450±47.35 gm and 1650±58.56 gm, respectively; the net body weight gain were 1310±43.79 gm and 1510±54.25 gm, respectively and economics of production were analyzed and found that net profit per broiler was Tk. 24.21 and Tk. 34.78, respectively. The body weight was significantly increased ($p<0.01$) in treatment group compared to control group A. The TEC, ESR and PCV value of treatment group showed significant difference ($P<.05$), while Hb estimation did not show significant difference in control group. The results suggest that better growth performance could be achieved in broilers supplemented with Neem, Nishyinda and Papaya extract.

Onyimonyi and Ernest (2009) conducted a feeding trial to evaluate the effect of dietary inclusion of Pawpaw Leaf Meal (PLM) on the performance of finishing broilers. Sixty 5 weeks old broilers were used for the study that lasted for 28 days. The sixty birds were assigned to four dietary treatments with fifteen birds per treatment in a Completely Randomized Design. Each treatment was replicated thrice. Four is nitrogenous and is caloric broiler finisher diets containing 21% CP and 2800kcalME/kg were formulated. PLM was incorporated at levels of 0.5, 1.5 and 2.0% in treatments 2, 3 and 4 respectively. Treatment 1 had no PLM and was used as the control diet. Results should that the effect of treatments on final body weight, weight gain, daily weight gain, feed conversion ratio and feed cost/kg gain were significant ($P<0.05$). Birds on treatment 4 had a final body weight of 2972.5 g which differed significantly (P<0.05) from the 2612.5, 2785.0 and 2875.0 g observed for birds on T , T , and T respectively. The same birds on treatment 4 gained significantly (P< 0.05) more weight of 1 2 3 2044.0 g during the study period. A feed cost/kg gain of N138.42 was also recorded for the birds on T as 4 against N167.28, N148.15 and N141.05 observed for birds on T, T and T respectively. Percentage of edible 1 2 3 cuts as represented by dressing percentage was also significantly (P<0.05) higher in the birds on T. The 4 meat of birds on T also had a significantly (P<0.05) general acceptability. It is concluded that a 2% inclusion 4 of PLM in the diet of finishing broilers could improve performance, carcass and organoleptic indices.

Unigwe *et al.* (2014) conducted a feeding trial was on sixty day-old Anak broiler chicks of average weight of 204.11+3.29g for eight weeks to evaluate the effect of sun-dried paw-paw leaf meal (PLM) on their growth performance as well as proximate composition analysis on the PLM. The birds were assigned to four treatment diets comprising T1 (0% PLM), T2 (5% PLM), T3 (10% PLM) and T4 (15% PLM) in a completely randomized design. They were replicated thrice with 5 chicks per replicate. All data collected were subjected to analysis of variance and difference in means separated using Duncan's New Multiple Range Test. The results showed that PLM contains crude protein (25.30%), crude fibre (8.86%), ether extract (0.81%), ash (8.88%), nitrogen-free extract (43.82%) and moisture (12.33%). The average daily weight gains and feed conversion ratios (FCR) were not significantly different (P>0.05) among all the treatments although demonstrated progressive numerical increase in weight gains as the PLM increased in the diets. Similar trend was also observed in the FCR where T4(4.01) was better than T3 (4.11) and T2 (4.12) but not T1 (3.92). The average daily feed intake showed a significant difference (P<0.05) when T4 (120.69g) was compared with T3 (119.27g), T2 (119.24g) and T1 (115.40g). Similarly, T1Showed a statistical difference (P<0.05) when compared with other treatments but no significant difference (P>0.05) when T2 and T3 were compared in this respect. Therefore, inclusion of PLM up to 15% in broilers' diet is recommended for improvement in weight gain.

2.2.2 Growth promoter

Growth promoters are chemical and biological substances which are added to livestock food with the aim to improve the growth of chickens in fattening, improve the utilization of food and in this way realize better production and financial results. Their mechanism of action varies. Positive effect can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality, regulation of the intestinal micro-flora, etc. In any case, expected results of the use of these additives are increased financial effects of production (Perić *et al.,* 2009).

Growth promoters include some antibiotics (bacitracin methylene disalicylate or virginiamycins), vitamins, minerals, amino acids and some herbal drugs and they perform best when birds are in poor health (Prescott and Baggot, 1993; Peric *et al.,* 2009, Rahman *et al.,* 2012). Growth promoters are getting popularity as feed additives due to their beneficial effect on gut health and immunity, and growth performance (Panda *et al.,* 2009). Though, their mechanism of action varies, positive effect can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality and regulation of the intestinal microflora (Peric *et al.,* 2009, Rahman *et al.,* 2012).

2.2.3 Chemical composition of papaya and papaya leaf

The phytochemical analysis of the *C. papaya* that it contains saponins, alkaloids, tannins, flavonoids, cardiac glycosides, anthraquinones, phlobatinins, anthocyanosides and phenols (Imaga *et al.,* 2010). These phytochemicals vary from one plant part to the other plant which is determined by the solvent used for extraction (Doughari *et al.,* 2009, Imaga *et al.,* 2010). The papaya is a highly nutritive fruit containing a small amount of proteins and the same amount of minerals consisting mainly of iron, calcium and phosphorus, vitamin A and C and is rich in the enzyme papain (Kumar *et al.,* 2013).

Alkaloids carpain, pseudocarpain and dehydrocarpain I and II, choline, carposide, Vitamin C and E have been isolated from the leaves of *C. papaya* (Krishna *et al.,* 2008).

Igwe (2015) studied the chemical constituents of the extract of the leaves of *C. papaya* using Gas Chromatography-Mass Spectrometry (GC/MS) technique and identified six compounds which include hexahydro-1-aH-naphtho[1, 8a-b]oxiren-2(3H)-one (2.17%), 3, 7-dimethyloct-7-en-1-ol (8.08%), 3-methyl-4-(phenylthio)-2-enyl-2, 5-dihydrothiophene-1, 1-dioxide (11.78%), cyclopentaneundecanoic acid methyl ester (12.02%), 3,7,11,15-tetramethyl-2- hexadecen-1-ol (37.78%) and 9-octadecenamide (28.18%).

The constituents of *C. papaya* leaf extract showed twenty constituents, dominated by oleic acid (28.98%) with molecular weight of 282, with the least compound Trans-Geranylacetone (0.17%), with molecular weight of 194 (Oche *et al.,* 2017).

2.2.4 Active component and their action of papaya and papaya leaf

Papaya contains many biologically important active compounds which include chymopapain, papain, caricain, and glycylendopeptidase and papaya lipase (Yogiraj *et al.,* 2014, Aravind *et al.,* 2013, Chaiwut *et al.,* 2007). Chaiwut *et al.* (2007) reported that papaya latex proteases are composed of cysteine proteases contributing 69- 89% of total protein: less than 10% papain, 26-30% chymopapain, 23-28% glycylendopeptidase, and 14-26% caricain. These four proteases have similar molecular weight of approximately 23 kDa. Poulter and Caygill (1985) reported that the proteolytic activities of proteases are activated by additions of small reducing agent such as cysteine and a chelating agent like EDTA. It has been reported that proteases from latex of the fruit vary from those of the non-fruit parts (Brocklehurst and Salih, 1985, Mckee and Smith, 1986) and even from latex of newly wounded fruits (Azarkan *et al.,* 2004, 2006). The major component found in the non-fruit enzymes is chymopapain and the proportions of other enzymes are greatly reduced from the latex proteases (Brocklehurst and Salih, 1985). A series of proteins with low molecular weight are found in the latex obtained from newly wounded fruits (Azarkan *et al.,* 2004). Whereas, repeatedly wounded fruits accumulate and/ or activate several enzymes such as papain, chymopapain and caricain (Azarkan *et al.,* 2006). Proteases in *Carica papaya* are cysteine proteases which need small reducing agents such as cysteine to activate them before catalysis of the reaction. These reducing agents convert reversibly inactive forms of enzymes to the active forms and protect their catalyzed essential thiol group from oxidation (Poulter and Caygill, 1985, Caygill, 1979).

The papaya leaves have been found to contain many active components and these active components can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenicglucosides and glucosinolates (Noriko Otsuki *et al.,* 2010).

2.2 Medicinal properties of papaya and papaya leaf

It has been reported that the various parts of the papaya plant have medicinal properties in the treatment of various ailments and human diseases (Okeniyi *et al.,* 2007, Owoyele *et al.,* 2008, Adigwe *et al.,* 2012). It has been shown that the aqueous seed extract of the unripe mature fruit of *Carica papaya* to possess nephron protective activity (Adeneye *et al.,* 2009, Olagunju *et al.,* 2009). The Papaya fruits are used as topical ulcer dressings in Jamaica (Hewitt *et al.,* 2002, Adigwe *et al.,* 2012). The seeds of Papaya have both antimicrobial and antihelmintic activities (Okeniyi *et al.,* 2007). The latex of Papaya and fluconazole has synergistic action on the inhibition of the growth of Candida albicans (Giordani *et al.,* 1997, Roshan *et al.,* 2014). *Carica papaya* plants produce natural compounds in leaf bark and twig tissues that possess both highly anti-tumourandpesticidal properties (Jaiswal *et al.,* 2010). It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural pesticides (Roshan *et al.,* 2014). (Kalou *et al.,* 2011) concluded that self-defence compounds in the tree makes it highly resistant to insect and disease infestation. (Kalou *et al.,* 2011) reported that *Carica papaya* L. leaf tea or extract has a reputation as a tumour-destroying agent. The seed is used for intestinal worms when chewed (Ayoola and Adeyeye, 2010). The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. Ayoola and Adeyeye, (2010) reported that unripe fruit is used as a remedy for ulcer and impotence. Fresh, green leaf is an antiseptic but the brown, dried pawpaw leaf is used as a tonic and blood purifier. It protects the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12) (Dev and Iqbal, 2015). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, (Elizabeth, 1994, Eleazu *et al.,* 2012). The green unripe pawpaw has a therapeutic value because of its antiseptic quality. The green papaya leaf tea helps to promote digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Saran and Chaudhary, 2013). Papaya seed is used as carminative, emmenagogue, vermifuge, abortifacient, counter irritant, paste in the treatment of ringworm and psoriasis, antifertility agents in males (Krishna *et al.,* 2008).

The leaves have been used traditionally for the treatment of wide range of diseases such as malaria, jaundice and antiviral activity (Vijay *et al.,* 2014).

Leaves contain large amounts of alkaloids, carpaine and pseudocarpine which creates positive effects on heart as well as on respiration (Perry and Metzger, 1980). Leaf extract of C. papaya is well known as an anti-tumor agent (Walter Last, 2008).

2.2.1 Antibacterial properties of papaya and papaya leaf

Papaya roots have been reported to contain antibacterial properties against *S. aureus. S. pyogenase, B. cereus, S. pneumonia, E. coli, P. mirabilis* and *S. typhi* (Doughari *et al.,* 2007).

The extract of the leaves of C. papaya showed potent antimicrobial activity against *Salmonella sp*., *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis* (Igwe, 2015).

2.2.2 Anthelmintic properties of papaya and papaya leaf

Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tape worm. Raju and Yesuf, (2010) observed that the worms reside in the gastrointestinal tract and also burrow into the liver and other organs. They produce harmful effect on host by depriving him of food, causing blood loss and by secreting toxins. Dwivedi *et al.* (2011) noted that anthelmintic are drugs that act locally to expel parasitic worm from gastrointestinal tract. Papaya seeds are used as anthelmintics. Recent years, papaya latex and its commercial products have been widely applied in baking and beverage industries, pharmacy and new chemicals synthesis (Ortega, 2011). Kanthal, (2012) reported the antehelminthic properties of papaya on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro.

Ethanolic leaf extract of *C. papaya* could be as effective as standard anti-coccidial drugs if used before the threshold level of parasitic infection (Nghonjuyi *et al.*, 2015).

2.2.3 Antifungal activity of papaya and papaya leaf

Kumar *et al.* (2013) reported that Antifungal activity of the *Carica papaya* plant against the pathogenic fungi viz. *Aspergillusniger, A. flavus, Candida albicans* and *Microsporum fulvum* and observed that with the increase in concentrations the rate of growth inhibition also increases. Observation further shows that like root extract growth is also inhibited in the presence of shoot and seed alcoholic extract under culture medium further shows that the growth of these fungi inhibits more in presence of higher concentrations as compared to lower concentrations of extract. Moulds and yeasts are so widely distributed in human environment that human beings are instantly exposed to them. Fortunately, because of the relative resistance of human beings and comparatively non pathogenic nature of fungi, most of these exposures do not lead to over infection (Kumar *et al.,* 2013). However, fungi are gaining importance with respect to increased incidence of chronic, often fatal, mycoses in immune compromised patients (Blanco and Garcia, 2008). The fungi present in soil, water and air constitute exogenous fungal opportunists. The roster of opportunistic fungal species continues to increase. However, some of the common ones include *Aspergillus fumigates, A.niger, A. terreus, A. flavus, Absida, Candida albicans, Cryptococous neoformis, Microsporum fulvum, Mucor, Rhizomucor, Rhizopus* and *Torulopsis globrata* (Kumar *et al.,* 2013). To find suitable drug for the management of fungal diseases is difficult because fungi, like human beings, are eukaryotes. Giordani *et al.* (1997) reported the antifungal activity of *C. papaya* latex sap against Candida albicans. *Carica papaya* latex sap inhibits the growth of Candida albicans when added to a culture during the exponential growth phase. Roshan *et al.* (2014) noted that a mixture of C. papaya latex (0.41 mg protein/ml) and fluconazole (2 µg/ml) also showed a synergistic action on the inhibition of *C. albicans* growth.

2.2.4 Antioxidant activity of papaya and papaya leaf

The major groups of phytochemicals that have been suggested as a natural source of antioxidants may contribute to the total antioxidant activity of plant materials including polyphenols, carotenoid and traditional antioxidant vitamins such as vitamin C and E (Maisarah *et al.,* 2013). Antioxidant is any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Maisarah *et al.,* 2013). Antioxidant functions are associated with decreased DNA damage, diminished lipid per oxidation, maintained immune function and inhibited malignant transformation of cells (Gropper *et al.,* 2009, Swathi *et al.,* 2016). Several studies showed that phenolic compounds are the major bioactive phytochemicals with human health benefits (Swathi *et al.,* 2016). Mehdipour *et al.* (2006) explored the toxicological and antioxidant potential of dried *Carica papaya* juice in vitro and in vivo. In vivo examination was performed after oral administration of dried papaya juice to rats for 2 weeks at doses of 100, 200 and 400 mg/kg (Jaiswal *et al.,* 2010). The acute toxicity test LD50 demonstrated that papaya juice is not lethal up to a dose of 1500 mg/kg after oral administration and thus is considered nontoxic. In treated groups, no sign of toxicity was observed. In vitro evaluation of the antioxidant effects of papaya showed that the highest antioxidant activity 80% was observed with a concentration of 17.6 mg/ml (Jaiswal *et al.,* 2010). Blood lipid peroxidation levels decreased significantly after administration of all doses of papaya juice (100, 200, 400 mg/kg/day) to 35.5, 39.5 and 40.86% of the control, respectively, compared with a value of 28.8% for vitamin E (Mehdipour *et al.* (2006). The blood total antioxidant power was increased significantly by all doses of papaya juice (100, 200, 400 mg/kg/day) to 11.11, 23.58 and 23.14% of the control, respectively. The value for vitamin E was 18.44%. This study indicates the safety and anti- oxidative stress potential of C. papaya juice, which was found to be comparable to the standard antioxidant compound alpha tocopherol (Jaiswal *et al.,* 2010). Nakamura (2007) reported that hexane extract of C. papaya seed homogenate is highly effective in inhibitory superoxide generation and apoptosis in H2-60 cells. Olagunju (2009) observed that the aqueous seed extract of the unripe mature fruits of *Carica papaya* has nephro protective. It is due to its antioxidant or oxidative free radical scavenging activities (Jaiswal *et al.,* 2010).

2.2.5 Anti-reproductive activity of papaya and papaya leaf

Srivastava (2013) reported that feeding of bait formulation of papain with attractant (starch or serine) have sufficient molluscicidal activity against *L. acuminata*. Sub-lethal (40% and 80% of 24h LC50) of feeding of bait containing plant molluscicides papain significantly reduced the fecundity of the snail *L. acuminata*. The CDC cells (caudo dorsal cells) in the brain of the snail *L. acuminata* release ovulation hormone (Roubos *et al.,* 1981). The cerebral neurosecretorycaudo dorsal cells (CDCS) of the fresh water pulmonates snail *Lymnaea stagnalis* control egg laying, an event that involves a pattern of stereotyped behaviour (Vreugdenhil, 1988). The CDCS synthesize and release multiple peptides, among which is the ovulation hormone (CDCH). It is thought that each peptide controls a specific aspect of the processes involved in egg laying (Vreugdenhil, 1988). It seems that after sublethal treatment of papain caused the decrease the level of serotonin and inhibits prostaglandins synthesis by inhibiting 5-lipoxygenase and leukotriene directly or indirectly CDCs. Possibly, the active molluscicidal component papain affect the CDCs and reduce the release of ovulation hormone, resulting a decrease in the fecundity of treated snail.

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The experiment was conducted for a period of 27 July to 18 September, 2019 to investigate the dietary effect of papaya leaves juice in different doses on production performance of sonali and microbial load of sonali specially *Salmonella* and *E. coli.*

3.2 Experimental site

The study was conducted at Hajee Mohammad Danesh Science and Technology University (HSTU) Poultry Shed, Dinajpur and the microbial load was analyzed at Hajee Mohammad Danesh Science and Technology University (HSTU) lab.

Fig. 3.1: Experimental site

3.3 Experimental Birds

A total of 180, 28 days sonali chicks were purchased from the dealer of govt, poultry farm, Dinajpur.

All birds were randomly allocated to five dietary treatment groups as T_0, T_1, $T_{2,}$ T_3 and T_4, each group consisted of 3 replications containing 12 birds in each replication. Feed and water were supplied ad libitum throughout the trial. All birds were fed with standard commercial (govt, poultry farm, Dinajpur.) ration throughout the experimental period.

The layout of the experiment is shown in table 3.1. There were five treatments consist of three replications in each dietary treatment with 12 birds in each replication. Thus total number of replication was 15.

Table 1: Table showing the distribution of broiler to different dietary treatment groups

Replication (R)	**Treatment (T)**				
	T_0	T_1	T_2	T_3	T_4
R_1	12	12	12	12	12
R_2	12	12	12	12	12
R_3	12	12	12	12	12

* T_0 = Control fed with commercial diet

* T_1 =Commercial diet with growth promoter (Amino solve) @1ml/1L drinking water

* T_2 = Commercial diet with 10% papaya leaf juice in drinking water

* T_3 = Commercial diet with 20% papaya leaf juice in drinking water

*T_4 =Commercial diet with 5% dried papaya leaf in feed

3.4 Preparation of research shed

The shed was cleaned and washed using fresh water and disinfectant (Iodin, Non Ionic Surfectant, Sulfuric Acid, Phosphoric Acid, FAM 30®, Renata animal health, Bangladesh). Then the shed was kept free for 15 days before placing experimental birds. All necessary equipment were disinfected and set properly to care the broiler chicks successfully.

3.5 Source of papaya leaf

Papaya leaf was collected from HSTU campus area and local area of Dinajpur district in Bangladesh.

3.5.1 Preparation of plant extract

Papaya leaves were selected for effectiveness as growth promoter on poultry. Mature and disease free papaya leaves were collected from Dinajpur district. For the preparation of juice, equal amount of green leaf and water was taken to pulminize with a blender. The mixer was preserved in air tight plastic container until they were directly used for screening.

Fig. 3.2: Papaya leaves

Fig. 3.3: Papaya juice

3.5.1.1 Layout of the Experiment

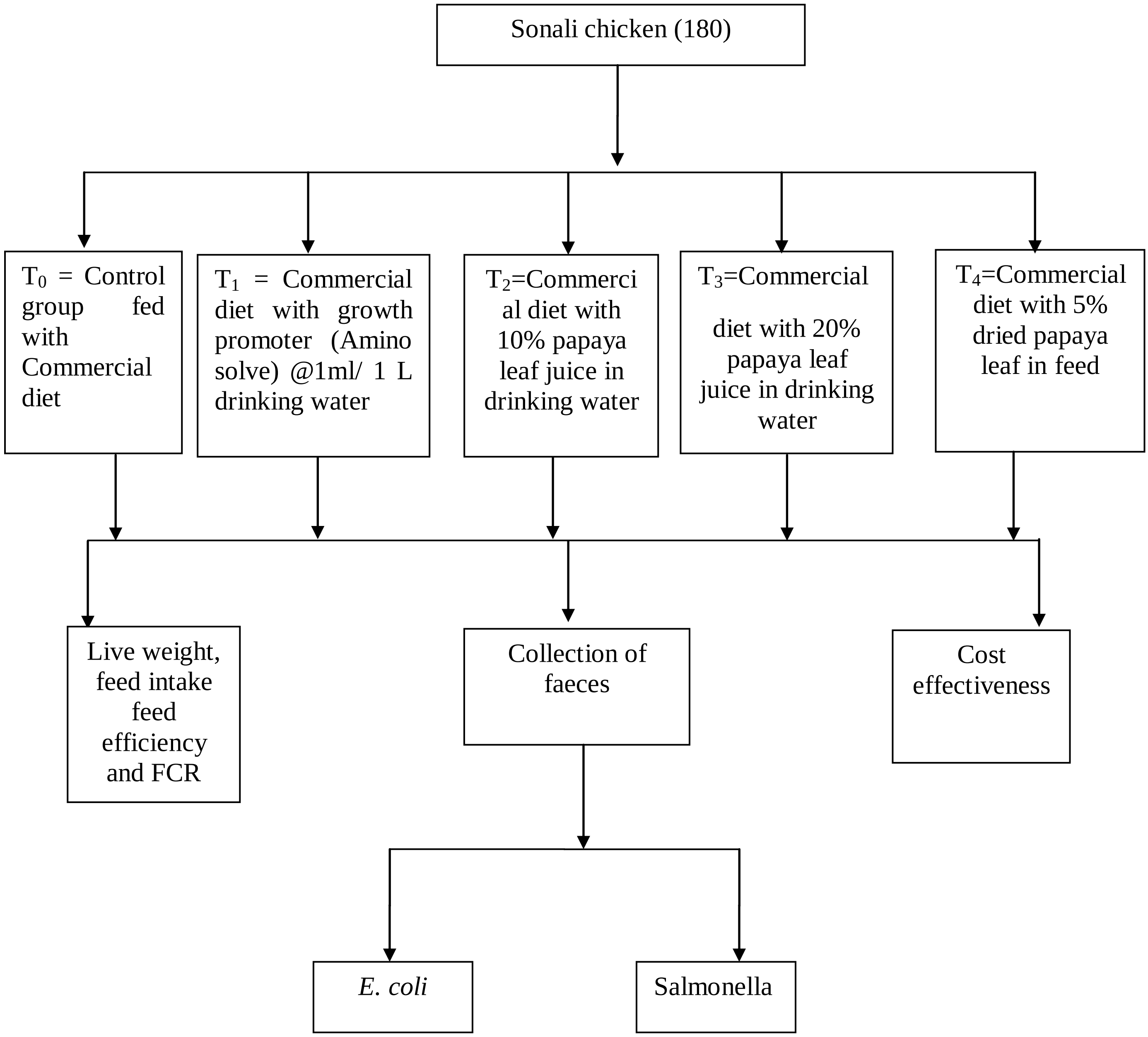

Fig.3.4: Layout of the experiment

3.5.1.2 Management of birds

A total of 180, 28 days sonali chicken were collected from govt, poultry farm, Dinajpur. The birds were kept on a floor litter system, each group in separate pens measuring 0.9×1.5 meters. The pens were thoroughly cleaned, white-washed and disinfected before use. All the groups were reared under the similar conditions of temperature, humidity, light, ventilation and floor space throughout the experimental period. Birds were fed commercial feed from Nourish Poultry Feed Limited and water adlibitum under strict biosecurity (Molla *et al*., 2012).

Fig. 3.5: Experimental birds

3.5.1.3 Biosecurity and sanitation

Proper hygienic and sanitation programs were followed during the experimental period. To prevent the outbreak of disease strict biosecurity was maintained during the experimental period. The following measures were taken to maintain the biosecurity: Visitors were not allowed to enter in the house, all equipment's in the experimental house were kept clean.

3.5.2 Experimental Procedure and Data Collection

The growth performance of sonali chicken was evaluated by using change in body weight (growth rate), feed intake and feed conversion ratio.

3.5.3 Live weight

The live weight of each bird was measured with the help of digital balance on initial weight, 5^{st} to 10^{th} week of experiment and recorded.

3.5.4 Body weight gain

The birds were weighed at start (initial body weight) and then at the end of the experiment (final body weight). Body weight gain/loss was calculated by the difference of initial body weight and final body weight.

Body weight gain = Final body weight – initial body weight

Fig. 3.6: Weight

3.5.5 Feed intake and feed conversion ratio

Feed intake was determined by weighing the amount of feed offered and feed refusal in each pen (replicate) on a weekly basis. Feed intake per pen was obtained by calculating the difference between the total weight of feed given and weight of the refusal feed (left over feed). However, Feed conversion ratio (FCR) was obtained by dividing the total feed intake with total weight gain of all birds in each pen. The feed intake and feed conversion ration were obtained by using following formula:

Feed intake (FI) = Feed offered – Feed refusal

Feed Conversion ratio (FCR) = Feed intake/ Average daily gain

Fig. 3.7: Feed preparation

3.5.6 Collection and transportation of faecal sample

Faeces from sonali of each group were collected aseptically and kept in phosphate buffered saline (PBS) solution containing collecting tubes and sent to the Mohammad Danesh Science and Technology University (HSTU) lab, Dinajpur.

3.5.7 Bacteriological reagents

The reagents used were phenol red, phosphate buffered saline (PBS), mineral oil, normal physiological saline solution, peptone water, 3% tri sodium citrate solution and other common laboratory chemicals and reagents as and when required during the experiment.

3.5.7.1 Bacteriological media preparation

3.5.7.1.1 Eosin Methylene Blue (EMB)

Lactose fermenting pink colony from MacConkey was sub cultured into EMB agar, used selective media for *E. coli* and incubated at 37^0C for 24 hours.

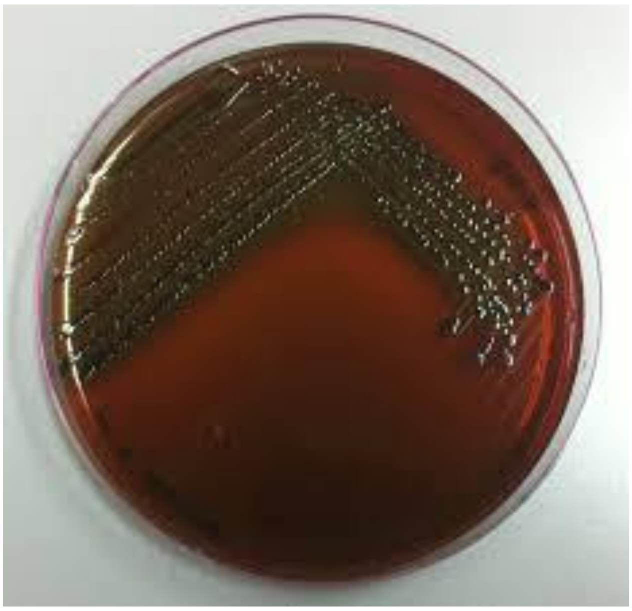

Fig. 3.8: Eosin Methylene Blue (EMB)

3.5.7.1.2 Salmonella-Shigella (SS) agar

The non-lactose fermenting colorless colony from the MacConkey agar was sub-cultured on SS agar media used as selective media for pathogenic *Salmonella* spp. and Shigella and incubated at 37^0C for 24 hours.

Fig. 3.9: Salmonella-Shigella (SS) agar

3.5.7.1.3 Isolation of *E. coli* and *Salmonella* in pure culture

All samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey and EMB agar and S-S agar by streak plate method to observe the morphology. The organism showing, characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies and the organism showing, characteristic colony morphology of *Salmonella* was repeatedly subcultured onto S-S agar agar until the pure culture with homogenous colonies.

Experimental Birds (Commercial broiler)
↓
Collection of sample (Cloacal feces)
↓
Preparation of samples
↓
Inoculation of samples into bacteriological media (Nutrient agar)
↓
Subculture
↓
S-S Agar → Suspected colony → *Salmonella*
E.M.B agar → Suspected colony → *E. coli*

Fig. 3.10: Schematic Illustration of Experiment

3.5.7.1.4 Examination of Plates (Identification of the isolates)

a) Gross colony study

Morphological characteristics (shape. size, surface texture, edge, elevation, colour., opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967) and recorded.

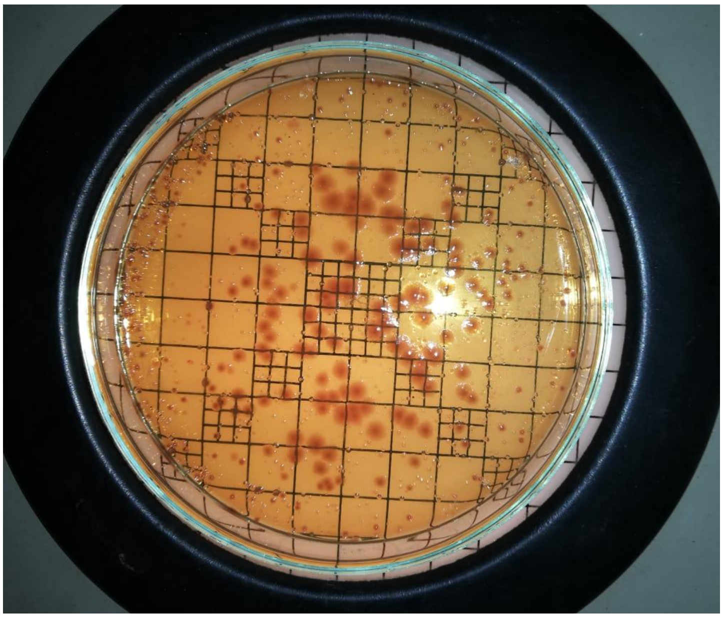

Fig. 3.11: Gross colony

3.5.8 Statistical analysis

Data were collected from each treatment and entered into the computer data base (excel sheet) ready for statistical analysis (SPSS, Version 22.0).

The data were analyzed analysed by one-way analysis of variance, followed by the Duncan post hoc test to determine significant differences in all the parameters among all groups using the SPSS computer program (Version 20.0; SPSS). Differences with values of $P<0.05$ were considered to be statistically significant.All data were expressed as Mean±Standard Error of Mean (SEM). Differences were considered statistically significant at least $P<0.05$.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Effect of papaya leaf juice on body weight (gm/bird) of sonali chicken

The effect of papaya leaf juice on body weight (gm/bird) of sonali chicken is presented on Table 2. The present study revealed that the average body weight (gm) of sonali chicken did not significantly (P>0.05) differed among the treatment groups at 5^{st} week, 6^{nd} week, 7^{rd} week and 8^{th} week of age but significantly (P<0.05) differed at 9^{th} and 10^{th} week of age. The average body weight (gm) at 1^{st} week in T_0, T_1, T_2, T_3 and T_4 was 164.83±8.80, 166.80±4.20, 169.31±8.44, 165.58±3.15 and 168.14±3.49 respectively. At the age of 6^{nd} week, the average body weight (gm) was highest in T_2 (274.36±9.71) and lowest in T_0 (243.71±5.99) whereas the body weight (gm) was T_1, T_3 and T_4 was 265.42±8.49, 261.78±5.90 and 268.19±7.37 respectively. Higher average body weight (gm) was found in T_4 (336.50±27.01), T_2 (404.31±19.01) and T_1 (495.56±23.41) at 7^{rd} and 8^{th} week of age. The average body weight (gm) was significantly (P<0.05) highest in T_1 (502.83±19.86), followed by T_2 (494.64±7.52), T_4 (475.11±4.98), T_3 (471.00±9.93) and T_0 (444.33±7.22) respectively at 9^{th} week of age. There was no significant difference between T_1 and T_2 treatment groups but they were differed from other groups. The final (10^{th} week age) average body weight (gm) was significantly (P<0.05) higher T_1 (605.00±7.64) and T_2 (601.88±5.82) which was followed by T_3 (572.22±8.92), T_4 (569.31±6.38) and lower in T_0 (539.33±14.85). The present study is supported by Rumokoy *et al*. (2016) who observed that papain crude extract level both in mash and pellet feed form were able to improve body weight of sonali. Onyimonyi and Ernest (2009) found that Pawpaw Leaf Meal (PLM) also increased body weight. The present result is disagreed with the findings of Sudjatinah *et al*. (2009) reported the use of papaya leaf extract to be mixed into the feed of poultry until a concentration of 25ml per liter of drinking water has not shown any significant influence on performance of broiler production.

Table 2: Effect of papaya leaf juice on body weight (gm/bird) of sonali chicken

Age	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
5st week	164.83±8.80	166.80±4.20	169.31±8.44	165.58±3.15	168.14±3.49	NS
6ndweek	243.71±5.99	265.42±8.49	274.36±9.71	261.78±5.90	268.19±7.37	NS
7rd week	289.57±4.84	330.44±10.58	336.47±13.92	327.47±15.59	336.50±27.01	NS
8th week	373.29±9.37	396.08±23.30	404.31±19.01	386.11±3.07	383.97±4.98	NS
9th week	444.33±7.22^a	502.83±19.86^c	494.64±7.52^c	471.00±9.93^b	475.11±4.98^b	*
10thweek	539.33±14.85^a	605.00±7.64^c	601.88±5.82^c	572.22±8.92^b	569.31±6.38^b	*

4.1.1 Effect of Papaya leaf juice on feed intake (gm/bird) of sonali chicken

Effect of papaya leaf juice on feed intake (gm/bird) of sonali chicken is shown in Table 3. The present study indicated that there was no significant (P>0.05) variation among the different treatment groups in case of feed intake (gm/bird) in all age of bird. The highest feed intake (gm/bird) was found in T_0 (194.06±5.89), T_3 (227.67±1.54), T_0 (258.76±4.24), T_0 (301.70±4.32), T_2 (309.69±3.29) and T_1 (326.15±2.50) in 5th, 6th, 7th, 8th, 9th and 10th week of age. Feed intake (gm/ bird) was more or less similar in all treatment groups. Rumokoy *et al*. (2016) observed that papain crude extract level both in mash and pellet feed form were able to improve feed intake of broiler

Table 3: Effect of papaya leaf juice on feed intake (gm/bird) of sonali chicken

Age	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
5st week	194.06±5.89	192.50±1.07	184.97±4.49	189.50±3.03	191.92±1.61	NS
6nd week	223.33±8.82	222.86±2.86	219.67±2.99	227.67±1.54	220.83±4.68	NS
7rd week	258.76±4.24	253.72±2.05	247.14±2.07	255.39±3.32	251.72±1.35	NS
8th week	301.70±4.32	291.11±3.89	289.16±4.29	293.47±1.52	295.63±3.05	NS
9th week	303.57±11.96	307.22±1.87	309.69±3.29	308.19±1.84	308.22±4.15	NS
10thweek	325.03±3.38	326.15±2.50	322.64±2.34	324.13±2.91	325.86±2.32	NS

4.1.2 Effect of Papaya leaf juice on total feed intake (gm/bird) and FCR of sonali chicken

Table 4 shows the effect of papaya leaf juice on total feed intake (gm/bird) and FCR of sonali chicken. It was revealed that there was no significant (P>0.05) difference among all treatment groups in case of total feed intake but significant (P<0.05) variation was found in case of FCR. The total feed intake (gm/bird) was highest in T_0 (1605.80±12.74), followed by T_3 (1598.35±3.09), T_4 (1594.18±6.26), T_1 (1593.57±1.13) and T_2 (1573.26±5.29) respectively. The best and lowest FCR was found in T_2 (2.63±0.04) and highest FCR was in T_0 (2.98±0.07) whereas in T_1, T_3, and T_4 was 2.63±0.04, 2.79±0.05 and 2.80±0.04 respectively. The FCR in T_1 and T_2 was statistically similar (P<0.05). Similar result is also observed by Rumokoy *et al.* (2016) and Mahejabin *et al.* (2015) who reported that papaya leaf extract improved the feed consumption and feed efficiency of the birds.

Table 4: Effect of Papaya leaf juice on total feed intake (gm/bird) and FCR of sonali chicken

Parameter	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
Total feed intake (gm)	1605.80±12.74	1593.57±1.13	1573.26±5.29	1598.35±3.09	1594.18±6.26	NS
FCR	2.98±0.07^c	2.63±0.04^a	2.61±0.03^a	2.79±0.05^b	2.80±0.04^b	*

4.2 Effect of papaya leaf juice on bacterial load of sonali chicken

Table 5 presents effect of papaya leaf juice on bacterial load in faeces of sonali chicken. The load of *Salmonella* sp. was significantly (P<0.05) lower in T_4 (111.00±7.23), followed by T_3 (148.33±7.26), T_2 (170.33±10.17), T_1 (244.33±26.77) and T_0 (250.33±24.91), respectively. The load of *E. coli* was significantly (P<0.05) lower in T_4 (118.67±4.10), followed by T_3 (124.33±5.49), T_2 (171.67±2.60), T_1 (230.33±22.81) and T_0 (252.67±11.29) respectively. The present study is similar to the reports of Mahejabin *et al.* (2015) who reported that papaya leaf extract helps to reduce the microbial load of birds. (Igwe, 2015) reported that the extract of the leaves of *C. papaya* showed potent antimicrobial activity against *Salmonella sp.*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*.

Table 5: Effect of papaya leaf juice on bacterial load in faeces of sonali chicken

Parameter	Dietary treatment groups					P value
	T_0	T_1	T_2	T_3	T_4	
Salmonella sp.	250.33±24.91^{c}	244.33±26.77^{c}	170.33±10.17^{b}	148.33±7.26^{ab}	111.00±7.23^{a}	*
E. coli	252.67±11.29^{c}	230.33±22.81^{c}	171.67±2.60^{b}	124.33±5.49^{a}	118.67±4.10^{a}	*

Table 6: Effect of papaya leaf juice on cost effective sonali production

Description	T_0	T_1	T_2	T_3	T_4
Cost / chicken (Taka)	40	40	40	40	40
Medicine	10	10	10	10	10
Cost of herbal growth promoters (Taka)	0	20	0	0	0
Miscellaneous cost (Taka)	8	8	8	8	8
Total feed cost (Taka)/ sonali	51.38	51.0	50.34	51.14	50.0
Total cost/sonali	109.2	129	108.34	109.14	108
Sale price/sonali (Taka)	108	121	120	114	113
Net profit/sonali (Taka)	1.2	8	11.66	4.86	5

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted to evaluate the efficacy of tulsi leaves extract on production performance, dressing yield and *E. coli* and *Salmonella* bacterial count of sonali chicken. For this purpose, one hundred eighty (180) vigorous 28 days sonali chicks were collected from govt. poultry farm, Dinajpur. The chick were randomly divided into five treatment groups namely (T_0, T_1, T_2, T_3 and T_4) having three replication in each treatment group. Experimental birds in T_2, T_3 and T_4 were provided papaya leaves juices @ 10%, 20% in drinking water and 5% dried papaya leaf in feed while T_0 was provided only plain water and T_1 with growth promoter (Amino solve) @1ml/1L drinking water. The data regarding body weight (growth rate), feed intake and feed conversion ratio on initial and 5^{st} to 10^{th} week of experiment were collected and recorded. At the end of experiment, bacterial loads on faecal sample were counted and recorded.

The present study indicated that the average body weight (gm) of sonali chicken did not significantly (P>0.05) differed among the treatment groups at 5^{st} week, 6^{nd} week, 7^{rd} week and 8^{th} week of age but significantly (P<0.05) differed at 9^{th} and 10^{th} week of age. Higher average body weight (gm) was found in T_2 (169.31±8.44), T_2 (274.36±9.71), T_4 (336.50±27.01), T_2 (404.31±19.01) and T_1 (495.56±23.41) at 5^{st}, 6nd 7^{rd}, 8^{th} and 9^{th} week of age compared to others. The final (10^{th} week age) average body weight (gm) was significantly (P<0.05) higher T_1 (605.00±7.64) and T_2 (601.88±5.82) which was followed by T_3 (572.22±8.92), T_4 (569.31±6.38) and lower in T_0 (539.33±14.85). It was found that there was no significant (P>0.05) difference among all treatment groups in case of feed intake but significant (P<0.05) variation was found in case of FCR. The total feed intake (gm/bird) was highest in T_0 (1605.80±12.74), followed by T_3 (1598.35±3.09), T_4 (1594.18±6.26), T_1 (1593.57±1.13) and T_2 (1573.26±5.29) respectively. The best and lowest FCR was found in T_2 (2.63±0.04) which was followed by T_1 (2.63±0.04), T_3 (2.79±0.05), T_4 (2.80±0.04) and T_0 (2.98±0.07) respectively. The load of *Salmonella* sp. and *E. coli* was significantly (P<0.05) lower in T_4 (111.00±7.23) and T_4 (118.67±4.10) respectively.

The result of this study suggests that supplementation of 10% papaya leaf juice in drinking water can be used as alternative to commercial growth promoter for the production of sonali chicken. The results suggest that better growth performance could be achieved in sonali chicken supplemented with 10% papaya leaf juice. Therefore, more studies are required to determine cost effective doses and form of use.

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