DIETARY INCLUSION OF PROBIOTIC (LEVU CELL) FOR THE PRODUCTION PERFORMANCE OF COMMERCIAL BROILER

A Thesis
By

MD. SHORIFUL ISLAM

Registration No. 1205105

Semester: January-June, 2014

Session: 2012-2013

MASTER OF SCIENCE (M.S.)

IN

DAIRY AND POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200

JUNE, 2014

DIETARY INCLUSION OF PROBIOTIC (LEVU CELL) FOR THE PRODUCTION PERFORMANCE OF COMMERCIAL BROILER

A Thesis
By

MD. SHORIFUL ISLAM

Registration No. 1205105

Semester: January-June, 2014

Session: 2012-2013

Submitted to the Department of Dairy and Poultry Science,
Hajee Mohammad Danesh Science and Technology University, Dinajpur
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE (M.S.)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200

JUNE, 2014

DIETARY INCLUSION OF PROBIOTIC (LEVU CELL) FOR THE PRODUCTION PERFORMANCE OF COMMERCIAL BROILER

A Thesis
By

MD. SHORIFUL ISLAM

Registration No. 1205105

Semester: January-June, 2014

Session: 2012-2013

Approved as to style and content by:

(Dr. Mst. Afroza Khatun)
Professor
Research Supervisor

(Dr. Tahera Yesmin)
Associate Professor
Research Co-supervisor

(Prof. Dr. Mst. Afroza Khatun)
Chairman
Examination Committee
and
Chairman, Department of Dairy and Poultry Science

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR-5200

JUNE, 2014

DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGEMENTS

All the praises, gratitude and thanks are due to Almighty ***ALLAH,*** *Who enabled me to complete this thesis for the degree of Master of Science (M.S.) in Poultry Science.*

The author expresses the deepest sense of gratitude and heartfelt indebtedness to his honorable and respected teacher and research supervisor, ***Prof. Dr. Mst. Afroza Khatun,*** *Chairman, Department of Dairy and Poultry Science, Faculty of Veterinary and Animal Sciences, Hajee Mohammod Danesh Science and Technology University, Dinajpur, for her scholastic guidance, cordial consistence, sympathetic supervision, valuable advice and radical investigation in all phases of research work and preparing this thesis.*

The author is ever grateful and immensely indebted to his respected teacher and research co-supervisor, ***Dr. Tahera Yaeasmin,*** *Associate Professor, Department of Dairy and Poultry Science, Faculty of Veterinary and Animal Sciences, Hajee Mohammod Danesh Science and Technology University, Dinajpur, for her sympathetic supervision, valuable advice and encouragement during the entire study period.*

The author would like to acknowledge his gratitude and sincere appreciation to respected teacher ***Prof. Dr. Uttam Kumar Majumder,*** *is the Department of Statistics under the Faculty of Agriculture, Hajee Mohammod Danesh Science and Technology University, Dinajpur, for his encouragement, constructive and informative suggestions in course of the research work period.*

The author also would like to acknowledge his thanks and appreciation to Dr. Elora Parvin, Dr. Bissojit Ghosh, Dr. Md. Saiful Islam, Dr. Al Helal Mondol, Dr. Abdul Momin, Tapan, Gofur for their cordial help and support in complesion this manuscript.

Finally, the author acknowledges his deepest sense of gratitude and heartfelt thanks to his beloved parents who sweated much to bring him up today's position; brothers, and relatives for their wishes and great sacrifices throughout the entire period of the study.

The author

ABSTRACT

The dietary effect of levu cell, a commercial probiotic on the broiler growth, meat yield and economics of production was studied for the period of 35 days. A total of One hundred twenty unsexed day-old commercial broiler chicks (Hubberd classic) were randomly divided into four dietary treatments having 3 replications in each treatment. The number of birds in each treatment was 30 while in each replicate 10. The birds were fed probiotic (levu cell) at dietary levels of T_0(0g), T_1(0.5g), T_2(1.0g) and T_3(1.5g) per kg of mixed feed. The body weight gain of different treatment groups ware as T_0(1196.28g), T_1(1186.88g), T_2(1251.58g) and T_3(1273.41g).Feed intake of different groups were T_0(2388.4g), T_1(2446.15g), T_2(2484.90g) and T_3(2502.80g) and feed conversion ratio of different group were T_0(1.99), T_1(2.06), T_2(1.98) and T_3(1.96). A little improvement was observed in body weight gain of broiler chicks at 35 days for T_2(1251.58g) and T_3(1273.41g) groups, although body weight gain, feed intake and feed conversion of broilers did not differ significantly ($P>0.05$) compared to control group. The abdominal fat weight of different group were T_0(1.11%), T_1(1.14%), T_2(1.0%) and T_3 (1.0%). The supplementation of probiotic in broiler diets was effective in reducing abdominal fat deposition ($P<0.05$) but had no significant effect on other meat yield parameters of broilers. The addition of probiotic in the diet of broilers at the levels studied could not aid in economizing broiler production. It was concluded that probiotic could not show beneficial effects on performance of broilers at the level tested but was effective in reducing abdominal fat.

Key word: Probiotic, feed conversion ratio, feed cost, abdominal fat weight.

CONTENTS

CONTENTS (CONTD.)

CONTENTS (CONTD.)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION AND SYMBOLS

AM	=Ante meridian
Av.	= Available
HSTU	= Hajee Mohammad Danesh Science and Technology University
Ca	= Calcium
CF	= Crude fiber
Cm	= Centimeter
cm^2	= Square centimeter
Contd.	=Continued
CP	= Crude protein
DM	=Dry matter
Dr.	=Doctor
et al.	= Associates
Fig.	= Figure
G	= Gram
i.e.	= That is
kcal	= kilo-calorie
Ltd.	= Limited
Lys.	= Lysine
ME	=Metabolizable energy
Met.	= Methionine
No.	= Number
°C	= Degree Celsius
P	= Probability
Total P	= Total Phosphorus
PM	=Post Meridian
Pp	= Page
Prof.	= Professor
SEM	= Standard Error of Means
Tk.	= Taka
Try.	= Tryptophan
UFFDA	=Users Friendly Feed Formulation Done Again
USFDA	= United States Food and Drug Administration
WHO	=World Health Organization
%	= Per cent
&	= and
@	=At the rate of
+	= Plus/and
/	= Per/or
>	= Greater than
<	= Less than
±	= Plus-minus

CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

The ultimate consumers of the end products of poultry are human beings and the major concern of all industries is the well-being of the mankind. People of today's world are very much conscious about their health and to the quality of the food items that they consider in daily dishes. As a result, therefore consumer´s demand for the improved quality of all poultry products continues to gather momentum but equally pressing & the requirement to offer products which have received no antibiotics, chemotherapy or growth promoters having detrimental effect on human health. The means of achieving this are to institute: (a) a program of vaccination to produce immunity to all relevant diseases (b) ensure strict biosecurity and (c) utilize the well-documented benefits of administering live beneficial microbes in poultry industry.

The broiler industry in Bangladesh is developing at a rapid pace and its success depends on how rapidly attains a maximum marketable age in a minimum period. The feed accounts about 65-70 percent of the total cost of poultry production. Hence it is necessary to improve the efficiency of feed at a minimum cost. Many farmers a number of feed additives like antibiotics, growth hormone etc. have been used to improve performance of poultry production. This excessive dependency of farmers on the medications threatens the mankind with the term 'cross resistency'. However, they are no longer permitted in advanced countries as growth promoters because of their residual effects on human health.

In recent years, some countries have banned the use of antibiotics in animal feeding, because continuous use of sub-therapeutic levels of antibiotics in animals feed may result the presence of antibiotic residues in animal products and the development drug resistant microorganisms in humans. Public disapproval and banning of antibiotics and growth hormones as feed additives in many parts of the world, has encouraged the use of probiotics (live beneficial microbes) in poultry feeding. Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989).

The term 'probiotic' is derived from a Greek word 'probios' meaning 'for life'. Havenaar *et al.* (1992) broadened Fuller's definition of probiotics as a mono or mixed culture of living microorganisms which (applied to animal or man) beneficially affect the host by improving the properties of indigenous microflora. Probiotics are organisms and substances which help to improve environment of the intestinal tract. It may also be defined as living microorganisms which, given to animals, assist in the establishment & of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes (Green and Sainsbury, 2001). The probiotics include enzymes, yeast, liver cultures, live bacteria, their metabolites and p^H adjusters which contribute to maintain balances in intestinal microflora (Tortuero, 1973).

The use of probiotics as a substitute for traditional antibiotics in poultry production has become an arena of great interest. The probiotic feeding assists in preventing colonization of pathogens in the intestinal tract and in producing certain enzyme like substances (Moses, 1992). Probiotics are claimed to exert beneficial effects on live weight gain, feed conversion ratio and reduce mortality (Mohan *et al.*, 1996). Feeding probiotic helps to stimulate immunity of broilers. The principle of poultry production is to achieve high levels of performance through efficient utilization of feed keeping survivability as maximum as possible. The biotechnology has a very important role in improving feed utilization capacity of birds and animals. Application of probiotics in the diet is one of the biotechnological tools to augment feed utilization in poultry. Chickens do not have the capacity to utilize dietary fibers properly due to lack of suitable microorganism in the gut, which is normally present in ruminant. These suitable organisms having fiber utilization ability when added to the feed would convert the indigestible cell-wall components into digestible components for the birds.

Probiotics, in general, maintain a better microbial environment in the digestive tract of birds, which may play a role in digestive process and in maintaining bird health. Among the biotechnological approaches, application of probiotics is the most important consideration for reducing the amount of harmful microorganisms in the gut as well as to enhance the utilization of nutrients by the birds. So it is imperative to the poultry nutritionists to use these resources i.e. feed additives especially probiotic in the diet of poultry to increase the efficiency of production. Feed additives play a vital role in the development of the poultry industry of Bangladesh through its innovative technologies,

which were backed by the know-how to use these technologies by the farmers. Probiotics influence the production of meat and egg without affecting the human health. To make up the equilibrium between the need of human food and the safe production of these foods by using the potentiality of the inputs, the role of probiotics has arose notably. In Bangladesh context, where farmers are not even aware of their own nutritional needs, probiotic may be one of the most important concerns to ensure the sustainability of poultry industry by helping the birds to fully utilize the nutritional worth of the feed not only for its own survival but also to provide safe and healthy end products to the consumers.

The probiotics are believed to exert beneficial effects on performance of broilers but controversy about the matter still exists. Several researchers claimed that probiotic has no beneficial effects on growth rate, feed intake and gain (Priyankarage *et al.*, 2003; Lima *el al.*, 2002 and Ergun *el al,.* 2000). Reports are also available that probiotics do not have positive effects on carcass characteristics of broilers (Mohan *et al.* (1996); Kalavathy *et al.*, 2003). Presently various probiotics preparations are available in the market and their indiscriminate uses are in practice without much scientific information. Levu cell is one of the commercial probiotics preparations containing a unique mixture of micro-organisms, which is marketed by Square Pharmaceutical (Bangladesh) Limited. The manufacturer of the product is Lallemand claims that it exerts its beneficial effects on the performance of broilers based on common principles of probiotics. Since levu cell appeared as a performance enhancer in the market, it could be interesting to conduct an experiment with this product to investigate its beneficial effects. Experimental results on the effect of levu cell in the diet of broiler chicks are not available under local condition.

Keeping all above points in view, the research was conducted with the following objectives:

1. To investigate the effect of different levels of a commercial probiotic, on the performance of broiler.
2. To investigate whether the probiotic (Levu cell) has effect on the carcass quality of broilers.
3. To recommended the optimum and economic level of inclusion of the probiotic in broiler diet.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

A considerable effort has been made by poultry scientists all across the globe for producing safe meat and egg from poultry through use of live beneficial microbes in the diet or drinking water of bird. Live beneficial microbes i.e., probiotic may be used as an alternative to the traditional antibiotic in the broiler diet in order to boost up the nutrients utilization as well as to reduce the risk of diseases. A good number of works have been conducted in abroad to find out the effect of probiotic on the performance of broiler. But limited information on probiotic under local condition is available. However, some of the literatures pertinent to the present tropic have been reviewed in this chapter.

2.1 History of probiotics

The concept of using bacteria to improve health is a hundred years old and the use of fermented foods (which involve bacteria) has a much longer history. Live beneficial bacteria which improve health are termed as probiotic. The term 'probiotic' was first used in 1965. Probiotics are live microbial feed supplement which beneficially affect the host animal by improving the intestinal microbial balance. Although the word 'probiotic' was only coined a few decades ago, but it has an aged and interesting history. The negative view of the colon and its content i.e. all bacteria present in the intestine are not harmful, shared by a key figure in the history of probiotics. The beneficial effect of probiotic was first recognized by Elie Metchnikoff (1907), an eminent Russian Zoologist. Metchnikoff who reported that the bacterial population of the intestine could be improved by adding beneficial bacteria. Metchnikoff's adoption of the idea of beneficial bacteria arose from his enquiries into how old age could be delayed and life be prolonged.

In this wide ranging enquiries Metchnikoff became interested in a population of mountain peasants in Bulgaria who were known for their longevity. He found that the peasants consumed more fermented milk. Then he thought that the fermented milk they consumed had a role to play in their long life. Metchnikoff reckoned that by consuming soured milk products the human microflora could be changed and improved. He found a

bacterium in the fermented milk consumed by peasants and named it *Bacillus bulgaricus*. Metchnikoff further speculated that detrimental microbial in the intestinal tract produce harmful substances to the host which could be neutralized by beneficial organisms in yoghurt. Because of inadequate records, it is not certain which bacterium Mctchnikolf identified. It may have been *Lactobacillus delhrueckii* sub spp. *bulgaricus*, a strain of bacteria commonly used today as a starter culture for yoghurt. In the United States, the Yale scientist Leo F. Rettger switched his attention away from *Lactobacillus bulgaricus* towards other lactic-acid bacteria, especially *Lactobacillus bulgaricus*. He found that various preparations using this bacterium helped to alleviate constipation and to improve diarrhoea. It was assumed that the beneficial effects of probiotics were due to the colonization of the gut by *Lactobacillus acidophilus* (Rettger and Chaplin, 1921).

From the early 1970s in the U.K., the ban on the use of certain antibiotics as growth promoters in farm animals had the effect of making some farmers more open to alternatives for keeping their intensively farm animals healthy. There was another factor that made farmer receptive to the concept of probiotics; the administration of antibiotics to farm livestock, particularly at sub-therapeutic levels possess certain hazards to human and animal health. In addition to recommending a reduction in the use of antibiotics, the WHO suggested the use of 'bacterial interference', an alternative phrase for probiotics. In 1989, the United States Food and Drug Administration (USFDA) instructed the manufactures to use the term direct-fed microbial (DFM) rather than probiotic (Miles and Bootwalla, 1991). Tortuero (1973) pioneered the use for poultry of preparations containing living bacteria. He demonstrated that implantation of lactobacilli produced results similar to those obtained when antibiotics were used, i.e. increased weight gain and better feed conversion. In the last decade of the twentieth century, interest in probiotics among the general public steadily increased. Probiotic food products, mostly milk based started to appear in supermarkets and probiotic supplements appeared in health food stores. The general public has been receptive to the idea of improving the intestine and the immune system by adding "friendly bacteria".

However, it is also fair to say that understanding of how probiotics work is poor and is little often more than a feeling that these products (probiotics) are "good for you". Having said that, the beginning of the twenty-first century has probably seen an end to the ridiculing of Metchnikoffs ideas on beneficial bacteria, which he first proposed one

hundred years ago. Now, the lay public and medics are receptive to the idea of probiotics, both in aiding good health and treating illness.

2.2 Biotechnology behind probiotics

Probiotics are beneficial bacteria that colonize in the intestinal tract and act to promote the efficient functioning of digestion, enhance growth or production and stimulate and maintain the natural immunity of the body of chicken. A most important characteristic of a well-functioning intestinal tract is the balance of its bacterial population. Probiotic bacteria are normal inhabitants of the intestinal Tract and are found in the healthy gut of the chicken. The way in which probiotics work is not well known. Extensive studies have been conducted to determine the effects of probiotics on the performance of chickens and the mechanisms involved. Some of the proposed modes of action of probiotics in poultry include:

(i) maintaining a beneficial microbial population in the alimentary tract
(ii) improving feed intake and digestion
(iii) altering bacterial metabolism
(iv) neutralization of enterotoxin
(v) stimulation of immune system

2.2.1 Maintaining beneficial microflora in the alimentary tract

Healthy animals are generally characterized as having a well-functioning intestinal tract. This is fundamental for the efficient conversion of feed for maintenance and for growth or production. Continuous feeding of probiotics to animals has been found to maintain the beneficial intestinal microflora in two ways:

(a) By competitive exclusion and
(b) By antagonistic activity towards pathogenic bacteria.

2.2.1.1 Competitive exclusion

Although several mechanisms by which the indigenous intestinal microflora of animals could inhibit the colonization of invading micro-organisms involved in exclusion of pathogenic bacteria by probiotics has yet to elucidate.

The proposed mechanisms include:

(i) Competition for adhesion sites
(ii) competition for nutrients
(iii) aggregation of lactic acid bacteria with pathogens

Adhering to adhesion sites along the wall of the gut is an important colonization factor and many intestinal pathogens rely on adhesion to the gut wall. Sissons (1989) has suggested that *Lactobacilli* compete with pathogens for sites of adherence on the intestinal surface. Attachment is necessary for proliferation and for reducing the rate of removal of organisms from specific sites in the gastrointestinal tract due to the movement digesta caused by peristalsis. An important function of probiotic bacteria is to prevent or limit the growth and colonization of potentially pathogenic bacteria such as *E. coli. Salmonella, listeria, Campylobacter* and *Clostridia* within the gut can hence help to reduce the risk of pathogenic challenge.

Competition for available nutrients as a means of controlling intestinal bacterial population is unlikely to be an effective competitive exclusion mechanism. Rolfe (1991) indicate that there are many environmental factors that either enhances availability of nutrients from the diet of the host or through manipulation of dietary ingredients, enhance the growth of certain microbial populations that may result in exclusion of other bacterial species. Within the gut (a rich source of nutrients), beneficial as well as pathogenic micro-organisms will be utilizing the same type of nutrients to grow and reproduce. Hence, the more gut is flooded with beneficial micro-organisms; the more competition is created between beneficial and pathogenic micro-organisms. Coaggregation between native gut bacteria and pathogens has been considered as one of the ways to exclude bacteria from their host. Spencer and Chesson (1994) reported that coaggregation between lactic acid bacteria and enteropathogens may play a protective role in excluding pathogens from the intestine. Reid *et al.* (1988) have suggested that the inhibitor-producing *Lactobacilli*, which coaggregate with pathogens of the urinary tract, may constitute an important host defense mechanism against infection.

2.2.1.2 Antagonistic activity

In vitro studies have demonstrated that lactic acid bacteria are able to inhibit the growth of poultry pathogens. Jin *et al.* (1996 a) found that all 12 *Lactobacillus* isolates studied had the ability to inhibit the growth of five Salmonella strains and three serotypes of *E. coli*. The antagonistic activity of lactic acid bacteria towards pathogens can be attributed to the production of bactericidal substances. Among those produced by Lactobacilli are bacteriocins, organic acids and hydrogen peroxide. Bacteriocins are compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action. Vincent *et al.* (1959) concluded that *Lactobacillus acidophilus* could play an important role in controlling undesirable microflora in the intestinal tract of animals including humans.

Antagonism by lactic acid bacteria has also been associated with major end products of their metabolism. Several by-products of *Lactobacillus* metabolism arc capable of antagonistic activities in vitro. The best known of these metabolic by-products are organic acids such as lactic and acetic acids (Sorrels and Speck, 1970) and hydrogen peroxide (Price and Lee, 1970). Sorrels and Speck (1970) demonstrated that lactic and acetic acids inhibit the growth of many bacteria including pathogenic Gram-negative organisms. Tramer (1966) showed that the inhibition of *E. coli* by *Lactobacillus acidophilus* could be related to the strong germicidal action of lactic acid at low p^H. Gilliland and Speck (1977) concluded that the antibacterial action produced by Lactobacillus acidophilus was probably due to a combination of Factors included acids, hydrogen peroxide and bacteriocins.

2.2.2 Increasing feed intake and digestion

Probiotic microorganism has an important role in the digestion and absorption of feed ingested by the host. The healthy microflora of the intestinal tract produces enzymes which aid the breakdown of polysaccharides such as carbohydrates to allow the absorption of the energy obtained from these nutrients by the gut. The microflora also ferments carbohydrates which have not been digested in the upper gut and produces vitamins which supply a secondary source to the host. Nahashon *et al.* (1996) found that supplementation of Lactobacillus culture in maize/soybean diets stimulated appetite and increased fat, nitrogen, calcium, phosphorus, copper and manganese retention in layers.

2.2.3 Digestive enzyme activity

Gut microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine (Sissons, 1989). Philips and Fuller (1983) reported that the proteolytic activity in the ceacum of conventional chicks was higher than that in germ-free chicks. Siddons and Coates (1972) also showed an increase in intestinal tissue of conventional chicks than that in germ-free chicks. *Lactobacillus* spp. have been shown to produce digestive enzymes in vitro and the enzymes may enrich the concentration of intestinal digestive enzyme. Amylase activity in the small intestine increased when the lactobacillus cultures were fed to the broilers (Jin *et al.*, 1997).

2.2.4 Stimulation of immunity

Immunity resulting from gut exposure to a variety of antigens, such as pathogenic bacteria and dietary protein, is important in the defense of young animals against enteric infection. *Lactobacilli* could be important in the development of immune competence in young animals, particularly when protection must be acquired against antigens likely to cause gut inflammatory reactions (Perdigon *et al.*, 1990). Oral inoculation of germ free animals with *Lactobacillus acidophilus* (probiotic microorganism) led to elevated levels of total serum protein, globulin rather than albumin, and increased white blood cells (Pollmann *et al.*, 1980). Dunham *et al.* (1993) reported that birds treated with *lactobacillus reuteri* exhibited longer ileal villi and deeper crypts, which is a response associated with enhanced T. cell function, and increased production of anti-*salmonella*1gM antibodies.

2.3 Levucell and its contents

Levucell® SB is concentrated live yeast specifically selected to enhance the nutrition & health of monogastrics. The strain (*Saccharomyces cerevisiae* CNCM I-1079) has been chosen for its specific properties:

2.3.1 Validate benefits in poultry

1. Scientifically validated against its actions on pathogens namely

    a) *Clostridium perfringens*
    b) *Clostridium defficile*
    c) *Salmonella gallinerum*
    d) *Salmonella typhimurium*
    e) Other *Salmonella* sp like *Enteritidis* (Patent)
    f) *E. coli* Pathogenic.

Also validated on its action on Clostridial Toxins.

2. Improvement in Zootechnical parameters like, reduced mortalities, better body weight and FCR.
3. Composition: *Saccharomyces cerevisiae boulardii* 2.0×10^{10}cfu/gm.

2.4 Functions of Levu cell® SB (Probiotic).

- Neutralizes bacterial toxins of *Clostridial spp.* Increases local immunity and has protective effect on intestinal v10×10^{10} illi
- Adherence of flgellate bacteria.
- *Saccharomyces cerevisiae boulardii* decrease pathogenic bacteria and increase concentration of beneficial bacteria and flora in gut which optimizes $p^{H.}$ of gut.
- Maturity of intestinal mucosa.
- *Saccharomyces cerevisiae* type *boulardii* improves maturity of intestinal cells,villous hight and crypt depth.
- Enhancing the assimilation of nutrients
- Decrease $p^{H.}$ of different segments of the intestine.
- Decrease mortality rate.

2.5 Effect of probiotic on live weight gain in broiler

Samanta and Biswas (1995) with one hundred twenty unsexed day-old commercial broiler chicks assessed the effect of feeding probiotic and lactic acid on the performance

of broiler. They had used *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and mixture of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* as probiotics in the drinking water. They reported that the body weight gain of the birds in 1 day to 5 weeks and 1 day to 7 weeks was slightly higher in all treated groups than that of control.

Mohan *et al.* (1996) studied that the effects of dietary probiotic (probiolac - a commercial probiotic mixture of lactic acid bacteria, *Aspergillus oryzae* and T*orulopsis*) supplementation on the growth, nitrogen utilization and serum cholesterol contents of broiler chickens. In the First experiment, they observed that the birds receiving the 0, 75, 100 and 125mg probiotic/kg diets had weight gains of 1204, 1272, 1268.3 and 1210.5g respectively at the end of 8 weeks of feeding. It was concluded that improvement in body weight gain was observed in broilers only after 4 weeks of feeding probiotic. They demonstrated that the probiotic plus antibiotic-supplemented group of birds had the maximum weight gain (1148.5g) followed by antibiotic (1141.3g), probiotic supplemented (1128.4g) and control birds (1045.6g) after 6 weeks.

Jin *et al.* (1996 a) used 10-day-old 200 Arbor Acres broiler chicks under a hot and humid environment and found that the weight gain in broilers given feeds incorporated with commercial Lactobacillus was significantly higher than that of the control birds $P<0.05$). Jin *et al.* (1996 b) also found that supplementation of commercial *Liictobacillus* or *Bacillus subtilis* probiotics could improve the weight gain of broilers. Yeo and Kim (1997) reported that feeding a diet containing probiotic (*Lactobacillus casei*) significantly increased average daily weight gain during the first 3 weeks ($P<0.05$) but not during weeks 4 to 6 of growth. Probiotic (*Lactobacillus acidophilus, Streptococcus faecium*, Betaglucanase and liver extract) fed broilers had significantly higher body weight than that of control ones ($P<0.05$) was also reported by Gohain and Sapcota (1998). Using adherent Lactobacillus cultures isolated from the intestine of chickens, Jin *et al.* (1998 a) reported that addition to the feed from 0 to 6 weeks of either a single strain of *Lactobacillus acidophilus* or a mixture of *Lactobacillus* significantly improved body weight. Jin *et al.* (1998 b) also found that the highest growth rate was obtained when broilers were fed a concentration of 1 % *Lactobacillus* cultures.

It was demonstrated by Panda *et al.* (1999) that there was no significant effect of probiotic (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, Streptococcus faccium, Asperillus oryzae, Torulopsis spp.*) on growth of broilers during the experimental period. The influence of *Lactobacillus acidophilus* and zinc bacitracin alone or in combination, the growth of broiler was monitored by Abdul Rahim *et al.* (1999) over a period of 8 weeks. They observed that the final body weights showed a response to the additives and bacitracin alone or in combination with *Lactobacillus acidophilus p*roduced significant improvements over the control. The improvements in weights 10.08% for the combined treatment and 9.1% for bacitracin alone.

Sing and Sharma (1999) conducted an experiment with 480 day-old commercial broiler chicks providing 0.02, 0.03 and 0.04 per cent probiotic (*Lactobacillus sporogenes*) in the diet of broiler to observe the performance of broiler chicks under different energy and probiotic levels during summer season. They reported that higher weight gain diet containing 0.02 per cent probiotic ($P<O.05$). An experiment was carried out by Mahajan *et al.* (1999) to investigate the effect of probiotic (Lacto-Sacc) feeding and seasons on growth performances and carcass characteristics of Vencob broilers. They observed that body weight gains were significantly higher for experimental birds as compared to control ones ($P<0.05$) during 1st, 2nd and 5th week in winter and in the 1st, 2nd, 3rd and 5th week in during the summer season. They also observed that the cumulative body weight gain for the entire period of six weeks was significantly higher in the birds fed with probiotics during the summer season only.

Jin *et al.* (2000) reported that significant improvement in body weight was observed in broilers fed the mixture of 12 *Lactobacillus* strains $P<0.05$). In another experiment, it was found that supplementation of probiotic with or without antibiotic, to the rations had no important effect on live weight gain of broilers. But it was found by Zulkifli *et al.* (2000) that after 3 weeks of heat exposure, birds receiving the *Lactobacillus* cultures diet had greater body weight gain than control chicks.

A 41-day feeding trial on broilers was conducted by Hamid and Aijazuddin (2001) and they observed that probiotic treated groups, at the rate of 1 g/litter in the drinking water, had higher average live weight gain (about 121g/bird). Ladukar *et al.* (2001) conducted an experiment involving 300 healthy day-old broiler chicks to investigate the effect of five commercially available probiotics (T_1- *Streptococcus faecium,*

Streptococcus thermophilus, Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium bifidum, Torulopsis, Acidophilus oryzol; T_2- *L. casei, L .atidophilus, B. bijìdum, S. faecium, TonIopsig* ; T_3- *Yeasacc 1026, L. acidophilus, S. fiecium*, T_4- Live yeast culture, *L sporqgens*, amino acids, Liver extract', T_5- *L. sporogens ,Saccharomyces cerevisiae*, SC-47, Alpha amylase) on growth performance of the chicks. They reported that the body weight gain was not affected by probiotic supplementation.

Bandy and Risam (2001) involving one hundred and sixty (160) day-old commercial broiler chicks investigated the growth performance of broiler chickens fed with probiotics. They had provided probiotics (Biospur) in the ration at the rate of 0, 25, 50, and 75g per 100 kg feed. They observed that body weight gain was significantly higher in the treated groups than the control groups ($P<0.05$). Shoeib and Madian (2002) assessed the effect of probiotic feed additives, pronifer or biogen, on the growth performance, feed utilization and intestinal flora of broiler chickens. They demonstrated that the addition of pronifer to the broiler diet significantly increased ($P<0.05$) the weight gain by 3.42 and 4.88% in groups II and III respectively, whereas in case of biogen supplementation, the level reached 1.40 and 6.83% in groups IV and V, respectively compared to the control group. Kwon *et al.* (2002) reported that there was no significant difference among different treatment groups for live weight gain. Lima *et al.* (2002) reported that the addition of probiotics (*Bacillus subtilis*) had no significant effect on the live weight gain of broiler for the whole period (1-42 days of age).

Priyankarage *et al.* (2003) conducted a feeding trial with 240 day-old broiler chicks to assess the efficacy of a commercial probiotic preparation protexin) containing *Lctobacillus bulgaricus, Lictobacillus acidophilus, Lactobacillus casei, Streptococcus faecium, Bifidobacterium bifidum, Aspergillus oryzae and Torulopsis spp.* on the growth performance of broilers fed on typical local diets based on rice by-products. They concluded that there was no significant treatment effect of probiotics on growth performance of the birds. Kalavathy *et al.* (2003) also carried out an experiment to assess the effects of a mixture of 12 *Lactobacillus* cultures (LC) on the growth performance of broilers chickens. They suggested that the supplementation of LC in broiler diets improved the body weight gain. They explained that initially, from 1 to 21 day of age there was no significant difference in the weight gain between the two treatments

although LC-fed chicks were heavier. However, from 22 to 42 or 1 to 42 days of age, broiler chicks fed LC gained more weight ($P<0.05$) than control chicks.

2.6 Feed consumption and feed conversion as influenced by probiotic

Samanta and Biswas (1995) reported that feed intake of broilers fed diets supplemented with probiotics (*Lactobacillus spp.*) did not differ significantly when compared with the control broiler chicks. They also suggested that feed conversion ratio for both 1 day to 5 weeks as well as 1 day to 7 weeks periods improved slightly due to addition of probiotic. Mohan *et al.* (1996) suggested that broilers fed probiotic (probiolac - a commercial probiotic mixture of lactic acid bacteria, *Aspergillus oryzae* and *Torulopsis* showed no significant improvement in the feed conversion ratio when compared with control chicks. Jin *et al.* (1996 b) showed that broilers chicks fed *Lactotobacilli* in the feed had a significantly lower feed to gain ratio. Broilers fed with *Lactobacillus casei* showed no significant improvement in the feed conversion ratio when compared with control chicks as reported by Yeo and Kim (1997). Gohain and Sapcota (1998) observed that the birds offered probiotic *(Lactobacillus acidophilus*, *Sitptococcus faecium*, Betaglucanase and Liver extract) supplemented diets consumed numerically less feed than their control counter parts. They also found that the difference in feed conversion ratio between the probiotic fed and control group was non-significant.

Singh and Sharma (1999) demonstrated that the *Lactobacillus* supplementation did not influence the feed consumption significantly ($P<0.05$) at all groups. They also showed that probiotic addition at the rate of 0.02 per cent resulted in improved feed efficiency at 0 to 6 and 0 to 8 weeks of age. It was reported by Mahajan *et al.* (1999) that feed consumption and feed conversion ratio on cumulative basis were significantly higher ($P\leq0.05$) in probiotic (Lacto-Sacc) fed broilers during both winter and summer and during winter only, respectively. But Panda *et al.* (1999) concluded that there was no significant effect of probiotic (*Lactobacillus acidophilus. Lactobacillus casel, Bifidobacterium bifidum, Streptococcus faecium, Aspergillus oryzae, Torulopsis spp.*) On feed consumption and feed efficiency. Zuilkifli *et al.* (2000) observed that broilers feed a diet containing *Lactobacillus* culture consumed less feed and had better feed efficiency ratios during the growing period (1 to 21 days), but found that the superior feed efficiency did not extend to the finishing period (22 to 42 days) during which the chicks

were subjected to 3- hours episodes of heat stress (36 ± 1°C) each day. Jin *et al.* (2000) found better (P <0.05) feed conversion ratio during the experimental period as a result of the supplementation of probiotics (*Lactobacillus* cultures) in the diet of broiler chickens. Supplementation of probiotic with or without antibiotic, to the rations had no significant effect on feed conversion ratio of broilers as reported by Ergun *et al.* (2000).

Bandy and Risam (2001) concluded that birds fed diets supplemented with probiotic (Biospur) at different levels consumed significantly higher feed (P<0.05) at the end of 28 days. But it was contradictory at the end of 42 days, the birds fed with diets containing 0.05 per cent and 0.075 per cent probiotic consumed significantly amount of feed when compared with those of the birds fed control diet They also reported that feed conversion ratio was significantly better in the group fed diet supplemented with 0.075 per cent probiotic, at the and 42 days of age. Hamid and Aijazuddin (2001) found that the probiotic at the rate of 1g/litter drinking water of broiler chicks improved feed conversion ratio. Ladukar *et al.* (2001) found that average feed intake during the experiment did not vary significantly among different treatments. They also observed that feed conversion ratio of the birds was not influenced by the supplementation of probiotic.

The amount of the feed intake was decreased significantly (P<0.05) as the level of probiotic either pronifer or biogen increased in the diet Shoeib and Madian, (2002). This study also showed that feed conversion was improved due to addition ofprobiotic either pronifer or biogen from 3.02 in control group to 2.84, 2.74, 2.79 and 2.56 in groups II, III, IV and V respectively. For the whole experimental period (1-42 days), statistically significant effects of the addition of probiotics (*Bacillus subtilis*) or enzyme in the broiler diet on feed intake and feed conversion ratio were not observed by Lima *et al.* (2002). Priyankarage *et al.* (2003) reported that probiotics had no significant treatment effects on feed conversion ratio of the broilers. Kalavathy *et al.* (2003) demonstrated that broiler chicks given *lactobacillus* cultures diet had better feed conversion ratio (P<0.05) during the growing (1 to 21 day) and finishing (22 to 42day) periods. The feed to gain ratios were improved by decreasing 0.10 (P<0.05) and 0.27 (P<0.05) units from 1 to 21 day of age and 22 to 42 days of age, respectively, in chicks supplemented with *Lactobacillus* cultures.

2.7 Influence of probiotic on mortality

Watkins *et al.* (1982) found that the mortality was lower in treated groups of broilers fed with feeds containing *Lactobacillus acidophilus* as compared to control groups. Watkins and Miller (1983) reported that the addition of probiotic (*Lactobacillus acidophilus* in the broiler diet decreased the mortality rate compared to control. The reduction in mortality in broiler chicks fed probiotic was also observed by Moses (1992). Elwinger *et al.* (1992) claimed that the addition of probiotic in the broiler diet reduced mortality rate than that of their counter parts. Broiler chicks supplemented with antibiotic or probiotics had higher viability than that of the control groups Lee *et al.* (1993). Samanta and Biswas (1995) found no mortality in probiotic (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and mixture of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*) fed groups, whereas it was 4.2 per cent in lactic acid fed groups and 8.3 per cent in control group. Alwan *et al.* (1997) involving Vedetta, Petra and Starboro broilers by feeding standard diet supplemented with probiotics up to 7 weeks of age. Found some interesting results. They observed decreased mortality in Petra and Starbro at 4 weeks, while the treatment caused an increased mortality in Vedetta at 7 weeks. This might be due to the inter-strain differences in disease resistance capability. Fabris *et al.* (1997) carried out an experiment involving 2400 male Cobb-500 day-old broilers fed diets containing probiotic (*Bacillus toyoi*) or antibiotic up to 53 days of age. They found that mortality was only 7% in probiotic fed groups as compared to 12.75% in control group.

Mortality in the probiotic (*Bacillus coagulans*) group was also lowered than the control or antibiotic treated group as reported by Cavazzoni *et al.* (1998). Reduced mortality was also observed in groups supplemented either by enzymes or yeast or a combination of enzymes and yeast (Piao *et al.* 1998). Mahajan *et al.* (1999) showed that per cent cumulative mortality during (0 to 6 weeks of age was lower in probiotic (Lacto-Sacc) fed broilers in both winter and summer seasons. Singh *et al.* (1999) conducted an experiment to know the influence of levels of probiotic and energy on mortality and economics of broilers in summer. They reported that the probiotic (*Lactobacillus sporogenes*) feeding decreased mortality from 11.67% in control to 6.67% in 0.02, and 10.83% in 0.03 and 9.17% in 0.04% *Lactobacillus* fed groups.

Zulkifli *et al.* (2000) conducted an experiment with Hubbard x Hubbard and Shaver x Shaver chicks given a dietary supplementation of either 50 mg/kg oxytetracycline (OTC) or 1 g/kg *Lactobacillus* cultures (LC) with exposure to 36 ± 1 °C for 3 hours daily from day 21 to 42. They observed higher mortality (2.2%) in *Lactobacillus* fed groups compared to control group (1.7%) but the highest percent of (4.2%) in OTC fed groups. Hamid and Aijazuddin (2001) reported that the probiotic @ of 1g/litter drinking water treated groups had lower mortality than control ones.

2.8 Carcass characteristics of broilers as influenced by probiotic

Chah *et al.* (1975) found that broilers supplemented with *Aspergillus oryza* fermented soyabeans had reduced carcass fat. Mandal *et al.* (1994) observed that feeding probiotic did not have any influence on the carcass yield. Santoso *et al.* (1995) found that abdominal fat contents were reduced in female broiler supplemented with *Bacillus subtilis* at 42 days of age. Gohain and Sapcota (1998) found no significant difference between the probiotic fed and control groups with regard to percent giblet weight and per cent dressed weight of broilers.

Mahajan *et al.* (1999) reported that significantly ($P \leq 0.05$) higher dressing percentage was observed for probiotic Lacto-Sacc fed broilers as compared to the control in both winter and summer season. They also demonstrated that the meat: bone ratio of all cut up parts and whole carcass was significantly higher in Lacto Sacc fed broilers. But Panda *et al.* (1999) observed no significant effect of probiotic (*Lactobadllus acidophillus, Lactobacillus casei, Bifidobacterium bifidum. Straptococcus faecium,Aipergilius oryzae, Torulopsis spp*) on dressing percentage. They also claimed that no significant differences were observed in weight of liver, heart, gizzard and fat due to the dietary treatments. Abdul Rahim *et al.* (1999) found that there was an increase in abdominal fat pads in female broilers fed with *Lactobacillus acidophillus* in combination with zinc bacitracin at 56 days of age.

No important effect of supplementation of probiotic, with or without antibiotic, to the rations on carcass yield and edible visceral organ weight of broilers, was observed by Ergun *et al.* (2000). Ladukar *et al.* (2001) observed that the average dressing percentage of broilers in control group was 67.59 whereas it was 64.66, 64.34, 63.65, 60.82 and 60.91% in T_1 (*Streptococcus faecium, Streptococcus thermophilus, Lactobacillus*

plantarum, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus acidophilus. Bifidobacterium bifidum, Torulopsis, Acidoplillus oryzol), T_2 *(L. casei, L. acidophilus, B. bifjidum, S. faecium, Torulopsis).* T_3 *(Yeasacc 1026, L.acidophilus, S.faecium),* T_4 (Live *yeast* culture, *L. sporogens*, amino acids, liver extract) and T_5 (*L. sporogens, Saccharomyces cerevisiae*, SC-47, Alpha amylase) groups, respectively. Dressing percentage was significantly higher ($P<0.05$) in control group than in T_3, T_4 and T_5 groups.

Bandy and Risam (2001) reported that there was a significant improvement ($P<0.05$) in the dressing, eviscerated and edible meat yields among the different dietary treatments. They also studied that the percent giblet yields were significantly higher ($P<0.05$) in the treatment groups fed diet supplemented with 0.05 percent and 0.075 per cent probiotic. Priyankarage *et al.* (2003) reported that dressing percentages and fat/meat ratios showed no indication of any advantages conferred by addition of probiotics. Kalavathy *et al.* (2003) observed that there was no significant difference in the weights of organs of control and *Lactobacillus* cultures (LC) fed broilers. They also found that the relative abdominal fat pad was reduced ($P<0.05$) in LC supplemented broilers at 28, 35 and 42 days of age when compared with the control broiler chicks.

2.9 Effect of probiotic on economics of broiler production

Khan *et al.* (1992) observed that there was an improvement in economics of broiler production as a result of addition of probiotic in broilers' ration. It was concluded by Buche *et al.* (1992) that the inclusion of lower level (0.02%) probiotic *(Lactobacillus sporogenes*) either alone or in combination with lower level of nitrofuran (0.05%) was beneficial for broiler production because the cost of feed per kg live weight gain was the lowest in T_4 (0.02% probiotic and 0.05% nitrofuran) group than other treatment groups. Lee *et al.* (1993) found that addition of probiotic in the broiler diet could not reduce the cost of production of broiler. Baidya *et al.* (1994) reported that the supplementation of probiotic in the diet of broiler chick had improved profitability in broiler production. Samanta and Biswas (1995) concluded that the average feed cost per kg live weight gain as well as net income per bird did not reveal any statistical variation among the groups. It was observed by Singh *et al.* (1999) that feeding of .02 per cent probiotic (*LactobaciIlus*

sporogens) reduced the production cost per kg live weight as compared to control and other treatments (0.03 and 0.04 percent).

Ladukar *et al.* (2001) observed that there was no significant difference in the cost of production of one kg live weight. They reported that probiotic could not aid in economizing the broiler production. But when economics was calculated for per kg dressed weight, they observed a significant increase ($P<0.0l$) in the cost of dressed meat production in T_3, T_4 and T_5 groups which was because of a significant reduction ($P<0.05$) in dressing percentage of these groups. Bandy and Risam (2001) studied that probiotic (Biospur) at the rate of 75g per 100kg fed broiler diet as growth promoter improved profitability in broiler raising.

2.10 Effect of probiotic on other parameters

Mohan *et al.* (1996) demonstrated that nitrogen retention was greatest in the antibiotic (48.5%) followed by the probiotic (46.5%), probiotic plus antibiotic-supplemented groups (46.3%) as compared to 40.2% in control birds. They also showed that serum cholesterol was significantly lower ($P<0.01$) in probiotic supplemented birds (86.lmg/dl) compared to 118.4mg/dl in control birds. Gohain and Sapcota (1998) found that the probiotic feeding did not play any significant role in changing the serum protein level of broilers. They also reported that the serum cholesterol level was numerically, not significantly reduced from174±8.3l mg/l00ml in the control group of broilers to a mean value of 149±2.88mg/l00ml in the probiotic fed group. Abdul Rahim *et al.* (1999) claimed that there was an increase in abdominal fat pads in female broilers fed with Lactobacillus acidophilus in combination with zinc bacitracin at 56 day of age. Ladukar *et al.* (2001) observed that protein efficiency ratio (PER) and nitrogen balance of the broilers did not differ significantly among different treatments. Shoeib and Machan (2002) observed that the level of probiotics (pronifer or biogen) to the chick diets had proportional effect in the reduction of the total viable count of E, coli in addition to the complete disappearance of clostridium. Kwon *et al.* (2002) reported that ammonia nitrogen (NH3-N) Concentration in faeces of PB1.0 (basal diet + 1.0% probiotics) treatment group was lower ($P<0.05$) than control or P130.5 (basal diet -I- 0.S% probiotics) treatment group. Priyankarage *et al.* (2003) demonstrated that a negative correlation between level of probiotics) in the diet and Salmonella occurrence was

observed in the birds (20% in T1 and 13% in T) and the differences were statistically significant ($P<0.05$). Here dietary treatment T1 was control diet + 0.1g probiotic per kg feed and dietary treatment T2 was control diet + 0.2g probiotic per kg feed. Dalloul *et al.* (2003) studied that the probiotic bacteria (Lactobacillus-based) impacted the local immune response as characterized by altered intestinal intra epithelial lymphocyte subpopulations and increased the birds' resistance to *Eimeria acervulina* as reflected by oocyst sheeding. Kalavathy *et al.* (2003) claimed that the supplementation of Lactobacillus cultures in broilers diets was effective in reducing abdominal fat pad deposition but only after 28 days of age. They also reported that the Lactobacillus cultures reduced serum cholesterol and low density lipoprotein cholesterol in broilers from 21 to 42 days of age.

2.11 Factors affecting efficacy of probiotics

It appears from the review of literatures that the probiotics exert their positive effects on growth performance, feed: gain ratio and mortality of broilers. Contradictory information is also available in this regard. The literature suggests that the effect might be variable between preparations as well as with several other environmental and management conditions (Gohain and Sapcota, 1998). Obviously, several factors must be considered if the desired results arc to be explored when using probiotics. Following factors might affect the responses of broiler birds to probiotic supplementation:

- Level of incorporation
- Composition of diet
- Strain of microbes present.
- Route of administration.
- Stress condition
- Health of the birds

2.11.1 Level of incorporation

It is necessary to incorporate probiotics in the feed or drinking water at an optimum level in order to obtain desirable responses. It may not be true that greater the number of beneficial microbes higher the expected result. Lyons (1987) suggested that the effectiveness of probiotics was related to the correct number of living bacteria.

2.11.2 Composition of diet

Composition of diet is an important factor to achieve better response from the addition of probiotics in the ration or in drinking water. In most cases, it is anticipated that the microorganisms need to survive and grow in the intestinal tract. The diet or drinking water containing detrimental component for the used beneficial microbes may hamper the positive effects of probiotics because that component will either suppress or destroy the probiotic bacteria.

2.11.3 Strain of microbes

Different microbes exert different functions depending on their inherent nature. The function of bacteria varies considerably among species and even among strains of the same species. Jin *et al.* (1996c) found that only 26% of the isolates of *Lactobacillus spp.* from chicken were able to attach moderately or strongly to the ileal epithelial cells of chickens. Lactobacillus tp. inhibits the growth of pathogenic bacteria by producing lactic acid which reduces the $p^{H.}$ Level of intestinal tract. On the other hand, when live yeast is added to the ration, it enhances the digestion of fiber components of diet. *Jin et al.* (1997) indicated that differences in the strains and forms of bacteria used, and concentrations of viable cells could produce discrepancies in results.

2.11.4 Route of administration

There are mainly two routes i.e. feed and water for the administration of beneficial bacteria in the gut of birds. To achieve desirable responses from supplementation of probiotics, it is necessary to provide correct concentration of viable cells in the intestine of birds through any route. Drinking water is an easy and better route for the attachment of beneficial bacteria in the intestine. The responses of probiotic through feed may vary due to the form of feed i.e. pellete and mash. In case of mash feed the results may also vary because of the fact that machine mixing is better than that of hand mixing. Mamun-Ur-Rashid (2003, unpublished) reported that the EM probiotic treated feed+ drinking water showed better performance than that EM treated feed or EM treated drinking water or control one.

2.11.5 Stress condition

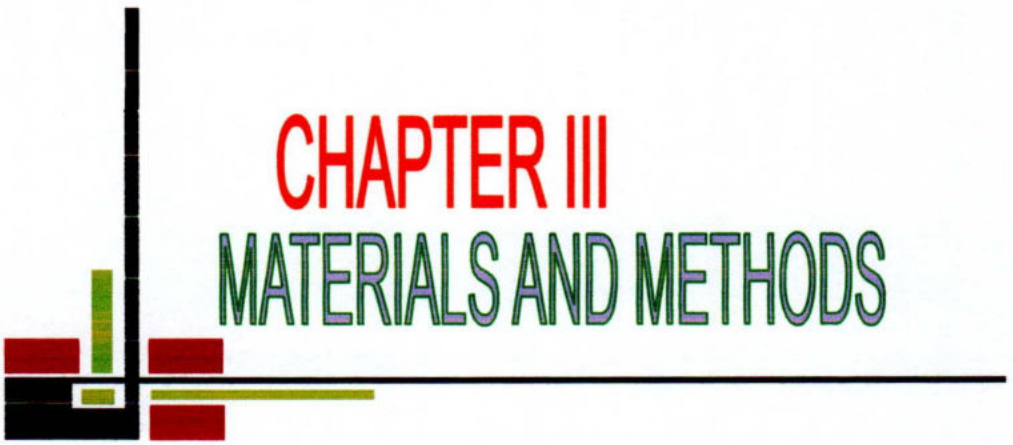
During the process of intensive production, chickens are stressed by several factors such as transportation to the growing site, overcrowding, vaccination, chilling or overheating. Lyons (1987) also suggested that the efficacy of probiotics was related to the presence of stress on the chicken. Numerous studies, under field and controlled conditions, over a period of years demonstrated that stress may alter both humoral and cell mediated immunity (Siegel, 1995). It has been suggested that probiotic supplementation is of greatest benefit when birds are exposed to stressful conditions Gin *et al.* (1997).

2.11.6 Health of the birds

Health status of the bird is one of the important factors that may affect the efficacy of probiotic. The responses of probiotics may vary due to differences in the load of pathogens in the healthy and diseased birds. There is no published information as to how beneficial bacteria containing probiotics react with health status of birds.

2.12 Research gaps and the present study

Recently, there is a trend among the poultry producers for using feed additives that have no residual effect. This is to provide safe poultry products to the human beings by discouraging the use of antibiotics or growth hormones or any other additives having residual effects. A large number of research works with probiotics (residual free dietary additives) has been conducted in abroad since 1970's. But the findings on the effect of probiotic supplementation in the broiler diet are still contradictory. Some researchers claimed that probiotics exert beneficial effects on the performance of broilers but some other workers proved just opposite to these findings. In this relation, a very little work has been conducted under Bangladesh condition specifically with layers. So, it seemed worthwhile to investigate whether or not probiotic exhibits beneficial effects on the performance of broilers. Levu cell was considered as it is one of the most widely used probiotics marketed in Bangladesh.



CHAPTER III
MATERIALS AND METHODS

CHAPTER III

METARIALS AND METHODS

3.1 Statement of the research work

To investigate the influence of a probiotic in the diet of broiler chicks, a 35-day feeding trial with 120 day-old Hubberd classic broilers was conducted spring season at Hajee Mohammod Danesh Science and Technology University Poultry Farm. The trial period continued from 05 April to 10 May, 2013.

3.2 Preparation of the experimental house and equipment

An- open-sided house was used for rearing the experimental birds. Each experimental room was partitioned into 03 separate pens of equal size by using wire net and bamboo materials with 04 pens on each side of a service area running along the middle of each room. The experimental rooms (ceiling, wall, floor and wire net) were properly brushed with broom and then washed and cleaned by forced water using hosepipe. After washing with clean water, the rooms were disinfected by using bleaching powder solution. Then the rooms were left vacant for 15 days. Later, the rooms were again disinfected and kept free to dry up properly. At the same time, all feeders, waterers and other necessary equipment were also properly cleaned, washed and disinfected with bleaching powder solution, subsequently dried and left them empty for one week before the arrival of chicks. Ceiling, walls and wire net were also thoroughly disinfected.

Three days before the arrival of chicks, the rooms were enclosed with curtains made of jute materials and fumigated with potassium permanganate (KMnO4) and formalin at double strength (2x). For 100 cubic feet area a mixture of 35g potassium permanganate $KMnO_4$ and 70cc formalin, which is equal to double strength, was used for fumigation. The rooms were fumigated for a period of 48 hours to destroy pathogenic bacteria and virus. The rooms were opened fully for proper aeration before 25 hours of the arrival of chicks. The chicks were allocated into the rooms on 05 April 2013, at 5.00 PM.

3.3 Collection of the experimental birds

One hundred and twenty straight-run day-old Hubbard classic broiler chicks were procured from Aftab Bahumukhi Farms Ltd, Bajithpur, Kishorgonj, Bangladesh.

3.4 Layout of the experiment

The day-old Hubbard classic hybrid broiler chicks were distributed randomly into 4 (four) dietary treatments, having 3 replicates in each treatment. The chicks were randomly picked up from chick boxes and allocated to respective replicate pens. There were 10 chicks in each replication. The layout of the experiment is shown in Table 3.1.

Table 3.1 Layout showing the distribution of experimental birds

Treatments (levu cell g/kg)	Number of birds in each replication			Total
	R_1	R_2	R_3	
T_0(without levu cell)	10	10	10	30
T_1 (0.5)	10	10	10	30
T_2(1.0)	10	10	10	30
T_3(1.5)	10	10	10	30
Total no. of birds	-	-	-	120

3.5 Procurement of feed ingredients and probiotic

Required feed ingredients for making experimental diets were procured from the local market of Dinajpur town. During procurement, ingredients were evaluated carefully for their freshness by observing its color with naked eye and smell with nose. A commercial probiotic preparation with a brand name Levu cell® SB was donated by the Animal Health Division of Square Pharmaceutical (Bangladesh) Ltd. To carry out the equipment.

3.6 Preparation of the experimental diet

The diets were formulated with least-cost principles by using computer software named UFFDA (1982). Nutrient composition of each ingredient was considered from the report of Chowdhury (2003) and the amount was calculated in such a way that the nutrients

composition per unit feed could fulfill the breeder's recommendation. The experimental diets were divided into two phases (broiler-starter and broiler-finisher). Broiler starter diet was provided between 0 and 14 days, whereas that of the broiler-finisher phase consists of 15 to 35 days.

The experimental diets were formulated with locally available feed ingredients. The ground ingredients were mixed thoroughly and properly. Then rice polish, micronutrients (vitamin-mineral-premix, lysine & methionine) and common salt were mixed thoroughly in a separate place. The required amounts of mixed rice polish were again weighed according to respective treatments. The required amount of levucell was weighed treatment-wise and it was then mixed with a small quantity of the previously weighed mixed rice polish and the quantity was increased gradually by adding remaining rice polish. After proper mixing, it was then thoroughly mixed with maize, wheat, soybean meal etc. properly. At last required amount of soybean oil was sprayed on the mixed feed and finally, it was mixed properly and thoroughly. Mixing was done manually and no coccidiostat or any other feed additives were added.

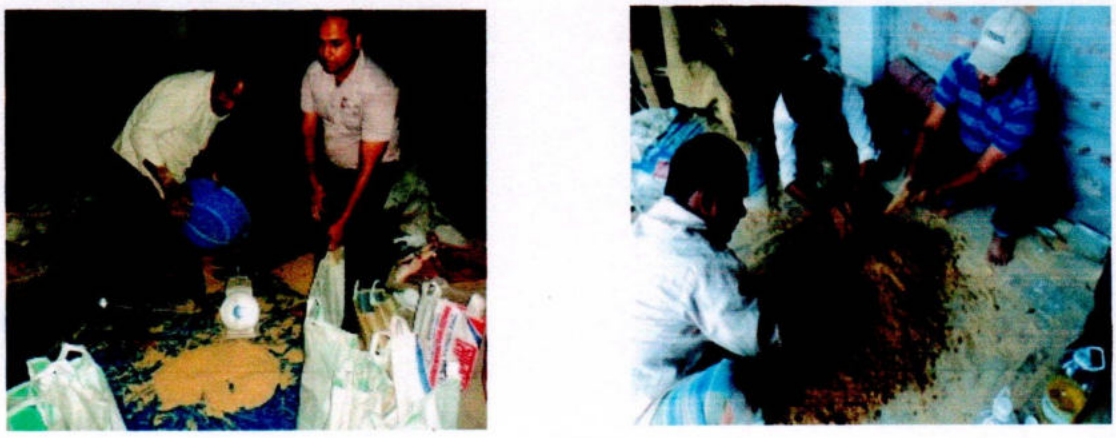


Fig 3.1 Preparation of the experimental diets

3.2 Ingredient and chemical composition of experimental diets

Feed ingredients	Amount (kg/100 kg feed)	
	Starter (0-14 days)	Finisher (15-35 days)
Maize	53.5	57.00
Rice polish (Auto)	10.00	10.00
Soybean meal (44)	23.00	18.00
Protein Concentrate	10.00	10.00
Oyester Shell	1.00	0.75
Soybean oil	1.50	3.00
DCP	0.5	0.75
**Vitamin-mineral premix	0.25	0.25
Common salt	0.25	0.25
Total	100kg	100kg

Nutrients	Calculated composition	
	Starter (0-14 days)	Finisher (15-35 days)
ME (kcal/kg)	2977	3074
CP (%)	21.21	19.40
CF (%)	5	5
Ca (%)	1.00	0.95
Available P (%)	0.74	0.75
Lysine (%)	1.02	0.89
Methionine (%)	0.35	0.35
Ash (%)	6	6

N.B. Levu cell was added according to each treatment as per the experimental design.

**Vitamin-mineral premix composition (each 2.5 kg contained): Vitamin A 12000000 IU, Vitamin D_3 2000000 IU, Vitamin E 15000 mg, Vitamin B_1 1000 mg, Vitamin B_2 4000 mg, Vitamin B_6 3000 mg, Vitamin B_{12} 10 mg, Vitamin K_3 201K) mg, Folic acid 1500 mg, Nicotinic acid 25000 mg, Pantothenic acid 11000 mg, Biotin 15 mg, Iron 32000 mg, Copper 8000 mg, Manganese 64000 mg, Cobalt 300 mg, Zinc 40000 mg, Iodine 800 mg, Selenium 200 mg, Lysine 30000 mg, Methionine 50000 mg, Antioxidant 10000 mg.

3.7 Routine management

The birds were exposed to identical care and management in all treatment groups throughout the experimental period. The following management practices were carried out during the entire experimental period.

3.7.1 Litter management

Fresh and dried rice husk was used as litter with a depth of about 3cm. After 3rd week of age the old litter was totally removed and new litter was provided. Again it was practiced after 4th week of age. The litter was stirred three times a week from 14 days and onwards to prevent accumulation of harmful gases.

3.7.2 Floor space

Each pen was 274.32cm × 101.50cm (27843.48cm^2) allocated for 10 birds. Therefore, each bird was provided with a floor space of 1031.24cm^2.

3.7.3 Brooding

Since experiment was done in spring season (March to April), the environmental temperature was sometimes lower and sometimes higher than the requirements. In the first week of experimental, the environmental temperature was lower than the required brooding temperature for all treatment groups, therefore, additional heat was provided to chicks during this time. Brooding of chicks was done by using 2 electric 100 watt bulbs were used in the respective pens. The bulbs were hanged just above the birds' level at the center of each pen. Brooding temperature was kept 34 °C at the beginning of first week and decreased gradually as shown in Table 3.4.

Table 3.3 Brooding temperature for experimental birds

Age of birds (days)	Brooding temperature (°C)
0-3	34
4-6	33
7-9	32
10-12	30
13-15	29
16-28	28

3.7.4 Lighting

The birds were exposed to 23.30 hours of lighting and a dark period of 0.30 hour per day throughout the experimental period. The dark period provision was kept to make broilers familiar with the possible darkness due to electricity failure. Two 100-watt electric bulbs were satisfactory for lighting.

Fig 3.2 Brooding and feeding management of birds during experimental period

3.7.5 Feeder and waterer management

For the first 7 days, feeds were given on the news paper and water was supplied in round waterer. After 7 days of age, one round feeder and one round waterer were provided for each replicate group of birds. One additional round feeder was provided to each replication after 18 days of age. Required feeding and drinking space were provided according to the number and age of the birds in each replication. The feeders and waterers were fixed in such a way that the birds were able to eat and drink conveniently. Feeders were cleaned at the end of each week and waterers were washed twice a day.

3.7.6 Feeding and drinking

Immediately after allocating the chicks in their respective pen, 5% glucose solution was provided to the chicks for 3 hours. Then fresh, clean and cool drinking water was supplied to the chicks. For the first seven days, feeds were given to the birds at two to three hours interval and water was provided three times a day. From the second week, feeds were supplied to the experimental birds three times every day; once in the morning, in the afternoon and again at night. Fresh cool drinkingwater was made available at all the times. Feeders and waters were not kept empty.

3.7.7 Immunization

The experimental birds were vaccinated to prevent Newcastle Disease and Infectious Bursal Disease (Gumboro). The vaccination schedule followed during the experimental period is given in Table 3.5.

Table 3.4 Vaccination schedule of birds

Age(days)	Disease	Name of vaccine*
3	Newcastle Disease	IB + ND
10	Gumboro	D-78
17	Gumboro	D-78

*Vaccine, prepared by Intervet International, Netherland, were applied as per recommendation of the manufecturer (one drop in each eye)

3.7.8 Medication and Sanitation

During the course of experiment no medication was provided.Proper hygienic measures and strict sanitation programs were followed. during the experimental period,the entrance point and veranda were kept clean and solution of PPM.In addition, the service area of the experimental rooms, outside wall of the experimental house and the feed room were kept clean throughout the experimental period.

3.7.9 Biosecurity

To prevent the outbreak of diseases, biosecurity was maintained during the experimental period. The following measures were taken to maintain biosecurity.Visitors were not allowed to enter the house. This was done by hanging billboard written as "RESTRICTED AREA - NO ENTRANCE WITHOUT PERMISSION" at entrance of the experimental shed.A footbath containing disinfectant solution (potassium permanganate or bleaching powder)was provided at the entrance point.All equipment and machinaries of the experimental house were kept clean.Dead and sick birds were removed promptly.Dead birds were buried far away from the experimental house.The entrance of cats, dogs and other wild flying birds were prevented inside the experimental house.

3.7.10 Postmortem examination of birds

Dead birds were diagnosed promptly at the Pathology Department under Veterinary Faculty, Hajee Mohammod Danesh Science and Technology University, Dinajpur. After posstmortem examination, the results were collected and necessary measures were taken to solve the problem without applying medicines.

3.8 Processing of broilers

The processing of broilers was done according to the procedure of Jones (1982). At the end of trial, the weight of birds was taken and average body weight was calculated. At 36 day of age, two birds weighing average from each replication were randomly selected for determining meat yield. To facilitate slaughter, all birds from each treatment group were kept without feed for 12 hours prior to killing, but water was supplied adlibitum. The birds were slaughtered and allowed to bleed for 2 minutes. After complete bleeding, birds were weighed individually. Then they were immersed in hot water (51 to 55°C) for 120 seconds for proper defeathering of carcass. The feathers were removed manually (by hand) and the birds were again individually weighed. Finally, processing was performed by removing head, shank, viscera, oil gland, kidney and giblets. As soonas these were removed, the gall bladder was cut off from the liver and pericardial sac and arteries were cut from the heart. After removal of gizzard from the intestine, it was split open with knife and the fecal materials were removed. Then it was washed with clean water and the lining was removed by hand.

3.9 Record keeping

Body weight of chicks was recorded initially and weekly replication-wise for each treatment. Feed intake was also recorded weekly replication-wise for each treatment. Mortality was recorded daily if occurred. During the whole experimental period, the temperature of the experimental house was recorded with the help of an automatic digital thermometer.Relative humidity was also recorded by using an automatic digital hygrometer. The different meat yield parameters like dressing weight, blood weight, featherweight, liver weight, gizzard weight, heart weight, shank weight, breast meat weight, thigh weight, drumstick weight, wing weight and dark meat weight for individual bird were recorded after slaughterin.

3.10 Calculation

On the basis of collected data, the required variables were calculated. The weight gain of each broiler was calculated by deducting initial body weight from the final body weight for each birds. Feed intake was also calculated as the total feed consumption in a replication divided by number of birds per replication. Necessary adjustme for the calculation of feed cosumption were made cosidering bird's mortality, if any. The feed conversion ratio was calculated as the total consumption of feed divided by live weight gain. Performance index was calculated by dividing the live weight (kg) by the feed conversion ratio and it was multiplied by 100. Survivability was calculated as the total number of birds survived divided by the total number of birds in each replication and multiplied by 100. The survived birds were calculated by deducting the number of dead birds from the total number of birds. The efficiency of performance was evaluated in terms of production number (PN) as follows (Euribrid, 1994):

$$\text{Production number (PN)} = \frac{\text{abw x \% liv}}{(\text{Days x FCR})10}$$

Here, abw = average live weight in gram

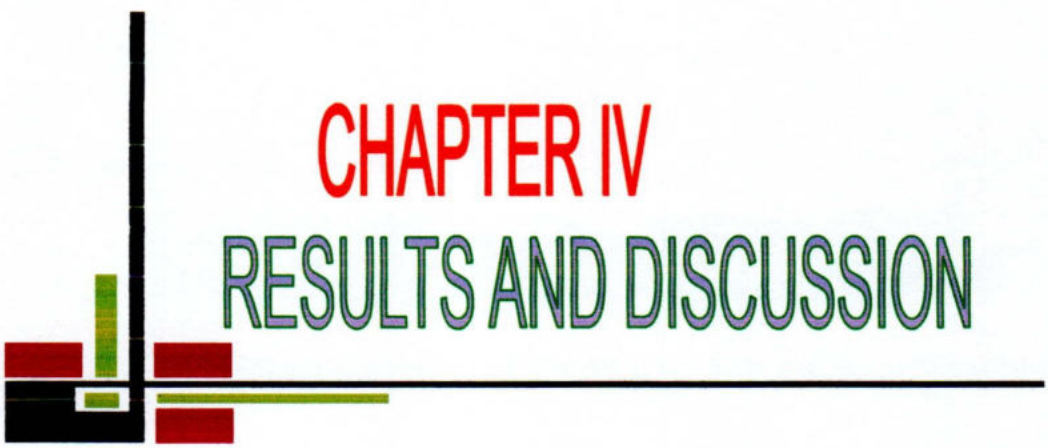
% liv = percent livability

days = duration of fattening in days

FCR = Feed Conversion Ratio

3.11 Statistical analysis

Data on performance were statistically analyzed by using analysis of variance (ANOVA) technique by a computer using SAS (1998) program in accordance with the principles of Completely Randomized Design (CRD). The meat yield parameters were analyzed by using a 2 (Sex) x 4 (diets) factorial experiment in a CRD. Least Significant Differences (LSD) were calculated to compare variation among treatments where ANOVA showed significant difference at 0.05 level of probability.



CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Performance of broiler

The performance in terms of live weight gain, feed intake and feed conversion of birds fed probiotic at different dietary levels is shown in Table 4.1. Survivability, performance index and production number of broilers are presented in Table 4.2.

4.1.1 Body weight gain

Initial body weight of day-old broiler chicks fed on different dietary treatments was similar ($P>0.05$). From 1 to 21 days of age and also from 1 to 35 days of age, the highest body weight gain was attained in birds that received the probiotic at the highest level (1.5 g probiotic per kg feed). This was followed by 1.0g probiotic per kg feed, control (without probiotic) and 0.5g probiotic per kg feed groups, respectively (Table 4.1). During 22 to 35 days of age, 1.0g/kg group gained more weight than that of other treatment groups. From 1 to 21 days of age and also from 22 to 35 days of age, there was no significant difference in weight gain of broilers among different dietary treatments ($P>0.05$). However, from 1 to 35 days of age, broiler chicks fed probiotic at 1.5g/kg feed group gained significant improvement in body weight ($P<0.05$) than group consumed diet supplemented with probiotic at 0.5g/kg. There was no significant improvement in treated groups compared to the control in the same period. The results revealed that addition of probiotic in the diet of broilers numerically increased weight gain by 2.28 and 3.00 per cent in 1.0g/kg and 1.5g/kg group respectively, compared to control group at the end of the feeding trial.

The non-significant effect of probiotic on body weight gain was in agreement with the findings of some previous studies (Ergun *et al.*, 2000; Ladukar *et al.*, 2001; Lima *et al.*, 2002; Priyankarage *et al.*, 2003). But these findings contradict the observation of Jin *et al* (2000); Bandy and Risar (2001); Shoeib and Madian (2002); Kalavathv *et al.* (2003) who found that supplementation of probiotics improved live weight gain of broilers. Jin *et al.* (1997) further explained that differences in the strains and forms of bacteria used, and

concentrations of viable cells could produce discrepancies in results. The effect of probiotic on body weight gain as obtained in this study might be due to some factors that affected the efficacy of probiotic such as composition of diet, stress condition, strain of microbes and concentration of microbes.

4.1.2 Feed intake

The average cumulative feed intake of broiler during the experimental period showed that except early period of rearing (1 to 21 days), probiotic supplemented groups tended to consume higher amounts of feed compared to control in other stages of age (from 22 to 35 days and from 1 to 35 days). Among different dietary treatments, 0.5g/kg group had higher intake than that of other treatment groups from 22 to 35 days of age and also from 1-21 days of age but from 1 to 35 days of age 1.5g/kg group consumed more feed (2502.8g) followed by 2446.15g, 2484.9g and 2388.4g in 0.5g/kg, 1.0g/kg and control groups respectively. However, there was nonsignificant difference ($P>0.05$) between the broilers fed on control diet and diets supplemented with probiotic at different levels 0.5g/kg, 1.0g/kg &1.5g/kg. At the end of trial, results of feed intake indicated that feed consumption of broilers were increased by 2.10, 2.62 and 2.03% with a supplementation of 0.5g/kg, 1.0g/kg & 1.5g/kg probiotic respectively, while compared to control group.

Higher feed intake in probiotic supplemented groups was in agreement with the results of some earlier studies (Samanta and Biswas, 1995; Panda *et al.*, 1999; Ladukar *et al*, 2001; Lima *el al*, 2002). In those studies, feed intake of different broiler groups did not differ significantly due to addition of probiotics. However, contrary to these observations, some workers have found that feed consumption differed significantly between the control and probiotic fed groups (Mahajan *et al.*, 1999; Zulkifli *et al.*, 2000; Bandy and Risam, 2001) explaining that the higher feed consumption in probiotic supplemented group might be due to the increase of digestive efficiency. Mohan *et al.* (1996) also indicated that probiotic supplemented diets improved the feed intake irrespective of seasons. The higher amounts of feed consumption although not significant as found in the present study, might be due to increased appetite and rate of enzymatic activity which enhances the digestive 'efficiency of broilers.

Table 4.1 Effect of supplementation of probiotic in the diet of broilers on body weight gain, feed intake and feed conversion ratio

Variables	Dietary probiotic (g/kg)				SEM	P value	Level of significant
	0 (control)	.05	1.0	1.5			
Initial body weight (g/kg)	45.19±0.39	44.7±0.43	43.81±0.13	44.24±0.41	0.39	0.731	NS
Body weight gain(g/broiler)							
1 to 21 days of age	517.38±12.2	506.96±4.5	522.10±14.2	552.03±12.5	11.74	0.000	**
22 to 35 days of age	678.9±14.48	679.92±21.85	729.48± 16.75	721.38±15.98	16.98	0.000	**
1 to 35 days of age	1196.28±21.6^{ab}	1186.88±23.6^{b}	1251.58±2.78^{a}	1273.41±3.96^{a}	19.39	0.000	**
Feed intake (g/broiler)							
1 to 21 days of age	885.31±6.63	853.88±1.59	907.12±29.05	924.30±18.28	16.06	0.000	**
22 to 35 days of age	1503.08±33.35	1592.26±35.28	1577.77±7.99	1578.5±23.15	29.43	0.000	**
1 to 35 days of age	2388.4±27.6	2446.15±36.88	2484.90±36.75	2502.80±27.31	37.80	0.000	**
Feed conversion ratio							
1 to 21 days of age	1.71±0.04	1.68±0.01	1.74±0.03	1.67±0.05	0.03	0.0659	NS
22 to 35 days of age	2.21±0.01	2.34±0.09	2.16±0.06	2.18±0.08	0.06	0.697	NS
1 to 35 days of age	1.99±0.01	2.06±0.04	1.98±0.03	1.96±0.02	0.03	0.018	*

* = Significant at 5% level of probability

** = Significant at 1% level of probability

NS = Non significant

Means within a row with uncommon superscript differ significantly (P<0.05). Means without superscript do not differ significantly at 5 per cent level of probability.

SEM = Standard errors of means.

4.1.3 Feed conversion

The feed conversion in different dietary treatments were very much close with each other in every stages of growth. At the end of the trial i.e. at 35 days of age, the feed conversion was better in 1.5g/kg (1.96) followed by 1.99, 1.98 and 2.06 in control, 1.0g/kg and 0.5g/kg groups respectively. The data pertaining to the feed conversion ratio in different dietary treatments at different stages of age indicated that addition of probiotics had no significant effect on feed conversion ($P>0.05$) at any stage (Table 4.1).

The non-significant effect of probiotic on feed conversion was in close agreement with the observations of some previous workers (Mohan *et al.*, 1996; Yeo and Kim1997; Lima *et al.*, 2002; Priyankarage *et al.*, 2003). In consistent with this result, Ergun *et al* (2000) reported that supplementation of probiotic with or without antibiotic in the rations had no significant effect on feed conversion of broilers. In contrast, broilers fed Biospur (Bandy and Risam, 2001), Lacto-Sacc (Mahajan *et at.*, 1999), Lictobacillus cultures (Zulkifli *et al.*, 2000) and Pronifer or Biogen (Shoeib and Madian, 2002) showed significant improvement in the feed conversion when compared with control chicks. Probiotic supplemented groups consumed more feed but could not show a significant increase in body weight gain, which might be the reason for comparable feed conversion in the present study.

4.1.4 Survivability

Survivability of broilers fed on different dietary treatments was very much acceptable during the study period. The survivability did not vary significantly ($P >0.05$) among different treatment groups during the whole experimental period.

Lower survivability of broilers fed diets supplemented with probiotics is available in the results of Zulkifly *et al* (2000). When broilers were given a dietary supplementation of probiotics (*Lactobacillus* cultures) and exposed to 36±1 °C for 3 hours daily from day 21 to 35. But the result of present study was inconsistent with the findings of some earlier studies (Samanta and Biswas, 1995; Fabris *et at.*, 1997; Singh *et al.*, 1999; Hamid and Aijazuddin, 2001). Where higher survivability in probiotic fed groups was found as compared to control. Since the result on survivability was quite acceptable in this study with little differences among the dietary groups, the beneficial effect could not be

detected over the control group. The birds, in the present study, felt discomfort due to high temperature of 32±1° C for 6 hours daily from 23 to 35 days of age. This could not, in any way, either depress or improve performance significantly in the probiotic fed birds. 'The superior performance index in 1.5g/kg group might be due to slightly higher body weight since feed conversion was comparable in all groups. The broilers of 1.5g/kg group attained highest production number probably because of more body weight compared to other dietary treatments since its livability (%) was close to the other treatment groups. The findings on performance index and production number of broilers in the present study could not be related with other findings due to lack of published information on these variables.

4.1.5 Performance index

At the end of the feeding trial, the differences in performance indices varied significantly ($P<0.05$) between treatments 1.5g/kg and 0.5g/kg but there was no significant difference with control and 1.0g/kg groups. The performance index in1.5g/kg group was observed to be 66.25%, the highest of all, whereas it was 64.08, 63.92 and 61.01% in 1.0g/kg, control and 0.5g/kg groups respectively from 1 to 35 days of age (Table 4.2).

4.1.6 Production number

The data pertaining to the production number (PN) in different dietary treatments from 1 to 35 days of age indicated that 1.5g/kg groups had higher production numbers similar to control group but other two groups had comparatively lower values. However, small differences in production number revealed no significant differences among the diet groups ($P>0.05$).

Table 4.2 Effect of supplementation of probiotic in the diet of broiler on survivability, performance index and production number

Variables	Dietary probiotic (g/kg)				SEM	Level of Significant
	T_0(control)	T_1 (0.5)	T_2 (1.0)	T_3(1.5)		
Survivability (%)						
1 to 21 days of age	99.38	98.15	96.92	96.91	97.84±0.52	NS
22 to 35 days of age	100.00	99.38	99.33	99.36	99.57±0.21	NS
1 to 35 days of age	99.38	97.53	96.30	96.30	97.37±0.65	NS
Performance index (%)						
1 to 35 days of age	63.92^{ab}	61.01^{b}	64.08^{ab}	66.25^{a}	63.81±0.81	NS
Production Number(PN)						
1 to 35 days of age	181.58	170.09	176.30	182.18	177.53±2.47	NS

Means within a row with uncommon superscript differ significantly ($P<0.05$).
Means without superscript do not differ significantly at 5 per cent level of probability.
SEM = Standard errors of means.

4.2 Meat yield parameters

The effects of diet, and interaction of diet on different meat yield parameters are presented in Table 4.3. The Table indicates that there was no significant difference ($P>0.05$) in the per cent weight of different organs and components of broilers except abdominal fat due to addition of probiotic in the diet of broilers. The differences in the per cent abdominal fat of broilers fed diet supplemented with probiotic varied significantly $P<0.05$) when compared with the control broiler chicks. Among different dietary treatments, abdominal fat percentage was lowest in 1.5g/kg group (1.0%) followed by 1.0, 1.14 and 1.11% in 1.0g/kg, 0.5g/kg and control groups respectively. There was no significant influence of diets on the per cent weight of different organs of broilers as well ($P>0.05$).

The observation of the present study with regard to meat yield was consistent with the findings of Mandal *et al.* (1994); Panda *et al.* (1999); Ergun *et al.* (2000); Kalavathy *et at* (2003) who found no significant difference in the weights of organs reports of Mahajan *et al.* (1999) and Bandy and Risam (2001). They claimed that there was a significant improvement in the dressing, eviscerated and edible meat yields due to addition of probiotics. Significant reduction in the abdominal fat compared to control one agreed with well the results of some previous workers (Chah *et al.*, 1975; Santoso *et al.*, 1995; Kalavathy *et al.*, 2003). They found that diet supplemented with probiotics reduced abdominal fat significantly in boilers. But this finding contradicted with the observation of Panda *et al.* (1999) who found no significant effect of probiotic on abdominal fat of broilers.

Table 4.3 Effect of supplementation of probiotic in diet of broilers on meat yield

Meat yield parameters	Dietary probiotic (g/kg)				SEM	Level of Sig.
	T_0 (control)	T_1 (0.5)	T_2 (1.0)	T_3 (1.5)		
Live weight (g)	1253.33±17.63	1250±26.45	1343.33±8.81	1343.33±8.81	22.24	**
Feather weight (%)	5.79±0.03	5.62±0.17	5.99±0.12	5.99±0.12	0.14	NS
Digestive tract weight (%)	8.99±0.11	9.3±0.25	8.59±0.33	8.59±0.33	0.20	NS
Heart weight (%)	0.53±0.008	0.52±0.01	0.54±0.03	0.54±0.03	0.03	NS
Gizzard weigh (%)	2.15±0.01	2.16±0.03	2.15±0.1	2.15±0.1	0.07	NS
Liver weight (%)	2.49±0.02	2.23±0.14	2.52±0.15	2.52±0.15	0.21	**
Abdominal fat Weight (%)	1.11 ± 0.03^{ab}	1.14 ± 0.06^{a}	1.00 ± 0.1^{b}	1.00 ± 0.1^{b}	0.09	NS
Head weight (%)	2.7±0.01	3.03±0.09	2.97±0.07	2.97±0.07	0.10	NS
Shank weight (%)	4.58±0.01	4.24±0.08	4.40±0.19	4.40±0.19	0.16	*
Dressed weight(g)	67.94±0.24	67.74±0.18	67.81±0.16	67.81±0.16	0.30	NS
Thigh weight (%)	11.49±0.02	11.36±0.05	11.60±0.07	11.60±0.07	0.19	**
Wing weight (%)	22.95±0.26	22.50±0.33	22.60±0.50	22.60±0.50	0.25	NS
Breast weight (%)	7.49±0.02	7.24±0.37	7.79±0.30	7.79±0.30	0.32	NS
Neck weight (%)	3.84±0.09	3.78±0.01	4.14±0.22	4.14±0.22	0.16	NS
Drumstick weight (%)	9.64±0.09	9.06±0.04	9.42±0.23	9.42±0.23	0.27	NS

Means within a row with uncommon superscript differ significantly ($P<0.01$).
Means without superscript do not differ significantly at 5 per cent level of probability.
SEM = Standard errors of means.
* = Significant at 5% level of probability
** = Significant at 1% level of probability
NS = Not significant

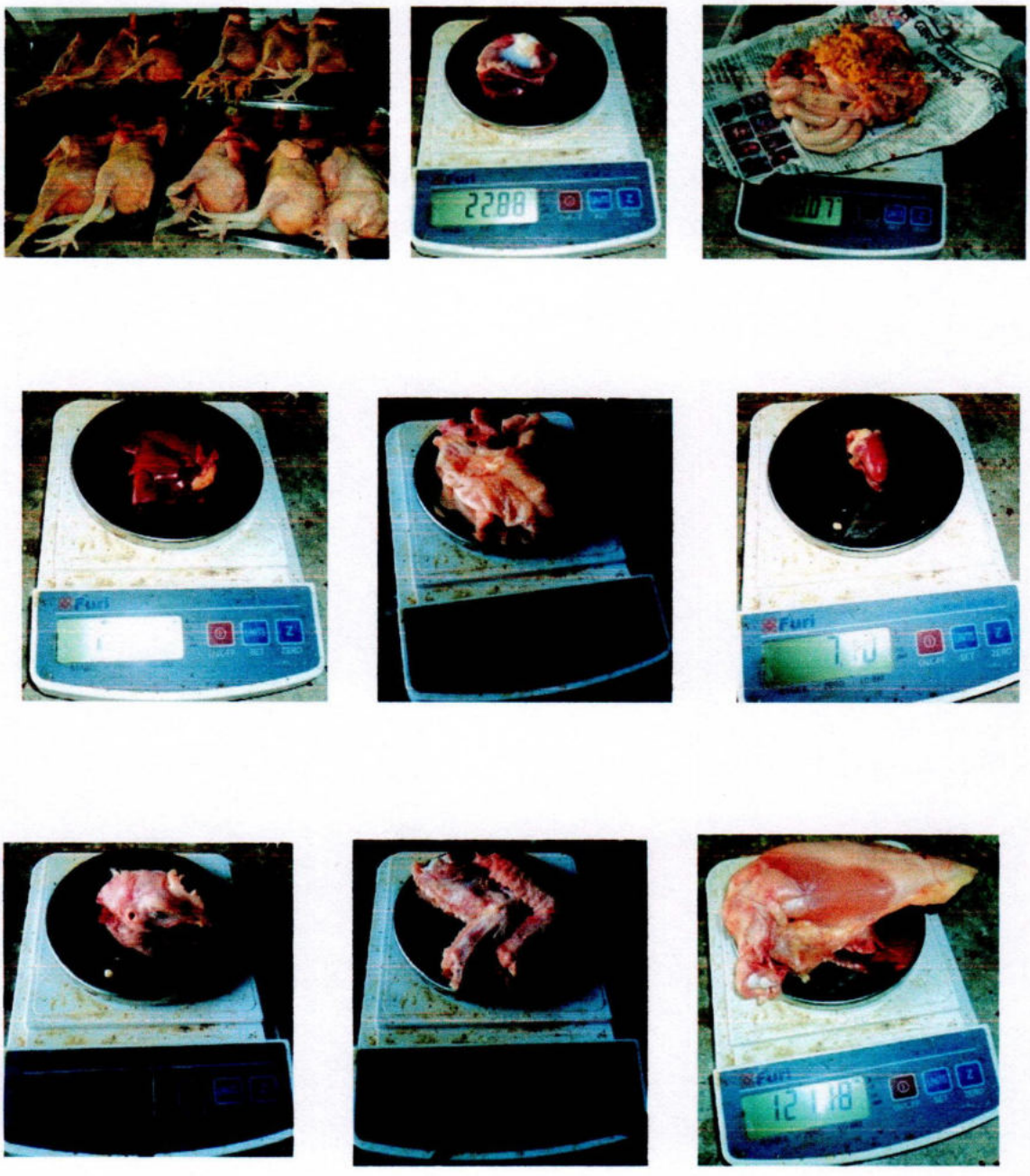


Fig 4.1 weighing the different parts of broiler during processing the meat yield

4.3 Economics of feed cost and income

The mean values on cost of feed due to addition of probiotic in relation to per kg live weight gain and per kg dressed weight are shown in Table 4.3. It was observed that the cost of probiotic supplementation for 1 kg live weight gain varied significantly ($P<0.01$) among different dietary groups. On the other hand, when cost was calculated for per kg dressed weight, a highly significant increase ($P<0.01$) in the cost of dressed meat

production was observed in 1.5g/kg, 1.0g/kg and 0.5g/kg groups compared to Control group. It was also observed that there was a significant difference in the cost of per kg live broiler when compared with the control broiler chicks ($P<0.01$). Control broiler chicks required lowest cost to produce one kg live broiler as compared to broilers fed diets supplemented with probiotic. When profit was considered for per kg live broiler, the same trend was observed in different treatment groups. The highest profit was obtained from control group which was Tk. 11.72 followed by Tk. 9.44, 9.44 & 8.28 in0.5g/kg,1.0g/kg and 1.5g/kg groups respectively. The profit per kg live broiler from probiotic treated groups differed significantly compared to control one ($P<0.01$).

Probiotic could not show its beneficial effects on the profitability of broiler raising. The findings of the present study is similar to the observations of Lee *et al.* (1993); Samanta and Biswas (1995); Laduar *el al.* (2001). They reported that probiotic supplementation per kg live weight gain could not reduce the cost of production of broiler as well as net income per bird did not reveal any statistical variation among the groups. But this finding is inconsistent with the results of Khan *et al.* (1992); Singh *et al.* (1999); Bandy and Risam (2001) who claimed that the addition of probiotics in the diet of broiler chicks had improved profitability in broiler production.

Table 4.4 Economic of supplementation of probiotic in the diet of broilers

Variables	Dietary treatments				SEM	P value	Level of Sig.
	T_0 (control)	T_1(0.5)	T_2(1.0)	T_3(1.5)			
Cost of total feed consumed per bird (Tk.) up to 35 days.	33.28	33.93	34.63	34.19	0.56	0.214	NS
Cost of probiotic consumed per bird (Tk.) up to 35 days	0^{d}	01.39^{c}	2.83^{b}	05.60^{a}	0.04	0.0024	**
Cost of probiotic+feed consumed per bird (Tk.) up to 35 days	33.28^{d}	35.32^{c}	37.47^{b}	39.79^{a}	0.49	0.0007	**
Cost of probiotic + feed per kg live weight gain (Tk.)	27.53^{c}	29.61^{b}	30.29^{b}	31.95^{a}	0.49	0.0038	**
Cost of probiotic + feed per kg dressed weight (Tk.)	49.21^{d}	52.09^{c}	55.45^{b}	58.64^{a}	0.17	0.0274	*
Cost (Tk.) per kg live broiler	46.28^{b}	48.56^{a}	48.56^{a}	50.05^{a}	0.76	0.0421	*
Income (Tk.) per kg live broiler	58.0	58.0	58.0	58.0	-	ND	-
Profit (Tk.) per kg live broiler	11.72^{a}	9.44^{b}	9.44^{b}	8.28^{b}	0.77	0.0008	**

Means within a row with uncommon superscript differ significantly ($P<0.01$).

Means without superscript do not differ significantly at 5 per cent level of probability.

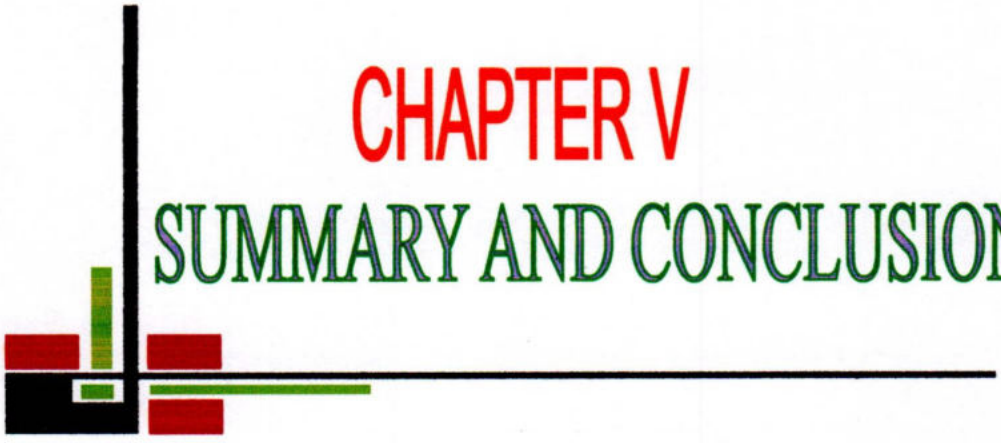
SEM = Standard errors of means.

* = Significant at 5% level of probability

** = Significant at 1% level of probability

NS = Not significant

Although statistically significant effect of probiotic was not found but slightly improvement in body weight gain was observed in 1.5g/kg and 1.0g/kg group compared to control group. Among the meat yield characteristics, reduced abdominal fat appeared as a positive outcome in the present study. It may be concluded on abdominal fat that higher the probiotic levels lower the abdominal fat. The economics of feeding probiotic clearly indicated that cost of production increases as the dietary level is increased. So, a minimum level needs to be determined. Dietary levels between 0.5 g and 1.5g per kg may be reinvestigated. In addition, use of probiotic in drinking water, a different route of administration may be examined in the future. An investigation into the existence, nature and viable cell counts of different species of gut microflora would be interesting to understand the mechanism of action of probiotic bacteria.



CHAPTER V

SUMMARY AND CONCLUSIONS

CHAPTER V

SUMMARY AND CONCLUSIONS

A feeding trial with 120 day-old Hubbard Classic broiler chicks was carried out at poultry shed of Hajee Mohammod Danesh Scince and Technology University, Dinajpur. The duration of the experimental period was 35 days from 05 April to 10 May, 2013. The chicks were randomly distributed to 4 different dietary treatments each having 3 replicates where each replication (pen) contained 10 birds. The 4 dietary treatments were T_0 (control), T_1 (control + 0.5g probiotic per kg feed), T_2 (control + 1.g probiotic per kg feed) and T_3 (control + 1.5g probiotic per kg feed). Feed and water were provided ad libitum to all birds throughout the experimental period. Identical care and management were followed for birds of all dietary groups.

At the end of the feeding trial, the cumulative body weight gain of different groups was 1196.28, 1186.88, 1251.58 & 1273.41 g in T_0 (control), T_1 (0.5g/kg), T_2 (1.0g/kg) and T_3 (1.5g/kg) groups, respectively. Birds that received probiotic at T_3 (1.5g/kg) gained more weight although body weight gain of broilers of different dietary groups did not differ significantly compared to T_0 (control) ones ($P>0.05$). The broilers of T_0 (control), T_1 (0.5g/kg), T_2 (1.0g/kg) and T_3 (1.5g/kg) groups consumed 2388.4, 2446.15, 2484.90 & 2502.8 g feed respectively, during the whole experimental period. From 1 to 35 days of age, the feed conversion ratio was 1.99, 2.06, 1.98 & 1.96 in T_0 (control), T_1 (0.5g/kg), T_2 (1.0g/kg) and T_3 (1.5g/kg) groups respectively. There were no significant differences in feed consumption and feed conversion of broilers among different dietary treatments ($P>0.05$).

The survivability of broilers ranged between 96.3 & 99.3% from 1 to 35 days of age. Survivability of broilers was quite acceptable in all dietary groups during the whole experimental period. The performance index as well as production number of broilers was highest in birds that received probiotic at T_3 (1.5g/kg) feed. Performance index and production number of broilers of different dietary groups did not vary significantly ($P>0.05$) compared to control ones. Investigation into meat yield characteristics revealed that probiotic could not show its beneficial effects on the per cent weight of different

organs of broilers except a reduction in abdominal fat. The per cent abdominal fat was lowest in T_3 (1.5g/kg) group followed by T_2 (1.0g/kg), T_1 (0.5g/kg) & T_0 (control) groups respectively.

The cost per kg live broiler was lowest in control group compared to probiotic supplemented groups. The profit per kg live broiler was highest in control group as well. The profit of broiler raising differed significantly ($P<0.001$) among different dietary groups due to addition of probiotic. Considering the results of this study, it may be concluded that, little improvement in body weight gain is achievable in birds that received probiotic at 1.0g and 1.5g per kg feed. The effects of supplementing probiotic on meat yield are comparable except that it may be effective to reduce the abdominal fat of broilers. And supplementation of probiotic in broiler diets between 0.5 and 1.5g/kg feed increases feed cost suggesting that cost of probiotic need to be minimized or responses of birds would have to be maximized.


REFERENCES

REFERENCES

Abdul Rahim, S.M., Haddadian, M.S.Y., Odetallah, N.H.M. and Robinson, R.K. (1999). Effect of *Lactobacillus acidophilus* and zinc bacitracin as dietary as dietary additives for broiler chickens. *British Poultry Science*, 40: 91-94.

Alwan, D., Swierczewska, E. and Riedel, J. (1997). Effect of probiotic (cerbiogalli) or antibiotic on performance variation of three broilers strains. Annals of Warsaw Agricultural University. *Animal Science,* 33: 37-46.

Baidya, N., Mandal, L., Sarkar, S.K and Banergee, G.C. (1994). Combined feeding of antibiotic and probiotic on the performance of broilers. *Indian Journal of Poultry Science*, 29: 228-231.

Bandy, M.T. and. Risam, K. S. (2001). Growth performance and carcass characteristics of broiler chicken fed with probiotics. *Indian Journal of Poultry Science*, 36: 252-255.

Buche, A.U., Gaffar, M.A., Kalbande, V.H. and Deshmukh, S.V. (1992). Influence of probiotic and nitrofuran on the performance of broilers. *Indian Journal of Poultry Science*, 27: 160-162.

Cavazzoni, V., Adami, A. and Castrovilli, C. (1998). Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. *British Poultry Science*, 39: 526-529.

Chah, C.C., Carlson, C.W., Semeniuk, G.., Palmer, I.S. and Hesseltine, C.W. (1975). Growth promoting effects of fermented soyabeans for broilers. *Poultry Science*, 54: 600-609.

Chiang, S.H. and Hsieh, W.M. (1995). Effect of direct-fed microorganisms on broiler growth performance and litter ammonia level. *Asian-Australian Journal of Animal Science,* 8: 159-162.

Chowdhury, S.D. (2003). Development of least cost feed formulae to improve productivity of semi-scavenging poultry birds. Participatory Livestock

Development Project (PLDP) Final Report. Department of Poultry Science. Bangladesh Agricultural University, Mymensingh.

Dalloul, R.A., Lillehoj, H.S., Shellem, T.A. and Doerr, J.A. (2003). Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a Lactobacillus-based probiotic. *Poultry Science*, 82: 62-66.

Dunham, H.J., Williams, C.,Edens, F.W., Casas, I.A. and Dobrogosz, W.J. (1993) *Lacto bacillusreuteri* immunomodulation of stressor-associated diseases in newly hatched chickens and turkeys. Poultry Science, 72 (suppl.): 103.

Elwinger, K., Schneitz, C., Berndtston, E., Fossum, O., Teglof, B. and Engstom, B. (1992). Factors affecting the incidence of necrotic enteritis, caecal carriage of (*Clostridium perfringens* and bird performance in broiler chicks. *Acta Veterinaria Scandinavica*, 33: 369-378.

Ergun, A., Yalcin, S. and Sacakli, P. (2000). the usage of probiotic and zinc bacitracin in broiler rations. *Ankara-Universitesi- Veteriner-Fakkultesi-Dergisi*. 47:271-280.

Euribrid, B.V. (1994). Euribrid Technical Information for Hybro Broilers. Euribrid Poultry Breeding Farms, Boxmer, the Netherlands.

Fabris, G., Cristofori, C., Padoa, E. and Franchini, A. (1997). Auxinic antibiotics and probiotics in the feeding of broiler chickens. *Rivistad. Avicoltura*, 66: 69-72.

Fuller, R. (1978). epithelial attachment and other factors controlling the colonization of the intestine of the probiotic chicken by *lactobacilli*. *Journal of Applied Bacteriology*, 45: 389-395.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology*, 66: 365-378.

Gilliland, S.E. and Speck, M.L. (1977). Antagonistic action of *Lactobacillus acidophilus* toward intestinal and Foodborne pathogens in associative cultures. *Journal of Food Protection*, 40: 820-823.

Gohain, A. K. and Sapcota, D. (1998). Effect of probiotics feeding on the performance of broilers. *Indian Journal of Poultry Science*, 33: 101-106.

Green, A. A. and Sainsbury, D. W.B. (2001). the role of probiotic in producing quality poultry products. *XV European Sjmposium on the quality of poultry meat*. 9-12 September 2001 Kusadasi Turkey, 245-251.

Hamid, M.S. and Aijazuddin, Q. (2001). Trial study on the efficacy of protexin (water soluble) on the performance of broilers. *Pakistan Veterinary Journal,* 21: 224-225.

Havenaar, R., Brink, B.T., Huis Veld, J.H.H. and Fuller, R. (1992). Selection of strains for probiotics use. In *probiotics: The scientific Basis* (Ed. Fuller, R.), Chapman and Hall, London, 209-224.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1996b). Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chickens. *Letters in Applied Microbiology,* 23: 67-71.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1997). Probiotics in poultry: modes of action. *World's Poultry Science Journal*, 53: 351-368.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1998a). Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Animal Feed Science and Technology*, 70: 197-209.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1998b). Growth performance, intestinal microbial populations and serum cholesterol of broilers' diets containing *Lactobacillus* cultures. *Poultry Science*, 77: 1259-1265.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (2000). Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. *Poultry Science*, 79: 886-891.

Jin, L.Z., Ho, Y.W., Ali, M. A., Abdullah, N., Ong, B. K. and Jalaiudin, S. (1996c). Adhesion of *Lactobacillus* isolates on intestinal epithelial cells of chickens. *Letters in Applied Microbiology*, 22: 229-232.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. (1996a). Influence of dried *Bacillus subtilis* and Lactobacilli cultures on intestinal microflora and performance in broilers. *Asian-Australian Journal of Animal Science*, 9: 397-404.

Jones, R. (1982). A standard method dissection of poultry for carcass analysis. The West of Scotland Agricultural Collage Technical Note, No. 222.

Kalavathy, R., Abdullah, N., Jalaluddin. 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. *British Poultry Science*, 44:139-144.

Khan, M.L., Ullah, I. and Javed, M.T. (1992). Comparative study of probiotics TM 50 Biovin-40, and Albac on the performance of broiler chicks. *Pakistan Veterinary Journal*, 12: 145-147.

Kwon, O.S., Kim, I.H., Hong, J.W., Han, Y.K., Lee, S.H. and Lee, J.M. (2002). Effects of probiotics supplementation on growth performance, blood composition, and fecal noxious gas of broiler chickens. *Korean Journal of Poultry Science*, 29:1-6.

Ladukar, M.D., Mehta, M.K. and Rane, A.S. (2001) Effect of commercial probiotic preparations on performance of broilers. *Indian Journal of Animal Nutrition*, 18:357-362.

Lee, S.J., Kim, S.S., Suh, O.S., Na, J.C., Lee, S.H. and Chung, S.B. (1993). Effect of dietary antibiotics and probiotics on the performance of broiler. RDA -*Journal of Agriculture Science-Livestock*, 35: 539-548.

Lima, A.C.F., Harnich, F.A.R., Macari, M. and Pizauro Junior, J.M. (2002). Evaluation of the performance of the broiler chickens feed with enzymatic or probiotic supplementation. *Ars-Veterinaria*, 18: 153-157.

Lyons, T.P. (1987). Probiotics: an alternative to antibiotics. Pig News Info, 8: 157-164.

Mahajan, P., Sahoo, J. and Panda, P. C. (1999). Effects of probiotic feeding and seasons on the growth performance and carcass quality of broiler. *Indian Journal of Poultry Science*, 34: 167-176.

Mandal, S.K., Biswas, I.K. and Mandal, L. (1994). Efficiency of different growth promoters on the performance of broilers. *Indian Journal of Poultry Science*, 92:13-17.

Metchnikoff, E. (1907). *Prolongation of life*, G.P. Putnam and Sons, New York.

Miles, R.D. and Bootwalla, S.M. (1991). Direct-fed microbials in animal production. In *Direct-fed Microbials in Animal production*: a review, National Feed Ingredient Association, West Des Moines, Iowa, USA, 117-132.

Mitchell, I.G. and Kenworthy, R. (1976). Investigations on a metabolite from *Lactobacillus bulgaricus* which neutralizes the effect of enterotoxin from *Escherichia coli* pathogenic for pigs. *Journal of Applied Bacteriology*, 41: 163-174.

Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A. (1996). Effect of probiotic supplementation on growth, nitrogen utilization and serum cholesterol in broilers. *British Poultry Science*, 37: 395-401.

Moses, J.S. (1992). Combined use of probiotics and antibiotics in broilers. Poultry Guide, 29: 58-63.

Panda, A. K., Rama Rao S. V., Reddy, M. R. and Praharaj, N. K. (1999). Effect of dietary inclusion of probiotics on growth, carcass traits and immune response in broilers. *Indian Journal of Poultry Science*, 34: 343-346.

Perdigon, G., Alverez, S., De Macias, M.E.N., Roux, M.E. and De Ruiz Holgado, A.A.P. (1990). the oral administration of lactic acid bacteria increase the mucosal intestinal immunity in response to enteropathogens. *Journal of Food protection*, 53: 404-410.

Philips, S. M. and Fuller, R. (1983). the activity of amylase and a trypsin-like protease in the gut contents of germ-free and conventional chickens. *British Poultry Science*, 24: 115-121.

Piao, X. S., Han, I. K., Kim, J. H., Cho, W. T., Kim, Y. H. and Liang, C. (1998). Effects of K-enzyme, phytase and yeast supplementation on the growth performance and pollution reduction of broiler chicks. *Asian-Australian Journal of Animal Sciences*, 12: 36-41.

Price, R.J. and Lee, J.S. (1970). Inhibition of *Pseudomonas* species by hydrogen peroxide producing *Lactobacilli. Journal of Milk Food Technology*, 33: 13-18.

Priyankarage, N., Silva, S. S. P., Gunaratne, S. P., Kothalawala, I-L, Palliyaguru, M.W. C. D. and Gunawardana, G. A. (2003). Efficacy of probiotics and their effects on performance, carcass characteristics, intestinal microflora and Salmonella incidence in broilers. *British Poultry Science*, 44 (suppl.): 26-27.

Reid, G., McGroarty, J.A., Angotti, R. and and Cook, RL. (1988) *Lactobacillus* inhibitor production against *Escherichia coli* and co aggregation ability with uropathogens. *Canadian Journal of Microbiology*, 34: 344-351.

Rettger, L.F. and Chaplin, H.A. (1992). Treatise on the transformation of the intestinal flora with special reference to the implantation of *Bacillus acidophilus*. Yale University Press, Connecticut.

Rolfe, RD. (1991). Population dynamics of the intestinal tract. In *Colonization Control of Human Bacterial Entreropathogens in poultry* (Ed. Blankenship, L.C.), Academic Press Inc., San Diego, 59-75.

Samanta, M. and Biswas, P. (1995). Effect of feeding probiotic and lactic acid on the performance of broiler. *Indian Journal of Poultry Science*, 30: 145-147.

Santoso, U., Tanaka, K. and Ohtani, S. (1995). Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks, *British Journal of Nutrition*, 74: 523-529.

SAS (1998). Statistical Analysis System Institute, *SAS User's Guide* (Cary, NC, SAS Institute).

Shoeib, H.K. and Madian, A.H. (2002). A study on the effect of feeding diets containing probiotics (pronifer&biogen) on growth performance, intestinal flora and haematological picture of broiler chicks. *Assiut Veterinary-Medical Journal*, 47: 112-125.

Siddons, R. C. and Coates, M. (1972). the influence of the intestinal microflora on disaccharidase activities in the chick. *British Poultry Science*, 27: 101-112.

Siegel, H.S. (1995). Stress, strains, and resistance. *British Poultry Science*, 36: 3-21.

Singh, S. and Sharma, V. P. (1999). Performance of broiler chicks under different energy and probiotics levels during summer season. *Indian Journal of Poultry Science*, 34: 34-37.

Singh, S., Sharma, V.P. and Panwar, V.S. (1999). Influence of levels of probiotic and energy on mortality and economics of broilers in summer. *Indian Journal of Animal Sciences*, 69: 830-831.

Sissons, J.W. (1989). Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals: a review. *Journal of Food and Agriculture Science*, 49: 1-13.

Sorrels, K.M. and Speck, M.L. (1970). Inhibition of *Salmonella gallinarum* by culture filtrates of *Leuconostoccitrovorum*. *Journal of Dairy Science*, 59: 338-343.

Spencer, R.J. and Chesson, A. (1994). the effect of *Lactobacillus* spp. on the attachment of enterotoxigenic *Escherichia coli* to isolated porcine enterocytes. *Journal of Applied Bacteriology*, 77: 215-220.

Tortuero, F. (1973). Influence of implantation of *Lactobacillus acidophilus* in chicks onthe growth, feed conversion, malabsorption of fats syndrome and intestinal flora. *Poultry Science*, 52: 197-203.


APPENDICES

APPENDICES

Appendix 1. Body weight (g/broiler) of broilers fed on different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (week)					
		Day old	1	2	3	4	5
	R_1	45.74	122.22	281.48	569.23	965.00	1277.04
T_0(control)	R_2	44.44	122.59	292.59	579.26	948.52	1245.19
	R_3	45.41	122.22	291.85	539.26	932.59	1156.81
Mean±SE		**45.19±0.39**	**122.34±0.12**	**288.64±3.58**	**562.58±12.01**	**948.7±9.35**	**1226.34±35.9**
	R_1	45.63	126.67	293.08	561.60	923.20	1249.60
T_1(0.5)	R_2	44.33	125.93	290.74	547.69	904.62	1260.77
	R_3	44.37	117.78	280.74	545.30	898.52	1184.62
Mean±SE		**44.7±0.43**	**123.46±2.85**	**288.18±3.78**	**551.53±5.08**	**908.78±7.42**	**1231.66±23.7**
	R_1	43.74	120.11	282.96	579.20	963.20	1290.83
T_2 (1.0)	R_2	44.07	122.22	277.04	537.78	928.15	1300.74
	R_3	43.63	122.59	290.00	580.77	996.15	1294.62
Mean±SE		**43.81±0.13**	**121.64±0.77**	**283.33±3.74**	**565.91±14.07**	**962.50±19.6**	**1295.39±2.88**
	R_1	43.74	125.93	297.78	576.92	962.31	1319.23
T_3 (1.5)	R_2	43.93	121.48	294.82	591.11	966.67	1322.96
	R_3	45.07	121.93	306.40	620.80	982.40	1310.80
Mean±SE		**44.24±0.41**	**123.11±1.41**	**299.66±3.47**	**596.27±12.92**	**970.46±6.1**	**1317.66±3.59**

Appendix 2. Cumulative body weight gain (g/broiler) of broilers different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (day)				
		0-7	0-14	0-21	0-28	0-35
	R_1	76.48	235.74	523.49	919.26	1231.30
T_0(control)	R_2	78.15	248.15	534.82	904.08	1200.75
	R_3	76.81	246.44	493.85	887.18	1156.81
Mean±SE		**77.14±0.51**	**243.44±3.88**	**517.38±12.2**	**903.50±9.26**	**1196.28±21.6**
	R_1	81.04	247.45	515.97	877.57	1203.97
T_1(0.5)	R_2	81.60	246.41	503.36	860.29	1216.44
	R_3	73.41	236.37	501.56	854.15	1140.25
Mean±SE		**78.68±2.64**	243.41±3.5	**506.96±4.5**	**864.00±7.01**	1186.88±23.6
	R_1	76.37	239.22	535.46	919.46	1247.09
T_2 (1.0)	R_2	78.15	232.97	493.71	884.08	1256.67
	R_3	78.96	246.37	537.14	952.52	1250.99
Mean±SE		**77.82±0.76**	**239.52±5.2**	**522.10±14.2**	**918.68±19.7**	**1251.58±2.78**
	R_1	82.19	254.04	533.18	918.57	1275.49
T_3 (1.5)	R_2	77.55	250.89	547.18	922.74	1279.03
	R_3	76.86	261.33	575.73	937.33	1265.73
Mean±SE		**78.86±1.67**	**255.42±3.09**	**552.03±12.5**	**926.21±5.7**	**1273.41±3.9**

Appendix 3. Feed consumption (g/broiler) of broilers fed on different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (week)				
		1	2	3	4	5
	R_1	101.36	281.47	489.23	719.25	844.41
T_0(control)	R_2	103.88	278.32	510.37	682.22	814.80
	R_3	120.19	282.24	488.88	683.41	765.17
Mean±SE		**108.47±5.9**	**280.67±1.2**	**496.12±7.1**	**694.96±12.1**	**808.12±23.1**
	R_1	110.53	280.56	465.92	789.60	871.99
T_1(0.5)	R_2	104.58	271.67	475.51	742.28	803.88
	R_3	108.43	277.76	466.69	742.42	826.63
Mean±SE		**107.84±1.74**	**276.66±2.6**	**469.52±3.07**	**758.1±15.75**	**834.16±20.01**
	R_1	115.22	271.67	510.37	739.97	840.00
T_2 (1.0)	R_2	117.25	278.52	466.69	748.16	814.80
	R_3	121.38	297.99	542.29	769.23	821.17
Mean±SE		**117.95±1.8**	**282.72±2.6**	**506.45±21.9**	**752.45±8.7**	**825.32±7.5**
	R_1	109.41	296.10	546.21	673.82	869.26
T_3 (1.5)	R_2	118.30	280.56	490.77	701.82	868.56
	R_3	122.36	289.17	520.03	744.03	878.01
Mean±SE		**116.69±3.82**	**288.68±4.5**	**519.00±16.01**	**706.55±20.4**	**871.94±3.04**

Appendix 4. Cumulative feed consumption (g/broiler) of broilers fed 011 different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (day)				
		0-7	0-14	0-21	0-28	0-35
	R_1	101.36	382.83	872.06	1591.31	2435.72
T_0(control)	R_2	103.88	382.20	892.57	1574.79	2389.59
	R_3	120.19	402.43	891.31	1574.72	2339.89
Mean±SE		**108.47±5.9**	**389.15±6.64**	**885.31±6.63**	**1580.27±5.5**	**2388.4±27.6**
	R_1	110.53	391.09	857.01	1646.61	2518.60
T_1(0.5)	R_2	104.58	376.25	851.76	1594.04	2397.92
	R_3	108.43	386.19	852.88	1595.30	2421.93
Mean±SE		**107.84±1.74**	**384.51±4.36**	**853.88±1.59**	**1611.98±17.31**	**2446.15±36.88**
	R_1	115.22	386.89	897.26	1637.23	2477.23
T_2 (1.0)	R_2	117.25	395.77	862.46	1610.62	2425.42
	R_3	121.38	419.37	961.66	1730.89	2552.06
Mean±SE		**117.95±1.81**	**400.67±9.69**	**907.12±29.05**	**1659.58±36.47**	**2484.90±36.75**
	R_1	109.41	405.51	951.72	1625.54	2494.80
T_3 (1.5)	R_2	118.30	398.86	889.63	1591.45	2460.01
	R_3	122.36	411.53	931.56	1675.59	2553.60
Mean±SE		**116.69±3.82**	**405.30±3.65**	**924.30±18.28**	**1630.86±24.43**	**2502.80±27.31**

Appendix 5. Feed conversion ratio of broilers fed on different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (day)				
		1	2	3	4	5
	R_1	1.32	1.77	1.70	1.82	1.71
T_0(control)	R_2	1.33	1.64	1.78	1.85	1.75
	R_3	1.56	1.66	1.98	1.74	2.84
Mean±SE		**1.40±0.07**	**1.69±0.04**	**1.82±0.08**	**1.80±0.03**	**2.10±0.37**
	R_1	1.36	1.69	1.74	2.18	2.67
T_1(0.5)	R_2	1.28	1.65	1.85	2.08	2.26
	R_3	1.48	1.70	1.76	2.11	2.89
Mean±SE		**1.37±0.05**	**1.68±0.01**	**1.78±0.03**	**2.12±0.02**	**2.60±0.18**
	R_1	1.51	1.67	1.72	1.93	2.56
T_2 (1.0)	R_2	1.50	1.80	1.79	1.92	2.19
	R_3	1.54	1.78	1.87	1.85	2.75
Mean±SE		**1.51±0.01**	**1.75±0.04**	**1.79±0.04**	**1.90±0.02**	**2.50±0.16**
	R_1	1.33	1.72	1.96	1.75	2.44
T_3 (1.5)	R_2	1.53	1.62	1.66	1.87	2.44
	R_3	1.59	1.57	1.65	2.06	2.67
Mean±SE		**1.48±0.07**	**1.63±0.04**	**1.75±0.1**	**1.89±0.09**	**2.51±0.07**

Appendix 6. Cumulative feed conversion ratio of broilers fed on different dietary treatments at different ages

Treatment (probiotic g/kg)	Replication	Age (day)				
		0-7	0-14	0-21	0-28	0-35
	R_1	1.32	1.62	1.67	1.73	1.96
T_0(control)	R_2	1.33	1.54	1.67	1.74	1.99
	R_3	1.56	1.63	1.80	1.77	2.02
Mean± SE		**1.40±0.07**	**1.59±0.02**	**1.71±0.04**	**1.74±0.01**	**1.99±0.01**
	R_1	1.36	1.58	1.66	1.88	2.09
T_1(0.5)	R_2	1.28	1.53	1.69	1.85	1.97
	R_3	1.48	1.63	1.70	1.88	2.12
Mean± SE		1.37±0.05	1.58±0.02	1.68±0.01	1.87±0.01	2.06±0.04
	R_1	1.51	1.62	1.68	1.78	1.99
T_2 (1.0)	R_2	1.50	1.70	1.75	1.82	1.93
	R_3	1.54	1.70	1.79	1.82	2.04
Mean± SE		**1.51±0.01**	**1.67±0.02**	**1.74±0.03**	**1.80±0.01**	**1.98±0.03**
	R_1	1.33	1.60	1.78	1.77	1.96
T_3 (1.5)	R_2	1.53	1.59	1.63	1.72	1.92
	R_3	1.59	1.57	1.62	1.79	2.02
Mean± SE		**1.48±0.07**	**1.58±0.008**	**1.67±0.05**	**1.76±0.02**	**1.96±0.02**

Appendix 7. Meat yield parameters of broilers fed control diet

Parameters	R_1	R_2	R_3	Mean± SE
Live weight (g)	1280	1260	1220	1253.33±17.63
Feather weight (%)	5.86	5.73	5.78	5.79±0.03
Digestive tract wt. (%)	9.14	8.77	9.07	8.99±0.11
Heart weight (%)	0.55	0.52	0.53	0.53±0.008
Gizzard weight (%)	2.16	2.12	2.17	2.15±0.01
Liver weight (%)	2.55	2.47	2.46	2.49±0.02
Abdominal far wt. (%)	1.10	1.19	1.06	1.11±0.03
Head weight (%)	2.73	2.71	2.67	2.7±0.01
Shank weight (%)	4.60	4.55	4.59	4.58±0.01
Dressed weight (%)	68.34	67.51	67.98	67.94±0.24
Thigh weight (%)	11.53	11.45	11.49	11.49±0.02
Breast weight (%)	23.44	22.52	22.91	22.95±0.26
Wing weight (%)	7.55	7.46	7.48	7.49±0.02
Neck weight (%)	4.02	3.70	3.80	3.84±0.09
Drumstick weight (%)	9.57	9.83	9.54	9.64±0.09

Appendix 8. Meat yield parameters of broilers fed control diet supplemented with probiotic at 0.5g per kg feed

Parameters	R_1	R_2	R_3	Mean± SE
Live weight (g)	1260	1290	1200	1250±26.45
Feather weight (%)	5.94	5.34	5.60	5.62±0.17
Digestive tract weight	9.21	9.78	8.93	9.3±0.25
Heart weight (%)	0.52	0.55	0.51	0.52±0.01
Gizzard weight (%)	2.17	2.21	2.10	2.16±0.03
Liver weight (%)	2.19	2.50	2.00	2.23±0.14
Abdominal fat weight (%)	1.22	1.18	1.02	1.14±0.06
Head weight (%)	2.92	3.23	2.96	3.03±0.09
Shank weight (%)	4.13	4.41	4.18	4.24±0.08
Dressed weight (%)	67.97	67.37	67.88	67.74±0.18
Thigh weight (%)	11.32	11.47	11.30	11.36±0.05
Breast weight (%)	22.69	21.86	22.97	22.50±0.33
Wing weight (%)	7.98	6.76	6.98	7.24±0.37
Neck weight (%)	3.78	3.75	3.81	3.78±0.01
Drumstick weight	9.12	8.97	9.09	9.06±0.04

Appendix 9. Meat yield parameters of broilers fed control diet supplemented with probiotic at 1.0g per kg feed

Parameters	R_1	R_2	R_3	Mean± SE
Live weight (g)	1300	1320	1310	1310±5.77
Feather weight (%)	3.13	6.11	5.91	5.05±0.96
Digestive tract weight (%)	9.27	9.33	9.29	9.29±0.01
Heart weight (%)	0.55	0.59	0.60	0.58±0.01
Gizzard weight (%)	2.29	2.13	2.04	2.15±0.07
Liver weight (%)	3.18	3.21	2.78	3.05±0.13
Abdominal fatweight (%)	1.03	0.95	1.11	1.03±0.04
Head weight (%)	3.19	3.01	2.75	2.98±0.12
Shank weight (%)	5.11	4.97	4.77	4.95±0.09
Dressed weight (%)	67.62	67.68	68.05	67.78±0.13
Thigh weight (%)	11.76	12.09	11.72	11.85±0.11
Breast weight (%)	23.20	22.77	23.26	23.07±0.15
Wing weight (%)	7.60	7.03	7.34	7.32±0.16
Neck weight (%)	4.27	3.16	3.23	3.55±0.35
Drumstick weight (%)	9.58	8.86	9.23	9.22±0.20

Appendix 10. Meat yield parameters of broilers fed control diet supplemented with probiotic at 1.5g per kg feed

Parameters	R_1	R_2	R_3	Mean± SE
Live weight (g)	1360	1340	1330	1343.33±8.81
Feather weight (%)	6.14	5.75	6.10	5.99±0.12
Digestive tract weight (%)	8.43	8.11	9.23	8.59±0.33
Heart weight (%)	0.58	0.47	0.57	0.54±0.03
Gizzard weight (%)	2.36	2.03	2.07	2.15±0.1
Liver weight (%)	2.71	2.22	2.65	2.52±0.15
Abdominal far weight (%)	1.19	0.81	1.00	1.00±0.1
Head weight (%)	3.09	2.83	3.01	2.97±0.07
Shank weight (%)	4.80	4.15	4.27	4.40±0.19
Dressed weight (%)	68.14	67.68	67.63	67.81±0.16
Thigh weight (%)	11.47	11.62	11.73	11.60±0.07
Breast weight (%)	22.02	23.61	22.18	22.60±0.50
Wing weight (%)	7.23	7.89	8.27	7.79±0.30
Neck weight (%)	3.74	4.18	4.50	4.14±0.22
Drumstick weight (%)	9.89	9.16	9.23	9.42±0.23