USES OF PREPARED PROBIOTICS AS GROWTH PROMOTER IN CATTLE PRODUCTION TO AVOID ADVERSE EFFECT ON HEALTH

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DECEMBER, 2019

USES OF PREPARED PROBIOTICS ASGROWTH PROMOTERS IN CATTLE PRODUCTION TO AVOID ADVERSE AFFECT ON HEALTH

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A Thesis
Submitted to the Department of Animal Production & Management
Sher-e-Bangla Agricultural University, Dhaka 1207
In Partial Fulfilment of the Requirements
for the degree of

MASTER OF SCIENCE (MS) IN ANIMAL SCIENCE

SEMESTER: December, 2019

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CERTIFICATE

This is to certify that the thesis entitled, "USES OF PREPARED PROBIOTICS INSTEAD OF HARMFUL GROWTH PROMOTERS IN CATTLE PRODUCTION TO AVOID ADVERSE AFFECT ON HEALTH" submitted to the Department of Animal Production and Management, Faculty of Animal science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment 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 Farzana Yasmin, Registration No. 18-09077 under my supervision and my 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.

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Dedicated to my beloved parents

ACKNOWLEDGEMENT

At first, all praises are due to the Omnipotent, the Almighty Allah, and Supreme Authority of this universe, who enabled the author to complete this thesis and to get the degree of MS in Animal Science. The author would like to express her deepest gratitude to the following people for guidance, support, consideration and friendship.

Professor ***Dr. Md. Jahangir Alam****, Chairman of the Department of Animal Production and Management, Sher-e-Bangla Agricultural University (SAU), respected teacher, enormously supportive & positive supervisor, for his warmth and indomitable guidance throughout the period of research work and sincere co-operation, inspiration and valuable suggestions and for the completion of the research work.*

The author finds it a great pleasure in expressing her heartfelt gratitude and immense indebtedness to her research co-supervisor ***Prof. Dr. Md. Ekramul Haque****, Chairman of the Department of Biotechnology, Sher-eBangla Agricultural University (SAU) Dhaka, for his sympathy, sincere cooperation and valuable suggestions for the successful completion of the research work and preparation of the manuscript, for scientific discussions, for support and for never closing his door.*

The author feels much pleasure to convey heart-felt gratitude and thanks to the staffs of the department of Animal Production and Management, SAU, Dhaka for their co-operation. The author deeply owes her whole hearted thanks to friends, well-wishers specially Abdullah Al Maruf (PhD fellow, Dept. of Medicine, BAU), Asraf Islam (MS fellow, Department of Animal Production and Management, SAU) and all of her classmates for their good wishes, constant friendly inspiration, support and spontaneous cooperation during the entire period of the study and the writing up the thesis.

Finally, the author is overwhelmed with great passion and emotion in expression her lifelong indebtedness and expresses her utmost gratitude to her family and all the relatives, specially to her beloved father Md. Zayedul Haque and mother Farida Haque for their everlasting love, patience, sacrifice, blessing and co-operation not only during the study period to complete her higher study but during whole period of author's life.

The Author

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ABBREVIATIONS

ANOVA	Analysis of Variance
BQ	Black Quarter
CBC	Complete Blood Count
CFU	Culture Forming Unit
DMI	Dry Matter Intake
EDTA	Ethylenediamine tetraacetic acid
FAO	Food and Agricultural University
FCR	Feed Conversion Ratio
FMD	Food And Mouth Disease
HCL	Hydrochloric Acid
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MRS	De Man S Rogosa and Sharpe
NaOH	Sodium Hydroxide
PCA	Plate Count Agar
PCV	Packed Cell Volume
PDA	Potato Dextrose Agar
RBC	Red Blood Cell

SAU	Sher-e-Bangla Agricultural University
SPSS	Statistical Package For Social Sciences
WBC	White Blood Cell
WHO	World Health Organization
YC	Yeast Culture

LIST OF SYMBOLS

< = less than

± = plus minus

> = greater than

% = percentage

/ = per

: = ratio

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ABSTRACT

The present study was conducted to evaluate the effect of probiotics as supplemented feed additives in cattle production. A total of 9 cross bred bull calves (three for each treatment) were assigned to the three experimental dietary treatments. Experimental groups consisted of control T_1 (basal diet with no probiotics), T_2 (fed probiotic containing bacteria culture) and T_3 which were fed probiotics with bacteria and yeast both culture throughout the experimental period. Compared to control, inclusion of probiotic increased ($P<0.05$) total live weight gain. Significantly ($P<0.05$) lowest glucose level found was in probiotic group which indicates better fiber digestion and better weight gain. Significant difference ($P<0.05$) was found in LDL concentration and triglyceride among the trial group. Probiotic groups had lower LDL concentration and triglyceride than the control one. In addition, inclusion of probiotic resulted significant ($P<0.05$) increases in haematological parameters like haemoglobin, white blood cell and Erythrocyte proportion as well as PCV and MCV. However, Probiotics had no effect on serum cholesterol and HDL levels. Results indicate that partial substitution of probiotics improve growth performance by improving fiber digestion and modulating immune status of cattle.

CHAPTER 1

INTRODUCTION

Bangladesh is an agriculture base country where about 80 percent of the population depends on agriculture. Livestock provide a major source of disposable income for disadvantaged and marginal populations in this country and livestock provides a major entry point to fight against rural poverty (FAO, 2016). Despite the benefits to many of increased livestock production, this has created a major public health issues and that is therapeutic and sub-therapeutic use of antibiotics as growth promoters in animal feed. For several decades, antibiotics and harmful growth promoters in prophylactic dosages have been used in animal feed to obtain economic benefits in terms of improved animal performance and reduced medication costs. However, there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both human and livestock linked to the therapeutic and sub therapeutic use of antibiotics in livestock. The use of antibiotics in livestock also make serious problems such as expansion of antibiotic-resistant bacteria in dairy foods, meat and milk (Niewold, 2007). Many local farms are still neglecting the harmful effects and using antibiotic growth promoters in Bangladesh. Such wide spread use of antibiotics for promoting growth could easily contribute to the already alarming pool of antibiotic resistant bacteria. Residues of antibiotics in meat and other products can directly harm

consumer's health and at the same time an indirect effect could be their role in producing resistance in several human pathogens (Emborg, Andersen, Seyfarth, & Wegener, 2004). Antibiotics also cause imbalance in intestinal normal flora (KOZASA, 1989). Steroid is another harmful growth promoter which is used for fattening animals. Excessive uses of steroid damaged the kidneys and intestines of cattle. It attacks the immune system and make vulnerable to diseases. People consuming meat of such animals may get kidney problem, cancer, liver failure, gastric ulcer, diabetes, pancreas diseases, high blood pressure, skin diseases and infertility in women. Consumers are looking for a better alternative and probiotics could be an amazing safe choice as many countries are already using them (Trafalska and Grzybowska, 2004; Griggs and Jacob, 2005; Nava et al., 2005). Probiotics reduce these problems and the use of some growth promoters like probiotics and prebiotics which have positive effect on animal's growth performance. Probiotics are being considered to fill this gap and already some farmers are using them in preference to antibiotics and hormones. (Trafalska and Grzybowska, 2004; Griggs and Jacob, 2005; Nava et al., 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. Previously it was considered that all bacteria are harmful, forgetting about the use of the organisms in food preparation and preservation, thus making probiotic concept somewhat difficult to accept. Scientists now a day 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. Public disapproval of the use of antibiotics and growth hormones in livestock production necessitates the use of probiotics in the feeding of farm animals. The application of probiotics and prebiotics in contemporary animal farm production systems has undoubtedly improved the animal production.(Rai, Yadav, & Lakhani, 2013). Probiotics are defined as live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora (Rai et al., 2013). Benefits of probiotics also include: strengthening of the immune system, prevention and treatment of diarrhea and enhancement of resistance against pathogens. The term "probiotics" comes from the Greek words "pro" (in favor) and "biotic" (life). Probiotics are de- fined as "living microorganism in feed which when taken at certain level, provide stability to intestinal micro flora" (Metchnikoff E,1908). Probiotics are defined as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora" (R Fuller; 1989). Prebiotics are defined as "a non-digestible but fermentable food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). According to the FAO/WHO, prebiotics are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria. Commonly used probiotics are

Lactobacillus acidophilus, Lactobacillus bifidus, Coccobacilli sp., Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Bifidobacterim bifidum, Enterococcus feccium, Streptococcus faecium and Streptococcus thermophiles etc. Benefits of probiotics include: strengthening of the immune system, prevention and treatment of diarrhea and enhancement of resistance against pathogens. Although the probiotics concept has been recognized for many years, their precise mode of action has not been fully elucidated. Principal microorganisms used as probiotics for ruminants are bacteria and yeasts (Abd El-Tawab, Youssef, Bakr, Fthenakis, & Giadinis, 2016). This work was performed to focus mainly in the role of probiotics in nutrition and health of cattle production with these following objectives:

OBJECTIVES:

- Preparation of probiotics for cattle and evaluation of their efficacy
- To study the effect of probiotic feeding on performance (Live weight gain, feed efficiency), blood profile and blood biochemistry in cattle

CHAPTER 2

REVIEW OF LITERATURE

2.1 Effect of antimicrobial growth promoters, steroid and growth hormone in ruminant production

Growth promoters are chemical and biological substances, which are added to livestock feed with the aim to improve the growth of animal in fattening, improve the utilization of feed and in this way realize better production and financial results. 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 into strong and healthy individuals (Chang, Wang, Regev-Yochay, Lipsitch, & Hanage, 2015). The extensive uses of antibiotics in animal farms to promote growth rate, increasing feed efficiency and prevention of intestinal infections have led to the development of antibiotic-resistant bacteria in the gastrointestinal tract and drug residuals in meat which are health hazardous when human consume them. Quality of food from animal products is widely concerning public health agencies around the world since veterinary drugs have played an important role in the field of animal husbandry and agro-industry, and increasing occurrence of residues, and resistance have become interesting issues. Resistant microorganism can get access to human, either through direct contact or indirectly via milk, meat, and or egg (Chang et al., 2015). There has been increasing concern that drugs as well as environmental chemicals may pose a potential hazard to the human population by production of gene mutagen or

chromosome breakage that may have adversely affects human fertility (Ture, Fentie, & Regassa, 2019). The bacteria that usually live in the intestine acts as a barrier to prevent incoming pathogen from being established and causing diseases. Antibiotics may reduce the total number of the bacteria or selectively kill some important species. The broad-spectrum antimicrobials may adversely affect a wide range of intestinal flora and selectively kill some important species and consequently cause gastrointestinal disturbance (Beyene, 2015). Some antimicrobial agents like (Virginiamycin, 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 (Phillips, 2001). limitation on the use of antimicrobial agents in food animals reduced antimicrobial resistance in those food animals and in humans. The majority of studies addressed the question indirectly – that is, they examined whether an increase in exposure to antimicrobials increases resistance (Scott et al., 2018).

Steroid is another drug which is used as beef fattening product by some farmers but it has very harmful effect on animals and also in humans. Steroid boost production of growth stimulating hormones that help the animal convert feed into muscle, fat and other tissues more efficiently than they would naturally (Nichols, Galyean, Thomson, & Hutcheson, 2002). The excessive use of steroid causes kidney damage, liver failure, pancreas disease, high blood pressure and makes the consumer bulky (Agarwal et al., 2002).

Hormones like Testosterone, progesterone, prolactin which are responsible for development of sex organs are also used alone or combination with other

substance to improve the weight and feed efficiency. But uses of these types of hormones as growth promoters causes congenital malformation (Carmichael et al., 2005).

2.2 Effect of Probiotics:

Probiotics are defined as live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora (Rai et al., 2013). According to the legislative framework of the FAO and WHO (Anonymous, 2002) probiotics are "live microorganisms that, administered in adequate amounts, confer health benefits to the host". 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). There was no effect of probiotic feeding on the log number of cells of lactic acid bacteria, yeast and coliform bacteria in the faeces and rumen liquor at any age. The activities of carboxymethyl cellulase, xylanase, b-glucosidase, aglucosidase, a-amylase, protease, urease and pH of the rumen liquor remained unaffected by probiotic feeding at all ages tested in this experiment (Agarwal et al., 2002). Probiotics/prebiotics have the ability to modulate the balance and activities of the gastrointestinal (GI) microbiota, and are thus considered beneficial to the host animal and have been used as functional foods (Uyeno,

Shigemori, & Shimosato, 2015). (F. Chaucheyras-Durand & Durand, 2010) also found that, Once ingested, the probiotic microorganisms can modulate the balance and activities of the gastrointestinal microbiota, whose role is fundamental to gut homeostasis. 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.

One of the most important probiotic, yeast culture (YC) supplements containing *Saccharomyces cerevisiae,* are known to be rich source of enzymes, vitamins, other nutrients and important co-factors, have been reported to produce a variety of beneficial production responses. These include growth rate, feed intake, feed efficiency, milk composition, egg production and reproduction in ruminants, poultry, pigs and horses. It inhibits the pathogen by competition for colonization sites or nutritional sources and production of toxic compounds. Benefits of probiotics also include: strengthening of the immune system, prevention and treatment of diarrhea and enhancement of resistance against pathogens. The addition of yeast culture in a dairy calf starter at 2% enhances dry matter intake and growth and slightly improves rumen development in dairy calves (Lesmeister, Heinrichs, & Gabler, 2004). One strain of *S. cerevisiae* could prevent pH decrease by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and thereby effectively compete with amylolytic, lactateproducing bacteria (Sarker et al., 2010). Regarding bacterial probiotics, lactateproducing bacteria (*Enterococcus, Lactobacillus*), which would sustain a

constant level of lactic acid, thus allowing the lactate-utilizing species to flourish (Nocek & Kautz, 2006) may represent a possible means to limit acidosis in highconcentrate fed animals. *M. elsdenii* or *Propionibacterium spp*., which utilize lactate as an energy source, could be administered as direct-fed microbials to avoid ruminal lactate engorgement (Klieve et al., 2003).

Feeding a yeast culture of *S. cerevisiae* improved yields of milk and milk components in heat-stressed multiparous Holstein cows (Lesmeister et al., 2004). Calves receiving *L. acidophilus* maintained initial BW, and the control calves lost BW until 2 weeks of age, at an average rate of 112 g/d. Starter intake, total DMI, feed efficiency, and occurrence of diarrhea were unaffected by treatment. Therefore, L. acidophilus supplementation for calves fed milk replacer may be beneficial during the first 2 weeks of life.(Cruywagen, Jordaan, & Venter, 1996).

In vitro studies have reported that live yeasts could influence the balance of lactate-metabolising bacteria, by limiting lactate production by *Streptococcus bovis* and favoring lactate uptake by *Megasphaera elsdenii* or *Selenomonas ruminantium* (F. Chaucheyras-Durand & Durand, 2010)

A growing interest for using probiotics is to reduce digestive carriage by adult ruminants of human pathogens, such as *Escherichia coli O157* or *Salmonella*. Certain strains of *Lactobacillus acidophilus* have shown to decrease numbers of E. coli O157 in feedlot cattle faeces (Tabe et al., 2008) or in vitro in sheep fecal suspensions (Frédérique Chaucheyras-Durand, Madic, Doudin, & Martin, 2006) and also appear to reduce shedding of *Salmonella enterica* (Stephens, Loneragan, Karunasena, & Brashears, 2007). Distribution of probiotics on farms would

represent a very practical strategy to limit pathogen release in the environment and thereby the risk of foodborne infections in humans (F. Chaucheyras-Durand & Durand, 2010).

In young ruminants, probiotics such as lactic acid bacteria (*Lactobacillus spp., Bifidobacterium spp., Enterococcus spp., Propionibacterium spp.*) or *Bacillus* spores generally target the small intestine, as the rumen is not yet developed, and they represent an interesting means to stabilise the gut microbiota and limit the risk of pathogen colonisation. However, live yeast distributed from the first days after birth have been reported to favor microbial colonisation and the set-up of fermentative capacities in the rumen (Frédérique Chaucheyras-Durand & Fonty, 2002). Improved weight gain and rumen development in young calves have been reported with several products (Abu-Tarbousch et al., 1996; Adams et al., 2008; Galvao et al., 2005).

2.2. 1. Effect of probiotics on growth performance of ruminants

There has been much interest recently in the use of fungal and bacterial cultures to improve productivity in livestock enterprises. The two most commonly used microbial additives are *Lactobacillus spp.* and *Saccharomyces cerevisiae*. These two microbes have specific roles in the host's body, the former is primarily responsible for the exclusion of enterotoxigenic bacteria, whereas the later mainly affects the functioning of rumen (Fuller., 1989). Feeding of S. cerevisiae resulted in increased nutrient digestibility (Panda et al. 1995), increased body

weight gain and better feed conversion efficiency (Singh et al. 1998) in calves (Panda, Singh, & Pathak, 1995).Higher milk yield has been recorded in dairy cattle and increase in live weight gain in growing calves due to addition of live yeast culture in their diets (Williams and Newbold., 1990). Supplemented with DFM produced 2.3 kg more milk/cow per day than did non supplemented cows. 1. Effect of probiotics on growth performance of ruminants (Nocek & Kautz, 2006). Feeding a yeast culture of *S. cerevisiae* improved yields of milk and milk components in heat-stressed multiparous Holstein cows (Bruno, Rutigliano, Cerri, Robinson, & Santos, 2009).

Dry matter intake was greater, and milk production tended to be higher, for cows supplemented with yeast culture, but milk composition was not affected.(Erasmus, Botha, & Kistner, 1992).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 while others researchers reported improved weight gain, feed consumption and (Agarwal et al., 2002),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 (Antunović et al., 2005). It has, in general, been reported that impact of probiotics in performance of animals may vary, as supplementation can increase feed intake, FCR (Khalid et al., 2011) or bodyweight gain (Jang et al., 2009). (Haddad & G-oussous, 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). Moreover, addition of yeast improved bodyweight gain in lambs fed low protein diets with no favorable effects on those fed high protein diets (Kawas et al., 2007). 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(Whitley, Cazac, Rude, Jackson-O'Brien, & Parveen, 2009). 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 & Kafilzadeh, 2007) or that it increased feed intake with no effect on growth and feed conversion or that it had no effect in any of growth, feed intake and feed conversion (Of et al., 2006). Feeding of *S. cerevisiae* or combination of *S. cerevisiae* and *L. sporogenes* to lambs also had no effect on bodyweight and daily weight (Soren, Tripathi, Bhatt, & Karim, 2012). 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 & Wilson, 1996), increased microbial protein synthesis leading to more amino-acid supply post-ruminally (Erasmus et al., 1992). Further, improved bodyweight gain may also be related to increased consumption and improved efficiency of feed utilisation in the probiotics

upplemented animals (Antunović et al., 2005). 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). 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 programs (Cruywagen et al., 1996) . Animal probiotics is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Uyeno et al., 2015), 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, Wallace, & Mcintosh, 1996).The development and growth during this period has important bearing on its future productive and reproductive performance. Some researchers have reported that these probiotics decrease the incidence of diarrhea, improved body weight gain and feed conversion and decreased mortality (Abe, Ishibashi, & Shimamura, 1995). 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 (El-waziry & Ibrahim, 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 cattle production.

2.2.2 Effect of probiotics on blood metabolites

Feeding YC did not influence plasma metabolites, insulin, or body condition score of cows, but urea N concentrations were reduced (Bruno et al., 2009). Published information on effects of probiotics on haematological and blood biochemical parameters of 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 (Machmüller et al., 2003). Smaller concentrations of BUN in probiotic supplemented lambs might be due to improved nitrogen utilization by ruminal bacteria (Bruno et al., 2009). Feeding probiotics with the lower CP level (14.5% vs. 16.5%; DM basis) resulted in lower concentrations of blood metabolites, urea nitrogen (19.9 mg/dl vs. 25.0

± 1.16 mg/dl; $P < 0.05$), rumen pH (5.99 ± vs. 6.22 ± 0.03; $P < 0.05$), and ruminal NH3-N (10.99 mg/dl vs. 11.22 ± 0.03 mg/dl; $P < 0.05$) (Vosooghi-poostindoz et al., 2014). Moreover 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 (Riis 1983) 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 and 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) (Hussein A.F, 2014). 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 (Antunović et al., 2005). On the other hand, (F. Chaucheyras-Durand & Durand, 2010) has reported a significant increase in glucose concentration in kids and lactating ewe after probiotic supplementation. Similar findings have been observed in lambs (Hussein A.F, 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 (Soren et al.,

2012). Nevertheless, some studies (Antunović et al., 2005) 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.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

The study was conducted at Animal Production and Management Lab. and Animal Farm house of the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from January to October, 2019.

3.2 Probiotic preparation

For probiotic preparation several conditions were considered to make it cost effective. The conditions which were considered are - growth media for bacteria and yeast, growing temperature and conditions, number of organism present in the probiotic and how long they survive. Sample yogurt like Milk vita yogurt, Krishibid yogurt, Arong yogurt were collected from different local markets in Dhaka and the yeast genotypes *saccharomyces cerevisiae* was isolated from maize.

3.2.1 Collection of bacterial samples, growth and identification

3.2.1.1 Sterilization of glass ware

Glass wares such as sample bottle, petri-dish, conical flask, test tubes, measuring cylinder etc. were sterilized at 121^{O}c for 21 minutes under 15lb pressure which

is according to the procedure given by Harrigan (1998) after washing them with detergent.

Fig. 1. Sterilization of glass wares by autoclave machine.

3.2.1.2 Media preparation

De Man,s Rosoga Agar and Sharpe agar and De Man,s Rosoga Agar and Sharpe broth used for bacterial broth were prepared according to the manufacturer's instruction. The pH lies between 6-0 and 6-5 after sterilizing (about 6-2 to 6.6 before). The pH was controlled by using 0.1N NaOH and 0.1N HCL. The composition of the media is given below:

Fig.2 MRS agar powder

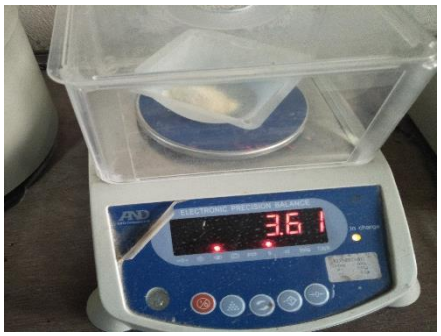

Fig.3. Weighing of agar powder

Table 1. Composition of MRS broth

Ingredients name	Quantity(g/l)
Oxoid peptone	10 g
Yeast extract (Difco or Oxoid)	5 g
Glucose	20 g
Meat extract	10g
triammonium citrate	2 g
Sodium acetate trihydrate	5g
Dipotassium hydrogen phosphate	2g
Magnesium sulfate heptahydrate	0.2g

3.2.1.3 Preparation of the MRS broth

Near about 55.5 gm media was dissolved in 1 liter distilled water by hitting and stirring on the magnetic hot plate stirrer and the pH was adjusted at 5.2 to 6.8 and then the media was sterilized at 121^{O}c for 21 minutes.

Fig.4. Hitting and stirring the media.

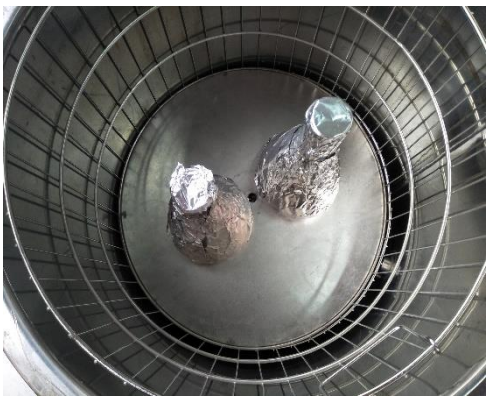

Fig.5. sterilizing the media.

3.2.1.4 Preparation of the MRS agar plates

For making about 35 plates 55g MRS broth powder and 15g Nutrient agar was dissolved in 1litre distilled water by hitting and stirring on the magnetic hot plate stirrer and then autoclaved for 21 minutes. After autoclaving cooled to 50-55^{o}c and poured the medium into sterile petri dishes (about 30 ml per plate) and allow the agar to solidify (about 30-60 min).

Fig. 6. MRS media and MRS broth.

Fig. 7. Pouring the media into plate.

3.2.1.5 Inoculation and incubation

1 gm of yogurt sample was taken in 9 ml of MRS Broth (Hi-Media, India) and incubated at 37^{o}c for 48 h. One loopful broth culture was streaked on MRS agar plates and incubated 48 hrs. Suspected single colonies were isolated and identified by gram staining and short biochemical tests (MacFaddin 2000; Bergey et al., 1994).

Fig. 8. Incubation of agar plates in Incubator.

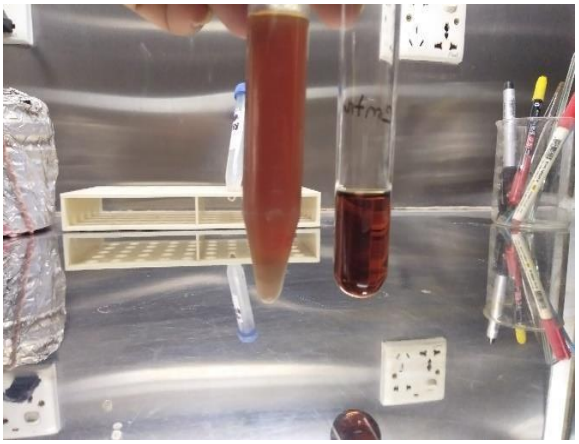

Fig. 9. Turbidity of broth after inoculation and incubation.

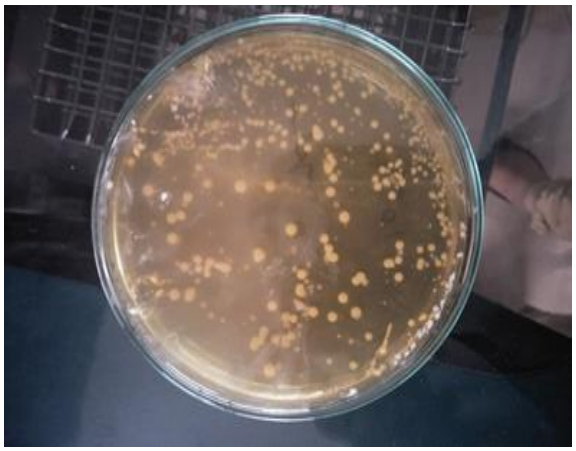

Fig. 10. Growth of bacteria on MRS agar after inoculation and incubation.

3.2.1.6 Gram staining:

Gram staining test was performed for all isolated strains according to the standard procedure. A smear of single colony was prepared on a clean glass slide and the smear was allowed to air-dry and then heat fixed. The heat fixed smear was flooded with crystal violet solution and after one minute, it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with

95% ethyl alcohol and rinsed with water. Then safranin was used as counter stains for 60-80 sec and washed with water, and examined under oil immersion (100X).

Fig. 11. Identification of bacteria under Microscope.

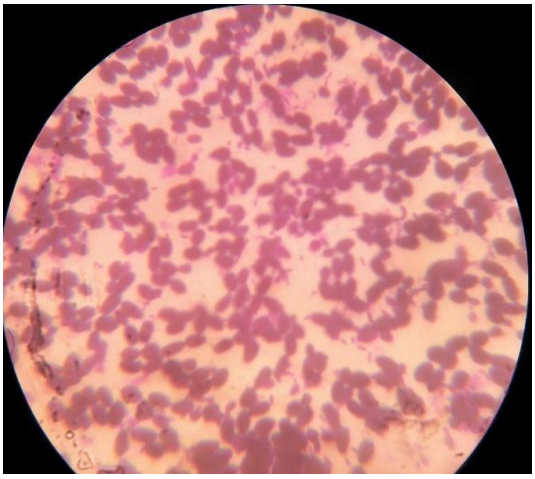

Fig. 12. *Lactobacilli sp*. and *Cocco bacilli sp*. under microscope.

3.2.1.7 NaCl tolerance test

NaCl tolerance of isolated Lactobacillus was determined by using MRS broth with 2%, 4% and 8% of NaCl concentration. Fresh culture was inoculated and incubated at 37ºC for 48 h. Only media was used as negative control. Results were determined by observing the turbidity after 24 h and 48 h and no growth was observed in negative control.

3.2.1.8 Determination of sugar fermentation

Sugar fermentation test was performed using 1% (w/v) sugar in MRS broth. Glucose, fructose, sucrose, xylose and lactose were used in this test. Phenol red solution was used as indicator. 10 ml media were dispensed and Durham's tube was inserted invertably in each of test tubes. Fresh culture was inoculated and incubated at 37°C for 24 h. Only media was used as negative control. Results were observed by color changing and gas formation.

3.2.1.9 Bacterial counting

Spread plate technique was used to count bacterial colony. 10 fold serial dilutions were done. Then samples from the culture containing broth were spread in PCA (plate count agar) agar plate and incubate at 37^{o}C for 24 hrs. After incubation period

Bacterial growth found on the plate and plate counting results 1.6×10^{8}CFU found in per ml of diluent.

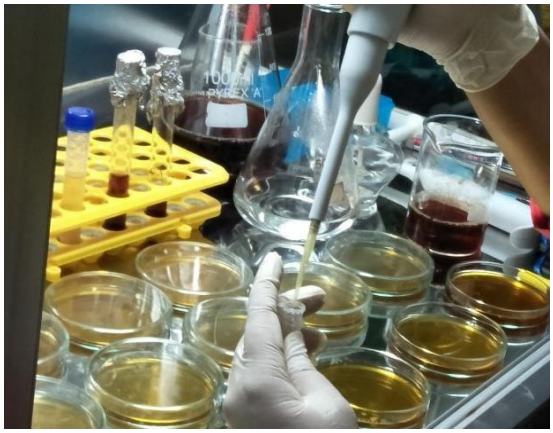

Fig. 13. Serial dilution of culture.

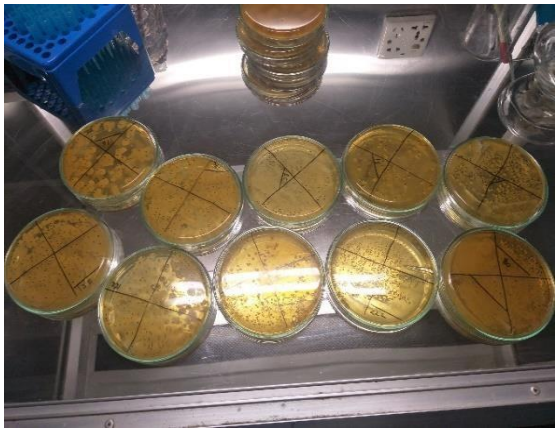

Figure.14. Counting of bacteria.

3.2.2 Probiotic mixture preparation

10 g skimmed milk, 5 g dextrose, 10 ml molasses and 90 ml distilled water were mixed for every 100 ml mixture and autoclaved according to the procedure Harrigan (1998) and then cooled to room temperature and mixed the bacterial culture 1ml / 100ml mixture and incubated them for 24 hours. Again spread plate technique was used to count bacterial colony and 2×10^{8}CFU found in per 100 ml of probiotic mixture. For yeast yeast and bacterial mixed probiotics 0.5gm live yeast was mixed per 100 ml of bacteria containing probiotics mixture and incubated for 24 hours.

3.2.3 Growth of *Saccharomyces cerevisiae* and identification

3.2.3.1 Sterilization of glass wares

Glass wares such as sample bottle, petridish, conical flask, test tubes, measuring cylinder etc. were sterilized at 121^{O}c for 21 miniutes under 15lb pressure according to the procedure given by Harrigan (1998) after washing them with detergent.

3.2.3.2 Media preparation

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3.2.3.3 Potato Dextrose Broth PDA (Potato Dextrose Agar) and PDB (Potato Dextrose Broth) used for yeast growth were prepared according to the methods

recommended by Harrigans (1998). The pH was controlled by using 0.1N NaOH and 0.1N HCL.

3.2.3.3 Potato dextrose broth:

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

Table 2. Composition of Potato Dextrose Broth

Ingredients	Quantity
Small pieces of potatoes	200g
Glucose	20g
Distilled water	1000ml

3.2.3.4 Potato dextrose agar

Potato dextrose agar was prepared by adding 1.5% agar to potato dextrose broth and then sterilized by autoclaving.

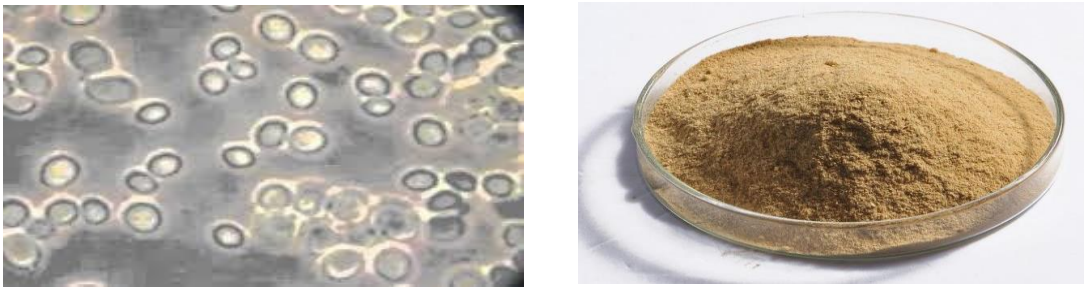

Figure. 15. Saccharomyces cerevisiae under microscope and in open eyes.

3.3 Preparation of experimental cattle and diets

Nine male cattle of animal farm under the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka, with a mean initial body weights of around 150kg and around 1.5 year aged 9 bull calves were selected for trial. The supplementations of 100ml probiotics mixture were used for every cattle. The control group- T_1 was fed a basal diet (un supplemented - control), whereas the experimental groups were fed the same basal diet but group T_2 was supplemented with probiotics containing multiple bacterial culture and group T_3 was provided probiotics containing bacterial culture and yeast culture. Group T_2 and group T_3 were fed 100 ml of particular probiotics mixture with their daily ration for 3 months.

Table 3. The layout of the experiment showing number of cattle allocated in each replication and treatment group

Trial group	Number of calves each replication			Total number of calves
	R1	R2	R3	
T1	1	1	1	3
T2	1	1	1	3
T3	1	1	1	3
Grand total				9

Where, T1= Control diet (No probiotics), T2= Diet with probiotics containing

bacterial culture, T3= Diet in which probiotics containing bacteria and yeast added.

3.4 Management of experimental Animals

The animals were allocated to three groups of 3 cattle each, balanced in terms of live weight and body condition scores. Each barn will be used to house 1 cattle and the feed trial for the experiment was conducted for 60 days. Throughout the experimental period, around 3-3.5kg concentrated feed was supplied to each cattle. The succulent, ad libitum native green grasses were supplied daily to each of the cattle and all animals will be provided with fresh clean drinking water. For immunization of FMD, BQ and anthrax vaccine was applied according to the commonly recognized schedule. In addition, anthelmintic (deworming) medicine was applied prior to trial. The stanchion barn, feeder, waterer, instruments and utensils were cleaned and dried daily. Disinfectant and strict bio-security for hygienic measures and sanitation programs were also employed in the experimental house throughout the research period.

Fig.16. Making plastic tag for marking.

Fig. 17. Tagging of cattle

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Fig. 18. Making concentrate mixture **Fig. 19. Feeding concentrate mixture**

Table 4. Mixed concentrate feed & composition supplied to the cattle during the experimental period.

Ingredients	**Diet %**
Wheat bran	**27**
Rice bran	**25**
Maize crust	**15**
Sesame oil cake	**20**
Molasses	**10**
DCP	**2**
Common salt	**1**
Total	**100**

3.5 Ethical issues

The experimental protocol was specifically approved and 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 the research period. The animals 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. Feed intake, body weight gain, FCR, survivability, blood profile were performed at final stage. The following parameters were recorded during the experimental period:

3.7 Study Parameter

Live weight gain of every week was recorded. After 3 months of trial period final live weight was recorded and then analysed. Blood samples were collected from jugular vein of cattle of both control and treated groups at the end of feeding

period of 3 months to study the CBC, PCV, MCV haemoglobin, glucose and lipid profile.

Formula used for live weight gain (kg/cattle) =

$$\frac{\text{Final live weight – initial live weight}}{\text{Period of time}}$$

3.8 Blood sample analysis

Blood sample were collected at 90th days of experiment (12 ml/ cattle) were collected and 2ml from each were taken in to EDTA tubes for analysis of CBC and haemoglobin level.

Blood samples for total serum cholesterol measurement, serum glucose measurement and lipid profile,10ml blood samples were collected in sterilized test tubes and these were allowed to clot for 1 hour at room temperature. After 1 hour, the serum was taken in a set of centrifuge tube and centrifuged at 3000 rpm for 15 minutes. The clear non hemolysed supernatant fresh serum was then carefully taken into a set of clean, dry Eppendorf tube. All the blood and serum sample were analysed in ACI animal health diagnostic laboratory, Gulshan, Dhaka.

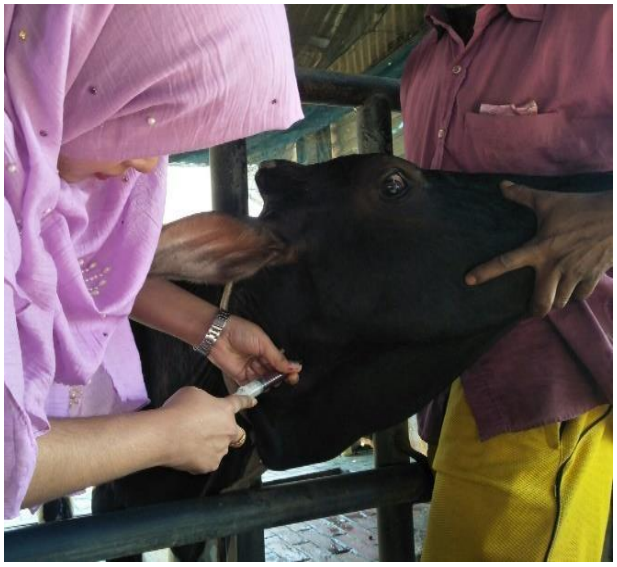

Fig. 20. Blood collection from jugular vein

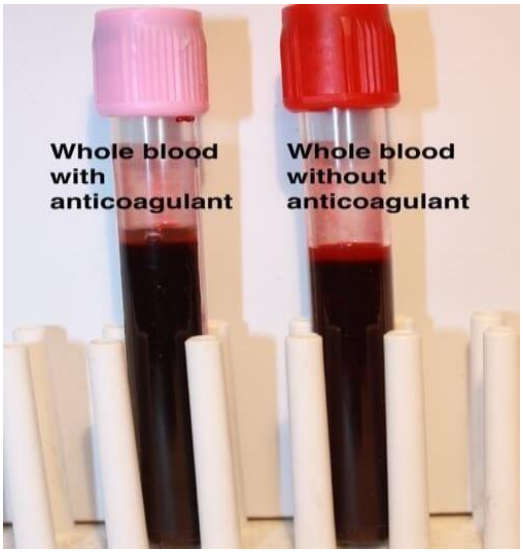

Fig. 21. Blood sample

.3.9 Statistical analysis

The results were presented as the means and the standard deviation of the means. Data were 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). $p<0.05$ will be considered to be statistically significant.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of probiotics on live weight gain

Significant difference ($P<0.05$) was found in live weight of cattle among the treatments and control group (Table 5). The significantly ($P<0.05$) highest live weight gain was found in T_3 group (Diet in which both bacterial and yeast probiotics added) 0.903 kg, T_2 group (Diet in which bacterial probiotics added) 0.88kg and T_1 group (Control diet) 0.78 kg. Significant ($P<0.05$) difference was found among probiotics supplement T_3 group. Highest live weight was found in T_3 group (Diet in which both bacterial and yeast probiotics added). This results supported by many researcher demonstration, like- (Cruywagen et al., 1996) reported that though there was no effect of Lactobacillus feeding on the performance of the calves and diarrhoea, it maintained the body weight at 2 weeks of age, at which control calves lost 4% body weight and could improve resistance against infections since the calves are highly susceptible and vulnerable at this age. Using probiotic yogurt resulted in a significant ($P < 0.05$) increase in starter intake (SI) at seventh and eighth weeks of trial and improve the growth when compared with control group (Noori, Alikhani, & Jahanian, 2016). 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 (Erasmus et al., 1992), increased microbial protein synthesis leading to more amino-acid supply post-ruminally (Erasmus et al., 1992).

Further, improved bodyweight gain may also be related to increased consumption and improved efficiency of feed utilisation in the probiotic supplemented animals (Antunović et al., 2005). 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). The increases in hip depth and wither height in yogurt-fed calves can be attributed to increase in mineral bioavailability such as calcium, phosphorus and magnesium (Khuntia Chaudhary, 2002) as well as higher DMI and weight gain in these calves (Noori et al., 2016). Consistent with our findings, (Chandra et al.,2009) found that dietary supplementation of probiotics resulted in an increase in wither height as compared with control calves.

Fig.22.T_1 group before trial.

Fig. 23. T_1 group after trial.

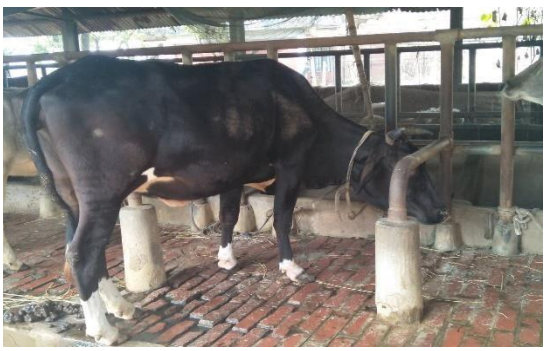

Fig. 24. T_3 group after trial.

Table.5. Effect of probiotics in production performance of cattle

Production performance	T_1 group	T_2 group	T_3 group	Level of significance
Initial live weight (kg)	131.14±11.3	146.31±1.76	164.41±6.1	NS
Final live weight (kg)	216.95^{b}±6.15	211.24^{a}±5.67	245.93^{a}±9.1	*
live weight gain (kg)	0.783^{b} ±0.027	0.886^{a} ±.008	0.903^{a}±0.018	*

Data presented as Mean±SE

*The mean difference is significant at 0.05 level.

Means within a column with different superscription differs significantly (P<0.05)

Means within a column with different superscription don't differs significantly (P>0.05)

SE = Standard Error, NS = Not significant, T1 = Control diet (no probiotics), T2 = Diet in which Probiotics of bacteria culture added, T3 = Diet in which Probiotics of bacteria and yeast culture added.

4.2 Effect of probiotics in haematological parameter

Table 6 shows the average hemoglobin percentage of different groups of cattle with different treatment. Average hemoglobin level of different groups were T_1 (Control diet) 10.30 g/dl, T_2 group (Diet in which bacteria containing probiotics added) 15.40 g/dl, T_3 group (Diet in which bacteria and yeast containing probiotics added) 14.85 g/dl. The significantly ($P<0.05$) highest haemoglobin percentage was found in T_2 group (Diet in which bacterial probiotics added) and T_3 group (Diet in which bacterial and yeast probiotics added) than T_1 group (Control diet).

4.3 Effect of probiotics on other blood parameter

RBC and WBC were affected by probiotics treatment and shows higher T_2 and T_3 group than T_1 group ($P<0.05$) in Table 6. The proportion of lymphocytes was noticeably ($P < 0.001$) higher in calves supplemented with probiotic yogurt than that of milk group and It has been demonstrated that probiotics stimulate and promote immunological responses in calves (Schiffrin & Blum, 2002). On the other hand (Roodposhti & Dabiri, 2012) observed that supplementation of probiotics did not affect leukocytes subpopulations which is contrast to our findings. No significant result found in Platelet count because of probiotic treatment. But in Table 6 effects found in PCV and MCV for probiotics feeding. The highest PCV found in T_2 group ($p<0.05$) and highest MCV found in T_3 group ($P<0.05$).

Table.6. Effect of probiotics on haematological performances of cattle

Haematological parameters	T_1 group	T_2 group	T_3 group	Level of significance
Haemoglobin (g/dl)	10.30^{b}±0.15	15.40^{a}±0.23	14.85^{a}±0.21	*
RBC (million/mm3)	4.3700^{b}±0.20	5.4833^{ab}±0.18	4.9300^{a}±0.04	*
WBC (per mm^3)	11450^{b}±229.12	12133.33^{b}±440.95	13750^{a}±250	*
Platelets (per/µl)	243630±1833.09	202000±14106.73	239500±9500	NS
PCV	32.79^{b}±2.36	47.08^{a}±.50	43.93^{a}±.93	*
MCV(%)	88.31^{b}±0.84	91.14^{ab}±0.67	92.25a±0.75	*

Data presented as Mean±SE

*The mean difference is significant at 0.05 level.

Means within a column with different superscription differs significantly (P<0.05)

Means within a column with different superscription don't differs significantly (P>0.05)

SE = Standard Error, NS = Not significant, T1 = Control diet (no probiotics), T2 = Diet in which Probiotics of bacteria culture added, T3 = Diet in which Probiotics of bacteria and yeast culture added.

4.3 Effect of probiotics on blood biochemistry

4.3.1 Effect in blood glucose level

In Table 7 significant difference ($P<0.05$) was found in glucose level cattle among the treatment and control group. The significantly ($P<0.05$) highest glucose level was found in T_1 group (Control diet) 3.41 mmol/L and lowest glucose level was found inT_3 group (Diet in which bacteria and yeast containing probiotics added) 2.29 mmol/L. This result supported by many researcher demonstration. Probiotics 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 (Antunović et al., 2005). But (Noori et al., 2016) noted that Serum concentrations of glucose and total protein were not affected by experimental dietary probiotic treatments.

4.3.2 Effect of probiotics on blood cholesterol level

The blood Cholesterol level of cattle presented in table 7 was not affected significantly ($P>0.05$). The Cholesterol level were 4.55 mmol/L, 4.74 mmol/L for group T_2, T_3, respectively and 5.09 mmol/L for control group (T_1 group). Previously, researchers have demonstrated that, probiotic supplementation had no effect in blood cholesterol concentration (Hussein A.F, 2014).

4.3.3 Effects on Triglyceride

In table 7 significant difference ($P<0.05$) was found in Triglyceride level cattle among the treatment and control group (Table 7). Average Triglyceride level of different groups were T_1 (Control diet) 68.33 mg/dl, T_2 group (Diet in which bacteria containing probiotics added) 63.01 mg/dl, T_3 group (Diet in which bacteria and yeast containing probiotics added) 58.15 mg/dl. The significantly ($P<0.05$) highest Triglyceride was found in T_1 group (control group). Serum triglycerides concentration was significantly ($P < 0.01$) influenced by inclusion of yogurt, especially 30% probiotic yogurt with pH value of 3.8 (Noori et al., 2016). This might be due to the effect of probiotics and lower pH values on the reduction of pathogenic bacteria in gastrointestinal tract. Decrease in pathogenic bacteria could reduce the conversion of primary bile acids to secondary ones, and in turn, increase fat absorption. In contrast to our results, Chiofalo et al. (2004) indicated that feeding lactobacillus caused a significant decrease in serum triglycerides level of kids (Machmüller et al., 2003)

Table.7. Effects of probiotics in blood biochemistry of cattle

Blood biochemistry Parameters	T_1 group	T_2 group	T_3 group	Level of significance
Glucose (mmol/l)	3.41^{a} ±0.22	2.76^{ab}±0.08	2.29^{b} ±0.10	*
Cholesterol (mmol/l)	5.09±0.07	4.55±0.16	4.74±0.18	NS
Triglyceride (mg/dl)	68.33^{a} ±0.88	63.01^{b}±1.67	58.15^{c} ±0.35	*
LDL (mg/dl)	150.76^{a}±1.13	141.43^{a}±4.12	124.31^{b}±4.78	*
HDL (mg/dl)	39.29±0.5 5	40.50±0.76	39.64±0.14	NS

Data presented as Mean±SE

*the mean difference is significant at 0.05 level.

Means within a column with different superscription differs significantly (P<0.05)

Means within a column with different superscription don't differs significantly (P>0.05)

SE = Standard Error, NS = Not significant, T1 = Control diet (no probiotics), T2 = Diet in which Probiotics of bacteria culture added, T3 = Diet in which Probiotics of bacteria and yeast culture added.

4.3.4 Effects of probiotics in HDL and LDL in cattle

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Probiotics has effect on LDL concentration of cattle. Significant result ($p<0.05$) was found among the trial group in Table 7. Lowest LDL concentration 138.83 mg/dl was found in T_3 group and highest LDL concentration was found in T_1 group 150.76mg/dl. Serum HDL concentration was not influenced by probiotics treatments. Also, feeding probiotic yogurt had no significant impact on HDL concentration when compared with control group. Similarly, there was no significant difference in HDL concentration between groups fed yogurt with either pH values (Noori et al., 2016). Our results are in agreement with those of Panda., et al 1995 who showed that the addition of probiotics had no significant effect on serum HDL concentration.

CHAPTER 5

SUMMARY AND CONCLUSION

The study was conducted at Animal Production and management Lab. and the Animal Farm under the department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka-1207. The research work was conducted for one year and the cattle rearing period was for three months (August-October,2019). Nine 1-1.5 years old cross bred bull calves were selected for this experiment. The supplement probiotics were used. The control group- T_1 was fed a basal diet (un supplemented - control), whereas the experimental groups were fed the same basal diet but supplemented with probiotics. A probiotic group- T_2 (probiotics prepared by bacteria) provide 50 ml per kg of feed and probiotic group- T_4 (probiotics prepared by bacteria and yeast) provide 50 ml per kg of feed. The probiotics groups of cattle showed significantly better result in live weight gain. So, probiotics can be used instead of harmful growth promoter because it increases production performance. All groups consumed same amount of feed but probiotics group produce highest live weight which had significant ($P< 0.05$) difference with cattle of control group.

The significantly ($P<0.05$) lowest glucose level was found in probiotics group. It indicates better fibre digestion and better weight gain. Similarly, haematological parameters like Haemoglobin, TEC, WBC, PCV and MCV were significantly affected ($P<0.05$) by probiotics supplement. Triglyceride and LDL had significant difference among the probiotic and control group.

Probiotics feeding as a feed additives causing better live weight gain by-competitive exclusion of pathogenic micro-organisms, and improved fibre digestion. It doesn't show any side effect like harmful growth promoter. So, probiotics can be used as a nutritious tool to reduce various complications and to improve animal production. The study therefore, recommends conducting field trail on cattle farm to use probiotics as a feed additive. In this line, future detailed research works should be conducted on gut microbial status and immune system of cattle.

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Appendix 1. Body weight of cattle before and after trial

Cattle ID	Initial body weight (kg)	Final live weight after trial (kg)
T_1R_1	148.8	221.00
T_1R_2	147.25	221.05
T_1R_3	142.90	208.80
T_2R_1	109.60	188.10
T_2R_2	148.33	229.53
T_2R_3	135.4	216.10
T_3R_1	175.40	260.50
T_3R_2	152. 23	232.83
T_3R_3	165.62	244.47

Apendix: 2. Different haematological parameter of experimental cattle

Cattle ID	**haemoglobin** gm/dl	**RBC million/ cumm**	**WBC million/ cumm**	**Platelets million/ cumm**
T_1R_1	10.23	4.41	11750.00	245000.00
T_1R_2	10.5	4.70	11000.00	240000.00
T_1R_3	9.15	4.00	11600	245890.00
T_2R_1	15.5	5.12	11300	191000.00
T_2R_2	15.8	5.71	12300	185000.00
T_2R_3	15	5.62	12800	230000.00
T_3R_1	14.9	4.97	13500	249000
T_3R_2	14	4.89	14000	230000
T_3R_3	15.05	5.19	14200	235000

Appendix: 3. PCV and MCV value of experimental cattle

Cattle ID	PCV%	MCV
T_1R_1	30.00	87.50
T_1R_2	30.89	4.70
T_1R_3	37.50	87.45
T_2R_1	47.00	91.00
T_2R_2	46.26	92.372
T_2R_3	48.00	90.05
T_3R_1	43.00	93.00
T_3R_2	44.87	91.50
T_3R_3	45.50	92.75

Appendix 4: Different Blood biochemistry parameter of cattle

Cattle ID	Glucose mmol/L	TRIGLYCERIDE mg/dl	LDL mg/dl	HDL mg/dl
T_1R_1	3.75	67	150	38.50
T_1R_2	3.00	68	153	39.00
T_1R_3	3.50	70	149.30	40.37
T_2R_1	2.93	60	134.80	41.00
T_2R_2	2.75	65.8	140.50	41.50
T_2R_3	2.62	63.24	149.00	39.00
T_3R_1	2.25	57.80	114.75	39.50
T_3R_2	2.5	58.5	129.50	39.78
T_3R_3	2.13	58	128.70	40.23