USES OF PREPARED PROBIOTICS INSTEAD OF HARMFUL GROWTH PROMOTERS IN SHEEP PRODUCTION TO AVOID ADVERSE EFFECT ON HEALTH

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CERTIFICATE

This is to certify that the thesis entitled "USES OF PREPARED PROBIOTICS INSTEAD OF HARMFUL GROWTH PROMOTERS IN SHEEP PRODUCTION TO AVOID ADVERSE EFFECT ON HEALTH" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Animal science, embodies the result of a piece of bona fide research work carried out by Mahfuza Shamima Akhter, Registration No. 17-08248 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: June, 2019
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Dedicated
To
My Beloved Parents

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ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
ACTH	=	Adreno Corticotropic Hormone
AGPs	=	Antibiotic growth promoters
AIA	=	Acid Insoluble Ash
ANOVA	=	Analysis of Variance
AO	=	Aspergillusoryza
AR	=	Androgen receptor
ATA	=	A-Tocopheryl Acetate
BLRI	=	Bangladesh Livestock Research Institute
BUN	=	Blood Urea Nitrogen
Ca	=	Calcium
CAT	=	Catalase
CBC	=	Complete Blood Count
CF	=	Crude Fiber
CF	=	Crude Fibre
CFU	=	Colony Forming Units
Cm	=	Centimeter
cm^2	=	Squre Centimeter
CONT'D	=	Continued
CP	=	Crude Protein
Cu	=	Copper
DHT	=	Dihydrotestosterone
DM	=	Dry Matter
DMD	=	Dry Matter Digestibilities
DP	=	Dressing Percentage
Dr.	=	Doctor
e.g.	=	For Example
EBM	=	Eosin Methylene Blue
EDTA	=	Ethylene Diethyle Tetraacitic Acid
EE	=	Crude Fat
ER	=	Erythocytic Reticular
ESR	=	Erythrocytes Sedimentation Rate
et al.	=	And others/Associates
FAO	=	Food and Agricultural Organization
FCR	=	Feed Conversion Ratio
FMD	=	Foot & Mouth Disease
FOS	=	Fructo-oligosaccharides
G.I.T	=	Gastrointestinal tract

gm	=	Gram
GSH	=	Glutathione Stimulating Hormone
Hb	=	Hemoglobin
HCl	=	Hydrochloric Acid
HCW	=	Half-Carcass Cut Weight
HDL	=	High Density Lipoproteins
hr	=	Hour
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
Kg	=	Kilogram
L	=	Litre
lb	=	Pound
LDL	=	Low Density Lipoproteins
LSD	=	Least Significant Difference
M.S.	=	Master of Science
MCHC	=	Mean Corpuscular Hemoglobin Concentration
MDA	=	Malondialdehyde
ME	=	Metabolizable Energy
ml	=	Mililitre
mm	=	Milimeter
mmol	=	Milimol
Mn	=	Manganese
MRS	=	De Man, S Rogosa And Sharpe
MT	=	Metric ton
NaOH	=	Sodium Hydroxide
NDV	=	Newcastle Disease Vaccine
NEFAs	=	Non-Esterified Fatty Acids
NGP	=	Natural Growth Promoter
No.	=	Number
NS	=	Non-significant
PBD	=	Potato Dextrose Broth
PCA	=	Plate Count Agar
PCV	=	Packed Cell Volume
PDA	=	Potato Dextrose Agar
PPR	=	Peste Des Petits Ruminant
RBC	=	White Blood Cell
RNS	=	Reactive Nitrogen Species
SAU	=	Sher-E-Bangla Agricultural University
SC	=	Saccharomyces Cerevisia
SOD	=	Superoxide Dismutase
SOD	=	Superoxide Dismutase
spp.	=	Species

SPSS	=	Statistical Package for Social Sciences
T_1	=	control
T_2	=	Antibiotic group
T_3	=	Bacterial probiotics group
T_4	=	Both bacterial and yeast probiotics group
TA	=	Total Ash
TBA	=	Trenbolone acetate
TEC	=	Total Erythrocytes Count
UK	=	United Kingdom
USA	=	United States of America
viz.	=	Such as
Vs	=	Versus
WBC	=	White Blood Cell
WHO	=	World Health Organization
Wt.	=	Weight
Zn	=	Zinc

LIST OF SYMBOLS

Symbols		Full meaning
®	=	Trade name
@	=	At the rate of
±	=	Plus Minus
<	=	Less than
>	=	Greater than
°C	=	Degree Celcius
°F	=	Degree Fahrenheit
%	=	Percentage
&	=	And
*	=	5% level of significance
**	=	1% level of significance
/	=	Per
:	=	Ratio

USES OF PREPARED PROBIOTICS INSTEAD OF HARMFUL GROWTH PROMOTERS IN SHEEP PRODUCTION TO AVOID ADVERSE EFFECT ON HEALTH

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ABSTRACT

The study was conducted at Environmental Biotechnology Laboratory and Animal Farm under the Department of Animal Production & Management, Sher-e-Bangla Agricultural University. The research was for one year, but the sheep rearing period was for three months and it was the month of March-May, 2018. Eight 8-9 month-old sheep were collected from BLRI, Savar, Dhaka. The sheep were group penned for each of the replication. The control group- T_1 was fed basal diet (unsupplemented or control). Antibiotic group- T_2 containing 14 mg/L of Renamycin and probiotic group- T_3 (probiotics prepared by bacteria) provide 10 ml/kg of feed and probiotic group- T_4 (probiotics prepared by bacteria and yeast) provide 10 ml/ kg of feed. Same amount of feed supplied to all group but probiotics group produced highest live weight which had significant ($P< 0.05$) difference with sheep of control and antibiotics group. Dressing percentage was not affected by probiotics. The significantly ($P<0.05$) lowest glucose level was found in probiotics group. That indicates better fiber digestion and better weight gain. But other hematological parameters like PCV, RBC,WBC, Platelates, cholesterol and ESR did not affected ($P> 0.05$) by probiotics. The number of harmful bacteria reduced in probiotics group which had significant ($P< 0.05$) difference with sheep of control group. Testicular weight and G.I.T weight affected by probiotics. No significant ($P> 0.05$) difference was found in weight of liver, spleen, lungs and heart. Again, fresh meat quality and chemical composition did not affected ($P> 0.05$) by probiotics supplement. From the findings, it is concluded that Probiotics feeding as a feed additives causing better live weight gain by competitive exclusion of pathogenic microorganism and improved fibre digestion.

CHAPTER 1

INTRODUCTION

Livestock farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. Research on meat production globally indicates livestock sector is the fastest growing sectors especially in developing countries. It has triggered the discovery and widespread use of a number of "Growth promoter". Farmer used growth promoter because it causes increase growth rates, improve product quality, more profit and quick return. Steroids and antibiotics are the most common harmful growth promoter used in livestock sector. Steroid mainly used for beef fattening. It boost production of growth-stimulating hormones that help the animal convert feed into muscle, fat, and other tissues more efficiently than they would naturally (Neumann, 1977). The excessive use of steroid damaged the kidneys and intestines of cattle. Discharge of water from their bodies is affected and the accumulated water is absorbed in to the flesh of the animal rendering it bulky. It attacks immune system and make vulnerable to diseases. People consuming meat of such cattle may get kidney problem, cancer, liver failure, gastric ulcer, diabetes, pancreas disease, high blood pressure, skin disease and infertility in women. Theoretically, the fetus and the prepubertal child are particularly sensitive to exposure to sex steroids" (Swan *et al*., 2007). Antibiotics is the another harmful growth promoter. Antibiotic-resistant and Antibiotic residue are the harmful effect of it. Sub-therapeutic level of antibiotics given to animal as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health (Sarica *et al.,* 2005). The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria which is administered at a low sub therapeutic dose. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005; Niewold, 2007). Growth stimulating antibiotics, by the spread of antibiotic resistant bacteria, are a threat to human health (Wray and Davies, 2000; Turnidge, 2004).

Alternative feed additives for farm animals are referred to as Natural Growth Promoters (NGP) which include acidifiers, probiotics, prebiotics, phytobiotics, feed enzymes, immune stimulants and antioxidants are gaining the attention (Steiner, 2006). Probiotics are being considered as growth promoter and already some farmers are using them in preference to antibiotics and hormones (Trafalska and Grzybowska, 2004; Griggs and Jacob, 2005). The concept of probiotics in recent year is no more confusing as was earlier thought in the developed countries. It now constitutes an important aspect of applied biotechnological research and therefore as opposed to antibiotics and chemotherapeutic agents can be employed for growth promotion in animal. In past years, men considered all bacteria as harmful, forgetting about the use of the organisms in food preparation and preservation, thus making probiotic concept somewhat difficult to accept. Scientists now are triggering effort to establish the delicate symbiotic relationship of animal with their bacteria, especially in the digestive tract, where they are very important to the well being of man and livestock. The present research was taken to investigate that probiotics could be successfully used as nutritional tools in animal feeds for promotion of growth, modulation of intestinal microflora and promoting meat quality of livestock as well as immense potential to become an alternative to antibiotics and other growth promoters. Probiotics are individual microorganisms or groups of microorganisms, which have favourable effect on host by improving the characteristics of intestinal microflora (Fuller, 1989). Certain species of bacteria, fungi and yeasts belong to the group of probiotics. Existing probiotics can be classified into colonizing species (*Lactobacillus sp., Enterococcus sp.* and *Streptococcus sp*.) and free, non-colonizing species (*Bacillus sp* and *Saccharomyces cerevisiae*s). Probiotics acts by inhibiting bacterial growth by secretion of products, which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide. The other way by which probiotics act is competitive exclusion, which represents competition for locations to

adhere to the intestinal mucous membranes and in this way pathogenic microorganisms are prevented from inhabiting the digestive tract and the third way is competition for nutritious substances (Patterson and Burkholder, 2003). In this way, they create conditions in intestines, which favour growth of useful bacteria and inhibit the development of pathogenic bacteria (Line *et al.*, 1998). They improve the function of the immune system (Zulkifli *et al.*, 2000; Kabir *et al.*, 2004) and exhibit significant influence on morpho-functional characteristics of intestines (Yang *et al.*, 2009). Under physiological conditions the reactive species figure a crucial role in primary immune defense (Diplock *et al.,* 1998). But prolonged excess of reactive species is highly damaging for the host biomolecules and cells, resulting in dysbalance of the functional antioxidative network of the organism and leading to substantial escalation of pathological inflammation (Petrof *et al.*, 2004). Several studies reported the antioxidant activity of probiotic bacteria using assays *in vitro* (Shen *et al.*, 2011). Lactic acid bacteria are evaluated as beneficial bacteria by their product of acids (lactic acid), bacteriocin-like substances or bacteriocins (Strus *et al.,* 2001). Widely accepted probiotics contain different lactic acid producing bacteria: *bifidobacteria*, *lactobacilli*or *enterococci* (Mikelsaar and Zilmer, 2009). Their efficiency was demonstrated for the treatment of gastrointestinal disorders, respiratory infections and allergic symptoms. In most cases, evidence for a beneficial effect was obtained by studies using animal models (Travers *et al.*, 2011).

With this background, the work was planned to explore the possibilities of probiotics in sheep production as a replacement for the antibiotic growth promoters, with the following specific objectives:

1. To study the effect of prepared probiotic on growth performance of sheep.
2. To study the effect of probiotics on meat quality, microbiological status, and hematological parameter in sheep.
3. To replacement of antibiotic growth promoters with probiotic.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Antibiotic growth promoters (AGPs)

Feed antibiotics were first applied in animal nutrition in 1946. The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibit bacteria and is administered at a low, sub therapeutic dose for the purpose of performance enhancement (Hughes and Heritage, 2002). Antibacterial growth promoters are used to help the animals to digest their food more efficiently, get maximum benefit from it and allow them to develop in to strong and healthy individuals (Ellin, 2001). They may produce improved growth rate because of thinning of mucous membrane of the gut, facilitating better absorption, altering gut motility to enhance better assimilation, producing favorable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria and partitioning proteins to muscle accretion by suppressing monokines (Prescott and Baggot, 1993). When used at sub-therapeutic levels, these antimicrobials improve overall performance (Falcao-e-Cunha *et al.,* 2007*)* through reduced normal intestinal flora (which compete with the host for nutrients) and harmful gut bacteria (which may reduce performance by causing sub clinical-diseases) (Jensen, 1998). But the antibiotics are specific to their spectrum of activity only in the active multiplying stage of bacteria and it will not provide overall protection. Large numbers of antimicrobials were banned due to residual effects on human health and cross-resistance to antimicrobial drugs used in human medicine (WHO, 1997). Some antimicrobial agents (Virginiamanycin, Zn bacitracin, etc.), which are not absorbed in the systemic circulation and exert their action locally in the gut are still used as growth promoters (Ian phillips, 1999). Administration of drugs to food-producing animals requires not only consideration of effects on the animal but also the effects on humans who ingest food from these animals.

In short, after food- producing animals have been exposed to drugs in order to cure or prevent disease or to promote growth, the effects of the residues of such treatment on humans should be known. In view of the above the use of antibiotic growth promoters (AGPs) in sheep production is under serious criticism by governmental policy makers and consumers because of the development of microbial resistance to these products and the potential harmful effects on human health. Thus, there is increasing public and government pressure in several countries to search for natural alternative to antibiotics (Botsoglou and Fletouris, 2001; Williams and Losa, 2001; McCartney, 2002).

2.1.1 Antimicrobial resistance

Bacterial resistance to antimicrobial drugs has become an issue of increased public concern and scientific interest during the last decade. This resulted from a growing concern that the use of antimicrobial drugs in veterinary medicine and animal husbandry may compromise human health if resistant bacteria develop in animals and are transferred to humans via the food chain or the environment. While there is still no consensus on the degree to which usage of antibiotics in animals contributes to the development and dissemination of antimicrobial resistance in human bacteria, experiential evidence and epidemiological and molecular studies point to a relationship between antimicrobial use and the emergence of resistant bacterial strains in animals and their spread to humans, especially via the food chain (Moritz, 2001). However, imprudent use of antibiotics in animal production can lead to increased antibiotic resistant bacteria in products. In general, when an antibiotic is applied in animal farming, the drug eliminates the susceptible bacterial strains, particularly at a therapeutic dose, leaving behind or selecting those variants with unusual traits that can resist it. These resistant bacteria thus become the predominant micro-organism in the population and they transmit their genetically defined resistance characteristics to subsequent progeny of the strains and to other bacterial species via mutation or plasmid-

mediated (Gould, 2008). According to WHO, the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Catry *et al.,* 2003). Potential transfer of resistant bacteria from animal products to human population may occur through consumption of inadequently cooked meat or handling meat contaminated with the pathogens (Van den Bogaard and Stobberingh, 2000). Studies have shown that animal enterococci are mostly different from human colonizers, although concerns for transient transfers of resistance remain (Apata, 2009).

2.1.2 Antimicrobial residues

In animal, antibiotic usage had facilitated their efficient production and also enhanced the health and well being by reducing the incidence of disease. But unfortunately, edible tissues may be contaminated with harmful concentrations of drug residues (Donoghue, 2003). Antibiotic residues in foods of animal origin are one of the sources of concern among the public and medical health professionals (Adams, 2001). Many authors carried out investigations of antibiotic residues in sheep meat and reported that antibiotic residues were identified in the sheep muscle, liver and kidney.

2.2 Steroidal effect

Steroid is called life saving emergency drug. But many dishonest people use it for beef fattening . It boost production of growth-stimulating hormones that help the animal convert feed into muscle, fat, and other tissues more efficiently than they would naturally (Neumann, 1977). The excessive use of steroid damaged the kidneys and intestines of cattle. Discharge of water from their bodies is affected and the accumulated water is absorbed in to the flesh of the animal rendering it bulky. It attacks immune system and make vulnerable to diseases. People consuming meat of such cattle may get kidney problem, cancer, liver failure, gastric ulcer, diabetes, pancreas disease, high blood pressure,

skin disease and infertility in women. Theoretically, the fetus and the prepubertal child are particularly sensitive to exposure to sex steroids" (Swan *et al.,* 2007).

2.2.1 Oestradiol

Oestradiol is the most active of the female sex hormones synthesized and secreted mainly by the ovary, the adrenals and the testis. Oestradiol is synthesized and secreted in early stages of embryogenesis and has an active role in the normal development of the female sex accessories during the lifetime of females. It has been used to induce parturition (birth) especially in sheep, a species in which an associated oestradiol-induced increase in mothering ability has also been recorded (Poindron, 2005). In non-pregnant animals, oestradiol has been used clinically to increase uterine contractions and cervical softening for the expulsion of unwanted uterine contents in the absence of a corpus luteum (i.e. to remove a dead fetus or infected material especially in cattle) (Elmore, 1992; Pepper and Dobson, 1987; Sheldon and Noakes, 1998). Oestradiol has been used in the past in turkeys and other poultry to castrate young birds. Implants would be placed subcutaneously at 5-6 weeks of age, or in slightly older birds, but certainly 4 weeks before killing. Alternatively, preparations were available as feed-additives. Another use of very low doses of oestradiol is as a growth promoter via appetite stimulating and increased food-conversion properties. Occasionally in the past, this approach has been taken to advance the onset of puberty and thus alleviate potential gynaecological problems in slower maturing species.

2.2.2 Testosterone

Testosterone and its more active metabolite, 5α -dihydrotestosterone (DHT), are the main sex hormones secreted by males. Testosterone is responsible for the early development, and the appearance and maintenance of male secondary sex accessory organs (prostate, secretory glands, penis size, etc.) during adulthood. Testosterone secretion is also affected by the complex interaction among all endocrine glands, especially with those in the brain.

Testosterone is metabolized and as a result, metabolites of different activity are generated. Some of these metabolites play a more active role in certain organs than in others. The actions of both testosterone and DHT are mediated through their high affinity and high specificity binding and activation of an intracellular protein, the androgen receptor (AR). This AR protein is a member of the steroid hormone superfamily. In animals, testosterone or testosterone propionate, alone or in combination with other hormonally active substances, is used primarily to improve the rate of weight gain and feed efficiency. This effect is most likely a consequence of the anabolic action of androgens.

2.2.3 Progesterone

Progesterone is synthesized and secreted mainly by the corpus luteum in the ovary of cycling females, and, during pregnancy, by the placenta. As all hormones, progesterone synthesis and secretion is regulated by a series of positive and negative feedback mechanisms in which polypeptidic hormones secreted by the brain (hypothalamus, pituitary) affect circulating progesterone levels. Progesterone and synthetic progestins are used pharmacologically in women in conjunction with ovulation stimulation drugs as well as during early pregnancy in cases of luteal phase dysfunction. Although results have been conflicting, some studies find an association between pregnancy-related intake of progestins and increased risk of hypospadias (congenital malformation of the urethral opening on the penis) in the male offspring (Carmichael *et al.,* 2005). It is well established that progesterone not only serves as the precursor of all the major steroid hormones (androgens, oestrogens, corticosteroids) in the gonads and adrenals, but also is converted into one or more metabolites by most tissues in the body (Wiebe, 2006).

2.2.4 Trenbolone acetate

Trenbolone acetate (TBA) is a synthetic steroid with an anabolic potency that may exceed that of testosterone. It is a prodrug that converts into its active form 17β-trenbolone, which isomerises into 17α-trenbolone.

17 β -trenbolone is the major form occurring in muscle tissue, whereas the 17 α -epimer is the major metabolite occurring in liver and in the excreta including bile. It is assumed to exert its anabolic action via interaction with androgen and glucocorticoid receptors (Danhaive and Rousseau, 1986). Experiments with cattle tissues have shown that 17 β – trenbolone binds to the androgen receptor with similar affinity as dihydrotestosterone. It also binds to the progesterone receptor with an affinity that exceeds that of progesterone (Bauer et al., 2000). Reports regarding the misuse of TBA as an anabolic agent in sports people describe several adverse effects, including liver cell injury with an increase in liver-specific enzymes in serum, cholestatic jaundice, peliosis hepatitis and various neoplastic lesions. Moreover, decreased endogenous testosterone production and spermatogenesis, oligospermia and testicular atrophy may be associated with the repeated use of TBA as anabolic (Bahrke and Yesalis, 2004; Maravelias *et al.,* 2005).

2.2.5 Zeranol

Zeranol is derived from the naturally occurring mycoestrogen zearalenone, and is a potent oestrogen receptor agonist in vivo and in vitro (Leffers *et al.,* 2001; Le Guevel and Pakdel, 2001; Takemura *et al.,* 2007; Yuri *et al.,* 2006). Its actions resemble those of oestradiol. (Leffers *et al.,* 2001). Zeranol stimulates the proliferation of ER-dependent cell proliferation in MCF-7 human breast cancer cells (which are widely used in the assessment of estrogenic activity) and in transfected cells (Leffers *et al.,* 2001; Le Guevel and Pakdel, 2001; Liu and Ling, 2004). It is used alone or in combination with TBA as a hormonal growth promoter in various products.

2.3 Alternatives to harmful growth promoters

In view of the concerns regarding the potential for selection of antibiotic resistant bacteria, residues and environmental effects attributed to the use of antimicrobial growth promoters, a host of non-antibiotic alternatives are available or under investigation. The currently available alternatives are reviewed here under.

2.3.1 Prebiotics

Prebiotics are defined as non-digestible food components, which have positive effect on host in their selective growth and activation of certain number of bacterial strains present in intestines (Gibson and Roberfroid, 1995). The most significant compounds, which belong to group of prebiotics, are fructo-oligosaccharides (FOS), gluco- oligosaccharides and mannan-oligosaccharides (MOS). Their advantage, compared to probiotics is that they promote growth of useful bacteria, which are already present in the host organism and are adapted to all conditions of the environment (Yang *et al.*, 2009). Similar to probiotics, results of the effects of prebiotics on broiler performance are contradictory. A study was conducted to analyze the effects of incorporation of FOS on broiler performances and the results showed improvement in body weight gain by 5-8% and improvement of feed conversion by 2-6% (Li *et al.*, 2008; Yang *et al.*, 2009). But, Biggs *et al.* (2007) obtained results showing decrease of body weight gain by 2% in-group fed FOS in diet. Application of MOS to fattening chicks resulted in improvement of body weight gain and feed conversion in fattening chickens by up to 6% (Roch, 1998; Newman, 1999). This proves that effect of application of prebiotics depends on the condition of animals, environment conditions, composition of food and level and type of prebiotic included in the mixtures.

2.3.2 Synbiotics

This is relatively recent term among additives used in poultry nutrition. Synbiotics are combination primarily of probiotics and prebiotics, as well as other promoting substances

which together exhibit joint effect with regard to health of digestive tract, digestibility and performances of broilers. Investigations showed that combinations used in synbiotics are often more efficient in relation to individual additives (Uscebrka *et al.*, 2005; Li *et al.*, 2008). Maiorka *et al.* (2001) suggest that the substitution of antibiotics by symbiotics in broiler chicken diets is an alternative to poultry industry, since no negative effect was found on performance. According to Cristina *et al.* (2012) the usage of probiotic- prebiotic-ficofytic compounds as feed additive generated better results related to hens performance, feed valorization, eggs yield and their quality. The administration of symbiotic to broiler chickens early in life increased significantly ($p<0.05$) the phagocytic activity, lysozyme activity and nitric oxide levels in a dose dependent manner and improved the oxidative state by increasing glutathione (GSH) and decreasing malondialdehyde (MDA). High concentration of symbiotic improves the antibody response to Newcastle Disease Vaccine (NDV) and Infectious Bronchitis Vaccines (IBV) (El-Sissi and Mohamed, 2011).

2.3.3 Enzymes

Supplementation of broiler feed with enzymes is applied in order to increase the efficiency of production of poultry meat. This is especially interesting if enzymes, which enable utilization of feeds of poorer nutritive value, are used. Numerous authors have reported that administration of enzymes can improve the production performances by 10% (Cowieson *et al.*, 2000; Cmiljanic *et al.*, 2001), whereas in some studies no positive effect has been reported (Peric *et al.*, 2002). It is obvious that the positive effect of application of additives depends on the quantity and quality of feeds included in the mixture, type of enzyme, as well as fattening conditions (Acamovic, 2001; Lukic *et al.*, 2002). Obtained results in some researches indicate that better effect is realized with utilization of two or more enzymes in food (Silversides and Bedford, 1999; Chesson, 2001). Therefore, new enzyme combinations are constantly analyzed, as well as their optimum doses, in order to realize positive financial effect through improved utilization of feeds.

Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995; Van der Klis *et al.*, 1995). Enzymes have been shown to improve performance and nutrient digestibility when added to poultry diets containing cereals, such as barley (Friesen *et al.,* 1992; Marquardt *et al.*, 1994), maize (Saleh *et al.*, 2003), oats (Friesen *et al.,* 1992), rye (Friesen *et al.,* 1991, 1992; Belford and Classen 1992; Marquardt *et al.*, 1994) and wheat (Friesen *et al.*, 1991; Marquardt *et al.,* 1994) and to those containing pulses, such as lupins (Brenes *et al.*, 1993). The effect of enzyme supplementation on dry matter digestibilities (DMD) in pigs and poultry depends on the type of diet and the type of animal: increases in DMD range from 0.9 (Schutte *et al.,* 1995) to 17% (Annison and Choct, 1993) in poultry.

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. According to Bharathidhasan *et al.* (2009) when Broilers were supplemented with enzyme level at 0, 250, 500, 750 and 1000 g/ton of feed there was no significant difference in carcass yield, dressing percentage, giblet weight, carcass weight, intestinal length and organoleptic characteristics of the meat.

2.3.4 Acidifiers

Acidifiers have been used in poultry nutrition for long time, in different forms and combinations, which are constantly changing. Organic acids reduce pH value of food and act as conserving agents and prevent microbial contamination of food in digestive tract of poultry (Freitag *et al.*, 1999). As a result of this there will be improved consumption of food, better-feed conversion and increased gain. Favourable effect of supplementation of individual organic acids to mixtures was established relatively long time ago for formic acid (Kirchgessner *et al.*, 1991) .n research published by Ao *et al.* (2009) it was established that citric acid in combination with α –galactosidase increased the effect of enzyme action, but also had negative effect on feed consumption and weight gain.

2.3.5 Antioxidants

Antioxidants are the agents, which donate free electron to reactive oxygen species (ROS) and reactive nitrogen species (RNS) and convert them to harmless substances and break the chain reaction (Dekkers *et al.*, 1996). After donating an electron, an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive.

Antioxidants are synthesized within the body and can also be extracted from the food that humans and animals eat, such as fruits, vegetables, seeds, nuts, meat, oil, leaves and grass (natural antioxidants). There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, *beta*-carotene and coenzyme-Q (Kaczmarski, 1999). Of these, vitamin E is considered to be the most potent chain-breaking antioxidant within the membrane of the cell. The second line, inside the cell consists of water soluble antioxidant scavengers that include vitamin C, glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) (Dekkers *et al.*, 1996). To maximize the oxidative stability of meat, antioxidants, mostly α-tocopheryl acetate (ATA), are added to feeds. The beneficial effect of dietary ATA supplementation for the enhanced stability of lipids in muscle foods has been extensively reported for poultry, beef cattle, veal calves and pigs (Gray *et al.*, 1996; Jensen *et al.*, 1998). Selenium is component of enzyme glutathione peroxidase, which prevents formation of free radicals, which are very harmful to cells as they disrupt their integrity (Kanacki *et al.*, 2008). Therefore, selenium and other antioxidants have favourable effect on quality of broiler meat (Surai, 2002; Peric *et al.*, 2007). Protective effect of selenium and vitamin E is also stated by Roch *et al.* (2000). One of the most accepted approaches for preservation of sensory properties of the meat is addition of antioxidants, such as selenium or vitamin E, directly to livestock food or during technological procedure of processing (Surai, 2002; Peric *et al.*, 2007b). Beside positive effect on quality of meat, Edens *et al.* (2000) and Peric *et al.* (2006) established

better feathering and body mass of chickens fed organic forms of selenium. Peric *et al.* (2008b) also stated that addition of organically bound selenium into feed for broiler parents significantly increases quality of one-day-old chickens. Lower plasma concentrations of antioxidant vitamins such as vitamin C, E and folic acid and minerals like zinc and chromium have been inversely correlated to increased oxidative damage in stressed poultry (Cheng *et al.,* 1990; Sahin *et al.,* 2002).

Super oxide dismutase (SOD), is a class of closely related enzymes that catalyze the breakdown of the highly reactive superoxide anion into oxygen and hydrogen peroxide. SOD proteins are present in almost all aerobic cells and in extra cellular fluids. Each molecule of superoxide dismutase contains atoms of copper, zinc, manganese or iron. SOD that is formed in the mitochondria contains manganese (Mn- SOD) and synthesized in the matrix of the mitochondria. SOD that is formed in the cytoplasm of the cell contains copper and zinc (Cu/Zn-SOD). The SOD is a specific catalyst of the reaction and decreases concentration of O_2 (Izumi *et al.*, 2002).

2.3.6 Herbal adaptogens

An adaptogen is a substance that shows some nonspecific effect, such as increasing body resistance to physical, chemical, or biological noxious agents and have a normalizing influence on pathological state, independent of the nature of that state . A vast number of plants have been recognized as valuable sources of natural antimicrobial compounds (Mahady, 2005). A wide range of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al.*, 2005). Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone and methanol are often used to extract bioactive compounds (Eloff, 1998).

Ethanol is the most commonly used organic solvent by herbal medicine manufactures because the finished products can be safely used internally by consumers. In terms of active ingredients, adaptogenic preparations can be divided into three groups.

a. Those that contain phenolic compounds such as phenylpropanoids, phenylethane derivatives and lignans, which structurally resemble catecholamines that activates sympatho-adrenal system and possibly imply an effect in the early stages of the stress response (Kochetkov *et al.,* 1962; Wagner, 1995).

b. Those that contain tetracyclic triterpenes, such as cucurbitacin R diglucoside, which structurally resemble the specific corticosteroids that inactivate the stress system to protect against overreaction to stressors (Munck, 1984; Panossian *et al.,* 1999).

c. Those that contain unsaturated trihydroxy or epoxy fatty acid ssuch as oxylipins structurally similar to leukotrienes and lipoxines (Panossian *et al.,* 1999).

Mechanism of action of these additives is not completely clear. Some plant extracts influence digestion and secretion of digestive enzymes and besides, they exhibit antibacterial, antiviral and antioxidant action (Ertas *et al.,* 2005; Cross *et al.,* 2007). There is extensive evidence that single-dose administration of adaptogens activates corticosteroid formation and repeated dosage with adaptogens normalizes the levels of stress hormones, such as adrenocorticotropic hormone (ACTH) (Panossian, 1999). The effects of adaptogens become somewhat more clear when it is recalled the stress is a defensive response to external factors and that it stimulates the formation of endogenous messenger substances such as catecholamines, prostaglandins, cytokines and platelet-activating factor, which inturn activate other factors that may either counteract stress or conversely, induce or facilitate disease.

Results of research of application of phytobiotics in nutrition of broiler chickens are not completely consistent. Some authors state significant positive effects on broiler performance (Ertas *et al.,* 2005; Cross *et al.,* 2007, Peric *et al.,* 2008a), whereas another group of authors established no influence on weight gain and consumption or conversion of food (Cross *et al.,* 2007; Ocak *et al.,* 2008). The differences in results are consequences of numerous factors, of which Yang *et al.* (2009) pointed out four- 1) type and part of plant used and their physical properties, 2) time of harvest, 3) preparation method of phytogenic additive and 4) compatibility with other food components. Feeding of Andrographis paniculatis to broiler chickens resulted in improved feed conversion ratio, increased live weight and decreased mortality rate and opined that the plant feeding could be an alternative to chlortetracycline in the broiler diet.

In the past two decades a number of ayurvedic preparations have been extensively used in poultry industry in India. Preparations like Livol® and Zeestress® have been found to possess hepatoprotective and immunopotentiative actions in vaccinated birds and reduced the stress in intensively housed chickens during summer (Parida *et al.,* 1995; Rao *et al.,* 1995).

2.4 Probiotics

The internationally endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Other definitions advanced through the years have been restrictive by specification of mechanisms, site of action, delivery format, method, or host. Probiotics have been shown to exert a wide range of effects. The mechanism of action of probiotics (e.g., having an impact on the intestinal microbiota or enhancing immune function) was dropped from the definition to encompass health effects due to novel mechanisms and to allow application of the term before the

mechanism is confirmed (Sanders *et al*., 2008). Probiotics are single or mixed cultures of live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (WHO, 2001). It was also defined as a live microbial feed supplement that beneficially affects the host animal by improving its microbial intestinal balance (Fuller, 1989). Probiotics stimulates the growth of beneficial microorganisms and reduces the amount of pathogens thus improving the intestinal microbial balance of the host (Fuller, 1989; Chiang; Pan, 2012). *Lactobacillus, Bifidobacterium, Leuconostoc, Enterococcus, Lactococcus, Bacillus, Saccharomyces, Aspergillus* and *Pediococcus* species are most commonly used probiotics (Getachew, 2016). Intake of Probiotic lowers the risk of gastro-intestinal diseases by stimulating the growth of beneficial microorganisms (Fuller, 1989; Chiang and Pan, 2012). Supplementation if probiotics alleviates the problem of lactose intolerance, the enhancement of nutrients bioavailability, and prevention or reduction of allergies in susceptible individuals (Isolauri, 2001; Chiang and Pan, 2012). Probiotics are reported to have also antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis, and immune modulatory effects (Chiang and Pan, 2012). Moreover, it has been shown that probiotics could protect broilers against pathogens by colonization in the gastrointestinal tract (Nisbet *et al.,* 1993; Hejlicek *et al.,* 1995; Pascual *et al.,* 1999) and stimulation of systemic immune responses (Muir *et al.,* 1998; Que´re´ and Girard, 1999). Probiotic is alive microscopic minutes given certain doses and to increase the effectiveness of the body, are usually non-existent bowel as an increase in these neighborhoods over the possibility of digestion and produce immune and reduce the incidence of certain diseases, and reduce the proportion of blood cholesterol, which materials are often certain nutrients given to the animal to stimulate beneficial micro flora.

Several studies have shown that probiotics improve the growth performance compared with non-supplemented diets, being as effectives as antibiotic growth promoters (Kalavathy *et al.,* 2003; Mountzouris *et al.,* 2010; Sher *et al.,* 2010). Some authors have investigated the effects of adding a single level of probiotics (Khosravi *et al.,* 2010; Mountzouris *et al.,* 2007; Zakeri and Kashefi, 2011), while others have tested two (Anjum *et al.,* 2005; Mehr *et al.,* 2007; Nayebpor *et al.,* 2007; Panda *et al.,* 2006) or three or more levels of probiotic supplementation (Apata , 2008; Li, *et al.,* 2008; Mountzouris *et al.,* 2010; Wang and Gu, 2010). Probiotics improve feed-intake, growth performance, meat quality, egg production, egg quality and have cholesterol lowering potential in poultry products. However, some studies reported no significant effect of probiotics on feed-intake, production traits, products' quality and cholesterol level (Getachew, 2016). The use of probiotic organisms in order to sustain appropriate homeostasis of the digestive tract and protect it against pathogenic microflora is a common practice in poultry production in some parts of the world (Verstegen and Williams, 2002). The main common characteristics of probiotics is their biological influence to effect the organism, in which they are stimulating physiological and biological functions and thereby induce an increasing productive potential upon the animals. To avoid the health hazardous growth promoters we want to introduce newly prepared cost effective probiotic in the chicken production sector so that farmer can get same benefit instead of antibiotics, steroids etc. Variations in the efficacy of probiotics can be due to the difference in microbial species or micro-organism strains used, or with the additive preparation methods (Jin *et al.,* 1998). Recently, emphasis has been given to the selection, preparation and application of probiotic strains, especially lactic acid bacteria (Wang & Gu, 2010). Natural adaptation of lactic acid bacteria to intestinal environment and the lactic acid produced by them have provided advantages for these organisms over other microorganisms used as probiotic (Guerra *et al.,* 2007).

Substitution of conventional and prohibited AGPs with probiotics has received much attention in the recent years. One of the major reasons for increased interest in the use of probiotics is because they are natural alternatives to antibiotics for growth promotion. However, very few studies have been conducted in this regard in Bangladesh.

2.4.1 Mode of action of probiotics

Proposed mechanism of probiotics health effect (Sanders *et al*., 2008).

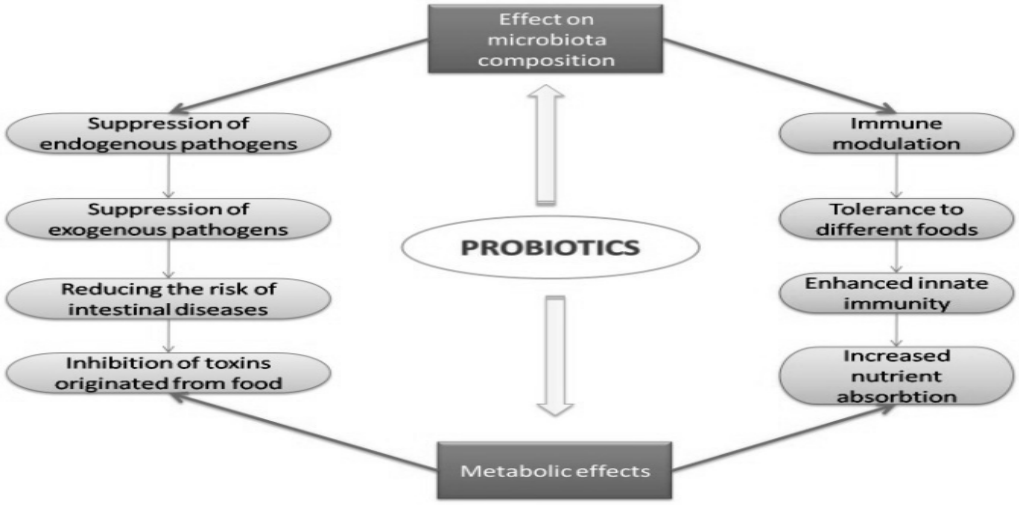

Figure 2.1 Proposed mechanism of probiotics health effect

2.4.2 Effect of probiotics on growth performance of small ruminants

Studies on performance responses of sheep and goats supplemented with yeast or yeast cultures have been variable. Growth rate and efficiency of bodyweight gain were found to be similar or reduced in some studies (Agarwal *et al.,* 2002; Erasmus *et al.,* 2005; Tripathi *et al.,* 2008; Tripathi and Karim, 2010), while others researchers reported improved weight gain, feed consumption and feed efficiency of gain after yeast supplementation (Lesmeister *et al.,* 2004). A positive effect of probiotic supplementation on nutrient intake, bodyweight gain and feed conversion rate (FCR) in small ruminants has been recorded by many researchers (Antunovic *et al.,* 2006, Whitley *et al.,* 2009).

It has, in general, been reported that impact of probiotics in performance of animals may vary, as supplementation can increase feed intake (Abd El-Ghani, 2004; Antunovic *et al.,* 2005; Desnoyers *et al.,* 2009), FCR (Khalid *et al.,* 2011) or bodyweight gain (Jang *et al.,* 2009; Hussein, 2014). Haddad and Goussous (2005) found that supplementation with yeast culture of diets of Awassi lambs had resulted in increased bodyweight gain compared to controls (266 *versus* 212 g daily). Similarly, Antunovic *et al.,* (2006) found increased bodyweight gain in kids given a probiotic supplement (curds) compared to controls (4.37 *versus* 3.15 kg and 44.6 *versus* 32.1 g daily). In contrast, Titi *et al.,* (2008) have reported that yeast supplementation had no effect on growth rate or DM intake in lambs and kids, these authors have explained a lack of beneficial effect of yeast supplementation by the high protein diet content. Moreover, Kawas *et al.,* (2007b) mentioned that addition of yeast improved bodyweight gain in lambs fed low protein diets with no favourable effects on those fed high protein diets. Whitley *et al.,* (2009) have found that growth performance of kids remained unaltered in cases of probiotic (dry yeast and lactic acid producing bacteria) supplementation, except in only one trial in which significant increase in bodyweight gain and improvement of FCR were observed in the supplemented animals. On the other hand, it was reported that supplementation of sheep diets with dry live *S. cerevisiae* had also conflicting results on performance data. This feed additive may contribute to increased growth and improvement of FCR, but it has no effect on feed intake (Haddad and Goussous, 2005). Other researchers found that it increased growth and feed intake with no effect on FCR (Payandeh and Kafilzadeh, 2007) or that it increased feed intake with no effect on growth and feed conversion (Khadem *et al.,* 2007) or that it had no effect in any of growth, feed intake and feed conversion (Macedo *et al.,* 2006; Kawas *et al.,* 2007a; Titi *et al.,* 2008). Soren *et al.,* (2013) observed that feeding of *S. cerevisiae* or combination of *S. cerevisiae* and *L. sporogenes* to lambs also had no effect on bodyweight and daily weight gain.

A possible positive effect of probiotics on bodyweight gain of lambs or kids might be the effect of improved cellulolytic activity resulting in improved fibre degradation (Russell and Wilson, 1996) increased microbial protein synthesis leading to more amino-acid supply post-ruminally (Erasmus *et al.,* 1992; Chaucheyras-Durand *et al.,* 2008). Further, improved bodyweight gain may also be related to increased consumption and improved efficiency of feed utilisation in the probiotic-supplemented animals (Antonovic *et al.,* 2006; Musa *et al.,* 2009; Papatsiros *et al.,* 2011). Additionally, probiotics attach onto the intestinal mucosa and prevent adhesion of potential pathogens, leading to improved nutrient digestion that may enhance dry matter intake (Seo *et al.,* 2010). Pankey *et al.,* (2014) reported that the feeding supplement of probiotics due to significantly ($P<0.01$) highly body weight gain in registered group as compared to control group in Marwari lamb. In this study on attempts has been made of generate data on impact of probiotics supplement in diet of growing kids at farmers flock under on farm trial programmes. Animal probiotics is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1999) and has been extracts, enzyme preparation or variation combinations of the above Saccharomyces cerevisia (SC) and Aspergillusoryza (AO) are the most widely use probiotics for enhancing the animal productivity (Newbold *et al.,* 1996). The development and growth during this period has important bearing on its future productive and reproductive performance. The purpose behind the use of probiotics has primarily to establish normal intestinal flora to prevent or minimize the disturbances caused by enteric pathogens and secondarily to serve has been so called mood against the use of antibiotic feed additives in diet of animals. Probiotics especially the lactobacilli and Bacillus cereus are important in the development of immune competence against enteric infections. Saccharomyces cerevisia release essential enzymes, vitamins and amino acids during digestion, all of which are thought to have positive effect on performance of ruminants (Waziry and Ibraher, 2007).

The low growth rate of growing goats is primarily due to poor genetic make-up, inadequate supply of nutrients or unscientific approach for feeding. In order to improve growth performance in goat there is a need to adopt scientific feeding strategies; however, limited reports of on farm trial are available to illustrate the beneficial effect of probiotic supplementation in small ruminants under Indian condition. Thus, present study was under taken to assess the beneficial effect of prepared probiotic supplementation on the performance of growing kids/lambs on growth at farmers flock under the on farm trail programme.

2.4.3 Effect of probiotics on blood metabolites of small ruminants

Published information on effects of probiotics on haematological and blood biochemical parametres of small ruminants is conflicting and controversial. With regard to protein metabolism, concentrations of blood urea nitrogen (BUN) and urea decreased in lambs given a probiotic-supplemented diet (Chiofalo *et al.,* 2004; Antunovic *et al.,* 2005; Antunovic *et al.,* 2006, Dimova *et al.,* 2013). Smaller concentrations of BUN in probiotic supplemented lambs might be due to improved nitrogen utilization by ruminal bacteria (Bruno *et al.,* 2009). Moreover, Chiofalo *et al.,* (2004) have attributed the reduction of blood urea concentration in lactobacilli probiotic (a mixture of *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus reuteri*) supplemented kids to the improved nutritional status of supplemented animals that do not resort to the amino-acid de-amination in order to acquire energy. With regard to other protein metabolites, it has been recorded that concentrations of total protein, albumin and globulin in probiotic supplemented lambs have not changed (Galip, 2006; Abas *et al.,* 2007; Dimova *et al.,* 2013; Soren *et al.,* 2013). Only Hussein (2014) has reported increased values of plasma total protein, albumin and globulin in lambs supplemented with probiotics (5 g and 10 g of probiotics per kg of diet; Biovet-YC + a concentrate feed mixture).

Probiotic supplementation can lead to decreased blood concentrations of glucose as the result of improvement in fibre digestion, which leads to increased acetic acid and reduction of propionic acid production in the rumen (Antunovic *et al.,* 2005; Bruno *et al.,* 2009). On the other hand, Sayed (2003) has reported a significant increase in glucose concentration in kids and lactating ewe after probiotic supplementation. Similar findings have been observed in lambs (Hussein, 2014). An increase in serum glucose levels in supplemented animals may be attributed to gluconeogenesis, as after probiotic supplementation there is improvement in gluconeogenesis due to increased propionate production, which is the main precursor of glucose with a decisive influence on the glucose blood concentration in small ruminants (Huntington and Eisemann, 1988). Nevertheless, some studies (Antunovic *et al.,* 2006; Galip, 2006; Ding *et al.,* 2008) have found that blood concentrations of glucose have not changed in lambs given diets containing probiotics. Many studies consider that probiotic supplementation may improve the lipid profile of animals. The concentrations of total lipids, non-esterified fatty acids (NEFAs), triglycerides and low density lipoproteins (LDL) were found to be decreased in probiotic-supplemented kids or lambs (Chiofalo *et al.,* 2004; Abas *et al.,* 2007; Baiomy, 2011). This may be attributed to an imporved metabolic status and a positive energy balance associated with probiotic supplementation. Chiofalo *et al.,* (2004) have reported a significant reduced concentration of NEFA (control 0.78 *versus* supplemented 0.40) and triglycerides and an increased one for high density lipoproteins (HDL) in growing kids supplemented with probiotics. Moreover, probiotic supplementation had no effect in blood cholesterol concentration in kids or lambs (Chiofalo *et al.,* 2004; Galip, 2006; Soren *et al.,* 2013; Hussein, 2014). Reduction in cholesterol concentration may be attributed to inhibition of cholesterol synthesis or direct assimilation of cholesterol (Zacconi *et al.,* 1992).

2.4.4 Effect of probiotics on carcass characteristics of small ruminants

Published data regarding effects of probiotic supplementation on carcass characteristics of sheep and goats are inconsistent. Abdelrahman and Hunaiti (2008) have reported increased dressing percentage (DP) by lambs fed diets supplemented with yeast and methionine (cyc-methionine). Similar results were recorded by Belewu and Jimoh (2005) in probiotic-supplemented goats. However, no changes were observed in weights and proportions of carcass cuts in Awassi lambs or Shami goat kids in response to probiotic supplementation (Titi *et al.,* 2008). Likewise, Whitley *et al.* (2009) reported that carcass weight and weights of fabricated cuts (shoulder, loin, leg, rack, shank and total parts), as well as carcass length, leg circumference, loin eye area and back fat thickness remained unaltered by probiotic supplementation in carcass of goats. Tripathi and Karim (2011) observed that pre-slaughter weight, empty live weight, hot carcass weight, dressing percentage, fore- and hind-quarter weight did not change by yeast culture supplementation to diets of growing lambs. Similarly, half-carcass cut weight (HCW) and carcass composition did not differ among control and yeast fed lambs. However, yeast culture-supplemented lambs had a trend of accelerated carcass composition (% of HCW) attributes of leg, neck and shoulder and breast and fork shank. Moreover, Soren *et al.* (2013) reported that pre-slaughter weight and hot carcass weight were similar in the control and probiotic supplemented lambs. The wholesale cuts (leg, loin, rack, neck, shoulder, breast, shank) were also similar among the groups with no difference. Similar results were also reported by Kawas *et al.* (2007b) in lambs fed finishing diet supplemented with either sodium bicarbonate or yeast. In their study, slaughter weight, hot carcass weight and dressed weight were not influenced by yeast supplementation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

The study was conducted at Environmental Biotechnology Laboratory and Animal Farm under the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for 1 year.

3.2 Probiotic preparation

Probiotics form selected strains were made previously. The bacterial organisms were isolated from milk and milk products, whereas yeast (*Saccharomyces cerevisiae*) was isolated from maize. Bacterial probiotics were made from four strain; *Lactobacillus gallinarum* JCM 2011(T), *Streptococcus infantarius* subsp. coli HDP90246(T), *Streptococcus salivarius* subsp. thermophilus ATCC 19258(T), and *Streptococcus equinus* ATCC 9812(T). Yeast probiotics were made from *Saccharomyces cerevisiae*_1.

3.3 Judgement of probiotics quality

Quality of probiotics was judged by growing the microorganism into suitable growth media and proper identification.

3.3.1 Growth of bacteria and identification

3.3.1.1 Sterilization of glass ware

The sterilization of glassware's such as sampling bottles, flasks, petridishes and test tubes after washing with detergent were autoclaved at 121°C, 15 lbs for 15 minutes according to the procedure given by Harrigan (1998).

Fig: Autoclave

Fig: Drying in oven

Figure 3.1 Sterilization of glass ware

3.3.1.2 Media preparation

Nutrient agar, nutrient broth, De Man,s Rogosa and Sharpe (MRS) agar and De Man,s Rogosa and Sharpe (MRS) broth, used for bacterial growth were prepared according to manufacturer's instruction. The pH of media was adjusted by using 0.1N NaOH and 0.1N HCl. The composition of various media is given below:

MRS broth

This was the selective medium used for the isolation and enumeration of *Lactobacillus* spp.

Table 1. Composition of MRS Broth

Ingredients name	**Ingredients quantity (g/l)**
Peptone	10
Meat extract	10
Yeast extract	5
D-glucose	20
Dipotassium hydrogen phosphate	2
Sodium acetate trihydrate	5
Triammonium citrate	2
Magnesium sulfate heptahydrate	0.2

55.5 g media was dissolved in 1000 ml distilled water by heating and stirring in magnetic stirrer hot plate and the pH was adjusted at 5.8 to 6.2 and then medium was

sterilized at 121°C for 15 minutes, under 15 lb pressure.

MRS agar

MRS agar was prepared by adding 1.5% agar in MRS broth (prepared above) and was then autoclaved.

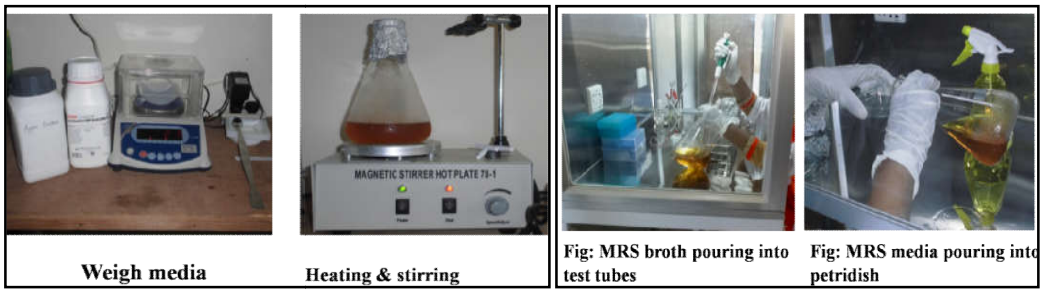

Figure 2.2 Preparation of agar media

3.3.1.3 Inoculation and incubation

The prepared probiotics after serial dilutions were inoculated into the nutrient broth and incubated at 37°C for 24-48 hours. From nutrient broth the growth was transferred to the nutrient agar for separation of colonies. The selected colonies from nutrient agar were then shifted to MRS broth subsequently to MRS agar for isolation and purification by following techniques of Harrigan (1998).

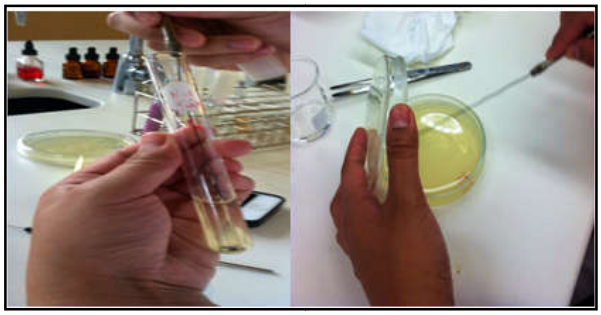

Figure 3.3 Inoculation of sample in Nutrient broth and agar

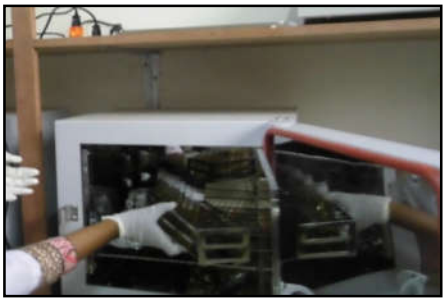

Figure3.4 Incubation of media

3.3.1.4 Identification of bacteria

The colony characteristics on solid medium and cellular morphology of culture isolates after Gram's staining were examined at each step of incubation according to the methods of Harrigan (1998) for identification. Lactobacilli appeared as large, round shape, off-white to cream color, shiny colonies embedded in or on MRS Agar or as turbidity in MRS Broth.

Figure 3.5 Colony morphology of *Lactobacillus* spp. on MRS broth

The Gram's staining after slide preparation and smear fixation was performed by the following procedure:

1. Crystal violet was applied for 2 minutes and washed with tap water by keeping the slide in tilt position at an angle of 45°.
2. Gram's iodine as mordant was applied for 1 minute, washed and blot dried.
3. The slide was then washed with 95% ethyl alcohol to decolorize by keeping the slide in tilt position at an angle of 45° until alcohol ran almost clear and then was rinsed with tap water.
4. The slide was counterstained with safranine (1% aqueous) for 15 seconds and washed with water.

5. Slides were blot dried and examined under microscope.
6. In Gram staining, rod shaped, short-medium chain and positive in Gram reaction (violet colour bacilli) bacteria found that indicates *Lactobacillus* spp. Small, coma shaped, violet colour Gram positive bacteria found that indicates *streptococcus* spp.

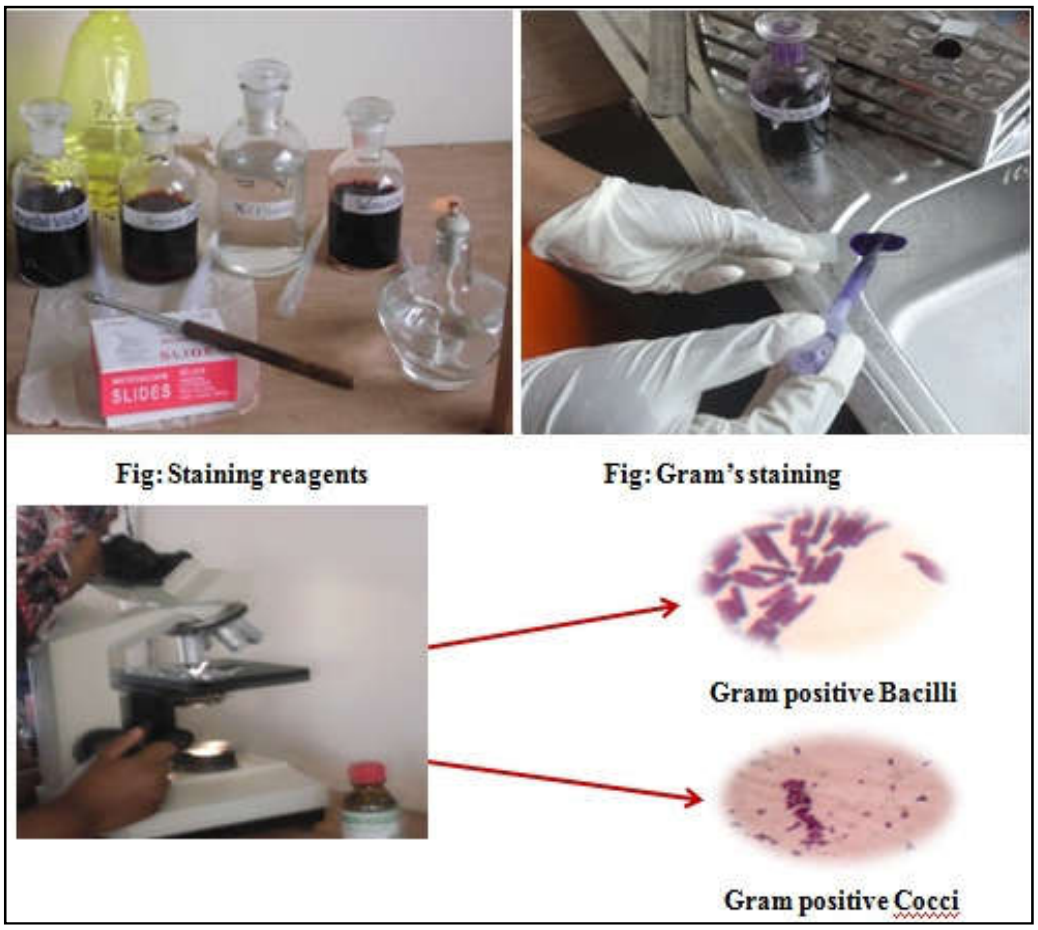

Figure 3.6 Identified bacteria by Gram's staining

3.3.2 Growth of *Saccharomyces cerevisiae* and identification

3.3.2.1 Sterilization of glass ware

The sterilization of glassware's such as sampling bottles, flasks, petridishes and test tubes after washing with detergent was autoclaved at 121°C, 15 lbs for 15 minutes according to the procedure given by Harrigan (1998).

3.3.2.2 Media preparation

Potato dextrose agar (PDA) and potato dextrose broth (PDB), used for yeast growth were prepared according to the methods recommended by Harrigan (1998). The pH of media was adjusted by using 0.1N NaOH and 0.1N HCl.

Potato dextrose broth

The diced potatoes were boiled in 1 L of distilled water for 1 hour and then filtered through muslin cloth. The volume of filtrate was made upto 1000 ml and then glucose was added. The medium was sterilized by autoclaving.

Table 2. Composition of potato dextrose broth

Ingredients	Ingredients Quantity
Potatoes peeled and diced into small pieces	200 g
Glucose	20 g
Distilled water	1000 ml

Potato dextrose agar

The potato dextrose agar (PDA) was prepared by adding 1.5% agar to potato dextrose broth and then sterilized by autoclaving.

3.3.2.3 Inoculation and incubation

The sample (water suspension) was first inoculated on to the PDA and incubated at 30°C for 72-96 hours and growth pattern was studied according to the suggestions and methods of Harrigan (1998). The selected colonies from PDA were further transferred

to the same media again and again to isolate *Saccaromyces cerevese*. The morphology of yeast colony was observed for identification.

3.3.2.4 Identification of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae was identified on the basis of morphology and growth pattern according to the methods recommend by Harrigan (1998). It was based on general examination of growth pattern of mycelia and spores under microscope after staining. In PDA agar *Saccharomyces cerevese* formed round, shining yeast cells. Spore and mycelia found under microscope.

Figure 3.7 Yeast on PDA agar

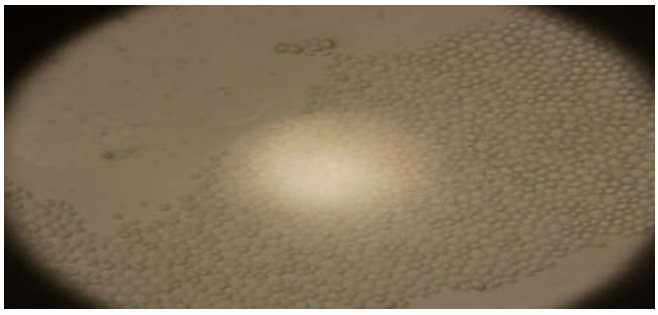

Figure 3.8 Microscopic view of *Saccharomyces cerevisiae*

3.4 Preparation of experimental sheep and diets

Eight 8-9 month-old sheep with a mean initial body weights of 9.0±0.3 kg were collected from Bangladesh Livestock Research Institute (BLRI), and used in the study during the period of December 2017 to November 2018. The sheep were group penned for each of the replication in grilled partition with concrete floor (1.5×1.5 m) that complied with welfare standards. The supplementations of antibiotics and probiotics was used. The control group- T_1 were fed a basal diet (unsupplemented - control), whereas the antibiotic and experimental groups were fed the same basal diet but supplemented with antibiotic group- T_2 containing 14 mg/L of Renamycin and probiotic group- T_3 (probiotics prepared by bacteria) provide 10 ml/kg of feed and probiotic group- T_4 (probiotics prepared by bacteria and yeast) provide 10 ml/kg of feed.

3.4.1 Management of experimental Animals

The sheep were given a 7-day acclimatization period to feed and housing before commencement of the experiment. The animals were allocated to four groups of two sheep each, balanced in terms of live weight and body condition scores. Each pen was used to house single sheep and the feed trial for the experiment was conducted for 90 days. Throughout the experimental period, concentrated feed (0.15 kg in the morning and 0.15 kg in evening) supplied to each animal daily according to their treatment. The succulent, *ad libitum* native green grasses supplied daily to each of the sheep and all animals provided with fresh clean drinking water *ad libitum.* For immunization of FMD, PPR and anthrax vaccine were applied according to the commonly recognized schedule. In addition, anthelminthes (Endex) medicine was applied after taking the sheep from BLRI and prior to trial. The sheep pens, feeder, waterer, instruments and utensils were cleaned and dried daily. Disinfectant and strict bio-security for hygienic

measures and sanitation programs was also employed in the experimental house throughout the research period.

3.4.2 Preparation of experimental diet

Table 3. Mixed concentrate feed & composition supplied to the sheep during the experimental period

Ingredients	**Diet (%)**
Wheat bran	21
Rice bran	20
Maize crust	20
Khesari bran	18
Sesame oil cake	10
Jasoprot protein	10
Common salt	1
Total	100
Composition:	
Protein in mixed feed (%)	16.25
Energy ME(kcal/100g) in mixed feed	258.35

Figure 3.9 Various feed ingredients

Figure 3.10 Mixing of feed ingredients

3.5 Ethical issues

The experimental protocol specifically approved and was in compliance with the Dept. of Animal Production & Management, Sher-e-Bangla Agricultural University on research in animals and the internationally accepted principles for animal use and care. Proper ventilation, temperature, light and hygienic management were present during research period. The sheep were examined by the University veterinarian on a weekly basis throughout the entire experimental period to ensure compliance to welfare requirements.

3.6 Record keeping and calculation of data

The different parameters were recorded throughout the experimental periods. Weekly body weight gain data recording were performed throughout the research trail period and meat quality, blood profile, bacterial count were performed at final stage.

3.7 Study parameters

Weekly live weight gain data were collected. After slaughtering, carcass weight was measured. Dressing percentage was calculated from here. Blood sample was analyzed from each replication to measure PCV, ESR, RBC count, WBC count, Platelets count, Hemoglobin, glucose and cholesterol level. Meat sample was analyzed for physical examination (colour, odor, presence of any infestation) and chemical examination (Moisture, Dry Matter, Crude Protein, Total Ash, Acid Insoluble Ash, Crude Fiber, Crude Fat). Microbial load was analyzed for determining the presence of harmful microbs.

Formulae used for calculation different parameter:

$$\text{Live weight gain (kg/sheep)} = \frac{\text{Total live wt. gain in a replication}}{\text{No. of sheep in a replication}}$$

$$\text{Dressing percentage (DP)} = \frac{\text{Total eviscerate carcass weight in a replication}}{\text{Total live weight in a replication}} \times 100$$

3.8 Blood sample analysis

Blood sample (5ml/sheep) were collected into EDTA tubes from jugular vein. This sample was transferred to laboratory for analysis of PCV, RBC count, WBC count, Platelets count, DLC and Hemoglobin level.

Serum sample (3ml/sheep) were collected into Eppendorf tube for analysis of blood cholesterol and glucose. Blood sample (2ml/sheep) were collected into ESR tubes for analysis of ESR level. All of these test were analyzed in ACI laboratory, Gulshan, Dhaka.

Figure 3.11 Blood collection from sheep

Figure 3.12 Blood sample for test

Figure 3.13 Serum collection in Eppendorf tube

3.9 Meat sample analysis

Meat sample (100gm) were collected after slaughter from thigh region of each carcass. All sample were preserved in cool box and send to DLS laboratory, Khamarbari, Dhaka for analysis.

3.10 Analysis of microbial load

Nutrient broth was prepared for microbial culture. Intestinal content were collected and grown in that culture media and finally send to ACI laboratory, Glshan, Dhaka for observing microbial load.

Figure 3.14 Autoclaving of culture media

Figure 3.15 Intestinal content collection

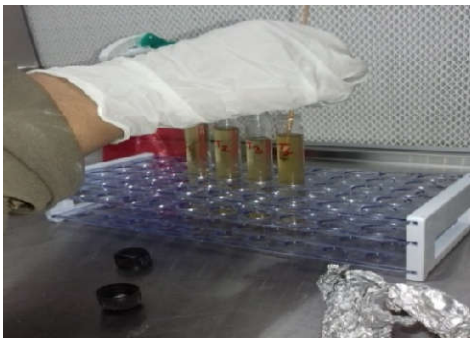

Figure 3.16 Intestinal content mixing into culture

3.11 Statistical analysis

The results were presented as the means and the standard deviation of the means (Means±SD). Data was statistically analyzed by one- way analysis of variance (ANOVA) using the COMPARE MEANS procedure (SPSS 7.5., 1999 software for windows, SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) was calculated to evaluate the variations between treatments. $P<0.05$ was considered to be statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Production performance of sheep

The production performance parameters like live weight gain, eviscerate weight, and dressing percentage data of sheep are presented in the table 4.

4.1.1 Effect of probiotics on live weight gain

In the table 4 significant difference ($P<0.05$) was found in live weight of sheep among the treatments and control group. The significantly ($P<0.05$) highest live weight was found in T_4 group (Diet in which both bacterial and yeast probiotics added) 5.25 kg, T_3 group (Diet in which bacterial probiotics added) 5.0 kg and T_2 group (Diet in which antibiotics added) 4.64 kg than T_1 group (Control diet) 3.35 kg. Significant ($P<0.05$) difference was found among probiotics supplement T_3 group and T_4 group. Highest live weight was found in T_4 group (Diet in which both bacterial and yeast probiotics added). This results supported by many researcher demonstration, like- A possible positive effect of probiotics on bodyweight gain of lambs or kids might be the effect of improved cellulolytic activity resulting in improved fibre degradation (Russell and Wilson, 1996), increased microbial protein synthesis leading to more amino-acid supply post-ruminally (Erasmus *et al.,* 1992; Chaucheyras-Durand *et al.,* 2008). Further, improved bodyweight gain may also be related to increased consumption and improved efficiency of feed utilisation in the probiotic-supplemented animals (Antonovic *et al.,* 2006; Musa *et al.,* 2009; Papatsiros *et al.,* 2011). Additionally, probiotics attach onto the intestinal mucosa and prevent adhesion of potential pathogens, leading to improved nutrient digestion that may enhance dry matter intake (Seo *et al.,* 2010). Pankey *et al.,* (2014) reported that the feeding

supplement of probiotics due to significantly (P<0.01) highly body weight gain in registered group as compared to control group in Marwari lamb.

4.1.2 Effect of probiotics on live weight and eviscerated weight

Both live weight and eviscerated weight significantly affected by probiotics. All treatment group viz. T_2 (Diet in which antibiotics added), T_3 (Diet in which bacterial probiotics added), and T_4 (Diet in which both bacterial and yeast probiotics added) gained more live weight then control T_1 group. These results are in accordance with the earlier findings of Haddad and Goussous (2005) found that supplementation with yeast culture of diets of Awassi lambs had resulted in increased body weight gain compared to controls (266 *vs* 212 g daily). Similarly, Anandan *et al.* (1999) found increased bodyweight gain in kids given a probiotic supplement (curds) compared to controls (4.37 *vs* 3.15 kg and 44.6 *vs* 32.1 g daily). Moreover, Kawas *et al.* (2007b) mentioned that addition of yeast improved bodyweight gain in lambs fed low protein diets with no favourable effects on those fed high protein diets.

4.1.3 Effect of probiotics on dressing percentage

The dressing percentage of sheep has been presented in Table 4 was not affected significantly (P>0.05) in control T_1 group and treatment group. The Dressing percentage were 54.18%, 53.37%, 54.05%, 53.26% for group T_1, T_2, T_3, T_4 respectively. These findings were supported by Soren *et al.* (2013) who reported that pre-slaughter weight and hot carcass weight were similar in the control and probiotic supplemented lambs. The wholesale cuts (leg, loin, rack, neck, shoulder, breast, shank) were also similar among the groups with no significant different.

Table 4. Effect of probiotics on production performance of sheep

Production performance	T_1 group	T_2 group	T_3 group	T_4 group	Mean ± SE	LSD value	Level of significance
Live weight gain (kg)	3.35^{b}± .30	4.64^{ab}± .35	5.00^{ab}± .43	5.25^{a}± .20	4.55±0.32	0.62	*
Final Live weight (kg)	12.10^{b}± .80	15.55^{a}±.15	15.45^{a}±.85	15.30^{a}±1.00	14.60±1.75	1.09	*
Eviscerated Weight(kg)	6.55^{b} ±.35	8.30^{a} ±.10	8.35^{a} ±.45	8.15^{a} ±.55	7.83^{a} ± .32	0.564	*
Dressing Percentage (%)	54.18±.69	53.37±.125	54.05±.06	53.26±.11	53.71± .20	0.503	NS

Means within a column with different superscripts differ significantly (P<0.05)
Means within a column with same superscripts don't differ significantly (P>0.05)
SE = Standard Error
LSD =Least Significant Difference
NS= Non significant
T_1= Control diet (No antibiotics and probiotics)
T_2= Diet in which antibiotics added
T_3= Diet in which bacterial probiotics added
T_4= Diet in which both bacterial and yeast probiotics added

4.2 Effect of probiotics on internal organ

4.2.1 Effect of probiotics on liver weight

The relative weight of liver (gm) of sheep in the dietary group T_1, T_2, T_3 and T_4 were 266.50±3.50, 266.00±1.00, 265.50±6.50 and 265.00±10.00 respectively. The highest results were obtain in T_1 and lowest was in T_4 group. However, there was no significant ($P>0.05$) difference in the relative weight of liver among the groups. (Table 5).

4.2.2 Effect of probiotics on heart weight

The comparative weight of heart (gm) of sheep in the dietary group T_1, T_2, T_3 and T_4 were 62.50±2.50, 63.50±4.50, 64.00±11.00 and 64.00±3.00 correspondingly. The qualified weight of heart of different groups showed that there was no significant ($P>0.05$) difference between the groups and the values were ranged from 62.50±2.50 to 64.00±11.00 (Table 5).

4.2.3 Effect of probiotics on spleen weight

The weight of Spleen (gm) of sheep in the dietary group T_1, T_2, T_3 and T_4 were 43.50±1.50, 47.00±4.00, 53.00±4.00 and 56.00±4.00 respectively. There was no significant ($P>0.05$) difference in the relative weight of liver among the groups. (Table 5).

4.2.4 Effect of probiotics on lungs weight

The weight of Lungs (gm) of sheep in the dietary group T_1, T_2, T_3 and T_4 were 151.00±2.00, 151.00±1.00, 149.50±1.50 and 148.50±3.50 respectively. There was no significant ($P>0.05$) difference in the relative weight of Lungs among the groups. (Table 5).

4.2.5 Effect of probiotics on G.I.T weight

G.I.T weight of sheep presented in Table 5 was affected significantly ($P<0.05$) by probiotics treatment. The G.I.T weight (kg) were 3.85 ±0.25, 4.07 ±0.12, 4.40 ±0.09 for group T_2, T_3, T_4 respectively and 3.60 ± 0.02 kg for control group (T_1 group). All treated group gained significantly better G.I.T weight than control group. Furthermore, both bacterial and yeast Probiotic group (T_4) were more weighted than other group.

4.2.6 Testicular weight

The Testicular weight of sheep presented in Table 5 was affected significantly ($P<0.05$) by probiotics treatment. The Testicular weight were, 133.5 ±4.5gm, 144.5±3.5gm, 146.0±3.0gm for group T_2, T_3, T_4 respectively and 129.5±2.5 gm for control group (T_1 group). All treated group gained significantly better testicular weight than control group. Furthermore , Probiotic group (T_3 and T_4) were more weighted than antibiotic T_2 group. It indicates quality semen. The present study give similar findings with the results of Zeitoun *et al*., (2014) found that the addition of 1:1 mixture (dandelion extract: probiotic) causing increased sperm concentration, less sperm motility, increased ejaculation volume, increased progressive motility and decreased abnormal sperm of ram.

Table 5. Effect of dietary supplementation of probiotics on internal organ weight of different treatment

Internal organ	T_1 group	T_2 group	T_3 group	T_4 group	Mean ± SE	LSD value	Level of significance
Liver wt. (gm)	266.50±3.50	266.00±1.00	265.50±6.50	265.00±10.00	265.75±2.36	8.817	NS
Heart wt. (gm)	62.50±2.50	63.50±4.50	64.00±11.00	64.00±3.00	63.50±2.37	8.845	NS
Spleen wt. (gm)	43.50±1.50	47.00±4.00	53.00±4.00	56.00±4.00	49.87±2.286	5.012	NS
lungs wt. (gm)	151.00±2.00	151.00±1.00	149.50±1.50	148.50±3.50	150.00±0.925	3.122	NS
G.I.T wt. (kg)	3.60^{b} ± 0.02	3.85^{ab} ±0.25	4.07^{ab} ±0.12	4.40^{a} ±0.09	3.98 ±0.12	0.209	*
Testicular weight (gm)	129.5^{b}±2.5	133.5^{ab} ±4.5	144.5^{a}±3.5	146.0^{a}±3.0	138.37±2.96	4.88	*

Means within a column with different superscripts differ significantly (P<0.05)
Means within a column with same superscripts don't differ significantly (P>0.05)
SE = Standard Error
LSD =Least Significant Difference.
NS= Non significant
T_1= Control diet (No antibiotics and probiotics)
T_2= Diet in which antibiotics added
T_3= Diet in which bacterial probiotics added
T_4= Diet in which both bacterial and yeast probiotics added

4.3 Haematological parameters of sheep: The average value of some hematological parameters of sheep are shown in Table 6.

4.3.1 Effect of probiotics on hemoglobin percentage

Table 6 shows the average hemoglobin percentage of different groups of sheep with different treatment. Average hemoglobin level of different groups were T_1 (Control diet) 10.26g/dl, T_2 group (Diet in which antibiotics added) 10.85g/dl, T_3 group (Diet in which bacterial probiotics added) 10.75 g/dl, T_4 group (Diet in which both bacterial and yeast probiotics added) 10.60 g/dl. The significantly ($P<0.05$) highest hemoglobin percentage was found in T_2 group (Diet in which antibiotics added) and T_3 group (Diet in which bacterial probiotics added) than T_1 group (Control diet). T_4 group (Diet in which both bacterial and yeast probiotics added) also having good hemoglobin level than control group.

4.3.2 Effect of probiotics on blood glucose level

In table 6 significant difference ($P<0.05$) was found in glucose level of sheep among the treatment and control group. The significantly ($P<0.05$) highest glucose level was found in T_1 group (Control diet) 3.90 mmol/L and lowest glucose level was found in T_3 group (Diet in which bacterial probiotics added) 2.63 mmol/L. This result supported by many researcher demonstration. Probiotic supplementation can lead to decreased blood concentrations of glucose as the result of improvement in fibre digestion, which leads to increased acetic acid and reduction of propionic acid production in the rumen (Antunovic *et al.,* 2005; Bruno *et al.,* 2009).

4.3.3 Effect of probiotics on blood cholesterol level

The blood Cholesterol level of sheep presented in table 6 was not affected significantly ($P>0.05$). The Cholesterol level were 0.630 mmol/L ,0.635 mmol/L, 0.645 mmol/L for group T_2, T_3, T_4 respectively and 0.635 mmol/L for control group (T_1 group). Previously, researchers have demonstrated that , probiotic supplementation had no effect in blood cholesterol concentration in kids or lambs (Chiofalo *et al.,* 2004; Galip, 2006; Soren *et al.,* 2013; Hussein, 2014).

4.3.4 Effect of probiotics on other blood parameter

TEC, WBC, Platelets, PCV, ESR all these Haematologicl parameters was not affected by probiotics treatment. All these remain normal value with less significant in every treatment and was not affected significantly. Results of this experiment were in agreement of other scientist's reports (Dibaji, *et al.*, 2012; Sharifi, *et al.*, 2011; Zarei, *et al.*, 2011)

Table 6. Effect of Probiotics on some Haematological parameter of sheep

Haematological parameters	T_1 group	T_2 group	T_3 group	T_4 group	Mean ± SE	LSD value	Level of significance
Hemoglobin (g/dl)	10.26^{b}	10.85^{a}	10.75^{a}	10.60^{ab}	10.61±0.09	0.134	*
Glucose (mmol/L)	3.90^{a}	3.73^{a}	2.63^{c}	3.18^{b}	3.36±0.19	0.123	*
cholesterol(mmol/L)	0.630	0.635	0.645	0.635	0.636±0.006	0.023	NS
TEC (million/Cumm)	4.12	4.14	4.13	4.16	4.14±0.008	0.022	NS
WBC (/Cumm)	14950	14650	14750	14750	14775±337.9	1254.9	NS
PCV (%)	31.35	31.4500	31.3000	31.3750	31.37±0.047	0.159	NS
ESR(mm in 1^{st} hour)	4.5	4.5	4.0	4.0	4.25±0.45316	1.658	NS

Means within a column with different superscripts differ significantly ($P<0.05$)
Means within a column with same superscripts don't differ significantly ($P>0.05$)
SE = Standard Error
LSD =Least Significant Difference.
NS= Non significant
T_1= Control diet (No antibiotics and probiotics)
T_2= Diet in which antibiotics added
T_3= Diet in which bacterial probiotics added
T_4= Diet in which both bacterial and yeast probiotics added

4.4 Analysis of sheep meat: The average value of meat quality parameters of sheep are shown in the Table 7.

Color and Odor of meat were normal in every treatment. The Moisture, DM, CP, TA,CF and EE level of sheep meat presented in Table 10. was not affected significantly (P>0.05). The Moisture level were 73.02%, 73.51%, 73.04% for group T_2, T_3, T_4 respectively and 73.17% for control group (T_1 group). Although the trends of moisture was comparatively higher in bacterial probiotics group than control. As like as moisture percentage, the percentage of DM, CP, TA,CF and EE were not affected significantly (P>0.05). Feeding probiotics did not have a negative effect on meat quality.

Table 7. Effect of Probiotics on some meat quality parameter of sheep (% on DM basis)

Meat quality parameter	T_1 group	T_2 group	T_3 group	T_4 group	Mean ± SE	LSD value	Level of significance
Color	Normal	Normal	Normal	Normal	NA	NA	NS
Odor	Normal	Normal	Normal	Normal	NA	NA	NS
Moisture	73.17	73.02	73.51	73.04	73.19±0.12	0.365	NS
Dry matter (DM)	26.82	26.97	26.48	26.94	26.80±0.12	0.368	NS
Crude protein(CP)	22.09	22.17	22.17	22.25	22.17±0.09	0.339	NS
Total ash(TA)	1.3450	1.3650	1.3650	1.3300	1.3513±0.01	0.055	NS
Acid insoluble ash (AIA)	Nill	Nill	Nill	Nill	NA	NA	NS
Crude fiber (CF)	0.610	0.630	0.625	0.615	0.6200±0.01	0.421	NS
Crude fat (EE)	4.360	4.405	4.505	4.4350	4.426±0.12	0.467	NS

Means within a column with different superscripts differ significantly (P<0.05)
Means within a column with same superscripts don't differ significantly (P>0.05)
SE = Standard Error
LSD =Least Significant Differenc
NS= Non significant

4.5 Analysis of microbial load of sheep GIT : The average value of microbial load of sheep GIT are shown in the Table 8.

The number of harmful bacteria reduced in probiotics groups (T_3 & T_4) and antibiotic groups (T_2) which had significant ($P< 0.05$) difference with sheep of control group. This result supported by many researchers demonstration. Probiotics responsible for competitive exclusion of pathogenic microorganism and adaptive immune system causing healthier body condition, Greenberg (1969). probiotics attach onto the intestinal mucosa and prevent adhesion of potential pathogens, leading to improved nutrient digestion that may enhance dry matter intake (Seo *et al.,* 2010). Highest levels of pathogen were owed to control group as compare to other experimental groups ($P < 0.05$).These results were in accordance to the (Awood, 2003).

Table 8. Effect of Probiotics on microbial load of sheep GIT

Parameter	T_1 group	T_2 group	T_3 group	T_4 group	Mean ± SE	LSD value	Level of significance
Microbial load in EMB (CFU/ml)	2.65×10^{8a}	4.45×10^{7b}	4.20×10^{7b}	8.85×10^{6b}	$9.008\times10^{7}\pm 0.38\times10^{8}$	0.158×10^{8}	*

Means within a column with different superscripts differ significantly ($P<0.05$)
Means within a column with same superscripts don't differ significantly ($P>0.05$)
SE = Standard Error
LSD =Least Significant Difference.
T_1= Control diet (No antibiotics and probiotics)
T_2= Diet in which antibiotics added
T_3= Diet in which bacterial probiotics added
T_4= Diet in which both bacterial and yeast probiotics added

CHAPTER 5

SUMMARY AND CONCLUSION

The study was conducted at Environmental Biotechnology Lab. and Animal Farm under the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka-1207. The research was for one year, but the sheep rearing period was for three months and it was the month of March-May,2018. Eight 8-9 month-old sheep were collected from Bangladesh Livestock Research Institute(BLRI), Savar, Dhaka. The sheep were group penned for each of the replication in grilled partition with concrete floor (1.5×1.5 m) that complied with welfare standards. The supplementations of antibiotics and probiotics was used. The control group- T_1 was fed a basal diet (unsupplemented - control), whereas the antibiotic and experimental groups was fed the same basal diet but supplemented with antibiotics and probiotics. Antibiotic group- T_2 containing 14 mg/L of Renamycin and probiotic group- T_3 (probiotics prepared by bacteria) provide 66.6 ml per kg of feed and probiotic group- T_4 (probiotics prepared by bacteria and yeast) provide 66.6 ml per kg of feed.

The probiotics groups of sheep showed significantly better result in live weight gain, FCR, dressing percentage, testicular weight, G.I.T weight, blood profile, microbial status; but no significant found in internal organ (liver, spleen, heart, lungs) and meat composition. So, probiotics can be used instead of harmful growth promoter because it increases production performance. All group consumed same amount of feed but probiotics group produce highest live weight which had significant ($P< 0.05$) difference with sheep of control and antibiotics group.

Testicular weight was significantly (P<0.05) highest in probiotics group. The significantly (P<0.05) lowest glucose level was found in probiotics group. That indicates better fiber digestion and better weight gain. The number of harmful bacteria reduced and number of friendly bacteria increased in probiotics group which had significant (P< 0.05) difference with sheep of control group. From the findings, it is concluded that the probiotics supplemented group achieved more friendly bacteria which are helpful for microbial digestion and microbial protein production; that results weight gain and better FCR. No significant (P> 0.05) difference was found in weight of liver weight, spleen, lungs and heart weight. Similarly, hematological parameters like cholesterol, PCV, TEC, WBC, Platelets and ESR did not affected (P> 0.05) by probiotics supplement. Again, fresh meat quality (colour, odour, WHC, texure), cooked meat quality (odour, tenderness, juiciness, taste) and chemical composition (% on DM basis) did not affected (P> 0.05) by probiotics supplement. Probiotics feeding as a feed additives causing better live weight gain by- competitive exclusion of pathogenic microorganism, and improved fibre digestion. It doesn't show any side effect like harmful growth promoter. So, probiotics can be use as a nutritious tool to reduce various complications and for improve animal production. The study therefore, recommends conducting field trail on sheep farm to use probiotics as a feed additive. In this line, future detail research works should be conducted on gut microbial status and immune system of sheep.

REFERENCES

Abas I., Kutay, H.C., Kahraman, R., Toker, N.Y, Ozcelik, D., Ates, F. and Kacakci, A. (2007). Effects of organic acid and bacterial direct-fed microbial on fattening performance of Kivircik-Male yearling lambs. *Pak. J. Nutr.* **6**: 149-154.

Abd El-Ghani, A.A. (2004). Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae)* on performance of Zaraibi goats. *Small Rumin. Res.* **24**: 53-70.

Abdelrahman, M.M. and Hunaiti, D.A. (2008). The effect of dietary yeast and protected methionine on performance and trace minerals status of growing Awassi lambs. J. Livest. Sci. 115: 235-241.

Acamovic, T. (2001). Enzymes for poultry. *World's Poultry Sci. J.* **57**: 225-242.

Adams, H.R. (2001). Veterinary pharmacology and therapeutics. 18th ed. Iowa state press, USA. pp. 310-311/ p. 310.

Agarwal, N., Kamra, D.N., Chaudhary, L.C., Agarwal, I., Sahoo, A. and Pathak, N.N. (2002). Microbial status and rumen enzyme profile of crossbred calves fed on different microbial feed additives. *Lett. Appl. Microbiol.* **34**(5): 329-336.

Anjum, M.I., Khan, A.G., Azim, A. and Afzal, M. (2005). Effect of dietary supplementation of multistrain probiotic on broiler growth performance. *Pak. Vet. J.* **25**(1): 25-29.

Annison, G. and Choct, M. (1993). Enzymes in poultry diets and Enzymes in Animal Nutrition. Proceedings 1st Symposium, Switzerland. Oct 13-16. pp. 61-63.

Antunovic, Z., Speranda, M., Amidzic, D., Seric, V., Steiner, Z., Domacinovic, N. and Boli, F. (2006). Probiotic application in lambs nutrition. Krmiva 48 : 175-180.

Antunovic, Z., Speranda, M., Liker, B., Seric, V., Sencic, D., Domacinovic, M. and Speranda, T. (2005). Influence of feeding the probiotic Pioneer PDFMR to growing lambs on performances and blood composition. Polish J. Vet. Sci. Acta Vet (Beograd) 55: 287-300.

Ao, T., Cantor, A.H., Pescatore, A.J., Ford, M.J., Pierce, J.L. and Dawson, K.A. (2009). Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *J. Poult. Sci*. **88**: 111-117.

Apata, D.F. (2008). Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *J Sci Food Agr.* **88**: 1253-1258.

Apata, D.F. (2009). Antibiotic resistance in poultry. *Int. J. Poult. Sci*. **8**: 404-408.

Armstrong, D. G. (1986). Gut active growth promoters: Control and manipulation of animal growth. 21-37.

Awood, M.H.H. (2003). Probiotics, perbiotics and immune-stimulation: More than an alternation for antibiotic growth promoters. *Asian Aus. J. Anim. Sci.* **1**(4): 12-18.

Bahrke, M.S. and Yesalis, C.E. (2004). Abuse of anabolic androgenic steroids and related substances in sport and exercise. Current Opinion in Pharmacology, 614-620.

Baiomy, A.A. (2011). Influence of live yeast culture on milk production, composition and some blood metabolites of Ossimi ewes during the milking period. *Am. J. Biochem. Mol. Biol.* **1**: 158-167.

Bauer, E.R., Daxenberger, A., Petri, T., Sauerwein, H., and Meyer, H.H. (2000). Characterization of the affinity of different anabolic and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. Acta Pathologica Microbiologica et Immunologica Scandinavic. **108**(12): 838-846.

Belewu, M.A. and Jimoh, N.O. (2005). Blood, carcass and organ measurements as influenced by *Aspergillus niger* treated Cassava waste in the diets of West African dwarf goat. *Glob. J. Agric. Sci*. **4**: 125-128.

Bellof, G. and Alarcon, S.C. (2013). Effect of *Spirulina platensis* in organic broiler production Archiv. Geflug. **77:** 73-80.

Berghmann, L.R., Abi-Ghanem, D., Wagnela, S.D. and Ricke, S.C. (2005). Antibodies: an alternative for antibiotics? *J. Poult. Sci.* **84**: 660–666.

Bharathidhasan, A., Chandrasekaran, D., Natarajan, A., Ravi, R. and Ezhilvalavan, S. (2009). Effect of enzyme supplementation on carcass quality, intestinal viscosity and ileal digestibilities of broilers to nutrient reduced diet. *Tamilnadu J. Vet. Anim. Sci.* **5** (6): 239-245.

Biggs, P., Parsons, C.M. and Fahey, G.C. (2007). Effects of several Oligosaccharides on growth performance, nutrient digestibilities, and caecal microbial populations in young chicks. *J. Poult. Sci*. **86**: 2327-2336.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Bioch. Phys.* **39**:911-917.

Bontempo, V.A., Giancamillo, G.P., Savoini, V.M. and Domeneghini, C.A. (2006). Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. *J. Anim. Feed Sci. Technol.* **129**: 224-236.

Botsoglu, N.A. and Fletouris, D.J. (2001). Drug resistant in food Pharmacology, Food safety and analysis. New York, Marcel Dekker, Inc. pp: 541-548.

Brenes, A., Guenter, W., Marquardt, R.R. and Rotter, B.A. (1993). Effect of glucanase pentosanase enzyme supplementation on the performance of chickens and laying hens fed wheat, barley, naked oats and rye diets. *Canadian J. of Ani. Sci*. **73**: 941–951.

Bruno, R.G., Rutigliano, H.M., Cerri, R.L., Robinson, P.H., Santos, J.E. (2009). Effect of feeding *Saccharomyces Cerevisiae* on performance of dairy cows during summer heat stress. *Anim. Feed Sci. Techn*. **150**: 175-186.

Carmichael, S.L., Shaw, G.M., Laurent, C., Croughan, M.S., Olney, R.S., and Lammer, E.J. (2005). Maternal progestin intake and risk of hypospadias. *Archives of Pediatrics and Adolescent Medicine.* **159**(10): 957-62.

Catry, B., Laevens, H., Devriese, L.A., Opsomer, G. and De Kruif, A. (2003). Antimcrobial resistance in livestock. *J. of Vet. Phar. Therapy.* **26**:81-93.

Chaucheyras-Durand, F., Walker, N.D. and Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Anim. Feed Sci. Techn.* **145**: 5-26.

Cheng, T.K., Coon, C.N. and Hamare, M.L. (1990). Effect of environmental stress on the ascorbic acid requirement of laying hens. *J. Poult. Sci*. **69**:774-80.

Chesson, A. (2001). Non-starch poly-saccharide degrading enzymes in poultry diets and influence of ingredients on selection of activities. *World's Poult. Sci. J.* **57**: 251-263.

Chiang, S.S. and Pan, T.M. (2012). Beneficial effects of Lactobacillus paracasei sub sp. paracasei NTU 101 and its fermented products. *Applied Microb. Biotech.* **93**(3): 903–16.

Chiofalo, V., Liotta, L., Chiofalo, B. (2004). Effects of the administration of Lactobacilli on body growth and on the metabolic profile in growing Maltese goat kids. *Reprod. Nutr. Dev.* **44**: 449-457.

Cmiljanic, R., Sretenovic, L.J., Trenkovski, S. and Marinkov, G. (2001). Systems of poultry nutrition and their effect on production traits and quality of product.

Biotech. Anim. Hus. **17**: 179-185.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbial. Rev*. **12:** 564- 582.

Cowieson, A.J., Acamovic, T. and Bedford, M.R. (2000). Enzyme supplementation of diets containing Camelina sativa meal for poultry. *Br. Poult. Sci.* **41**: 689-690.

Cristiana, G.R., Pop, I.M. and Simeanu, D. (2012). Effect of a Symbiotic Feed Additive Supplementation on Laying Hens Performance and Eggs Quality. Lucrări Ştiinţifice - vol. **53**: Seria Zootehnie.

Cross, D.E., Mcdevitt, R.M., Hillman, K. and 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. *Br. Poult. Sci*. **48**: 496-506.

Czerucka, D. and Rampal, P. (2002). Experimental effects of *Saccharomyces boulardii* on diarrheal pathogens. *J. Vet. Medi.* **4**(1): 733-739.

Danhaive, P.A. and Rousseau, G.G. (1986). Binding of glucocorticoid antagonists to androgen and glucocorticoid hormone receptors in rat skeletal muscle. *J. Steroid Bioch. Mol. Bio.* **24**: 481-487.

Dekkers, J.C., Van Doornen, L.J.P., and Han, C.G.K. (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Medicine. **21**: 213-238.

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C. and Sauvant, D. (2009). Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. *J Dairy Sci.* **92**: 1620-1632.

Dibaji, S.M., Seidavi, A. and Asadpour, L. (2012). Effect of dietary inclusion of the synbiotic biomin IMBO on broilers performance and some blood metabolites, research opinions. *J. Anim. Vet. Sci.* **2**: 10-13.

Dibner, J.J. and Richards, J.D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *J. Poult. Sci*. **84**: 634–643.

Dimova, N., Baltadjieva, M., Karabashev, V., Laleva, S., Popova, Y., Slavova, P., Krastanov, J. and Kalaydjiev, G. (2013). Effect of adding of probiotic "Zoovit" at feeding of lambs from breed synthetic population Bulgarian milk. *Bulg. J. Agric. Sci.* **19** : 98-101.

Ding, J., Zhou, Z.M., Ren, L.P. and Meng, Q.X. (2008). Effect of monensin and live yeast supplementation on growth per- formance, nutrient digestibility, carcass characteristics and ruminal fermentation parameters in lambs fed steam-flaked corn-based diets. *Asian-Aust. J. Anim. Sci.* **21**: 547-554.

Diplock, A.T., Charleux, J.L., Crozier-Willi, G., Kok, F.J., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. (1998). Functional food science and defense against reactive oxidative species. *British J. of Nutr*. **80**: 77-112.

Donoghue, D.J. (2003). Antibiotic residues in poultry tissues and eggs: human health concerns. *Poult. Sci*. **82**: 618–621.

Edens, F.W., Carter, T. A., Parkhurst, C. R. and Sefton, A. E. (2000). Effect of selenium source and litter type on broiler feathering. *J. App. Poult. Res.* **9**: 407-413.

Ellin D.M. (2001). Alternative to antibiotic use for growth promotion in animal Husbandary.

Elmore, R.G. (1992). Focus on bovine reproductive disorders: managing cases of fetal mummification. *J.Vet. Medi.* **87**: 155-159.

Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants. *J. Ethanopharmacol*. **60**: 1-8.

El-Sissi, A. Fand, S. and Mohamed, H. (2011). Impact of Symbiotic on the Immune Response of Broiler Chickens Against NDV and IBV Vaccines. *Global J. of Bio. & Biochem.* **6** (4): 186-191.

Erasmus, L.J., Botha, P.M. and Kistner, A. (1992). Effect of yeast culture supplement on production, rumen fermentation and duodenal nitrogen flow in dairy cows. *J. Dairy Sci.* **75**: 3056-3065.

Erasmus, L.J., Robinson, P.H., Ahmadi, A., Hinders, R. and Garrett, J.E. (2005). Influence of pre-partum and post partum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. *J. Anim. Feed Sci. Techn.* 122: 219-239.

Ertas, O.N., Guler, T., Ciftci, M., Dalkilic, B. and Simsek, U.G. (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *Int. J. Poult. Sci*. **4**: 879-884.

Falcao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J. and Mourao, J. L. (2007). Alternatives to antibiotic growth promoters in rabbit feeding. A review: *World Rabbit Sci.* **15**: 127-140.

FAO. Expert consultation report: Cordoba, Argentina : food and Agriculture organization of the united nations and world health organization,2001.

Freitag, M., Hensche, H.U., Schulte-Sienbeck, H. and Reichelt, B. (1999). Biologische Effekte konventioneller and alternativer Leistungs förderer. Kraftfutter. 49-57.

Friesen, O.D., Guenter, W., Marquardt, R.R. and Rotter, B.A. (1992). The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for the young broiler chick. *Poult. Sci*. **71**: 1710–1721.

Fuller R. (1989). Probiotics in man and animals. *J. App. Bacteriology*. **66**(5): 365–378.

Fuller R. (1995). Probiotics, their development and use. In: Old Herborn University Seminar.

Fuller, R. (1989). Probiotics in man and animals. *J. of App. Bact*. **66**: 365–378.

Galip, N. (2006). Effect of supplemental yeast culture and sodium bicarbonate on ruminal fermentation and blood variables in rams. *J. Anim. Physiol. Anim. Nutr. (Berl)*. **90**: 446-452.

Getachew, T. (2016). A Review on Effects of Probiotic Supplementation in Poultry Performance and Cholesterol Levels of Egg and Meat. *J. World Poult. Res.* **6**(1): 31-36.

Gibson, G.R. and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nut.* **125:** 1401-1412.

Gould, I.M. (2008). The epidemiology of antibiotic resistance. *Int. J. Antimicrob*.

Gray, J.I., Gomma, E.A. and Buckley, D.J. (1996). Oxidative quality and shelf life of meats. *J. Meat Sci.* **43**: S111-S123.

Griggs, J.P. and Jacob, J.P. (2005). Alternatives to antibiotics for organic poultry production. *J. Appl. Poult.* **14**: 750-756.

Guerra, N.P., Bern'ardez, P.F., M'endes, J., Cachaldora, P. and Castro, L.P. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *J. Anim. Feed Sci. and Tech.* **134**(1): 89-107.

Haddad, S.G. and Goussous, S.N. (2005). Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. *J. Anim. Feed Sci. Techn.* **118**: 343-348.

Harrigan, W.F. (1998). Laboratory methods in food microbiology. 3rd Ed. Academic

Press Limited, London.

Hejlicek, K., Soukupova, A. and Moltasova, M. (1995). Salmonellosis in 1-day-old chicks caused by Salmonella enteritidis. *Veterinarstvi*. **45**:16–18.

https:// www.drugs.com

Hughes, P. and Heritage, J. (2002). *Antibiotic Growth Promoters in Food Animals*, FoodandAgriculturalOrganization:http://www.fao.org/DOCREP/ARTICLE/AGRIPP A/555_EN. HTM.

Huntington, G.B., Eisemann, J.H. (1988). Regulation of nutrient supply bygut and liver tissues. *J. Anim. Sci.* 66 Suppl: 3-35.

Hussein, A.F. (2014). Effect of biological additives on growth indices and physiological responses of weaned Najdi ram lambs. *J. Exp. Biol. Agricl. Sci.* **2**: 597- 607.

Ian P.I. (1999). Assessing the evidence that antibiotic growth promoters influence human infections. *J. Hospital Infect.* **43**: 173-178.

Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H. and Salminen, S. (2001). Probiotics: Effects on immunity. *American J. Clinical Nutr.* **73**: 444-450.

Izumi, M., McDonald, M.A., Chatterjee, P.K. and Thiemermann, C. (2002). Superoxide dismutase mimetics with catalase activity reduces the organ injury in hamerrhogic shock. **18** (3): 230-235.

Jang, Y.D., Oh, H.K., Piao, L.G., Choi, H.B., Yun, J.H.and Kim, Y.Y. (2009). Evaluation of Probiotics as an Alternative to Antibiotic on Growth Performance, Nutrient Digestibility, Occurrence of Diarrhea and Immune Response in Weaning Pigs. *J. Anim. Sci. Techn.* **51**: 25-32.

Jensen, B.B. (1998). The impact of feed additives on the microbial ecology of young pigs. *J. of Ani. and Feed Sci.* **7**: 45-64.

Jensen, C., Lauridsen, C. and Bertelsen, G. (1998). Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science and Technology* **9**: 62-72.

Jin, L. Z., Ho, Y. W., Abdullah, N. and Jalaludin, S. (1998). Acid and bile tolerance of Lactobacillus isolated from chicken intestine. Lett. App. Microb. **27**(3): 183-185.

Kabir, S.M.L., Rahman, M.M., Rahman, M.B. and Ahmed, S.U. (2004). The dynamics of probiotics on growth performance and immune response in broilers. *Int. J. Poult. Sci*. **3** (5): 361-364.

Kaczmarski, M., Wojicicki, J., Samochowiee, L., Dutkiewicz, T. and Sych, Z. (1999).

The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. *Die Pharmaize* **54**: 303-306.

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

Kanacki, Z., Krnic, J., Uscebrka, G., Peric, L. and Stojanovic, S. (2008). Uticaj razlicitih izvora selena u ishrani tovnih pilica na pojedine proizvodane i biohemijske parameter. Savremena poljoprivreda. **57**, 1-2, 160-165.

Kawas, J.R., Garcia-Castillo, R., Fimbres-Durazo, H., Garza-Cazares, F., Hernandez-Vidal, J.F., Olivares-Saenz, E. and Lu, C.D. (2007a). Effects of sodium bicarbonate and yeast on nutrient intake digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. *Small Rumin. Res*. **67**: 149-156.

Kawas, J.R., Garcia-Castillo, R., Garza-Cazares, F., Fimbres-Durazo, H., Olivares-Saenz, E., Hernandez-Vidal, G. and Lu, C.D. (2007b). Effects of yeast and sodiumbicarbonate on productive performance and carcass characteristics of light-weight lambs fed finishing diets. *Small Rumin. Res.* **67**: 157-163.

Khadem, A.A., Pahlavan, M., Afzalzadeh, A. and Rezaeian, M. (2007). Effects of live yeast *Saccharomyces cerevisiae* on fermentation parameters and microbial populations of rumen, total tract digestibility of diet nutrients and on the in situ degradability of alfalfa hay in Iranian Chall sheep. *Pak. J. Biol. Sci.* **10**: 590-597.

Khadour, M. and Travers, G.T. (2015). Probiotics and prebiotics in animal feeding for safe food production. *Interl. J. Food Microb.* **141**: 15-28

Khalid, M.F., Shahzad, M.A., Sarwar, M., Rehman, A.U., Sharif, M. and Mukhtar, N. (2011). Probiotics and lamb performance: A review *Afr. J. Agr. Res.* **6**: 5198-5203.

Khosravi, A., Boldaji, F., Dastar, B. and Hasani, S. (2010). Immune response and performance of broiler chicks fed Protexin and propionic acid. *Int J Poultry Sci*. **9**: 188-191.

Kirchgessner, M., Roth, F. X. and Steinruck, U. (1991). Nutritive Wirkung von Fumarsäure bei Änderung der Protein qualität und des Proteingehaltes im Futter auf die Mastleistung von Broilern. Archiv für Geflügelkunde. **55**: 224-232.

Kochetkov, N.K., Khorlin, A.Y., Chizhov, O.S. and Sheichenko. (1962). Chemical

investigation of *Schisandra chinensis*: Communication Structure of Schizandrin. Izvestiyia Akademii Nauk SSSR, Seriya Khericheskaya; No. **5**: 850-856.

Lawrence, T.L.J. and Fowler, V.R. (2012). Growth of farm animal**. In: '**Growth Promoters', Performance Enhancers, Feed Additives and Alternative Approaches. Aberdeen, UK. pp. 320-346 / p. 321.

Le Guevel, R. and Pakdel, F. (2001). Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. *Human Repro.* **16**: 1030-6.

Leffers, H., Naesby, M., Vendelbo, B., Skakkebaek, N.E. and Jorgensen, M. (2001). Oestrogenic potencies of zeranol, oestradiol, diethylstilboestrol, bisphenol-A and genistein: implications for exposure assessment of potential endocrine disrupters. *Human Repro*. 16: 1037-45.

Lesmeister, K.E., Henrichs, A.J., Gabler, M.T. (2004). Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics and blood parameters in neonatal dairy calves. *J. Dairy Sci.* **87**: 1832-1839.

Li, L.L., Hou, Z.P., Li, T.J., Wu, G.Y., Huang, R.L., Tang, Z.R., Yang, C.B., Gong, J., Yu, H. and Kong, X.F. (2008). Effects of dietary probiotic supplementation on ileal digestibility of nutrients and growth performance in 1- to 42-day-old broilers. *J. Sci. Food Agr.* **88**: 35-42.

Li, X. Qiang, L. and Liu, C.H. (2008). Effects of supplementation of fructooligosaccharide and *Bacillus subtilis* to diets on performance and on intestinal microflora in broilers. Archiv fur Tierzucht. **511**(1): 64-70.

Line, E.J., Bailey, S.J., Cox, N.A., Stern, N.J. and Tompkins, T. (1998). Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. *J. Poult. Sci.* **77**: 405-410.

Liu, S. and Lin, Y.C. (2004). Transformation of MCF-10A human breast epithelial cells by zeranol and estradiol-17beta. Breast J. **10**(6): 514-21.

Lukic, M., Sinovec, Z., Pavlovski, Z., Cmiljanic, R. and Spasojevic, I. (2002). Effect of microbial phytase in nutrition of broilers on production performance and carcass quality. European Poultry Conference, September, Bremen, Archiv für Geflugelkunde, Band. **66**: 138.

Macedo, R., Arredondo, V. and Beauregard, J. (2006). Influence of yeast culture on productive performance of intensively fattened Pelibuey lambs in colima. *Mex*

Rev AIA. **10**: 59-67.

Mahady, G.B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. *Curr. Pharm. Des.* **11**: 2405-2427.

Maiorka, A., Santin, E., Sugeta, S.M., Almeida, J.G. and Macari, M. (2001). Utilizacao de prebioticos, probioticos ou simbioticos em dietas para frangos. Revista Brasileira de Ciencia Avicola. **3**: 75-82.

Maravelias, C., Dona, A., Stefanidou, M. and Spiliopoulou, C. (2005). Adverse effects of anabolic steroids in athletes. Toxicology Letters. 158: 167-175.

Marquardt, R.R., Boros, D., Guenter, W. and Crow, G. (1994). The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a Trichoderma reesei enzyme preparation. *Anim. Feed Sci. Techn.* **45**: 363–378.

McCartney, E. (2002). The natural empire strcks book. *poult. Int.* **41** (1): 36-42.

Medina, A.L, Lucero, M.E. Holguin, F.O. Estell, R.E. Posakony, J.F. Simon, J. and O‟Connell, M. (2005). Composition and antimicrobial activity of *Anemopsis califofornica* leaf oil. *J. agr. Food Chem.* **53**: 8694-8698.

Mehr, M.A., Shargh, M.S., Dastar, B., Hassani, S. and Akbari, M.R. (2007). Effect of different levels of protein and Protexin on broiler performance. *Int. J. Poult. Sci.* **6**:573-577.

Mikelsaar, M. and Zilmer, M. (2009). *Lactobacillus fermentum* ME-3 - an antimicrobial and antioxidative probiotic. **In**: Microbial Ecology in Health and Disease, vol. **21**. pp.1-27.

Mohammadian, A., Mehdizadeh, S.M., Lotfollahian. H., Mirzaei, F. and Noroozian, H. (2013). Influence of dietary probiotic (Biomin IMBO) on performance of laying hen. *J. Agric. Sci.* **4**(2): 23-26.

Mohan, I.K., Khan, M., Shobha, J.C., Naidu, M.U.C., Prayag, A. and Kuppusamy, P. (2006). Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats. *Cancer Chem. Pharm.* **58**(6): 802–808.

Morgan, A.J. and Bedford, M.R. (1995). Advances in the development and application of feed enzymes. Australian Poultry Science Symposium **7:** 109–115.

Moritz, V.V. (2001). Antibiotic resistance, with special reference to poultry production,conf. OIE 2001.

Mountzouris, K.C., Tsitrsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. and Fegeros, K. (2007). Evaluation of the efficacy of a probiotic containing Lactobacillus,

Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Sci.* **86**: 309-317.

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

Muir, W.I., Bryden, W.L. and Husband, A.J. (1998). Evaluation of the efficacy of intraperitoneal immunization in reducing Salmonella typherurium infection in chickens. *Poult Sci*. **77**:1874–1883.

Munck, A., Guyre, P.M. and Holbrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Rev. **5**: 25–44.

Musa, H.H., Wu, S.L., Zhu, C.H., Seri, H.I. and Zhu, G.Q. (2009). The Potential benefits of Probiotics in animal production and health. *J. Anim. Vet. Adv.* **8**: 313-321.

Nayebpor, M., Farhomand, P. and Hashemi, A. (2007). Effects of different levels of direct fed microbial (Primalac) on growth performance and humoral immune response in broiler chickens. *J Anim Vet Adv*. **6**: 1308-1313.

Neuman, L. (1997). Beef Cattle. **In**: Growth promoter: production of beef cattle. New York, USA. pp. 8-11/ pp. 9.

Newbold, C.J., Wallace, R.J. and Mclntosh, F.M. (1996). Mode of action of yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Br. J. Nutr.* **76**: 249-261.

Newman, E.K. (1999). Feeds with antibiotic growth promoters – The oligosaccharide alternative, Biotechnology responds. Alltech's 1999 European, Middle Eastern and African Lecture Toure.

Niewold, T. A. (2007). The nonantibiotic and inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *J. Poult. Sci*. **86**: 605–609.

Nisbet, D.J., Corrier, D.E., Scanlan, C.M., Hollister, A.G., Beier, R.C. and Deloach, J.R. (1993). Effect of a defined continuous flow derived bacterial culture and dietary lactose on Salmonella colonization in broiler chickens. *J. Avian Dis.* **37**: 1017–1025.

Ocak, N., Erener, G., Burak, A.K.F., Sungu, M., Altop, A. and 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. J. Ani. Sci.* **53**(4): 169-175.

Panda, A.K., Rao, S.V.R., Raju, M.V. and Sharma, S.R. (2006). Dietary supplementation of Lactobacillus sporogenes on performance and serum biochemico-lipid profile of broiler chickens. *J. Poult. Sci.* **43**: 235-240.

Pankay, L., Jingar, S.C. and Kantwa, S.C. (2014). Effect of probiotic supplementation on growth performance of Lambs. *Indian Vet. J*. **91**(05): 76-77.

Panossian, A., Wikman, G. and Wagner, H. (1999). Plant adaptogens III Earlier and More recent aspects and concepts on their modes of action. Phytomedicine. **6**(4): 287–300.

Papatsiros, V.G., Tassis, P.D., Tzika, E.D., Papaioannou, D.S., Petridou, E., Alexopoulos, C. and Kyriakis, S.C. (2011). Effect of benzoic acid and combination of benzoic acid with a probiotic containing Bacillus cereus var. Toyoi in weaned pig nutrition. *Pol. J. Vet. Sci.* **14**: 117-125.

Parida, R.N.S., Bisoi, P.C., Sahoo, P.K. and Mohapatra, M. (1995). Protective effect of Livol during experimental aflatoxicosis in chicks: a histomorphological approach. *Indian J. Indg. Med*. **17**: 19-25.

Partridge, G. and Wyatt, C. (1995). More flexibility with new generation of enzymes. *World Poult. J.* **11**: 17-21.

Pascual, M., Hugas, M., Badiola, J.I., Monfort, J.M. and Garriga, M. (1999). *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. *App. Envil. Microb.* **65**: 4981–4986.

Patterson, J. A. and Burkholder, M. K. (2003). Application of prebiotics and probiotics in poultry production. *J. Poult. Sci.* **82:** 627-631.

Payandeh, S. and Kafilzadeh, F. (2007). The effect of yeast (*Saccharomyces cerevisiae*) on nutrient intake, digestibility and finishing on performance of lambs fed a diet based on dried molasses sugar beet-pulp. *Pak. J. Biol. Sci*. **10**: 4426-4431.

Pepper, R. and Dobson, H. (1987). Preliminary results of treatment and endocrinology of chronic endometritis in the dairy cow. The Veterinary Record. 120: 53-56

Perić, L., Kovčin, S., Stanaćev, V. and Milošević, N. (2002). Effect of enzymes on broiler chick performance. Buletinul Usamv. **57**: 245-249.

Peric, L., Milic, D., Milosevic, N., Zikic, D., Nollet, L. and Spring, P. (2008b). Effect of different dietary selenium sources on performance of broiler breeders and progeny chick quality. Savremena poljoprivreda. **57**: 3-4, 238-243.

Peric, L., Milosevic, N. and Zikic, D. (2006). Effect of Sel-Plex and Bioplex substitution of inorganic trace mineral sources on performance, feathering and hock condition of broilers. World‟s Poultry Science Journal, XII European Poultry Conference. Book of Abstracts. **62**: 346-347.

Perić, L., Milošević, N., Đukić-Stojčić, M. and Bjedov, S. (2008a). Effect of phytogenic products on performance of broiler chicken. World Nutrition Forum, Mayrhofen, Austria: Nottingham University Press. 18-20, 325.

Perić, L., Nollet, L., Milošević, N. and Žikić, D. (2007b). Effect of Bioplex and Sel-Plex substituting inorganic trace mineral sources on performance of broilers. Archiv für Geflügelkunde. **71**: 3, 122-129.

Petrof, E.O., Kojima, K., Ropeleski, M.J., Musch, M.W., Tao, Y. De Simone, C. and Chang, E. B. (2004). Probiotics inhibit nuclear factor kappa-B and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. **In**: Gastroenterology. vol. **127**: 2004, p. 1474-1487.

Poindron, P. (2005). Mechanisms of activation of maternal behaviour in mammals. *Reprod. Nutri. Devl*. 45: 341-51.

Prescott, J.F. and Baggot, J.D. (1993). Antimicrobial Therapy in Veterinary Medicine. 2nd edition. Iowa State University Press. pp 564-565.

Que´re´, P. and Girard, F. (1999). Systemic adjuvant effect of cholera toxin in the chicken. *Vet. Immun. Immunopath*. **70**:135–141.

Rao, A.T., Panda, S.K. and Sahu, R.K. (1995). Effect of zeetress on infectious bursal disease vaccinated & challenged birds. *Indian J. Indg. Med.* **17**: 43-6.

Roch, G., Boulianne, M. and De Roth, L. (1998). Dietary antioxidants reduce ascites in broilers. World Poultry. 11: 16.

Russell, J.B. and Wilson, D.B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J. Dairy Sci.* **79**: 1503-1509.

Sahin, K., Sahin, N. and Yaralioglu, S. (2002). Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites, and mineral concentrations of laying hens reared at high ambient temperature. *Biol. Trace Elem. Res.* **85**(1): 35-45.

Sahin, T., Kaya, I., Unal, Y. and Email, D.A. (2008). Dietary supplementation of probiotics and prebiotics combination on performance, carcass quality and blood parameters in growing quaile. *J. Anim. Vet.* **7**: 1370-1373.

Saleh, F., Ohtsuka, A., Tanaka, T. and Hayashi, K. (2003). Effect of enzymes of microbial origin on in vitro digestibilities of dry matter and crude protein in maize. *J. Poult. Sci*. **40**: 274-281.

Sanders, G., and Ley, A. (2008). The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. *J. Dairy Sci.* **92**(1): 343-351.

Sarica, J., Akre, C., van Ginkel, L., Le Bizec, B., De Brabander, H., Korth, W., Points, J., Teale, P. and Kay, J. (2005). Presence and metabolism of endogenousandrogenic-anabolic steroid hormones in meat-producing animals. *J. Anim. Sci*. **26**(5): 640-71.

Sayed, A.S. (2003). Studies on the influences of pronifer as a probiotic on the clinical, hematological and biochemical status of goat kids. *Assiut. Vet. Med. J.* **99**: 131-143.

Schutte, J.B., de Jong, J. and Langhout, D.J. (1995). Effect of a xylanase enzyme supplementation to wheat-based diets in broiler chicks in relation to dietary factors. Proceedings of the Second European Symposium on Feed Enzymes, Noordwijkerhout, Netherlands. pp. 95–101.

Seo, J.K., Kim, S.W., Kim, M.H., Santi, D., Kam, D.K. and Ha, J.K. (2010). Direct-fed microbials for ruminant animals. *Asian-Aust. J. Anim. Sci.* **23**: 1657-1667.

Shams, M., Azadeghan M.M., Dastar, B. and Hosseini, S. (2008). Effect of different levels of protein and probiotics on production traits and some blood parameters in broiler. *J. Agricl. Sci.* **14**(5): 15-17.

Sharifi, M.R., Shams, M., Dastar, B. and Hosseini, S. (2011). The effect of dietary protein and symbiotic on performance parameters, blood characteristics and carcass yields of Japanese quail (coturnix coturnix japonica). *Italian J. Anim. Sci.* **10**: 17-21.

Sheldon, I.M. and Noakes, D.E. (1998). Comparison of three treatments for bovine endometritis. The Veterinary Record. pp. 142/ 575-579.

Shen, Q., Shang, N. and Li, P. (2011). Invitro and invivo antioxidant activity of

Bifidobacterium animals isolated from centenarians. **In**: Current Microbiology, vol. **62**(4): 1097-1103.

Sher, Y.H., Shinde, P.L., Choi, J.Y., Kim, J.S., Seo, D.K., Pak, J.I., Chae, B.J. and Kwon, I.K. (2010). Evaluation of multimicrobial probiotics produced by submerged liquid and solid substrate fermentation methods in broilers. *Asian Aust. J. Anim. Sci.* **23**: 521-529.

Shoeib, H.K. and Madian, A.H. (2000). A study on the effect of breeding diets containing probiotics (pronifer and biogen) on growth performance, intestinal flora and hematological picture of broiler chicks. *J. Assiut. Vet. Medi.* **47**: 112-125.

Shreedhar, J.N., Patil, M., and Kumar, P. (2016). Effect of probiotics supplementation on milk yield and its composition in lactating holstein fresien and deoni cross bred cows. *J. Medil. Bio.* **5**(1): 47-49.

Silversides, F.G. and Bedford, M.R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poult. Sci*. **78**: 1184-1190.

Soliman, A. M., and Shinnawy, A.M. (2016). Effects of Supplementation of Probiotics on the Performance, Nutrient Digestibility and Faecal Microflora in lamb. *Asian Aust. J. Anim. Sci.* **26**(3): 57-61.

Soren, N.M., Tripathi, M.K., Bhatt, R.S. and Karim, S.A. (2013). Effect of yeast supplementation on the growth performance of Malpura lambs. *Trop. Anim. Health Prod.* **45**: 547-554.

Steiner, T. (2006). The potential benefits of Natural Growth Promoters. *J. Feed Tech.* **10**(2): 26– 28.

Stobbering, E., Bogaard, A.E., London, N., Driessen, C., Top, J. and Williams, R. (1999). Enterococci with glycopetidic resistance in turkeys, turkey farmers, turkey slaughterers and (sub) urban residents in the South of The Netherlands: evidence for transmission of vancomycin resistance from animals to humans. Antimicrob. Agents Chemotherapy. **43**: 2215-2221.

Strus, M., Pakosz, K., Gościniak, H., Przondo-Mordarska, A., Rozynek, E., Pituch, H., Meisel-Mikolajczyk, F. and Heczko, P.B. (2001). Antagonistic activity of Lactobacillus bacteria strains against anaerobic gastrointestinal tract pathogens

(Helicobacter pylori, Campylobacter coli, Campylobacter jejuni, Clostridium difficile). **In**: Medycyna Doswiadczalna i Mikrobiologia, vol. **53**: p. 133-142.
substances in sport and exercise. Current Opinion in Pharmacology. 4: 614-620.

Surai, P. (2002). Natural antioxidants in Avian Nutrition and Reproduction. Nottingha University Press, Nottingham, UK, 621.

Swan, S.H., Liu, J.W., Brazil, C. and Skakkebaek, N.E. (2007). Semen quality of fertile US males in relation to their mothers beef consumption during pregnancy. *J. App. Livestock.* **12**(2): 23-25.

Takemura, H., Sher, J.Y., Sayama, K., Tsubura, A., Zhu, B.T. and Sheroi, K. (2007). Characterization of the estrogenic activities of zearalenone and zeranol in vivo and in vitro. *J. Steroid Biochem. Mol. Bio.* **103**(2): 170.

Titi, H.H., Dmour, R.O. and Abdullah, A.Y. (2008). Growth performance and carcass characteristics of Awassi lambs and Shami goat kids fed yeast culture in their finishing diet. *Anim. Feed Sci. Techn.* **142**: 33-43.

Trafalska, E. and Grzybowska, K. (2004). Probiotics-An alternative for antibiotics? . *J. Appl. Poult.* **57**: 491-498.

Travers, M.A., Florent, I., Kohl, L. and Grellier, P. (2011). Probiotics for the control of parasites. *J. Anim. Poul. Prod. 7*(10): 369- 376.

Tripathi, M.K. and Karim, S.A. (2010). Effect of individual and mixed live yeast culture feeding on growth performance, nutrient utilization and microbial crude protein synthesis in lambs. *Anim. Feed Sci. Techn.* **155**: 163-171.

Tripathi, M.K. and Karim, S.A. (2011). Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. *J. Livest. Sci.* 135: 17-25.

Turnidge, J. (2004). Antibiotic use in animals-prejudices, perceptions and realities. *J. Antimicrob. Chemother.* **53**: 26-27.

Uščebrka, G., Žikić, D., Perić, L., Jurcoane, S. and Milošević, N. (2005). Effect of probiotic supplementation on the small intestinal morphology of broiler chickens. Proceedings of the 34th International session of scientific communications of the faculty of animal science, Bucharest, Romania. 67-71.

van den Bogaard, A.E. and Stobberingh, E.E. (2000) . Epidemiology of resistance to antibiotics Links between animals and humans. *Int. J. of Anti. Agents.* **14**: 327–

335.

Van der Klis, J.D., Schelle, C. and Kwakernaak, C. (1995). Wheat characteristics related to its feeding value and to the response of enzymes. Proceedings 10th European Symposium on Poultry Nutrition, Antalya, Turkey. World's Poultry Science Association pp. 160–168.

Verstegen M.W.A. and Williams B.A. (2002). Alternatives to the use of antibiotics as growth promoters for monogastric animals. *Anim. Biotechn.* **13**: 113–127.

Wagner, H. (1995). Immunostimulants and adaptogens from plants. In Amason JT *et al.,* eds., Phytochemistry of Medicinal Plants. New York: Plenum Press. 1-18.

Wang, Y. and Gu, Q. (2010). Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. *Res. Vet. Sci.* **89**: 163-167.

Waziry, A.M. and Ibraher H.R. (2007). Effect of *Saccharomyces cerevisiae* of yeast on fiber digestion in sheep fed berseem (Trifoliumalexandrium) hay and cellulose activity. *Aust. J. Basic Appl.Sci.* **1**:379-385

Whitley, N.C., Cazac, D., Rude, B.J., Jackson-O'Brien, D. and Parveen, S. (2009). Use of commercial probiotics supplement in meat goats. *J. Anim. Sci.* **87**: 723-728.

WHO. (1997). The medical impact of the use of antimicrobials in food animals. Report from a WHO meeting; Berlin, Germany Oct 13-17. Geneva: world Health Organisation.

Wiebe, J.P. (2006). Progesterone metabolites in breast cancer. Endocrine-Related Cancer. **13**: 717-738.

Williams, P. and Losa, R. (2001). The use of essential oils and their compounds in poultry nutrition. *World Poult. Elsevier.* **17** (4): 14-15.

Wray, C. and Davies, R.H. (2000). Competitive exclusion-an alternative to antibiotics. *J. Vet. Sci.* **159**: 107-108.

Yang, Y., Iji, P.A. and 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 Poult.Sci. J.* **65:** 97-114.

Yirga, H. (2015). The use of probiotics in animal nutrition. *J. Prob. Health.* **3**: 132.

Yuri, T., Tsukamoto, R., Miki, K., Uehara, N., Matsuoka, Y. and Tsubura, A. (2006). Biphasic effects of zeranol on the growth of estrogen receptor-positive human breast carcinoma cells. *Oncology Reports*. **16**(6): 1307-12.

Zacconi, C., Bottazzi, V., Rebecchi, A., Bosi, E., Sarra, P.G. and Tagliaferri, L. (1992).

Serum cholesterol levels in axenic mice colonized with *Enterococcus faecium* and *Lactobacillus acidophilus*. Microbiologica **15**: 413-417.

Zakeri, A. and Kashefi, P. (2011). The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. *J. Anim. Vet. Adv*. **10**: 1097-1101.

Zarei, M., Ehsani, M. and Torki, M. (2011). Dietary inclusion of probiotics, prebiotics and synbiotic and evaluating performance of laying hens. *American J. Agricl. Biol. Sci.* **6**: 249-255.

Zeitoun, S.M., Shinnawy, A.M. and Soliman, A. M. (2014). Impact of the aqueous extract of dandelion, probiotic and their synbiotic on male lamb's testicular histopathology relative to semen characteristics. *J. Anim. Sci.* **4**(1): 23-30.

Zulkifli, I., Abdullah, N., Azrin, N. M., , Ho, Y.W. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracy-cline under heat stress conditions. *J . Br. Poult. Sci.* **41**: pp.593–597.

Appendix 1. Initial weight, weight just before slaughter, weight gain of different replication of sheep under different treatment

Treatment	Replication	Initial wt. (kg)	Wt. just before slaughter (kg)	Weight gain (kg)
T_1	R_1	7.8	11.3	3.5
	R_2	9.7	12.9	3.2
T_2	R_1	11.0	15.7	4.7
	R_2	10.9	15.4	4.5
T_3	R_1	11.4	16.3	4.9
	R_2	9.5	14.6	5.1
T_4	R_1	10.2	16.3	6.1
	R_2	9.9	14.3	4.4

Appendix 2. Live weight, Eviscerated weight and Dressing percentage of different replication of sheep under different treatment

Treatment	Replication	Live weight (kg)	Eviscerated Weight(kg)	Dressing Percentage (%)
T_1	R_1	11.3	6.2	54.87
	R_2	12.9	6.9	53.49
T_2	R_1	15.7	8.4	53.50
	R_2	15.4	8.2	53.25
T_3	R_1	16.3	8.8	53.99
	R_2	14.6	7.9	54.11
T_4	R_1	16.3	8.7	53.37
	R_2	14.3	7.6	53.15

Appendix 3. Haematological value of different replication of sheep under different treatment

Treatment	T_1		T_2		T_3		T_4	
Replication	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Hemoglobin (g/dl)	10.23	10.30	10.90	10.80	10.60	10.90	10.70	10.50
Glucose (mmol/L)	3.91	3.89	3.71	3.75	2.66	2.60	3.35	3.01
cholesterol(mmol/L)	0.61	0.65	0.64	0.63	0.64	0.65	0.61	0.66
TEC (million/Cumm)	4.10	4.14	4.15	4.13	4.15	4.11	4.15	4.17
WBC (/Cumm)	15300	14600	13800	15500	14300	15200	16200	13300
PCV (%)	31.5	31.2	31.5	31.4	31.2	31.4	31.25	31.5
ESR(mm in 1st hour)	05	04	04	05	03	05	02	06

Appendix 4. Microbial load of different replication of sheep under different treatment

Treatment	Replication	Microbial load
T_1	R_1	2.5×10^8
	R_2	2.8×10^8
T_2	R_1	3.2×10^7
	R_2	5.7×10^7
T_3	R_1	5.3×10^7
	R_2	3.1×10^7
T_4	R_1	9.0×10^6
	R_2	8.7×10^6

Appendix 5. Meat quality parameter of different replication of sheep under different treatment

Treatment	T_1		T_2		T_3		T_4	
Replication	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Moisture	72.9	73.45	73.10	72.95	73.13	73.90	73.24	72.85
DM	27.10	26.55	26.90	27.05	26.87	26.10	26.74	27.15
CP	21.70	22.48	22.2	22.15	22.37	21.98	22.45	22.05
Total Ash	1.30	1.39	1.33	1.40	1.41	1.32	1.36	1.30
CF	0.60	0.62	0.65	0.61	0.57	0.68	0.62	0.61
EE	3.99	4.73	4.76	4.05	4.81	4.2	4.72	4.15

Appendix 6. Weight of testicle of different replication of sheep under different treatment

Treatment	Replication	Testicular weight (gm)
T_1	R_1	132
	R_2	127
T_2	R_1	129
	R_2	138
T_3	R_1	148
	R_2	141
T_4	R_1	143
	R_2	149

Appendix 7. Effect of dietary supplementation of probiotics on Liver, heart, spleen, lungs, and G.I.T weight of different Treatment

Treatment	T_1		T_2		T_3		T_4	
Replication	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2
Liver wt. (gm)	270	263	267	265	259	272	255	275
Heart wt. (gm)	65	60	68	59	75	53	67	61
Spleen wt. (gm)	45	42	51	43	57	49	60	52
lungs wt. (gm)	149	153	152	150	151	148	152	145
G.I.T wt. (kg)	3.58	3.62	3.60	4.10	4.20	3.95	4.31	4.5