CHAPTER I: INTRODUCTION

Antibiotics used at sub therapeutic doses also the use of antimicrobial growth promoters have been found beneficial for rapid growth performance in animal production level and prevention of disease occurrence **(Barton *et al.*, 2000 and Snel *et al.*, 2002)**. Though the presence of low levels of antibiotics in animal feeds sensitive microorganisms are removed but resistant cells survive and grow producing an antibiotic resistant population in the final products **(Mehdi *et al.*, 2011)**. While growth stimulating antibiotics agents are used in poultry breeding industry by the spread of antibiotics resistant bacteria they are a threat to human health **(Wray *et al.*, 2000 and Turnidge *et al.*, 2004).** However, because of the development of resistance by pathogenic bacteria, which can impact on public health, antibiotics are being taken out of poultry and pig diets around the world, beginning in Sweden in the year 1986 **(Dibner *et al.*, 2005)**. Therefore a call for worldwide limited use of antimicrobial growth promoters was initiated by **Bogaard and Stobberingh (2000)** and **Snel *et al.* (2002)**. On a consequence of this initiation the application of antibiotics as growth promoters (AGP) in the animal feed has been limited in the European Union since January 2006 (**Mehdi *et al.*, 2011**).

In these circumstances, it was found necessary to develop alternatives using either beneficial microorganisms or to use non-digestible ingredients that retard microbial growth (**Awad *et al.*, 2009**). The search for alternatives to replace in feed additives (IFA) was a key concern for animal nutrition in recent years. **Bedford *et al.* (2000)** established in his study that the growth-promoting effects of antibiotics in animal diets have a close relation to the gut micro-flora as they exert no benefits on the performance of germ-free (GF) animals. Micro-flora from guts has significant effects on host nutrition, health, and growth performance through interaction with nutrient utilization and the development of gut system of the host (**Barrow *et al.*, 1992**).

It is becoming increasingly evident that to achieve the aims to significantly reduce the use of antibiotics, a combination of intervention strategies as genetic selection of resistant animals along with sanitation practices, vaccinations, and applications of

suitable feed additives is necessary (**Doyle and Erickson, 2006**). In this sense, the choice of the scientists was prebiotics and probiotics, classified as zoo-technical, a feed additive (**European Commission, 2003**) that comprises a functional nutritional approach, where by a healthy gastrointestinal (GI) environment is maintained and improvement of intestinal function is also pursued through the intake of adequate quantities of live beneficial microorganisms (**Fuller *et al.*, 1989 and FAO/WHO, 2002**).

This seems to be the best way of potentiating the efficacy of probiotics and is widely used in practice. A probiotic was defined as feed supplement with live microbes that beneficially affects the host animal by improving its microbial intestinal balance (**Fuller *et al.*, 1989**). On the other hand, a prebiotic was defined as non-digestible food ingredient that beneficially affects the host, selectively stimulating the growth or activity, or both, of one or a limited number of bacteria in the colon (**Gibson and Roberfroid, 1995**). The way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, this may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (**Awad *et al.*, 2009**).

Use of prebiotics or fermentable sugars instead of antibiotics is going to be popular in birds in order to improve the useful microbial population of GIT (**Kermanshahi *et al.*, 2006**). Prebiotics have been shown to alter gastrointestinal micro-flora, alter the immune system, prevent colonic cancer, reduce pathogen invasion including pathogens such as *Salmonella*, *Entritidis* and *E. coli* and reduce cholesterol and odor compounds (**Cummings *et al.*, 2002**). Also, prebiotics supplementation of broilers diet result in an increase of the pH of the GIT and use full bacteria population such as *lactobacillus* and *bifidobacteria*, due to increasing production of volatile fatty acids (**Ziggers *et al.*, 2000**).

Now, new commercial feed additives of plant origin considered to be natural products that consumers would accept, have been proposed to livestock producers. Herbs, spices, various plant extracts, prebiotics, and probiotics have received increased attention as possible antibiotic growth promoter substitutions (**Mehdi *et al.*, 2011**). Research on establishing new plant originated feed additives

effective on bird growth those will be free from harmful side effects on birds as well as public health concern is still on move.

Therefore the objectives of this study was to investigate the effects of different levels of *Spondias* tree leaves with or without fermentation using beneficial bacteria on growth performance, carcass characteristics, meat quality and blood parameters in broiler.

CHAPTER-II: REVIEW OF LITERATURE

2.1 *Spondias mombin* leaf

Medicinal plants have been recognized to be of great importance to the health of individuals and communities. Among these medicinal plants, *Spondias mombin* has been reported to have significant medicinal and economic values (**Maduka *et al.*, 2014**).

Spondias mombin Linn is a fructiferous tree that belongs to the family Anacardiacae. It grows in the coastal areas and in the rain forest into a big tree of up to 15–22mm in height. It is readily common in Nigeria, Brazil and several other tropical forests of the world with high genetic variability among populations (**Ayoka *et al.*, 2008**). It is called Hog plum in English, akika in Yoruba, ijikara in Igbo, tsader maser in Hausa, chabbuli in Fulani and nsukakara in Efik (**Gill *et al.*, 1992**).

2.1.1 Compositions

Preliminary phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, tannins, oxalate, phytate and cyanogenic glycosides, while their quantitative estimations (in percentages, %) gave saponins (4.80 ± 0.35), alkaloids (3.40 ± 0.10), flavonoids (2.80 ± 0.36), tannins (1.47 ± 0.06), oxalate (0.92 ± 0.09), phytate (1.73 ± 0.19) and cyanogenic glycosides (0.01 ± 0.00) (**Igwe *et al.*, 2010**).

The quantitative estimations (in percentages) of the proximate compositions are shown in Table 2. The total carbohydrate content was 68.92 ± 2.00%, moisture 15.13 ± 0.57%, crude protein 11.04 ± 0.71%, crude fibre 10.51 ± 0.84%, crude fat 4.82 ± 0.34% and ash content 0.09 ± 0.009% (**Igwe *et al.*, 2010**).

The plant has been shown to have a wide range of phytoconstituents such as tannins, saponins and anthraquinone glycosides.

The leaves contain saponins, alkaloids and tannins in all the extraction mediums while flavonoids, alkaloids and tannins were detected in the extraction medium of the stem bark. The leaf extract contains more vitamin C and E compared to the

stem bark extracts. The ethanol extract exhibited increased activity against *Staphylococcus aureus* as compared to the inhibitions seen with the other tested microorganisms. The results, therefore, showed that the anti-microbial principle in the plant was mostly extracted with ethanol (**Maduka *et al*., 2014**).

To evaluate the effect of flavonoids on cell–cell communication system, the bioluminescence production in reporterstrains V. harveyi BB886 and MM32 in the presence of flavonoids were measured. All the flavonoids except hesperidin inhibited either HAI-1- or AI-2-mediated bioluminescence, significantly. Naringenin, kaempferol, quercetin and apigenin significantly inhibited both HAI-1- and AI-2-induced bioluminescence. Growth rate of V. harveyi BB120 was measured at OD600 over a period of 16 h to determine the inhibitory effect of flavonoids . Apigenin, quercetinand kaempferol inhibited the growth of V. harveyi BB120 significantly, whereas neoeriocitrin induced the growth rate significantly. Naringenin, naringin, neohesperedin, sinensetin, hesperidin and rutin did not show inhibitory effect on V. harveyi BB120 growth (P > 0Æ05). To further confirm the influence of naringenin, quercetin and kaempferol, V. harveyi was grown upto 16 h in the presence of these flavonoids, and viable cell count was determined by plating on LM agar plates every 2 h . Quercetin and kaempferol demonstrated significant effect on V. harveyi growth, whereas naringenin was ineffective at 100 lg ml (**Vikram *et al*., 2008**).

The flavonoids are known to protect against allergies and inflammation, and represent the most common and widely distributed groups of plant phenolics that serve as flavoring ingredients of spices and vegetables. Flavonoids and other derivatives have been identified in *Spondias mombin* plant with anti-herpes, antioxidant and anti-aging properties. Flavonoids have been reported to be free radical scavengers, super antioxidants and with strong anticancer activity. They also provide anti-inflammatory activity as antioxidants. This could be the reason for the use of the plant in the treatment of intestinal troubles in herbal medicine.

Alkaloids are of therapeutic significance. The pure substance is used to repel parasites and predators. When ingested by animals, they affect glucagon, thyroid stimulatory hormone and inhibit some mammalian enzymatic activities. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal

agents due to their analgesic antispasmodic and antibacterial potentials. Tannins are known to improve wound healing and inflamed mucus membrane. The study showed high presence of tannins in both leaf and stem bark extracts supporting the strong use of *Spondias mombin* in healing wounds, various ulcers, frost-bite and burns in traditional herbal medicine. The tannins, flavonoids and alkaloids as seen in the results give credence to the reported anti- microbial, anti-viral, and anhelminthic properties of *Spondias mombin*. The stem back and leave extracts also contained the antioxidant vitamins C and E. Antioxidants repair free radical damages to the cells. The presence of antioxidant molecules suggests that *Spondias mombin* can be used as vitamin supplement probably during oxidative stressed conditions. The results of the study showed that the plant has high nutritive value which could attenuate physiological oxidative stress due to its high concentration of vitamin E and C as well as flavonoids contents. The presence of vitamin C in the extracts may confirm the reported wound healing property of the plant (28). The presence of vitamin C in *Spondias mombin* leaves and stem bark, implies that it can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer (**Maduka *et al.*, 2014**).

2.1.2 Use of *Spondias mombin*

The tree is commonly used for living fences, in farmlands and shelter by artisans. The fruits are edible. The extracted juice is used to prepare ice cream, cool beverages and jelly in Costa Rica and Brazil. In Amazon, the fruit is used mainly to produce wine sold as 'Vinho de Taperiba', while in Guatemala, it is made into a cider-like drink. It is used in Panama, Peru and Mexico in fairly large quantities as jams (**Ayoka *et al.*, 2008**). Thus, it has been evaluated as an unconventional source of vitamins A and C (**Keshinro *et al.*, 1985**).

The anti-bacterial, antimicrobial, antiviral and anti-fungal potentials of the plant have been reported. The plant was recommended for use by pregnant women after five months of pregnancy. The abortifacient activity of an aqueous extract of *Spondias mombin* was reported. The plant has been reported to be used in the treatment of many disease conditions in the Eastern part of Nigeria by the natives (**Maduka *et al.*, 2014**).

Some reported pharmacological activities include antibacterial (**Corthout *et al.*, 1994**), antiviral (**Corthout *et al.*, 1992**), anti-microbial (**Abo *et al.*, 1999**), anti-malarial (**Carabalo *et al.*, 2004**), anti-helmintic (**Ademola *et al.*, 2005**), molluscicidal (**Corthout *et al.*, 1994**), anti-diarrhoea (**Akubue *et al.*, 1983**), antiinflammation (**Abad *et al.*, 1996**), haemostatic (**Kone-Bamba *et al.*, 1987**), abortifacient (**Offiah and Anyanwu, 1989**), purgative (**Akubue *et al.*, 1983**), hypnotic (**Ayoka *et al.*, 2005**), wound-healing (**Villegas *et al.*, 1997)**.

Tannins are known to improve wound healing and inflamed mucus membrane. The study showed high presence of tannins in both leaf and stem bark extracts supporting the strong use of *Spondias mombin* in healing wounds, various ulcers, frost-bite and burns in traditional herbal medicine. The tannins, flavonoids and alkaloids as seen in the results give credence to the reported anti-microbial, anti-viral, and antihelminthic properties of *Spondias mombin*.

The stem back and leave extracts also contained the antioxidant vitamins C and E. Antioxidants repair free radical damages to the cells. The presence of antioxidant molecules suggests that *Spondias mombin* can be used as vitamin supplement probably during oxidative stressed conditions. The results of the study showed that the plant has high nutritive value which could attenuate physiological oxidative stress due to its high concentration of vitamin E and C as well as flavonoids contents.

The presence of vitamin C in the extracts may confirm the reported wound healing property of the plant. The presence of vitamin C in *Spondias mombin* leaves and stem bark implies that it can be used in herbal medicine for the treatment of common cold and other diseases like prostate cancer (**Maduka *et al.*, 2014**).

2.2 Distillers grains in poultry diets

Increased supplies of distiller's dried grains with solubles (DDGS) in the Midwest have rekindled the interest in utilization of this by-product in animal feeds. With increasing numbers of chicken layers and a large turkey industry in the Midwest, use of DDGS in poultry diets appears to have potential (**Noll *et al.*, 2001**).

It's a byproduct from beverage alcohol production and more recently from production of fuel alcohol, is an acceptable feed in poultry industry (**Wang *et al.*, 2007**).

In the dry mill production of ethanol two products are produced – liquid solubles and grain residue. Each could be dried separately but are mixed together to form DDGS as a dry ingredient (**Noll *et al.*, 2001**).

The principle reason for broiler producers to select dietary ingredients is economy, because feed represents approximately 70% of the live production cost (**Wang *et al.*, 2007**).

2.2.1 Composition

DDGS as a feed ingredient has a moderate protein content and energy level similar to soybean meal (**Noll *et al.*, 2001**).

As a sole source of protein in diet, **Parsons and coworkers (1983)** found DDGS to be limiting in tryptophan and arginine after lysine (**Noll *et al.*, 2001**).

An early use of DDGS in poultry diets was primarily as a source of unidentified factors that promote growth and hatchability. Distillers dried soluble (DDS) or DDGS were used in diets at low levels of inclusion usually less than 10% (**Couch *et al.*, 1957**) found 5% inclusion of DDS variably improved turkey growth rates with the response ranging from 17-32% (**Noll *et al.*, 2001**).

2.2.2 Functions

Broiler body weight improvements were recorded responsive to DDS and DDGS in broiler diets at 2.5 and 5% in one of 3 trials. Improved reproductive performance has also been indicated for turkey breeder hens. (**Couch *et al.*, 1957**) found improvements in turkey breeder hatchability during the second half of lay with inclusion of dried alfalfa meal, condensed fish solubles, and DDS. **Manley *et al.*** (**1978**) found 3% DDGS improved egg production in hens late in lay and experiencing a low rate of egg production. In diets low in phosphorus DDGS was particularly valuable in improving egg production (**Noll *et al.*, 2001**).

Alenier *et al.* (**1981**) noted chicken layer hens preferred rations containing 10% DDGS or 15% DDS over a corn-soy diet without DDGS (**Noll *et al.*, 2001**).

Use of DDGS has also been examined at high levels of inclusion. When lysine levels were adjusted in turkey diets, similar body weights were obtained with DDGS inclusion up to 20% of the diet to 8 wks of age; but feed conversion worsened (**Potter *et al.*, 1966**). **Parsons *et al.* (1983**) found that DDGS could replace up to 40% of soybean meal protein when lysine content was adjusted without an effect on body weight. When energy is also adjusted body weights and feed conversions are not affected by inclusion of distillers to high levels (**Noll *et al.*, 2001**).

Waldroup *et al.* (1981) included DDGS to 25% of diet for broilers. When adjusted for lysine and energy level, performance was not affected. Without adjustment for energy, growth was maintained but feed conversion decreased (**Noll *et al.*, 2001**).

As distiller grains undergo heating to produce the dried product, concern exists over amino acid digestibility especially for heating of lysine in the presence of sugars. Indeed, the limited literature citations indicate poorer availability of lysine. **Combs *et al.* (1969**) found lysine availability to range from 71-93% by chick growth assay. **Parsons *et al.* (1983**) found slightly lower availability of 66% by chick growth assay. Lysine digestibility with roosters was found to be 82%. Other sources also assign a low digestibility to DDGS (**Noll *et al.*, 2001**).

Digestible amino acid content of the DDGS used in this project was much better than reported elsewhere (**Noll *et al.*, 2001**).

2.3 *Bacillus subtilis*

Probiotics are defined as viable microorganisms (bacteria or yeasts) that exhibit a beneficial effect on the health of the host when they are ingested. The use of these products in poultry feeds has been extensively reviewed.

Increased awareness of the potential problems associated with the use of antibiotics has stimulated research efforts to identify alternatives to their use as feed additives. Probiotics (direct-fed microbial) have been suggested as alternatives to the use of antibiotics in food animals (**Murry *et al.*, 2006**).

Probiotics are characterized as live microorganisms (e.g., bacteria and fungi) that when ingested by animals have beneficial effects in the prevention and treatment of diseases (**Fuller *et al.*, 989**; **Miles *et al.*, 1991** and **Havenaar *et al.*, 1992**).

2.3.1 Composition

The composition of probiotics most frequently used contains strains of lactic acid bacteria *(L. acidophilus, L. casei L. helveticus, L. lactis, L. salivarius, and L. plantarum*), all of which originally were natural intestinal strains (**Murry *et al.*, 2006**).

2.3.2 Use

Plausible reasons for the selection of lactic acid bacteria are that they have been demonstrated to inhibit the in vitro growth of many enteric human pathogens, including Salmonella Typhimurium, Staphylococcus aureus, Escherichia coli, Clostridium perfringens, and Clostridium difficile. Lactic acid bacteria have also been used in both humans and animals to treat a broad range of gastrointestinal disorders (**Murry *et al.*, 2006**).

Draw back from the review

1. Most of the studies were conducted on Livestock but not in poutry species more precisely on broiler chicken.
2. In addition benefits of using fermented products and dry leaves in the poultry ration and their additional merits on carcass have not been seen in recent researches in context of Bangladesh.
3. Unconventional feed ingredients effects and their alternative uses in commercial and backyard system has not evaluated properly based on their advantageous effects.

CHAPTER-III: METHODOLOGY

3.1 Trail area

The study was conducted in experimental poultry shed of Department of the Animal Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh.

3.2 Study Period

The overall research work was designed and performed from July 2017 to March 2018. July was considered as a representation of monsoon seasons (**Islam *et al.*, 2006**). In July, average maximum temperature records cited as 29 to 32°C and humidity was 78 to 82% (**BMD, 2017**).

3.3 Leaves Preparation

3.3.1 Collection of *Spondias* leaves:

Creampic, limp *Spondias* leaves were collected from several locality of Chittagong region from1st September to 15th October, 2017. The leaves samples were kept in 26°c and 47% humidity in a stainless steel jar at Nutrition Laboratory at Chittagong Veterinary and Animal Sciences.

3.3.2 Drying of leaves:

Shed -dry were performed at well maintained ventilated condition due to reservation of absolute dimension of ingredients of the leaves whereas temperature and relative humidity had been maintained 31°C and 47% gradually.

3.3.3 Grinding of leaves:

Dry leaves were grinded and then sterilized the grinded particles after ensuring that the moisture was 30%. Electrical grinder was used to perform the grinding by 3-12 µm particle size. This process has a significant effect as to invasion and utilization by microbes.

3.4 Preparation of probiotic

3.4.1 Isolation of bacteria and fungus

Five types of samples as the source of organism including Branded yogurt (Banoful having 5% fat contained coagulated milk and sugar) local branded yogurt (Genuine yogurt contained more than 3% fat and sugar), non-branded yogurt, a commercial probiotic and a sample that was imported from South Korea.

3.4.2 Media preparation and isolation for *Lactobaccilus* spp

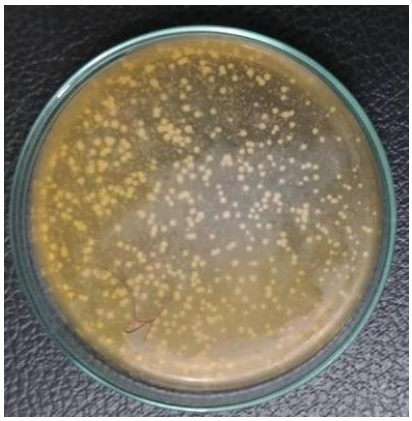


Figure 1. Isolation of

De Man, Rogosa and Sharpe agar (MRS agar®) (Company: Hi-Media Laboratories Pvt. Ltd. A-516, Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India) were dissolved in double distilled water and gently heated in electric heater and then autoclave it subsequently at 121°Cfor 15 minutes .Then sterile petridish were used to make media where following solution is conferred whole works were done under laminar flow (Biosafety cabinet class-II, vertical type., company: Labnics equipments) to ensure aseptic condition during lab work.

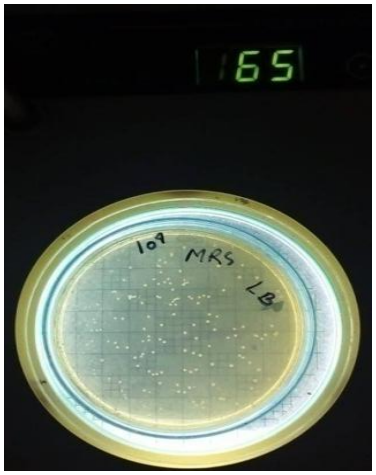


Figure 2. Counting of bacteria

After-that, Incubation was achieved at 37°C for 24 hours in incubator (Labnics equipments, Model-LGI-150T) (media preparation procedure were followed according to the instruction of level) .Then the sample was inoculated into MRS agar. Again, incubation performed at 37°Cfor duration of 72 hours. Then the result had been interpreted by MPN (Most probable number) technique through counting bacteria (*Lactobacillus* spp) with a digital colony counter (Brand: JP Selecta., Spain, Model: member, Spain. After confirmation of identifying the *Lactobaccilus* spp, the organisms were stocked in Brain-heart infusion broth.

3.4.3 Preparation and procedure for stocking the culture of *Lactobaccilus* spp

About 5 ml of broth was taken in a test tube. Then, autoclaved elaborated (BHI) at 121°Cfor 15 minutes. After cooling at room temperature, 2-3 colonies are mixed with BHI broth. After-that, incubation was done at 37°C for 12 hours. Consequently, bacterial growth properly stocked with 50% glycerol in an eppendorf tube. Then, 700 µl bacterial broth and 300µl of 50% glycerol were mix gently in a cryovial. Finally, preserved it at -80°C in a refrigerator (Esco , Model: UUs-4398-1, USA).

3.4.4 Media preparation for *Sacaromyces*

Potato dextrose agar (PDA) were dissolved in double distilled water and gently heated in electric heater and autoclaved it subsequently at 121°C for 15 minutes. Sterile petridish were used to make a media where following solution were conferred. All works were done under laminar flow to avoid the contamination. Again incubated at 37°C for 24 hours (media preparation procedure was followed according to the instruction of the manufacturer). Then, the samples were spreading in PDA agar by spreader and incubate at 37°Cfor 72 hours. Finally, the results were interpreted by counting of bacteria (*Sacaromyces* spp) with digital colony counter. After observing organisms (*Sacaromyces* spp.*)* were stocked in Brain-heart infusion broth.

3.4.5 Sample preparation

Figure 3. Isolation of *Sacaromysis*

Desired organisms source such branded, non-branded and local yogurt, commercial proboitics and exported proboitics were taken at the rate of 1ml. These were mixed with 9ml phosphate buffer saline (PBS) separately. However, MRS ager plates were used for *Lactobaccilus* and PDA for *Sacaromyces.* Preparation of nutrient broth was done for multiplication of bacteria and 4-5 colonies were added with 10 ml nutrient broth and incubated it at 37°C for 24hours in a shaking incubator .After observing considerable amount of bacterial colony and fungal growth, attempts were done to mixed 10 ml broth with 1littre broth individually. Shaking incubator was used for better growth of microbes at 37°C for 48 hours and serial dilution has performed for counting organism.

3.4.6 Serial dilution:

Firstly, 10 autoclaved and consecutive numbered glass test tubes were taken and poured with 9 ml phosphate buffer solution (PBS) individually, add 1ml bacterial broth in first one and mixed rapidly and 1ml mixed solution is taken to mixed with 2nd and the procedure had been followed for the rest, separate tips were used for each dilution to get accurate count and avoidance of the contamination. 1ml solution had been discarded from the 10thtest tube.

However, streaking of the diluted cultures in the MRS and PDA media plate, 24 hours' incubation were done at 37°c and finally the number of bacteria has been identified by counting bacterial colonies. Similar procedure was followed for *Saccharomyces* except counting. *Sacharomyces* were identified by cultural characters.

Figure 4. Brooding of Chicks

3.5 Probiotics trial mixure:

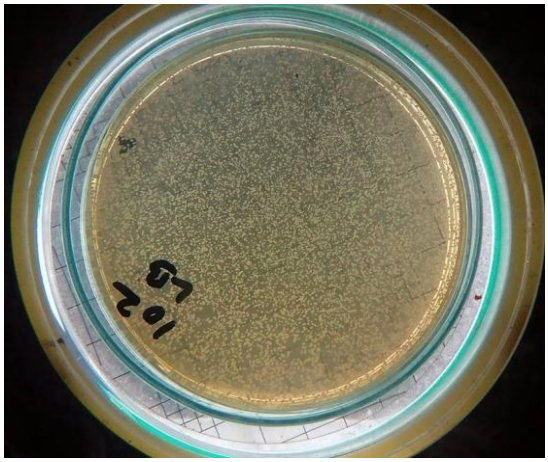


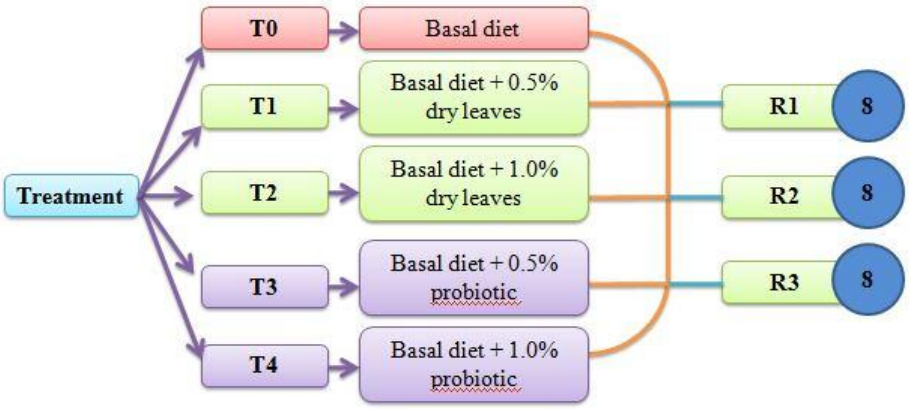
Figure 5 : Two fold dilution of bacteria

35% DDGS® (Distilled dried grains with soluble, Prorich Agro Foods, SCO 35, Distt: Mohali 140603 Punjab) + 35% DFRB®(Defatted rice bran, Prorich Agro Foods) + 30% Dry leaves grinded powder were mixed homogenously in sterile stainless steel jar. 100ml culture of *Lactobacillus*, About 100 ml culture of *Sacharomyces* and adequate amount of chlorination free water were added with the mixture. Then it was poured into a plastic bag. Incubation was done the mixture at 37°C for 72 hours in shaking incubator and counting the number of organism by serial dilution. Then preserved the incubated mixture at room temperature after shed drying .

3.6 Design of the experiment

A total of 120 birds were randomly distributedinto five dietary treatment groups: T_0 = Control (basal diet), T_3: fermented leaves (basal diet + 0.5% on DM basis), T_4: fermented leaves (basal diet + 1.0% on DM basis), T_1: dry leaves (basal diet + 0.5% on DM basis) and T_2: dry leaves (basal diet + 1.0% on DM basis) consisting 3 replications having 8 birds in a completely randomized design for 28 days.

Figure 6. Temperature and humidity maintain in Brooding period



3.7Animals and housing

One hundred and twenty days old unsexed broiler chicks of Ross 308® were purchased from Nahar Agro Complex Limited, address Chittagong, Bangladesh. All the chicks were examined for abnormalities and uniform size. Average body weight of the chicks was 40.74±0.26 g. The experimental shed was brick cemented with corrugated metal wiring. Floor space for each bird was 0.17 square feet in brooding box and 0.75 square feet in the cage. The cages were further divided into 20 pens. The pens were selected in an unbiased way for uniform distribution of chicks. The chicks were brooded for two weeks. Each pen was allocated for 8 birds. Dry and clean newspaper was placed in the brooding box and changed for every 6 hours. Room temperature and humidity was maintained using 200 watt incandescent lamps and

ceiling fans. The birds were exposed to continuous lighting. During brooding period, chicks were brooded at a temperature of 95 °F, 90 °F, 85 °F and 80 °F for the 1st, 2nd, 3rd and 4th weeks, respectively with the help of incandescent bulbs. Temperatures were measured by using thermometer.

3.8 Cleaning and sanitation

The shed was thoroughly cleaned and washed by using tap water with caustic soda. For disinfection, phenyl solution (1% v/v) was sprayed on the floor, corners and ceiling. Following spray, cleaning was done by using brush and clean water. Brooding boxes, rearing cages and pens were cleaned in the same manner. After cleaning and disinfection, the house was left one week for proper drying. After drying, all doors and windows were closed. The room was fumigated (Adding 35 ml of formalin to 10 g potassium permanganate per cubic meter) and sealed for 24 hours. On the next day, lime was spread on the floor and around the shed. Footbath containing potassium permanganate (1% w/v) was kept at the entrance of the poultry shed and changed daily. Feeders were cleaned and washed with Temsen® solution (0.3% v/v) weekly before being used further. Drinkers were washed with potassium permanganate (1% w/v) and dried up daily in the morning.

3.9 Experimental diets

Feed ingredients were purchased from Pahartali market, Chittagong, Bangladesh. During purchase, cleanliness and date of expiry were checked. T_0 = Control (basal diet), T_2: dry leaves (basal diet + 1.0% on DM basis), T_3: fermented leaves (basal diet + 0.5% on DM basis), T_1: dry leaves (basal diet + 0.5% on DM basis) and T_4: fermented leaves (basal diet + 1.0% on DM basis) consisting 3 replications having 8 birds in a completely randomized design for 28 days. Five different types of rations were formulated. Each ration had two different types i.e., starter (0 to 14 days) and finisher (15 to 28 days). All rations were iso-caloric and iso-nitrogenous. The composition of different feed ingredients and nutritive value of starter and grower rations are given in Table 1 and

Table 1. Ingredients names with their chemical composition of the broiler starter ration (1-14 days)

Ingredients (as percent feed basis)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Maize	59.50	59.25	59.00	59.25	59.00
Rice polish	1.50	1.25	1.00	1.25	1.00
Soyabean meal	33.20	33.20	33.20	33.20	33.20
Vegetable oil	2.25	2.25	2.25	2.25	2.25
Supplement	0.00	0.50	1.00	0.50	1.00
Molasses	0.30	0.30	0.30	0.30	0.30
Limestone	1.50	1.50	1.50	1.50	1.50
Vit-min premix[1]	0.20	0.20	0.20	0.20	0.20
Common salt	0.25	0.25	0.25	0.25	0.25
DCP[2]	1.00	1.00	1.00	1.00	1.00
DL-Methionine[3]	0.25	0.25	0.25	0.25	0.25
L-Lysine[4]	0.02	0.02	0.02	0.02	0.02
Toxin binder[5]	0.03	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.0
Estimated chemical composition					
Met. Energy[6](kcal/kg)	2965.82	2965.75	2965.13	2966.23	2966.30
Crude protein (%)	20.65	20.63	20.63	20.68	20.70
Crude fiber (%)	3.90	4.10	4.50	4.00	4.40
Calcium (%)	0.94	0.98	1.00	1.10	1.30
Phosphorus (%)	0.75	0.65	0.60	0.80	0.84
Lysine (%)	1.20	1.14	1.17	1.17	1.60
Methione (%)	0.54	0.57	0.60	0.65	0.67
Cysteine and Methionine (%)	0.74	0.75	0.73	0.70	0.67
Tryptophan(%)	0.25	0.23	0.22	0.32	0.29

T_0=Diet without treatment; T_1=Diet containing 0.5% dry leaves; T_2=Diet containing 1% dry leaves; T_3=Diet containing 0.5% probiotics; T_4=Diet containing 1% probiotics; [1]Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D3 1000 IU, K 1.6 mg, B1 1 mg, B2 2 mg, B3 16 mg, B6 1.6 mg, B9 320 µg, B12 4.8 µg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 µg); [2]DCP (18% P, 23% Ca); [3]DL-Methionine (Purity 99.0%); [4]L-Lysine (Purity 99.0%); [5]Toxin Binder (Purity 98%, all imported from Poland); [6]Metabolizable energy (kcal/kg).

Table 2. Ingredients names with chemical composition of the broiler grower ration (15-28 days)

Ingredients (as percent feed basis**)**	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**
Maize	60.10	59.95	59.60	59.95	59.60
Rice polish	1.85	1.60	1.35	1.60	1.35
Soyabean meal	31.00	31.00	31.00	31.00	31.00
Vegetable oil	3.50	3.50	3.50	3.50	3.50
Suppliment	0.00	0.50	1.00	0.50	1.00
Molasses	0.30	0.30	0.30	0.30	0.30
Limestone	1.50	1.50	1.50	1.50	1.50
Vit-min premix[1]	0.20	0.20	0.20	0.20	0.20
Common salt	0.25	0.25	0.25	0.25	0.25
DCP[2]	1.00	1.00	1.00	1.00	1.00
DL-Methionine[3]	0.25	0.25	0.25	0.25	0.25
L-Lysine[4]	0.02	0.02	0.02	0.02	0.02
Toxin binder[5]	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100
Estimated chemical composition					
Met. Energy[6](kcal/kg)	3057.9	3057	30.57.5	3057.75	3058
Crude protein (%)	19.75	19.59	19.45	19.74	19.78
Crude fiber (%)	6.17	6.22	6.25	6.21	6.23
Calcium (%)	0.94	0.92	0.9	0.97	0.99
Phosphorus (%)	0.73	0.7	0.68	0.8	0.89
Lysine (%)	1.05	1.04	1.02	1.25	1.35
Methione (%)	0.53	0.52	0.51	0.6	0.68
Cysteine and Methionine (%)	0.74	0.73	0.72	0.67	0.64
Tryptophan (%)	0.23	0.22	0.21	0.22	0.25

T_0=Diet without treatment; T_1=Diet containing 0.5% dry leaves; T_2=Diet containing 1% dry leaves; T_3=Diet containing 0.5% probiotics; T_4=Diet containing 1% probiotics; [1]Vitamin-mineral premix (Per kg vitamin mineral premix provided-Vitamin A 5000 IU, D3 1000 IU, K 1.6 mg, B1 1 mg, B2 2 mg, B3 16 mg, B6 1.6 mg, B9 320 µg, B12 4.8 µg, Cu 4 mg, Mn 40 mg, Zn 20 mg, Fe 2.4 mg, I 160 µg); [2]DCP (18% P, 23% Ca); [3]DL-Methionine (Purity 99.0%); [4]L-Lysine (Purity 99.0%); [5]Toxin Binder (Purity 98%, all imported from Poland); [6]Metabolizable energy (kcal/kg).

3.10 Feeding of birds

Feed was prepared manually and supplied ad-libitum to the birds on round small feeder and waterer for 0-7 days. After 7th day, small round feeders and waterers were replaced by medium linear feeders (2.21 ft X 0.25 ft) and round waterers. At 15th day, large linear feeder (3.5 ft X 0.38 ft) and round waterers (3-liter capacity) were provided for feeding and drinking of the birds.

3.11 Medications

All birds were vaccinated against Newcastle disease (BCRDV live) and Infectious Bursal Disease on the 4th day followed by a booster dose on 14th day. After each vaccination, multivitamin (Rena-WS, Renata; 1g/ 5liter of drinking water) was supplied along with Vitamin-C to overcome the effect of stress due to vaccination and cold shock.

Figure 7: Vaccination of the birds

3.12 Carcass measurement

At the end of the 28 day trail, four birds were randomly selected from each replicate and killed by severing the jugular vein and carotid artery. Once a bird was adequately bleed out, it was scalded and feather was removed. After de-feathering, the birds were eviscerated and the head and feet were removed as per technique described by Jones (1984). During evisceration process, abdominal fat, lung, liver, kidney, spleen, gizzard and proventriculus were excised separately and weighed. Dressed birds were weighed to obtain a dressed carcass weight.

Figure 8 : Separating organ

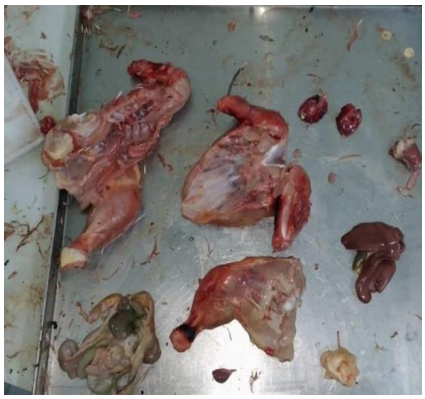


Figure 9 : Parts of carcass

Figure 10 : Weighing viscera

Figure 11 : Meat sample preparation

Figure 12 : Packing meat sample

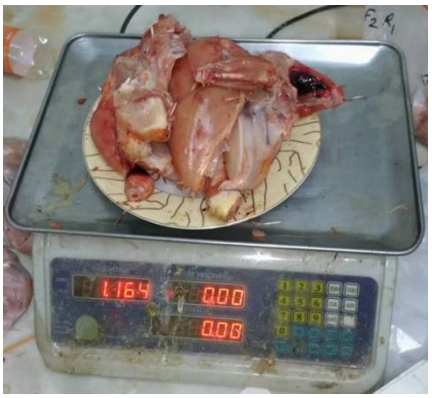


Figure 13: Carcass measurement

3.13 Analysis of feed and meat

From each treatment, 100 g of prepared feed was taken and preserved in an air tight bag to carry them in the laboratory for analysis during the experimental period. After slaughter, 120 g of meat was collected in the air tight bag from each carcass for estimation of the chemical composition of meat. Feed and meat samples were kept at 5°C in air tight bag. After that, chemical analyses of the feed and meat samples were carried out in triplicate for dry matter (DM), crude protein (CP), crude fiber (CF), nitrogen free extracts (NFE), ether extracts (EE) and total ash (TA) in the animal nutrition laboratory, Chittagong Veterinary and Animal Sciences University, Chittagong as per AOAC (2006).

3.14 Hematological analysis

Blood samples were collected from the brachial vein of four birds from each group (Two birds from each replicate) using a 3 ml sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant, ethylene diamine tetra acetic acid and 5ml blood was kept without anticoagulant.

3.15 Serum analysis

Blood was collected without anticoagulant from a total of four birds from each group at 28th days of age. Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the ependroff tube by micropipette. Sera samples were marked and stored in -20°C until being analyzed for LDL, HDL, total cholesterol. Randox® veterinary reagent kits were used for determination of the blood parameter of interest. Serum sample was mixed with the respective reagents in an ependroff tube. The serum with reagent was aspired by spectrophoto-metric method which measured the target parameter and immediately the printed result was recorded.

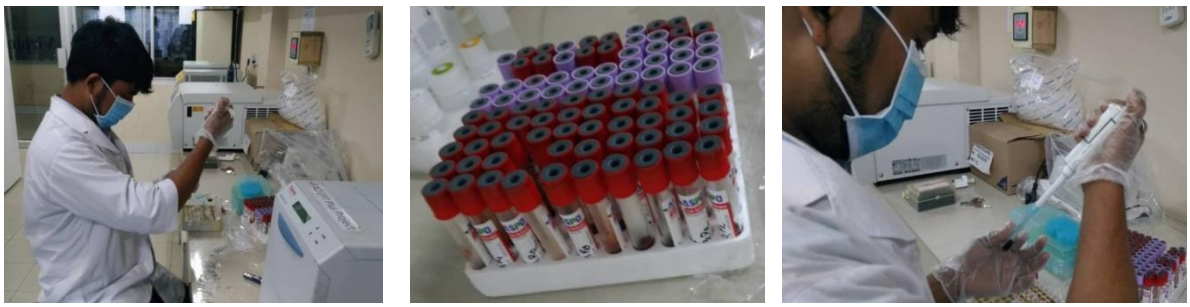


Figure 8 : Hematological sample preparation

3.16 Data collection

Weight gain, feed intake and FCR were recorded at weekly intervals. Carcass characteristics, hematological and biochemical parameters were recorded at 4th weeks. Weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed intake was calculated by deducting leftover from the total amounts of feed supplied to the birds. FCR was calculated dividing feed intake by the weight gain.

Figure 9: Measurement of different aged bird

Figure 10: Counting weight of birds weekly

3.17 Data analysis

Data were compiled in MS Excel. Raw data related to weight gain, feed intake, FCR, carcass characteristics, hematological and biochemical parameters were tested for normality by using normal probability plot and analyzed for ANOVA by using **STATA (2017).** Means showing significant differences were compared by Duncan's Multiple Range Test **(Duncan, 1955).** Statistical significance was accepted at $p<0.05$ for F-tests.

CHAPTER-IV: RESULTS

The objective of the conducted study was to observe and interpret effects of fermented probiotics and *Spodias* leves by observing growth performance, carcass characteristics and biochemical parameters of Ross-308 broilers.

Composition used in the treatment groups

Table 3. Organisms concentration used in T_3 and T_4 treatment group with probiotics.

Microbs(cfu/g)	**Solid substrate fermentations(SSF)**	**Submerged liquid fermentation (SLF)**
Lactobacillus acidophilus	1.1×10^{9}	4.0×10^{8}
Sacharomyces cerevisiae	1.5×10^{7}	1.0×10^{4}

Table 4. Estimated chemical composition (DM basis) of *Spondias* leaves

Parameters	Amount
Metabolizable Energy (Kcal/kg)	6565.26
Moisture (%)	15.13
Crude Protein (gm/100gm)	11.04
Crude Fiber (gm/100gm)	10.51
Calcium (gm/100gm)	10.85
Phosphorous (gm/100gm)	9.50
Vitamin.-C	58.05
Vitamin –A	5.60

Table 5. Estimated chemical composition (DM basis) of Probiotic

Parameter	**Amount**
Metabolizable Energy (Kcal/kg)	2957.35
Moisture (%)	29.50
Crude Protein (gm/100gm)	20.04
Crude Fiber (gm/100gm)	4.51
Calcium (gm/100gm)	8.85
Phosphorous (gm/100gm)	6.50

Table 6. Estimated chemical composition (DM basis) of *Spondias monbins* leaves

Component	Amount	Component	Amount
Sponins (%)	4.8	CF (%)	10.51
Alkaloids (%)	3.4	Ca (mg/100g)	10.85
Flavonoids (%)	2.8	K (mg/100g)	9.5
Tanins (%)	1.47	Na (mg/100g)	0.81
Oxalates (%)	0.92	Mg (mg/100g)	0.39
Phytates (%)	1.73	P (mg/100g)	1.1
CHO	68.92	Se (mg/100g)	0.24
Moisture (%)	15.13	Vit.C (mg/100g)	58.05
CP (%)	11.04	Vit. A (mg/100g)	5

4.1 Live Weight

The results shows that there is a significant difference ($p < 0.05$) among the different dietary groups (Table 6). During 3rd and 4th week of trial, all treatment groups carries significant improvement of live weight compared to control whereas T2 and T1 shows decline performance at period of 2nd week and all the treatment groups drastically decrease than control at 1st week.

Table 7. Effects of *Spondias mobins* tree leaves with or without probiotics on growth performance of broiler

Variables	Age birds of	Treatments					SEM	Level of Sig
		T_0	T_1	T_2	T_3	T_4		
Live weight (g)	1st week	218.09	187.64	171.34	187.96	179.08	0.34	NS
	2nd week	418.96^{c}	402.47^{e}	418.86^{d}	456.96^{a}	419.34^{b}	0.48	**
	3rd week	879.39^{e}	999.26^{d}	1087.31^{b}	1090.85^{a}	1025.42^{c}	1.82	***
	4th week	1873.99^{a}	1977.94^{d}	1986.04^{c}	1995.67^{b}	2071.17^{a}	2.64	***
Weight gain (g/bird/week)	1st week	177.99	147.64	131.34	147.96	139.08	0.34	NS
	2nd week	200.88^{e}	214.83^{d}	247.51^{b}	269.00^{a}	240.26^{c}	0.57	***
	3rd week	478.44^{e}	596.79^{d}	668.46^{a}	633.89^{b}	606.07^{c}	1.82	***
	4th week	976.59^{c}	978.68^{b}	898.72^{e}	904.82^{d}	1045.75^{a}	2.44	***
	Overall	1833.99^{e}	1937.95^{d}	1946.19^{c}	1955.67^{b}	2031.17^{a}	2.63	***
Feed intake (g)	1st week	268.52	186.18	176.087	175.75	173.13	0.59	NS
	2nd week	280.06	281.9	330.54	344.06	306.46	0.69	NS
	3rd week	647.24^{b}	783.31^{d}	877.19^{e}	768.37^{c}	555.89^{a}	2.27	***
	4th week	1347.08	1310.57	1212.49	1193.94	1376.99	0.001	NS
	Overall	2542.07^{b}	2561.95^{c}	2594.72^{d}	2434.17^{a}	2678.26^{e}	2.81	***
FCR	1st week	1.39^{e}	1.26^{c}	1.34^{d}	1.19^{a}	1.25^{b}	0.001	***
	2nd week	1.40^{e}	1.31^{b}	1.34^{d}	1.28^{a}	1.33^{c}	0.0009	***
	3rd week	1.37^{d}	1.31^{c}	1.31^{c}	1.21^{a}	1.27^{b}	0.001	***
	4th week	1.39^{e}	1.34^{c}	1.35^{d}	1.32^{b}	1.31^{a}	0.001	***
	Overall	1.39^{e}	1.31^{c}	1.33^{d}	1.27^{a}	1.30^{b}	0.001	***

^{abcde} Means with different superscipts in the same row differ significantly.

Data indicate means of 3 replications consisting of 8 birds per treatment (n=24).

T_0=Control; T_1= 0.5% dry leaves; T_2= 1.0% dry leaves; T_3= 0.5% probiotics; T_4= 1.0% probiotics; FCR = Feed conversion ratio, SEM=Standard error of means; NS = Nonsignificant ($p>0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)

4.2 Weight gain

Significant differences ($p<0.05$) were found in average weekly weight gain among different treatment groups (Table 6). On 3rd week, the highest and lowest weight gain were found between the T2 and C groups respectively. Maximum average weight gain was found at 4th week of age at T4 treatment group and lowest was in T1 group respectively. Whereas, T1, T2, T3, T4 groups showed increased weight gain at dramatically significant in 2nd, 3rd, 4th weeks respectively. Overall weight gain increased significantly compared to control.

4.3 Feed Intake

In case of feed intake there are also significant changes with different treatment groups **(**Table 6**)**. During the 3rd week, T2 and T4 groups showed the higher and lower feed intake while at 4th week of age C showed the higher feed intake and T3 indicated the lower feed intake per bird on 3rd and 4th week. In case of feed intake in total result assert that, T3 group is comparatively better the others including control group when other treatment groups told sorry tale.

4.4 Feed Conversion Ratio (FCR)

The FCR of different weeks of birds are shown in (Table 6). Remarkable difference also obtained for FCR among the treatment groups. On 3rd week, T3 treatment group showed the lowest FCR and C treatment group showed the higher FCR. At 4th week of age, T4 group showed lowest FCR and C treatment group showed higher FCR. The overall FCR value was also significantly decreased as compared to control (Table 6).

4.5 Serum Parameters

Different serum parameters estimated have been presented. HDL value ($p>0.05$) was found higher in T4 treatment group while in LDL ($p>0.05$), T1 group shows the lower average value and in case of total cholesterol ($p<0.005$) T1 group shows the lowest value that is statistically significant.

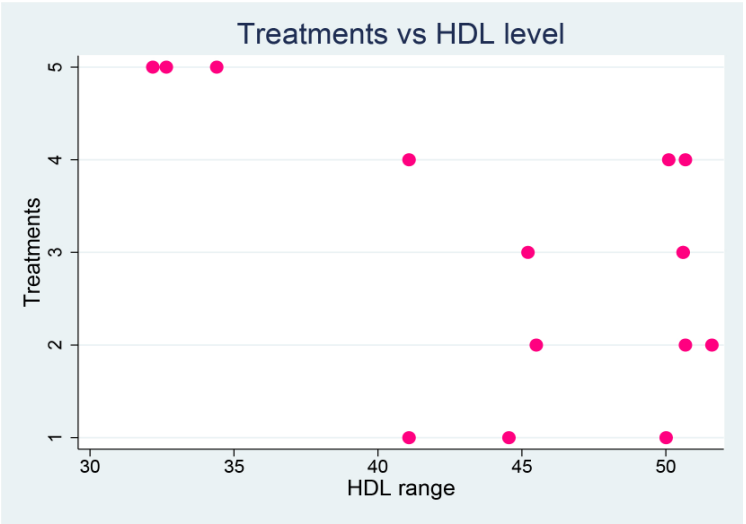
Table8 .Effects on HDL, LDL, and Total cholesterol for the corresponding feed trial

Parameter	Treatments					SEM	Level of Sig
	C	T_1	T_2	T_3	T_4		
HDL	33.08^{e}	48.80^{b}	47.29^{c}	45.21^{d}	49.26^{a}	1.1	***
LDL	122.71^{e}	79.78^{a}	100.93^{c}	102.87^{d}	89.03^{b}	3.37	***
Total cholesterol	177.8^{e}	138.43^{a}	143.33^{c}	145.6^{d}	140.1^{b}	2.62	***

^{abcde} Means with different superscipts in the same row differ significantly.

Data indicate means of 3 replications consisting of 8 birds per treatment (n=24)

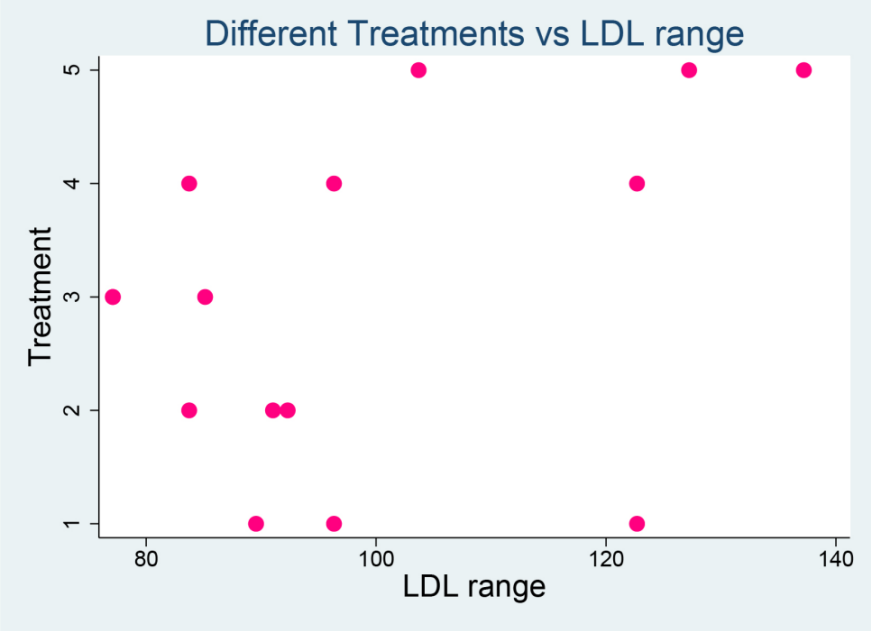
T_0=Control; T1= 0.5% dry leaves; T2= 1.0% dry leaves; T3= 0.5% probiotics; T4= 1.0% probiotics; FCR=Feed conversion ratio, SEM=Standard error of means; NS = Non significant ($p>0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)



At Y axis

5=T_0 (Control group)
1= T_3 treatment group
2=T_4 Treatment group
3=T_1 treatment group
4=T_2 treatment group

Figure 11. Level of cholesterol in comparison with different treatment group

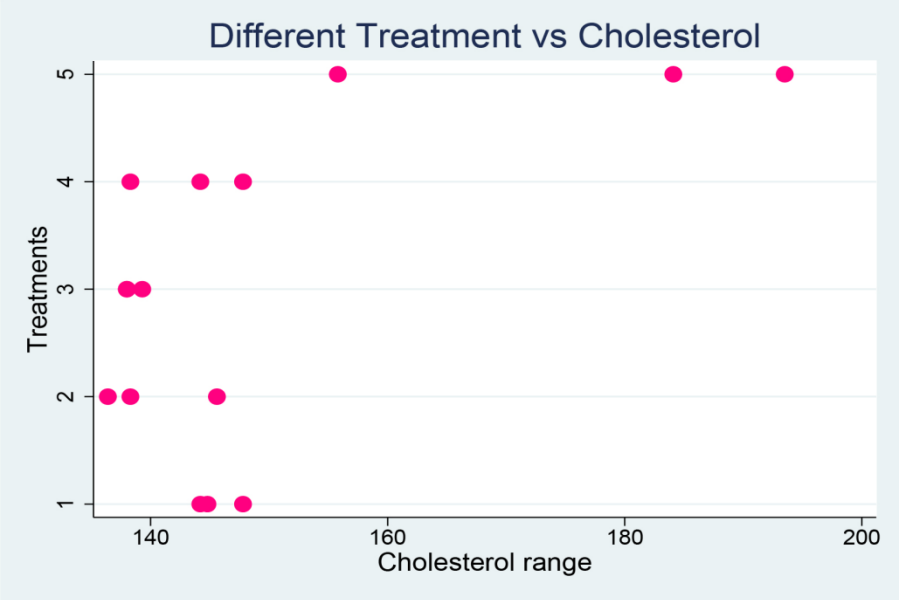


At Y axis

5=T_0 (Control group)

1= T_3 treatment group
2=T_4 Treatment group
3=T_1 treatment group
4=T_2 treatment group

Figure 12. Level of cholesterol in comparison with different treatment group



At Y axis

5=T_0 (Control group)
1= T_3 treatment group
2=T_4 Treatment group
3=T_1 treatment group
4=T_2 treatment group

Figure 13. Level of cholesterol in comparison with different treatment group

4.6 Chemical Composition of meat

Value of moisture, ether extract (EE), crude protein (CP) and ashes have been estimated from the lab tests of carcass of different treatment groups. The biochemical lab tests indicated that T_4 treatment group showed the lower moisture ($p> 0.05$). While higher CP ($p<0.005$) and EE values ($p<0.005$) were obtained from T_4 treatment group and T_1 treatment group respectively. Ashes showed that T_3 and T_4 group hold the highest proportion of total minerals ($p>0.05$) in comparison to other groups.

Table 9. Effects of *Spondias mobins* tree leaves with or without probiotics on meat proximate components in broiler

Parameters	Treatments					SEM	Level of Sig
	T_0	T_1	T_2	T_3	T_4		
Moisture (%)	77.76	76.27	77.13	76.68	76.46	0.848	NS
CP (%)	21.11^{d}	20.25^{e}	21.25^{c}	21.53^{b}	22^{a}	0.133	***
EE (%)	2.68^{c}	2.83^{e}	2.66^{b}	2.16^{a}	2.79^{d}	0.091	***
Total minerals (%)	0.88	0.903	0.86	0.903	0.903	0.006	NS

^{abcde} Means with different superscipts in the same row differ significantly. Data indicate means of 3 replications consisting of 8 birds per treatment (n=24). T_0=Control; T_1= 0.5% dry leaves; T_2= 1.0% dry leaves; T_3= 0.5% probiotics; T_4= 1.0% probiotics; SEM=Standard error of mean; NS=Nonsignificant ($P>0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)

4.7 Carcass Characteristics

Different carcass characteristics have been presented under this section. Comparative weights of different treatment groups among the carcass component (meat) and weights of different organs have been sequentially described. Live weights, spleen and breast meat were increased markedly ($p<0.005$) where T_4 and T_2 groups shows better result. Although other collected parts of carcass had no significant difference compared to control.

Table 10. Effects of *Spondias mombins* tree leaves with or without probiotics on carcass characteristics and organs weight in broilers

Parameters	Treatments					SEM	Level of Sig
	T_0	T_1	T_2	T_3	T_4		
Live weight	1874^{d}	1977.33^{c}	1998^{b}	1995.67^{c}	2075.33^{a}	23.74	***
Dressing (%)	64.75	65.25	65.01	63.73	64.36	0.44	NS
Drumstick (%)	9.44	10.06	1.011	9.57	9.69	0.125	NS
Thigh meat (%)	8.94	7.94	8.05	7.36	7.74	0.07	NS
Breast (%)	10.82^{d}	11.45^{b}	11.86^{a}	11.24^{c}	11.45^{b}	0.17	***
Intestinal (%)	5.37	5.07	5.10	5.66	5.51	0.27	NS
Abdominal fat (%)	0.83	0.59	0.70	1.15	0.64	0.06	NS
Bursa (%)	0.22	0.23	0.22	0.18	0.15	0.01	NS
Liver (%)	2.49	2.22	2.31	2.27	2.09	0.38	NS
Spleen (%)	0.11^{b}	0.10^{c}	0.12^{a}	0.07^{d}	0.10^{c}	0.01	***

[abcde] Means with different superscipts in the same row differ significantly. Data indicate means of 3 replications consisting of 3 birds per treatment (n=9). T_0=Control; T_1= 0.5% dry leaves; T_2= 1.0% dry leaves; T_3= 0.5% probiotics; T_4= 1.0% probiotics; SEM=Standard error of mean; NS=Nonsignificant (P>0.05); **=Significant (p<0.01); ***=Significant (p<0.001).

Table 11. Effects of Spondias mombins leaves with or without probiotics Cost-benefit analysis in broilers (BDT=Bangladeshi taka)

Parameter/bird	T_0	T_1	T_2	T_3	T_4
Live weight (kg)	1.87	1.97	1.98	1.99	2.07
Selling price	224.4	236.4	237.6	238.8	248.4
Total feed intake (kg)	2.5	2.56	2.5	2.4	2.67
Total Feed cost (BDT)	107.5	110.08	107.5	103.2	114.81
other cost (BDT)	50	50	50	50	50
Total cost (BDT)	157.5	160.08	157.5	153.2	164.81
Net profit (BDT)	66.9	76.32	80.1	85.6	83.59
Net profit /kg (BDT)	35.78	38.74	40.46	43.02	40.38

CHAPTER-V: DISCUSSION

Effects on performance of different probiotics and dry leaves treated chicks have been discussed during these 28 days of feed trial under this chapter. Weight gain, feed intake, feed conversion ratio, serological parameters, biochemical changes and carcass characteristics has been given more emphasis.

5.1 Weight gain

In the current study it was indicated that the weight gain in different treatment group shows a remarkable level of improvement in weight gaining in comparison with the control group. A study by **Mehedi *et al.* (2011)** showed quite relevance with this information, they used *Lactobacillus spp* as a probiotics organism that also suits with the objective of current study. They have found that weight gain performance significantly increased in probiotics treated groups in relation to the control group. Present study can also verify the same findings except 1^{st} week performance of the chicks. This might be due to the feed intake effect at very early stage. The newly carried chick needs to adjust some days with the dry leaves and probiotics treated feeds.

Between the dry leaves and probiotics treated groups, the groups with 1% probiotics mixure (*lactobacillus* and *saccharomyces* with spondias leaf of 30% of the composition of probiotics and 35% DDGS of the probiotics) showed the more improved weight gain performance. **Awad *et al.* (2015)** also complies with the same finding that at 35 days of treatment. The probiotics treated group performance is quite satisfactory than other groups (**Yang *et al.*, 2008)** also had given emphasis on the gut flora induced probiotics treatment efficacy on the chicks. **Zouris *et al.* (2010)** made the interpretation that probiotics (10^7 CFU) treated groups showed decreased weight gain performance. This might be due to difference in production process or presence of incompatible feed residues in the probiotics component. **Cark *et al.* (2008)** showed that weight gain increased in 7-21days among the dry leaves treated treatment flocks that also satisfy the objective of current study findings.

5.2 Feed intake

In present study, gradual increase in feed intake has been observable in 1.0% probiotics treated group that is more prominent figure than of control group. This finding also shows resemblance with study by **Mehdi *et al.* (2011).** According to this finding feed intake promptly increase at 28^{th} days at probiotics treated groups than control groups (**Mountzouris *et al.*, 2010)** implies that feed intake increased among the probiotics treated groups in 14^{th} day of age and at 28^{th} day; feed intake also increased at corresponding probiotics treated groups (**Ocark *et al.*, 2008)** indicated that feed intake is less in control group up to 7^{th} days of age and persisted up to 28^{th} days of age. So, feed intake is superiorly increasing among the dry leaves treated groups.

5.3 Feed conversion ratio

FCR is found more effective during 3^{rd} week in 0.5% probiotics treated groups than control groups while in comparison at 4^{th} week 1% probiotics treated groups showed decreased FCR than the control group. **Mehdi *et al.* (2011)** suggested that FCR was more increasing in control group during 7^{th} days of age but 14^{th} to 28^{th} days of age the performance of probiotics treated groups were much higher than of control groups. This finding also validate with the present study finding about the FCR relation with the probiotics treated groups.

5.4 Serum parameters

Estimation of serum parameters in the current study shows that there are significant changes in serum parameters among the various treatment groups. HDL concentration is found higher in 1% probiotics treated groups while found lower value in control group. In case, LDL, it's higher in control groups and found lower in 0.5% dry leaves treated group. Total cholesterol is also found higher in control groups and lowest value was found 0.5% dry leaves treated diet group. It might be due to synergistic effects of probiotics and plant phytochemicals. Decreased level of LDL and Total cholesterol components in treatment groups makes the efficacy of dry leaves treated groups while the increased level of HDL components also validate the efficacy of same treatment groups on serological components (**Yang *et al.*, 2009**) .

5.5 Biochemical composition of meat

Chemical estimation of the meat composition shows that moisture components is less in 0.5% dry leaves treated groups that the other treatment groups while Crude protein percentage is increased among the 1% probiotics treated groups while EE and Ashes are both higher among the 0.5% dry leaves treated groups. This proximate analysis shows both the 0.5% dry leaves and 1% probiotics treated groups' results more efficacy (**Anjum *et al.*, 2005**).

5.6 Carcass component

Carcass characteristics shows that, weight percentage shows increasing in 1% probiotics treated groups except, in thigh muscle weights more in control group whether drumstick shows higher weights in 1% dry leaves treated chick groups. Intestinal components also increased in T4 groups that will favor increased digestion (**Mehdi *et al.*, 2011)** also interpreted similarly in the study that more intestinal content is measured from the chicks of probiotics treated groups (**Anjum *et al.*, 2005**).

CHAPTER-VI: CONCLUSION

The study investigated the effects of *Spondias* leaves and probiotic supplementation on the performance parameters, carcass characteristics and blood parameters in commercial broiler under intensive rearing system. Highest weight gain, optimum feed intake and better FCR were observed in birds fed diet containing 1% fermented probiotic supplement. There were drastically changes in the serum parameters in comparison with the reference level especially in LDL, HDL, Total cholesterol. Similar to performance parameter, carcass characteristics were improved in terms of breast muscles yield in *Spondias* leaves and probiotic supplemented group. The study, therefore, suggests that, dry leave and probiotic are a potential feed supplement with basal diet at an inclusion level of 1%. However, a long term investigation with larger sample size and multi-dimensional temporal pattern is suggested for increasing sensitivity and validity of the study under field condition. This is profitable from economic point of view as well as considering the improved survivability this might be adopted in commercial and backyard rearing as well.

CHAPTER-VII: RECOMMENDATIONS

The use of *Spondias* leaves and probiotic in feed is a relatively recent development in poultry production. In tropical production systems, this may play a pivotal role in providing antibiotic free feed and reducing pathogen load, thus having enormous potential as a sole component of a successful alternative programme of antibiotic. However, supplementation of dry leaves at 1.0% and probiotic at 1.0% level is recommended in regular feed of broiler for better growth but the long term effect of supplementation on productive performance of broilers should be investigated in future.

Due to some unavoidable constraints and technical limitations, some vital blood parameters like glucose, calcium, phosphorus and other trace minerals both in meat and feed were not analyzed. These parameters could have vital impact on human health and will explore new horizon for investigating those parameters as future study.

CHAPTER-VIII: REFERENCES

Abbas, H., Rizal, Y., Dharma, A., & Kompiang, I. P. (2007). Selection and identification of cellulase-producing bacteria isolated from the litter of mountain and swampy forest. *Microbiology Indonesia*, *1*(3), 7.

Abbas, H., Rizal, Y., Djulardi, A., & Muis, H. (2012). The effect of supplementation of micro nutrient on nutrient rice bran which fermented by Bacillus amyloliquefaciens. *Pakistan Journal of Nutrition*.

Abou-Elela, G. M., Abd-Elnaby, H., Ibrahim, H. A., & Okbah, M. A. (2009). Marine natural products and their potential applications as anti-infective agents. *World Applied Sciences Journal*, *7*(7), 872-880.

Ammor, S., Tauveron, G., Dufour, E., & Chevallier, I. (2006). Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: 1—Screening and characterization of the antibacterial compounds. *Food Control*, *17*(6), 454-461.

Anjum, M. I., Khan, A. G., Azim, A., & Afzal, M. (2005). Effect of dietary supplementation of multi-strain probiotic on broiler growth performance. *Pakista. Veterinary Journal*, *25*(1), 25-29.

Awad, W. A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, *88*(1), 49-56.

Ayoka, A. O., Akomolafe, R. O., Akinsomisoye, O. S., & Ukponmwan, O. E. (2008). Medicinal and economic value of Spondias mombin. *African Journal of Biomedical Research*, *11*(2).

Babu, K. R., & Satyanarayana, T. (1995). α-Amylase production by thermophilic Bacillus coagulans in solid state fermentation. *Process Biochemistry*, *30*(4), 305-309.

Bampidis, V. A., Christodoulou, V., Florou-Paneri, P., Christaki, E., Chatzopoulou, P. S., Tsiligianni, T., & Spais, A. B. (2005). Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. *British Poultry Science*, *46*(5), 595-601.

Barton, M. D. (2000). Antibiotic use in animal feed and its impact on human healt. *Nutrition Research Reviews*, *13*(2), 279-299.

Battan, B., Sharma, J., & Kuhad, R. C. (2006). High-level xylanase production by alkaliphilic Bacillus pumilus ASH under solid-state fermentation. *World Journal of Microbiology and Biotechnology*, *22*(12), 1281-1287.

Bedford, M. (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. *World's Poultry Science Journal*, *56*(4), 347-365.

Benedito de Barber, C., & Barber, S. (1978). Toxic constituents of rice bran, 2: Hemagglutinating activity of raw and heat-treated bran. *Revista de Agroquimica y Tecnologia de Alimentos*.

Besong, S., Jackson, J. A., Hicks, C. L., & Hemken, R. W. (1996). Effects of a Supplemental Liquid Yeast Product on Feed Intake, Ruminal Profiles, and Yield, Composition, and Organoleptic Characteristics of Milk from Lactating Holstein Cows1. *Journal of Dairy Science*, *79*(9), 1654-1658.

Bradley, G. L., & Savage, T. F. (1995). The effect of autoclaving a yeast culture of Saccharomyces cerevisiae on turkey poult performance and the retention of gross energy, and selected minerals. *Animal Feed Science and Technology*, *55*(1-2), 1-7.

Byczkowski, J. Z., & Gessner, T. (1988). Biological role of superoxide ion-radical. *International Journal of Biochemistry*, *20*(6), 569-580.

Chen, K. L., Kho, W. L., You, S. H., Yeh, R. H., Tang, S. W., & Hsieh, C. W. (2009). Effects of Bacillus subtilis var. natto and Saccharomyces cerevisiae mixed fermented feed on the enhanced growth performance of broilers. *Poultry Science*, *88*(2), 309-315.

Çınar, M., Çatlı, A. U., Küçükyılmaz, K., & Bozkurt, M. (2009). The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. *South African Journal of Animal Science*, *39*(3).

Cross, D. E., Acamovic, T., Deans, S. G., & McDevitt, R. M. (2002). The effects of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. *British Poultry Science*, *43*, S33-S35.

Cross, D. E., McDevitt, R. M., Hillman, K., & Acamovic, T. (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science*, *48*(4), 496-506.

Cumby, T. R. (1986). Design requirements of liquid feeding systems for pigs: A review. *Journal of Agricultural Engineering Research*, *34*(3), 153-172.

Cummings,J.H.,& Macfarlane, G. T. (2002). Gastrointestinal effects of prebiotics. *British Journal of Nutrition*, *87*(S2), S145-S151.

Daeschel, M. A. (1989). Antimicrobial substances from lactic acid bacteria for use food preservatives. *Food Technol*, *43*, 164-167.

Demir, E., Sarica, Ş., Özcan, M. A., & Sui Mez, M. (2003). The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *British Poultry Science*, *44*(S1), 44-45.

Deniz, G., Orhan, F., Gencoglu, H., Eren, M., Gezen, S. S., & Turkmen, I. I. (2007). Effects of different levels of rice bran with and without enzyme on performance and size of the digestive organs of broiler chickens. *Revue De Médecine Vétérinaire*, *158*(7), 336.

Doyle, M. P., & Erickson, M. C. (2006). Reducing the carriage of foodborne pathogens in livestock and poultry. *Poultry Science*, *85*(6), 960-973.

Ehrlich, G. G., Goerlitz, D. F., Bourell, J. H., Eisen, G. V., & Godsy, E. M. (1981). Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. *Applied and Environmental Microbiology*, *42*(5), 878-885.

Ehrmann, M. A., Kurzak, P., Bauer, J., & Vogel, R. F. (2002). Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. *Journal of Applied Microbiology*, *92*(5), 966-975.

El Bendary, M. A. (2006). Bacillus thuringiensis and Bacillus sphaericus biopesticides production. *Journal of Basic Microbiology*, *46*(2), 158-170.

European Food Safety Authority (EFSA). (2008). Safety and efficacy of Ecobiol®(Bacillus amyloliquefaciens) as feed additive for chickens for fattening- Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed. *EFSA Journal*, *6*(8), 773.

Food, J. (2002). Agriculture Organization of the United Nations and World Health Organization Working Group: Report on Drafting Guidelines for the Evaluation of Probiotics in Food.

Fuller, R. (1989). Probiotic in man and animals. *Journal Applied Bacteriology*, *66*, 131-139.

Gao, J., Zhang, H. J., Yu, S. H., Wu, S. G., Yoon, I., Quigley, J., ... & Qi, G. H. (2008). Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poultry Science*, *87*(7), 1377-1384.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, *125*(6), 1401-1412.

Hayat, J., Savage, T. F., & Mirosh, L. W. (1993). The reproductive performance of two genetically distinct lines of medium white turkey hens when fed breeder diets with and without a yeast culture containing Saccharomyces cerevisiae. *Animal Feed Science and Technology*, *43*(3-4), 291-301.

Hu, J., Lu, W., Wang, C., Zhu, R., & Qiao, J. (2008). Characteristics of solid-state fermented feed and its effects on performance and nutrient digestibility in growing-finishing pigs. *Asian-Aust. Journal of Animal Science*, *21*(11), 1635-1641.

Hunt, J. H., Lyons, J. J., & Vandepopuliere, J. M. (1997). Corn stillage as a feedstuff for broilers and turkeys. *Journal of Applied Poultry Research*, *6*(3), 310-318.

Jay, J. M. (1982). Antimicrobial properties of diacetyl. *Applied and Environmental Microbiology*, *44*(3), 525-532.

Jensen, B. B. (1998). The impact of feed additives on the microbial ecology of the gut in young pigs. *Journal of Animal Feed Science*, *7*(Suppl 1), 45-64.

Kalavathy, R., Abdullah, N., Jalaludin, S., & Ho, Y. W. (2003). Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science*, *44*(1), 139-144.

Karaoglu, M., & Durdag, H. (2005). The influence of dietary probiotic (Saccharomyces cerevisiae) supplementation and different slaughter age on the performance, slaughter and carcass properties of broilers. *International Journal of Poultry Science*, *4*(5), 309-316.

Kashket, E. R. (1987). Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. *FEMS Microbiology Reviews*, *3*(3), 233-244.

Kermanshahi, H., & Rostami, H. (2006). Influence of supplemental dried whey on broiler performance and cecal flora.International. *Journal of poultry Science* ,(6), 538-543.

Keshinro, O. C. (1985). The unconventional sources of ascorbic acid in the tropics. *Nutrition Reports International*.

Koc, F., Samli, H., Okur, A., Ozduven, M., Akyurek, H., & Senkoylu, N. (2010). Effects of Saccharomyces cerevisiae and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulgarian Journal of Agricultural Science*, *16*(5), 643-650.

Koc, F., Samli, H., Okur, A., Ozduven, M., Akyurek, H., & Senkoylu, N. (2010). Effects of Saccharomyces cerevisiae and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. *Bulgarian Journal of Agricultural Science*, *16*(5), 643-650.

Kompiang, I. P., Sinurat, A. P., Purwadaria, T., & Darma, J. (1995). Cassapro in broiler ration: Interaction with rice bran. *Indonesian Journal of Animal and Veterinary Sciences*, *1*(2), 86-88.

Kong, S., & Davison, A. J. (1980). The role of interactions between O2, H2O2,· OH, e− and O2− in free radical damage to biological systems. *Archives of Biochemistry and Biophysics*, *204*(1), 18-29.

Lindgren, S. E., & Dobrogosz, W. J. (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentations. *FEMS Microbiology Letters*, *87*(1- 2), 149-164.

Lu, M. Y., Maddox, I. S., & Brooks, J. D. (1998). Application of a multi-layer packed-bed reactor to citric acid production in solid-state fermentation using Aspergillus niger. *Process biochemistry*, *33*(2), 117-123.

Maduka, H. C. C., Okpogba, A. N., Ugwu, C. E., Dike, C. C., Ogueche, P. N., Onwuzurike, D. T., & Ibe, D. C. (2014). Phytochemical, antioxidant and microbial inhibitory effects of Spondias mombin leaf and stem bark extracts. *Journal of Pharmaceutical and Biological Science*, *9*(2), 14-17.

Mathew, A. G., Chattin, S. E., Robbins, C. M., & Golden, D. A. (1998). Effects of a direct-fed yeast culture on enteric microbial populations, fermentation acids, and performance of weanling pigs. *Journal of Animal Science*, *76*(8), 2138-2145.

Miles, R. D., & Bootwalla, S. M. (1991). Direct-fed microbials in animal production. *Direct-fed microbials in animal production. A review*, 117-132.

Mitchell, D. A. (1992). Difinition, characteristics and potential. *Solid substrate cultivation*, 1-16.

Miyamoto, T., Horie, T., Fujiwara, T., Fukata, T., Sasai, K., & Baba, E. (2000). Lactobacillus flora in the cloaca and vagina of hens and its inhibitory activity against Salmonella enteritidis in vitro. *Poultry Science*, *79*(1), 7-11.

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., & Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, *89*(1), 58-67.

Mujahid, A., Asif, M., Ul Haq, I., Abdullah, M., & Gilani, A. H. (2003). Nutrient digestibility of broiler feeds containing different levels of variously processed rice bran stored for different periods. *Poultry Science*, *82*(9), 1438-1443.

Nemcova, R., Laukova, A., Gancarcikova, S., & Kastel, R. (1997). In vitro studies of porcine lactobacilli for possible probiotic use. *Berliner und Munchener Tierarztliche Wochenschrift*, *110*(11-12), 413-417.

Noll, S., Stangeland, V., Speers, G., & Brannon, J. (2001, September). Distillers grains in poultry diets. In *62nd Minnesota Nutrition Conference and Minnesota Corn Growers Association Technical Symposium, Bloomington, MN*.

Ocak, N., Erener, G., Burak Ak, F., Sungu, M., Altop, A., & Ozmen, A. (2008). Performance of broilers fed diets supplemented with dry peppermint (Mentha piperita L.) or thyme (Thymus vulgaris L.) leaves as growth promoter source. *Czech Journal of Animal Science*, *53*(4), 169.

Olejnik, M. A. Ł. G. O. R. Z. A. T. A., Szprengier-Juszkiewicz, T. E. R. E. S. A., & Jedziniak, P. I. O. T. R. (2010). Confirmatory method for determination of coccidiostats in eggs. *Bulletin of the Veterinary Instritute in Pulawy*, *54*, 327-333.

Panda, K., Rao, S. R., & Raju, M. V. L. N. (2006). Natural growth promoters have potential in poultry feeding systems. *Feed Technology*, *8*, 23-26.

Parks, C. W., Grimes, J. L., Ferket, P. R., & Fairchild, A. S. (2001). The effect of mannanoligosaccharides, bambermycins, and virginiamycin on performance of large white male market turkeys. *Poultry Science*, *80*(6), 718-723.

Parsons, C. M., Baker, D. H., & Harter, J. M. (1983). Distillers dried grains with solubles as a protein source for the chick. *Poultry Science*, *62*(12), 2445-2451.

Paryad, A., & Mahmoudi, M. (2008). Effect of different levels of supplemental yeast (Saccharomyces cerevisiae) on performance, blood constituents and carcass characteristics of broiler chicks. *African Journal of Agricultural Research*, *3*(12), 835-842.

Patel, H. M., Wang, R., Chandrashekar, O., Pandiella, S. S., & Webb, C. (2004). Proliferation of Lactobacillus plantarum in solid- state fermentation of oats. *Biotechnology Progress*, *20*(1), 110-116.

Podolak, R. K., Zayas, J. F., Kastner, C. L., & Fung, D. Y. C. (1996). Inhibition of Listeria monocytogenes and Escherichia coli O157: H7 on beef by application of organic acids. *Journal of Food Protection*, *59*(4), 370-373.

Potter, L. M. (1966). Studies with distillers feeds in turkey rations. In *Proc. Distillers Resolution Conference* (Vol. 21, pp. 47-51).

Rodríguez, D. A., Sulabo, R. C., González-Vega, J. C., & Stein, H. H. (2013). Energy concentration and phosphorus digestibility in canola, cottonseed, and sunflower products fed to growing pigs. *Canadian Journal of Animal Science*, *93*(4), 493-503.

Rompala, R. E., & Madsen, F. C. (1991). Diet impact mass, functional work load of animal gut. *Feedstuffs*.

Santin, E., Maiorka, A., Macari, M., Grecco, M., Sanchez, J. C., Okada, T. M., & Myasaka, A. M. (2001). Performance and intestinal mucosa development of broiler chickens fed diets containing Saccharomyces cerevisiae cell wall. *Journal of Applied Poultry Research*, *10*(3), 236-244.

Sauvant, D., Perez, J. M., & Tran, G. (2004). *Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish*. Wageningen Academic Publishers.

Shashidhara, R. G., & Devegowda, G. (2003). Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. *Poultry Science*, *82*(8), 1319-1325.

Snel, J., Harmsen, H. J. M., Van der Wielen, P. W. J. J., & Williams, B. A. (2002). Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. *Nutrition and health on the gastrointestinal tract*, 37-69.

Snijders, J. M. A., Van Logtestijn, J. G., Mossel, D. A. A., & Smulderst, F. J. M. (1985). Lactic acid as a decontaminant in slaughter and processing procedures. *Veterinary Quarterly*, *7*(4), 277-282.

Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poultry Science*, *79*(2), 205-211.

Steyaert, P., Buldgen, A., & Compere, R. (1989). Influence of the rice bran content in mash on growth performance of broiler chickens in Senegal. *Bulletin des Recherches Agronomiques de Gembloux*, *24*, 385-388.

Supriyati, T. H., Susanti, T., & Susana, I. W. R. (2015). Nutritional value of rice bran fermented by Bacillus amyloliquefaciens and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *28*(2), 231.

Supriyati, T. H., Susanti, T., & Susana, I. W. R. (2015). Nutritional value of rice bran fermented by Bacillus amyloliquefaciens and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *28*(2), 231.

Tangendjaja, B. (1993). Effect of Fermacto upon the utilization of broiler diets containing normal and high levels of rice bran. *Ilmu dan Peternakan*, *7*(7), 16-19.

Thayer, R. H., & Jackson, C. D. (1975). Improving phytate phosphorus utilization by poultry with live yeast culture. *Miscellaneous Publication of Agricultural Experiment Station of Oklahoma- State-University (USDA)*.

Thongsong, B., Kalandakanond-Thongsong, S., & Chavananikul, V. (2008). Effects of the addition of probiotic containing both bacteria and yeast or an antibiotic on performance parameters, mortality rate and antibiotic residue in broilers. *The Thai Journal of Veterinary Medicine*, *38*(1), 17-26.

Tirajoh, S., Piliang, W. G., & Ketaren, P. P. (2010). The suplementation of fibre degrading enzymes and phytase in poultry diet on the performance of broiler chickens. *Indonesian Journal of Animal and Veterinary Sciences*, *15*(1), 40-46.

Toghyani, M., Toghyani, M., & Tabeidian, S. A. (2011, May). Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. In *international conference on food engineering and biotechnology. Singapura., Anais… Singapura, v09* (pp. 168-184).

van den Bogaard, A. E., & Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. *International journal of Antimicrobial Agents*, *14*(4), 327-335.

Vescovo, M., Bottazzi, V., Torriani, S., & Dellaglio, F. (1993). Basic characteristics, ecology and application of Lactobacillus plantarum [in the production of fermented foods of animal and plant origin]: a review. *Annali di Microbiologia ed Enzimologia (Italy).*

Villegas, L. F., Fernández, I. D., Maldonado, H., Torres, R., Zavaleta, A., Vaisberg, A. J., & Hammond, G. B. (1997). Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. *Journal of Ethnopharmacology*, *55*(3), 193-200.

Waldroup, P. W., Owen, J. A., Ramsey, B. E., & Whelchel, D. L. (1981). The use of high levels of distillers dried grains plus solubles in broiler diets. *Poultry Science*, *60*(7), 1479-1484.

Wallace, R. J. (1994). Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. *Journal of Animal Science*, *72*(11), 2992-3003.

Warren, B. E., & Farrell, D. J. (1990). The nutritive value of full-fat and defatted Australian rice bran. IV. Egg production of hens on diets with defatted rice bran. *Animal Feed Science and Technology*, *27*(3), 259-268.

Warren, B. E., & Farrell, D. J. (1990). The nutritive value of full-fat and defatted Australian rice bran. I. Chemical composition. *Animal Feed Science and Technology*, *27*(3), 219-228.

Wizna, H. A., Rizal, Y., Dharma, A., & Kompiang, I. P. (2009). Improving the quality of tapioca by-products (onggok) as poultry feed through fermentation by Bacillus amyloliquefaciens. *Pakistan Journal of Nutrition*, *8*(10), 1636-1640.

Wray, C., & Davies, R. H. (2000). Competitive exclusion--an alternative to antibiotics. *Veterinary journal (London, England: 1997)*, *159*(2), 107.

Yang, Y., Iji, P. A., & Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*, *65*(1), 97-114.

Yang, Y., Iji, P. A., & Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*, *65*(1), 97-114.

Yao, L., Lee, S. L., Wang, T., de Moura, J. M., & Johnson, L. A. (2012). Effects of fermentation substrate conditions on corn–soy co-fermentation for fuel ethanol production. *Bioresource technology*, *120*, 140-148.

Ziggers, D. (2000). Tos, a new prebiotic derived from whey. *Animal Feed Science and Technology*, *5*, 34-36.

Wang, Z., Cerrate, S., Coto, C., Yan, F., & Waldroup, P. W. (2007). Effect of rapid and multiple changes in level of distillers dried grain with solubles (DDGS) in broiler diets on performance and carcass characteristics. *International Journal of Poultry Science*, *6*, 725-731.