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**Response of Broiler Chicks to Commercial Dietary Microbial
Probiotic (Dexflor-PR) As Natural Growth Promoter**

إستجابة الدجاج اللحم لمنتج البروباويوتك المايكروبي التجاري (دكسفلور بي آر)

كمحفز طبيعي للنمو

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(وَلَحْمِ طَيْرٍ مِّمَّا يَشْتَهُونَ)

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Dedication

To the soul of my Father,

To my dear Mother,

To my Brothers and Sisters,

To my beloved Husband,

To my Sons and Daughter,

To all of my friends.

IGBAL

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Abstract

This experiment was conducted to evaluate the response of broiler chicks to diet containing various levels of dietary bacterial probiotic (BP) as natural growth promoter alternative to antibiotic. Experiment parameters covered growth performance, slaughter, carcass values, commercial cuts and giblets percentage, subjective meat quality, carcass dressing percentage and economical appraisal were calculated. The experimental design used was complete randomize design (CRD). A total of 200, five days old 125 gm. initial weight, un sexed Arbor Acres strain broiler chicks were used in this experiment. The chicks were divided into five experimental groups with five replicates, each of eight chicks (5x5x8). The first group (A) fed on basal diet without feed additives as negative control diet (NC), the second group (B), fed on basal diet with antibiotic (Neomycin 20 mg /kg) as positive control (PC), the other groups C, D, and E were fed on basal diet supplemented with bacterial probiotic (BP) at levels 1, 2, and 3 gm/kg respectively. The basal diet was formulated to meet the nutrients requirements of broilers according to (NRC, 1994). Experimental diets were fed for five weeks.

The results showed that, the addition of dietary (BP) at all inclusion levels improved significantly ($P < 0.05$) the value of body weight gain (BWG) and feed conversion ratio (FCR) compared to (NC) without any effect on feed intake of broiler chicks. No mortalities were recorded throughout the experimental period. The results indicated that, the carcass dressing percentage were increased significantly ($p < 0.05$) in birds fed on 2 and 3 gm/kg dietary (BP) compared to those fed on (NC) diet .Whereas, the differences were not significant ($p > 0.05$) among the other treatment groups. No significant differences ($p > 0.05$) were observed among all treatment groups in the percent of giblets (gizzard, liver, and heart), and the subjective meat quality values (color, juiciness, tenderness, and flavor) of broiler chicks. The results showed that, all levels of (BP) added to the broiler diets were

improved significantly ($p < 0.05$) commercial cuts (breast, thigh and drumstick) and their percentage of separable tissue compared to (NC).

The results of economical evaluation of experimental diets, showed that the addition of (BP) at various levels to the broiler diets caused more net profit compared to (NC), but the value of profitability ratio (1.23) of group E (3 gm/kg dietary BP) was the highest of the tested groups .

According to the results of this study, dietary (BP) appeared to be superior compared to antibiotic. It thus shows that dietary (BP) can be used as replacement for antibiotic in broiler diets.

الملخص

أُجريت هذه التجربة لتقييم مدى إستجابة كتاكيت الدجاج اللاحم للعلائق المحتوية على مستويات مختلفة من البروبايتوك (المعزز الحيوي) الباكثيري كمحفز طبيعي للنمو بديلا للمضادات الحيوية . شملت قياسات التجربة الأداء الإنتاجي ، قيم الذبح والذبيحة ، نسب الأعضاء الداخلية والقطع التجارية ونسب اللحم ، الصفات الإنطباعية النوعية للحم كذلك حساب نسبة التصافي للذبيحة والتقييم الإقتصادي بنهاية التجربة . صممت هذه التجربة بإستخدام النظام العشوائي الكامل . تم إستخدام ٢٠٠ كتكوت عمر ٥ أيام بوزن ابتدائي ١٢٥ جم غير مجنسة من سلالة Arbor Acres ، تم تقسيمها عشوائيا الي ٥ مجموعات تجريبية ، إحتوت كل مجموعة علي ٥ مكررات بكل مكرر ٨ كتاكيت ، تمت تغذية المجموعة الأولى (A) علي عليقة أساسية بدون أي إضافة علفية (عليقة قياسية سالبة) ، المجموعة الثانية (B) تمت تغذيتها علي العليقة القياسية مع المضاد الحيوي (النيومايسين ٢٠ ملجرام لكل كيلوجرام) كعليقة قياسية موجبة ، اما المجموعات C،D،E فقد تمت تغذيتها علي العليقة الأساسية مضاف إليها المعزز الحيوي البكتيري بالمستويات التالية : ١ ، ٢ و ٣ جرام لكل كيلوجرام علي التوالي . تم تكوين العليقة القياسية لتقابل الإحتياجات الغذائية للدجاج اللاحم الصادر من (1994, NRC)، تمت التغذية علي العلائق التجريبية لمدة ٥ أسابيع .

اوضحت النتائج المتحصل عليها أن إضافة البروبايتوك (المعزز الحيوي البكتيري) بالمستويات المختلفة الي العلائق أدت الي تحسين معنوي ($P<0.05$) في قيم وزن الجسم المكتسب ومعدل التحويل الغذائي مقارنة بالعليقة القياسية السالبة بينما لم يكن له أي تأثير معنوي ($P>0.05$) في معدل إستهلاك العليقة للدجاج اللاحم .

لم تسجل أي حالات للنفوق خلال فترة التجربة .

دلت النتائج بأن نسبة التصافي قد زادت معنويا ($P<0.05$) وذلك بأضافة المعزز الحيوي البكتيري الي العليقة القياسية بمستوي ٢ و ٣ جرام لكل كيلوجرام مقارنة بالعليقة القياسية السالبة بينما كانت الفروق غير معنوية ($P>0.05$) بين المجموعات التجريبية الأخرى .

أوضحت النتائج ان إضافة المعزز الحيوي البكتيري بالمستويات المختلفة الي العلائق أدت الي تحسين معنوي ($P<0.05$) في نسب القطع التجارية (الصدر ، الفخذ والساق) ونسبة اللحم مقارنة بالعليقة القياسية السالبة .

لم تلاحظ أي فروقات معنوية بين المجموعات التجريبية المختلفة في نسب الاعضاء الداخلية (القانصة ، الكبد والقلب) وقياسات اللحم الإنطباعية النوعية (اللون ، الرائحة ، العصيرية والطراوة) للدجاج اللّاحم.

أظهر التقييم الإقتصادي للعلائق التجريبية بأن إضافة المعزز الحيوي البكتيري بجميع المستويات الي علائق الدجاج اللّاحم قد أحدث ربحية صافية أعلي مقارنة بالعليقة القياسية السالبة ولكن القيمة الربحية النسبية (١,٢٣) في المجموعة E (٣ جرام لكل كيلوجرام معزز حيوي بكتيري) كانت الأعلى بين المجموعات المختبرة .

إستناداً لنتائج هذه التجربة إتضح أن المعزز الحيوي البكتيري كان أفضل مقارنة بالمضاد الحيوي .
وعليه فإنه يمكن إستخدام المعزز الحيوي البكتيري كبديل للمضادات الحيوية في علائق الدجاج اللّاحم.

CHAPTER ONE

INTRODUCTION

Poultry industry is under increasing pressure to produce high quantity and quality products for consumers. Antibacterial feed additives as antibiotics have been used worldwide for years as growth promoters to control and prevent pathogenic bacteria in the gut mucosa so as to improve meat and egg production. However, the sub-therapeutic use of antibiotics in poultry production has become undesirable because of residuals in meat products (**Burgat, 1999**) and development of antibiotic resistant bacteria population in humans (**Sahin *et al.*, 2002**). Since January 2006 the use of antibiotic as growth promoter was prohibited by the European Union (**Eckert, *et al.*, 2010**). Currently, many parts of the world are experimenting alternative feed additives that be used to elevate the problems associated with the withdrawal of antibiotics from feed. In this view, the use of probiotic products as substitute for antibiotic in poultry production has become an area of great interests. A probiotic, which means (for life) in Greek (**Gibson and Fuller, 2000**), has been defined as alive microbial feed supplement which beneficially affects the host animal by improving its intestinal balance, (**Fuller, 1989; Dahiya *et al.*, 2006; Callaway *et al.*, 2008**). Probiotics have shown promise as an alternative to in-feed antibiotics in reducing enteric diseases and eliminating subsequent contamination of poultry products (**Lee *et al.*, 2010**). Unlike antibiotics, the probiotic are living organisms and their mode of action relies on replication and survival in the gastro intestinal tracts.(**Fuller, 1989; Guillot, 1998**). The most important advantage of probiotic is that it doesn't have any residues in animal products (**Abe *et al.*, 1995 and Rowghani *et al.*, 2007**).

The common probiotic used as feed supplements are the live bacteria and yeast (**Patterson and Burkholder, 2003**). Bacteria frequently used as

probiotic in chicken's diets include species of *Bacillus*, *Enterococcus*, *Escherchia*, *Lactobacillus*, *Lactococcus*, and *Streptococcus*. More recently there has been an interest in the use of live yeast cultures as probiotics. Such yeast cultures are usually dried from *Sacharomyces* species, in particular *Saccharomyces cerevisiae*, (**Huang *et al.*, 2004; Kabir *et al.*, 2004; Karaoglu *et al.*, 2004; Ahmad, 2006; Mountzouris *et al.*, 2007**). It is advisable to notice that among the bacterial species used as probiotic, the *Bacillus* and the *Lactobacillus* differ in many characteristics. Moreover, *Lactobacillus* and the *Entrococcus* are bacterial families present in great quantities 10^8 and $10^5/10^6$ per gram respectively, in the digestive microflora of animals. On the other hand, the *Bacillus* and the yeast (*Saccharomyces servisiae*) are not usual component of the gut microflora (**Ducluzaeue and Raibaud, 1979; Gillot and Ruckebusch, 1994**). They are two main mechanisms that have been proposed to explain how probiotic products work; (1) Nutritional effect: which include: Reduction of metabolic reaction that produce toxic substances; stimulate indigenous enzymes (better digestability of nutrients); production of vitamins and antimicrobial substances. (2) Health effects which include: competition with pathogens for gut surface adhesion; increase resistance to colonization by competitive exclusion; stimulate formation of epithelial cells, decrease inflammation of intestinal mucosa; stimulation of immune response (reinforcing host defense), (**Fuller, 1989; Nahanshon, *et al.*, 1992; 1993; Jin *et al.*, 1997; Anadon, 2006; Ng *et al.*, 2009; Awad and Ghareeb, 2010**). However, an ambiguous application of probiotics in broiler nutrition is still far from being possible. This may be due to probiotic efficiency may depend on multi-factors such as microbial species composition e.g, single or multistrain and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene and environmental stress factors (**Mountzouris *et al.*, 2010**).

Therefore, this work has the objective to assess the effect of graded levels of dietary bacterial probiotic (*Bacillus cereus* var. *Toyoi*) commercial products (Dexflor-PR) as natural growth promoter alternative to antibiotics on the performance and carcass characteristics of broiler chicks.

CHAPTER TWO

LITERATURE REVIEW

2.1 Feed additives:

Feed for broilers and laying hens is formulated to contain an optimum nutrient concentration obtainable at reasonable cost for desirable growth, production and efficiency of feed utilization. The diet of poultry contains a wide variety of additives, these additives are primarily intended to improve the efficiency of the bird's growth and/or laying capacity, prevent disease and improve feed utilization, they are generally used to improve feed intake and to increase the growth rate in broilers (Scott *et al.*, 1982; Fadlalla *et al.*, 2010; and Abouelfetouh *et al.*, 2012). In some instances additives are added to the animal's diet in order to enhance their value for human consumption and digestive enzymes production and activities improvement (Lee *et al.*, 2004). The feed additives are falling into two groups: The first group comprises those additives that have a specific nutritional role, and includes fifteen or more growth promoting substances alone. The second group covers those compounds concerned with the prevention and control of disease, and here the number used has so far topped sixty. Antibiotics may be included in both groups (Ray and Fox, 1979). The most common types of feed additives used are:

(1)Antibiotics and arsenicals, which have been used at low levels to help protect feeds from microbial destruction and to prevent production of toxic products by the intestinal microflora; (2)Anticoccidials, which are routinely used in broiler feeds and also (usually at lower levels) in diets for rearing replacement pullets; (3)Antifungal, have been used to prevent growth of harmful molds and fungi in feeds or in the digestive tract of the chicken; (4)Worming drugs, which are periodically added to feed for protection against internal parasites; (5) Antioxidant, are used to protect poly-unsaturated fatty

acids and that fat soluble vitamins from destruction by peroxidation; (6) Probiotics, which can be used to influence the intestinal microflora; (7) Enzymes, which under certain condition, to improve the digestibility of specific nutrients; (8) Pellet binders, which effect texture and firmness of pelleted feeds; (9) Flavoring agents, have been used in an effort to improve the palatability of feed; (10) Carotenoid, which are added to many feeds to improve pigmentation of broiler or egg yolk (**Parks *et al.*, 2000; Allam, 2000 and Sreenivasaiah, 2006**).

2.2 Growth promoters:

Growth promoters are molecules that are added at low rate to animal feeds without changing considerably their composition. And require very careful weighing, handling and mixing. They speed up the growth and consequently increase the body size and weight of animals (**Biovet, 2005**). Most of broilers industry practioners have been given a growth promoter as additive in ration (**Menten, 2001**). Their mechanism of action varies. Positive effect in ration can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality, regulating the intestinal micro-flora, etc. **Peric *et al.*, (2009)**.

2.2.1 Antibiotics:

Antibiotics represent a group of chemicals compounds produced biologically by certain plants or microorganism, usually a fungus and bacteria. Antibiotic is a drug that kills or slows the growth of bacteria. Drugs that kill bacteria are referred to as bacteriocidal, and those that slow the growth of bacteria are referred to as bacterio-static, and at the effective levels, are not toxic to chickens or other host animals (**Parks *et al.*, 2000**). There are many different kinds of antibiotic, and they destroy bacteria in different ways. The antibiotics with in a class generally have similar effectiveness and mechanisms of action and resistance and they tend to attack the same types of bacteria. Some

antibiotics, referred to as broad- spectrum antibiotics, treat a wide range of infections both gram positive and gram negative bacteria. Other, called narrow-spectrum antibiotics, are effective against only a few types of bacteria, gram positive or gram negative bacteria. Although antibiotics are some- times used in conventional animal feeds, some of the antibiotics can be used only under the supervision of veterinarian (**Moore *et al.*, 1946**). During the last decade, antibiotic resistance by various mechanisms had been increased worldwide in human and animal infectious diseases (**Earss, 2005; Harbarth and Samore, 2005; and WHO 2007**).

2.2.1.1 Using antibiotics in animals:

Antibiotics have long been used to treat illnesses in humans and farm animals. The use of antibiotics as growth promoters in poultry diets was started around 65 years ago, when the first indication of beneficial effects on production efficiency in poultry was reported by (**Moore *et al.*, 1946**). By 1949, antibiotics had been approved for growth promotion in subtherapeutic levels, 5-10 ppm/ton in experimental, and many different groups of antibacterial have subsequently been approved form on –farm use as growth promoter in many European countries and United States of America (**Inbarr, 2000; Leasons and Summers, 2001; Nasir and Grashorn, 2006**). Dietary antibiotics are reported to have beneficial effects on animal and poultry growth, feed conversion efficiency and inhibition of pathogen growth (**Gaskins *et al*, 2002**). The antibiotics as growth promoter may produce one or more of the following effects: (1)They may favor the growth nutrients-synthesizing microbes or inhibit that of nutrient destroying microorganism; (2) Antibiotic may inhibit the growth of organisms that produced excessive amount of ammonia and other toxic nitrogenous waste products in the intestines; (3)They may improve availability or absorption of certain nutrient (**Roozbeh *et al.*, 2012**); (4) They may improve feed or water consumption or both; (5)Antibiotic may instances prevent or cure actual pathological disease

which occur either in the intestinal tract or systemically; (6) They may reduce the maintenance cost associated with turnover of the intestinal epithelium (**Kahn *et al.*, 2005; Miles *et al.*, 2006; Sreenivasaiah, 2006**).

2.2.1.2 Ban of antibiotics:

The use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria, (**Yoshimura *et al.*, 2000**). Many scientific findings suggested that antibacterials used for animal feeding as growth promoters become risky for human and animal health (**Manning *et al.*, 1994; Sahin *et al.*, 2002; Thorns, 2000**). However, the Swan committee report (1969) was the first to suggest that the use of subtherapeutic levels of antibiotics for growth promotion and disease prevention could increase the risk of bacteria acquiring resistance to specific antibiotics (**Nasir and Grashorn, 2006**). Susceptible bacteria at the time of contact with the antibiotic are suppressed in growth or destroyed, while the resistant bacteria present in the gut flora can multiply to a higher or lower degree. Suppression of antibiotics, sensitive bacteria created an opportunity for colonization by resistant bacteria derived from external sources. Frequent use of antibiotics not only conducive to the formation, but also fortification of resistance in bacteria (**Dankowialowska and Marek, 2013**).

As early as the 1950s, concern was being expressed that continued use of antibiotics to promote growth of poultry and other food animals might result in antimicrobial resistance of pathogenic bacteria in humans. **Starr and Reynolds's (1951)** reported on the resistant bacteria in turkeys after they had been fed streptomycin, may have been the first report of resistant bacteria in food animals fed an antibiotic. The bacteria had not caused disease in the turkeys, but the authors mentioned its possibility and also the possibility of spread of resistant *Salmonella* from poultry to humans. Resistant bacteria in poultry have been characterised and both horizontal transmission and vertical

transmission of some of them, especially *Escherichia coli*, from breeder flocks to poultry houses documented (**Dierikx *et al.*, 2013; Kemmett *et al.*, 2013**). These transferred, resistant strains can cause infection in young broiler chicks (**Kemmett *et al.*, 2014**). Colibacillosis in young chicks also is caused by antibiotic-susceptible strains, so the frequency of infections with resistant strain is not known. The report of (**Huijdens *et al.*, 2006**) involved *Staphylococcus aureus*, and the others involved *Salmonella*. A currently ongoing outbreak of multidrug-resistant *Salmonella* Heidelberg infections has been linked to poultry meat from Foster farms in California (**CDCP, 2013**). **Silbergeld *et al.*, (2008)** have summarised the extensive literature calling for prohibition of the use of antibiotic growth promoters (AGP) by the food animal industry. The scientific rationale for the claim that it is a major source of antimicrobial –resistant bacteria in human infections was detailed. They presented the various ways genetic resistance to antibiotics can be transmitted among bacteria, emphasised the presence of reservoirs of resistant bacteria in the vicinity of facilities where animals are fed antibiotics, and pointed out that people living in the same vicinity carry a large number of resistant bacteria, but the presence of infectious disease caused by these bacteria was limited. The authors acknowledged that while an abundance of data implies that the use of antibiotics in animals contributes to antimicrobial-resistant infections in humans, it might not be possible to determine an accurate risk for agricultural antibiotics in the incidence of resistant human infections. The united kingdom banned the use of penicillin and tetracycline for growth promotion in the 1970s. Sweden and Denmark banned all growth promotion antibiotic in 1986 and 1999 respectively (**FMI, 2006**). Also world health organization (WHO) has recommended (1997) that antibiotic should be phased and replaced by alternatives, (**Bywater, 2005**). In 1999, European Union banned four antibiotic growth promoters Virginamycin, Spiramycin, Tylosin, and zinc bacitracin which are commonly used in feed around the world. The United States banned the use of entrofloxacin in 2005, (**Colligon,**

1999). Since 1st January 2006 the use of antibiotic growth promoters is prohibited in the European Union (**Buchanan *et al.*, 2008**).

After the use of most antibiotics growth promoters as feed additives has been banned by EU, scientists searched for alternatives to antibiotics, in this view, variety of substances are used in conjunction with or as alternatives to antibiotics in poultry diets. Herbs and spices, essential oils extracted from aromatic plants, enzymes, hormones, organic acid, probiotics, prebiotic, all shown promising results for use in organic poultry production (**Grigge and Jacob, 2005**). Several alternatives to growth –promoting antimicrobials have been investigated in recent years (**Huyghebaert *et al.*, 2011**). In modern poultry production, different types of growth promoters were used: 1) probiotic: defined as a live microbial feed supplement which beneficially affects the host animal (**Fuller, 1989**). 2) prebiotic: defined as a non-digestible food ingredients that induce the growth or activity of beneficial microorganism (**Gibson and Fuller, 2000**). 3) synbiotic: defined as a combination of probiotics and prebiotics (**Gibson and Fuller, 2000**). 4) phytogetic: defined as a group of natural growth promoters derived from herbs, spices or other plants (**Dhama *et al.*, 2014**). Those strategies have focused on preventing the proliferation of pathogenic bacteria and modulating beneficial gut microflora so that the health, immune status and performance are improved (**Adil and Magray, 2012**). This property is the basis for the mechanism of ‘competitive exclusion.’ (CE) (**Elijah and Ruth, 2012**).

2.2.2 Probiotics:

2.2.2.1 Definition of probiotics:

The term probiotic, means “for life” in Greek (**Gibson and Fuller, 2000**), has been defined as “ alive microbial feed supplement which beneficially affects the host animal by improving its intestinal balance.” (**Fuller, 1989; Dahiya *et al.*, 2006; Callaway *et al.*, 2008**).

Crawford, (1979), defined probiotics as “aculture of specific living micro-organisms (primarily *Lactobacillus* spp.) implants in the animal to ensure the effective establishment of intestinal populations of both beneficial and pathogenic organisms. The US National Food Ingredient Association presented, probiotic (direct feed microbial) as source of live naturally occurring microorganisms and this includes bacteria, fungi, and yeast (**Miles and Bootwalla, 1991**). According to the currently adopted definition by **FAO/WHO (2001)**, probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” More precisely, probiotics are live microorganisms of non pathogenic and non toxic in nature, which when administered through the digestive route, are favorable to the host’s health (**Guillot, 1998**). **Havenaar and Huisin’t (1992)**, modified the definition for probiotics offered by (**Fuller, 1992**), and that definition is as follows: “a mono-or defined mixed-culture of live microorganisms which, applied to animal or man, beneficially affect the host by improving the properties of the indigenous gastrointestinal microbiota, but restricted to products that: a) contain live microorganisms e.g., as freeze drier cells or in fresh or fermented product. b) Improve the health and well being of animals or man including growth promoting of animals. and c) Can have their effect on all host mucosal surfaces, including the mouth and gastrointestinal tract e.g., applied in food, pill, or capsule form and the upper respiratory tract e.g., applied as an aerosol, or in the urogenital tract local application.” Probiotics are a live micro-organisms that claim to be beneficial to humans and animals and maintain a balance of microflora in the digestive tract (**Goldin, 1998**).

The definition is very broad provides a basis for the use of numerous bacteria and yeast for the enhancement of health and well being in host animals. However, there might be some misunderstanding of the definition because there are other terms that describe similar concepts and these include direct-fed microbials competitive exclusion agents, and synbiosis. Probiotics, have

shown promise as an alternative to in-feed antibiotics in reducing enteric disease and eliminating subsequent contamination of poultry products (**Lee *et al.*, 2010**). Unlike antibiotics, the probiotics are living organisms and their mode of action relies on replication and survival in the gastro intestinal tracts (**Fuller, 1989 and Guillot, 1998**). It has been reported recently that utilization of probiotics in animal nutrition is of economic and health benefits (**Azza *et al.*, 2012**).

Probiotics can be classified into two major types namely viable microbial cultures and microbial fermentation products (**Jerigan and Miles, 1985**). Probiotics efficiency may depend on factors such as: microbial species composition e.g, single or multistrain, viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene and environmental stress factors (**Mountzouris *et al.*, 2010**). The most important advantage of probiotic is that doesn't have any residues in animal production (**Abe *et al.*, 1995; Rowghani *et al.*, 2007**).

The common probiotics used as feed supplements are the live bacteria and yeast (**Patterson and Burkholder, 2003**). Bacteria frequently used as probiotic in chicken's diets include species of Bacillus, Enterococcus, Escherchia, Lactococcus, and Streptococcus, Bifidobacterium, Lactobacillus. Several fungal genera, which include Asperigillus, Oryzae, Saccaromyces cerevisiae and Saccaromyces cidophilum, have also been reported as probiotics (**Huang *et al.*, 2004; Tortuero, 1973 and Pelicano *et al.*, 2003**). More recently there has been an interest in the use of live yeast cultures as probiotics. Such yeast cultures are usually dried from Sccharomyces species, in particular, Sccharomyces cerevisiae , (**Huang *et al.*, 2004; Kabir *et al.*, 2004; Karaoglu *et al.*, 2004; Ahmad, 2006; Mountzouris *et al.*, 2007**). It is advisable to notice that among the bacterial species as probiotic, the Bacillus and the Lactobacillus differ in many characteristics. Moreover, Lactobacillus and the Enterococcus are bacterial families present in great quantities 10^8 and $10^5/10^6$ per gram respectively, in the digestive microflora of animals. On the

other hand, the Bacillus and the yeast (*Sccharomyces cerevisiae*) are not usual component of the gut microflora (**Ducluzeau and Raibaud, 1979; Gillot and Ruckebusch, 1994**).

2.2.2.2 Characteristics of effective probiotics:

Just as not all strains of bacteria are the same, not all probiotics are the same. The effectiveness of a probiotic supplement depends upon what it contains. A good probiotic should have the following characteristics:

*The culture should be acid and bile resistant and should contain a minimum of 30, 10⁹ CFU (**Patterson and Burkholder, 2003; Choudhari et al., 2008**).

* It should be strain specific. The culture should possess survival ability and multiply fast in the conditions within the poultry gut (**Choudhari et al., 2008**).

* The culture should not have any side effects. It should be neither pathogenic nor toxic to the host (**Patterson and Burkholder, 2003; Choudhari et al., 2008**).

* The culture should have strong adhesive capability with the digestive tract of the poultry (**Patterson and Burkholder, 2003**).

* Be durable enough to with stand the duress of commercial manufacturing, processing and distribution (**Patterson and Burkholder, 2003**).

* The culture should have the ability to reduce pathogenic microorganisms (**Patterson and Burkholder, 2003**).

* It should be able to modulate immune response (**Patterson and Burkholder, 2003**).

2.2.2.3 Beneficial effects of probiotics:

Agrowing body of scientific research supports the role of probiotics as effective alternative to use of antibiotic growth promoters in animal nutrition (**Ghadban, 2002; Patterson and Burkholders, 2003**). More recently, beneficial effects of probiotics on : i) Broiler performance (**Kabir et al.,**

2004; Mountzouris *et al.*, 2007; Apata, 2008; Awad and Ghareeb, 2010), ii) Nutrient digestibility, iii) Modulation of intestinal microflora (Mountzouris *et al.*, 2007), iv) Pathogens inhibition (Dalloul *et al.*, 2005; Higgins *et al.*, 2007; Mountzouris *et al.*, 2007). v) Immune modulation and gut mucosal immunity (Kabir *et al.*, 2004; Chichlowski *et al.*, 2007), also meat quality and sensory characteristics have been reported (Kabir *et al.*, 2005).

2.2.2.4 Mode of action of probiotics:

The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism (Nurmi and Rantala, 1973; Jin *et al.*, 1998; Line *et al.*, 1998; Kabir *et al.*, 2005; Rantala and Nurmi, 1973 and Fuller, 1989). (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacteria enzyme activity and ammonia production (Cole *et al.*, 1987 and Yoon *et al.*, 2004). (iii) improving feed intake and digestion (Dierck, 1989 and Awad *et al.*, 2006). (iv) stimulating the immune system (Kabir *et al.*, 2004; Nayebpor *et al.*, 2007; Apata, 2008; Haghghi *et al.*, 2005; Mathivanan and Kalaiarasi, 2007; McCracken and Gaskins, 1999; and Brisbin *et al.*, 2008).

The beneficial effects of probiotics are mediated by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria. The most common manner of inhibition is by lowering the pH of the gut during in vitro studies it was found that primary metabolites, such as organic acids and hydrogen peroxide, are involved in the suppression of bacteria cultures (Fuller, 1989). Later volatile fatty acids (VFAs) were found to be equally effective in the suppression of pathogenic gut flora (Chichlowski *et al.*, 2007). Probiotics produce VFAs and organic acids as a part of their natural breakdown and metabolism of nutrients in the gut digesta. These organic acids lower the pH below that essential for the survival of pathogenic bacteria such as *E. coli* and *Salmonella* spp. It is now well

established that the observed beneficial effects of probiotics is accomplished via lowering the pH through the production of VFAs which inhibit the growth of harmful bacteria (**Fuller, 1989; Pascual *et al.*, 1999; Yoruk *et al.*, 2004; O’Dea *et al.*, 2006; Chichlowski *et al.*, 2007; Choudhari *et al.*, 2008**). Another mechanism is through the competition for adhesion sites on the intestinal epithelium, thus preventing colonies of pathogenic bacteria forming (**Guillot, 2003; O’Dea *et al.*, 2006; Revolledo *et al.*, 2006; Chichlowski *et al.*, 2007; Choudhari *et al.*, 2008**). This ‘competitive exclusion’ of harmful bacteria is achieved through colonisation of favourable sites of adhesion such as the intestinal villus and colonic crypts, or excretion of the mucins (MUC2 and MUC3) from goblet cells which inhibits the adherence of entropathogenic bacteria (**Chichlowski *et al.*, 2007**). Competitive exclusion via probiotics depends upon the ability of the strain to adhere to the gut surface which is a host specific phenomenon and varies from strain within the same species (**Fuller, 1989**). Lactic acid bacteria are well known to colonise the caecal wall in the chicken and their competitive exclusion effect has been explained (**Starvic, 1987; Fuller, 1989; Yoruk *et al.*, 2004**). This stresses the point that a strain adhering well to the gut should be chosen while selecting a probiotic. Another important mechanism involved in producing beneficial impacts on the host’s body is the stimulation of the immune system. An accumulated body of evidence has shown that the protective effect of probiotics is associated with elevated humoral and cellular immune responses, which is achieved through increased production of T-lymphocytes, CD⁺ cells and antibody secreting cells, expression of pro- and anti- inflammatory cytokines, interleukins, IFN- gamma, natural killer cells, antibody production, respiratory burst in macrophages and delayed type hypersensitivity reactions (**Panda *et al.*, 2003; Oyetayo and Oyetayo, 2005; Chichlowski *et al.*, 2007; Musa *et al.*, 2009**). Another mode of action of probiotics is lowering the activities of the intestinal and faecal B-glucosidase and B-glucuronidase bacteria enzymes. These enzymes are involved in the formation of toxins in

the body. The lactobacillus culture may reduce B-glucosidase and B-glucuronidase activities by attaching themselves along the chicken intestine, thus preventing colonisation of the bacteria with toxicant-promoting enzymes (**Jin *et al.*, 2000**). Additionally, lysozyme produced by Bifidobacteria, has been reported to alter the pathogenic activities of bacteria, reduce antibiotic-induced side-effects, inhibits mammary and liver tumours and in conjunction with oligofructose decrease 1, 2-dimethylhydrazine induced carcinogenesis (**Chichlowski *et al.*, 2007**).

Competition for nutrients in the gut, especially carbohydrate, is well recognised (**Fuller, 1989 and Choudhari *et al.*, 2008**). Probiotics organisms compete with pathogens for nutrients thus preventing them from acquiring energy for growth and function in the gut (**Chichlowski *et al.*, 2007**). In vitro studies have demonstrated competition for carbon sources between the gut flora and *Shigella flexneri* (**Fuller, 1989**). Inhibition of bacterial toxins by probiotics has also been reported (**Brandao *et al.*, 1998 and Musa *et al.*, 2009**), which involve several mechanisms. Firstly, probiotics produce 54-kDa protease which digests the toxin and its receptor, through which the toxin attaches to the enterocyte (**Pouthoulakis *et al.*, 1993; Brandao *et al.*, 1998**). Secondly, probiotic bacteria reduce the formation of cyclic AMP (cAMP) of the intestine. *E. coli* and cholera toxins catalyse the activation of adenyle cyclase causing a rise in cAMP that triggers active secretion of chloride and bicarbonate in crypt cells and inhibits water absorption in the villus resulting in diarrhoea. *S. boulardii* was demonstrated to produce a 120-kDa protein, which reduces the formation of cAMP by intestinal cells to which *E. coli* thermo labile toxins has been added (**Czerucka *et al.*, 1994**). Thirdly, the specific toxin may adhere to the probiotic surface. If specific receptors of the toxin are similar to the surface receptor of *S. boulardii* membrane, there is a likelihood that the toxin may bind to the probiotic bacteria (**Brandao *et al.*, 1998**). It has also been demonstrated that probiotics produce antimicrobial substances which prevent the pathogenic bacteria from localising in the

animal gut (**Fuller, 1989; Vandenberg, 1993**). This class of small antimicrobial molecules, referred to as bacteriocins, defensins and cathelicidines, act to combat the pathogenic bacteria or impede their colonisation. These are protein or protein complexes which have an antagonistic effect against the pathogenic bacteria. The polyamine derived piperidine, yielded by the intestinal microflora as a result of amino acid degradation, has been shown to inhibit the binding of Salmonella and Shigella to the intestinal epithelial cells (**Chichlowski *et al.*, 2007**).

Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract. These microorganisms have been reputed to modify the intestinal milieu and to deliver enzymes and other beneficial substances into the intestines (**Marteau and Rambaud, 1993**). Supplementation of *L. acidophilus* or a mixture of *Lactobacillus* cultures to chickens significantly increased ($p < 0.05$) the levels of amylase after 40 d of feeding (**Jin *et al.*, 2000**). This result is similar to the findings of (**Collington *et al.*, 1990**), who reported that inclusion of a probiotic a mixture of multiple strains of *Lactobacillus* spp. and *Streptococcus faecium* resulted in significantly higher carbohydrase enzyme activities in the small intestine of piglets. The lactobacilli colonizing the intestine may secrete the enzyme, thus increasing the intestinal amylase activity (**Duke, 1977 and Sissons, 1989**). It is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients (**Dierck, 1989**). The effect of *Aspergillus oryzae* on macronutrients metabolism in laying hens was observed (**Schneitz, 2005**), of which findings might be of practical relevance. They postulated that active amylolytic and proteolytic enzymes residing in *Aspergillus oryzae* may influence the digested nutrients. Similarly, it was reported that an increase in the digestibility of dry matter was closely related to the enzymes released by yeast (**Han *et al.*, 1999**). In addition, probiotics may contribute to the improvement of health status of

birds by reducing ammonia production in the intestines (**Chiang and Hsieh, 1995**).

Probiotic is a generic term, and products can contain yeast cells, bacterial cultures, or both that stimulate microorganisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency (**Dierck, 1989**). In addition, others have reported that yeast products affect nutrient digestibility and intestinal mucosal development (**Santin *et al.*, 2001 and Zhang *et al.*, 2005**). Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (**Yeo and Kim, 1997**). Therefore, the major outcomes from using probiotics include improvement in growth (**Yeo and Kim, 1997**), reduction in mortality (**Kumprecht and Zobac, 1998**), and improvement in feed conversion efficiency (**Yeo and Kim, 1997**). These results are consistent with previous experiment of (**Tortuero and Fernandez, 1995**), who observed improved feed conversion efficiency with the supplementation of probiotic to the diet.

2.2.2.5 Evaluating probiotic effects on the intestinal microbiota and intestinal morphology:

Kabir₂ *et al.*, (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total lactobacillus count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the 6th week of age. Their result revealed competitive antagonism. The result of their study also evidenced that probiotic organisms inhibited some nonbeneficial pathogens by occupying intestinal wall space. They also demonstrated that broilers fed with probiotics had a tendency to display pronounced intestinal

histological changes such as active impetus in cell mitosis and increased nuclear size of cells, than the controls. This results of histological changes support the findings of (**Samanya and Yamauchi, 2002**) and they indicated that birds who were fed dietary *B. subtilis* var. natto for 28 days had a tendency to display greater growth performance and pronounced intestinal histologies, such as prominent villus height, extended cell area and consistent cell mitosis, than the controls. On the other hand, (**Chichlowski *et al.*, 2007**) compared the effects of providing a direct-fed microbial (DFM) with the feeding of Salinomycin on intestinal histomorphometrics, and microarchitecture and they found less mucous thickness in DFM –treated chickens and the density of bacteria embedded in the mucous blanket appeared to be lower in DFM – treated chickens than in the control in all intestinal segments. **Watkins and Kratzer (1983)**, reported that chicks dosed with *Lactobacillus* strains had lower numbers of Coliforms in cecal macerates than the control. **Francis *et al.*, (1978)**, also reported that the addition of *Lactobacillus* product at 75mg/kg of feed significantly decreased the Coliform counts in the ceca and small intestine of turkeys. Using gnotobiotic chicks, (**Fuller, 1977**), found that host- specific *Lactobacillus* strains were able to decrease *Escherichia coli* in the crop and small intestine. **Kizerwetter-swida and Binek, (2009)**, demonstrated that *L. salivarius* 3d strain reduced the number of *Salmonella enteritidis* and *Clostridium perfringens* in the group of chickens treated with *Lactobacillus*. **Watkins *et al.*, (1982)**, similarly observed that competitive exclusion of pathogenic *E. coli* occurred in the gastrointestinal tract of gnotobiotic chicks dosed with *L. acidophilus*. Recently (**Yaman *et al.*, 2006; Mountzouris *et al.*, 2007 and Higgins *et al.*, 2007**), demonstrated that probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces* have a potential effect on modulation of intestinal microflora and pathogen inhibition.

2.2.2.6 Evaluating probiotic effect on food borne bacteria reduction:

Intensive genetic selection in broilers and layers in recent years for high performance traits has resulted in an increased susceptibility to infectious diseases. Poultry meat has been associated with the transmission of enteric pathogens, including Salmonella and Campylobacter spp (Cox and Pavic, 2009). Callaway *et al.*, (2008), stated that the 'link between human Salmonella and host animals is most clear in poultry' and that raw eggs and undercooked poultry are considered to be hazardous. Eggs have been implicated as vehicles in numerous outbreaks of Salmonella in particular, eggs are major vehicle of transmission of Salmonella enteritidis (Cox and Pavic, 2009). Probiotics have been extensively used to control pathogenic Salmonella in chickens to reduce mortality. Salmonella is one of the most important food borne zoonotic diseases around the world (Pascual *et al.*, 1999). Salmonella spp. contamination of poultry products primarily originates from the GIT (Gastro-intestinal tract) of poultry, specifically the caeca, where there is high microbial activity. To produce Salmonella- free meat and eggs, recent research has focused on reducing Samonella infection through competitive exclusion. The specific strain of Lactobacillus spp.adhere to the wall of the intestines of the host and competitively eject the Salmonella from the gut. Hassanein and Soliman (2010), found that live yeast culture of Saccharomyces cerevisiae at the level of 0.4% and 0.8% decreased the intestinal load of Escherichia coli, Klebsiella sp., Staphylococcus sp., Micrococcus sp., Campylobacter sp., and Clostridium perfringens in layers. When poultry meat and eggs were recognised as a vehicle for human Salmonella, the application of probiotics as a tool for preventing this disease was actively explored. Cox and Pavic, (2009), reported that increased numbers of Lactobacillus and Bifadobacterium spp. correlated with reduced Salmonella spp. prevalence. Starvic (1987), treated newly hatched chicks with different strains of bacteria belonging to Lactobacillus, Bacteroides,

Escherichia and Streptococcus spp., and observed an increased inhibition of Salmonella spp. colonisation. **Pascual *et al.*, (1999)**, reported that a single strain of Lactobacillus salivarius was capable alone of eliminating Salmonella enteritidis from the gut of one day old chicks. The immunological properties of probiotics have been extensively studied, demonstrating that certain Lactobacilli augment systemic and mucosal immunity against enteropathogens, leading to the production of secretory IgA (**Revolledo *et al.*, 2006**). The beneficial effects of probiotics, however, depend upon the health of the birds, which determine the extent of colonisation by enteropathogens (**Pascual *et al.*, 1999**).

Probiotics have been applied for the prevention of Campylobacter jejuni in poultry. C. jejuni is considered to be one of the major causes of food borne bacteria. Researchers have explored the ability of Lactobacillus spp. in producing anti-Campylobacter jejuni compounds to reduce infection. **Doyle and Schoeni (1986)**, reported on the selection of bacteria from chickens with the ability to produce anti-C.jejuni metabolites. They concluded that chicks treated with probiotics had an average protection of 64% against C. jejuni when compared to the control group. In the same study, the effect of probiotic supplementation with lactose, mannose and fructooligosaccharides on the extent of inhibition of C. jejuni was explored. These compounds were found to enhance the effectiveness of probiotics. Recently, (**Stern *et al.*, 2008**), fed 250 mg of purified bacteriocins per kg feed to broiler chicks and found that bacteriocins (obtained from Lactobacillus salivarius and Paenibacillus polymyxa) substantially reduced C. jejuni colonisation in live birds. **Cox and Pavic (2009)**, reported that competitive exclusion through probiotics may provide the best tool to exclude Salmonella spp., however, under commercial conditions, degree of exclusion of Salmonella spp. has been highly variable as the efficacy of competitive exclusion requires Salmonella-free chicks, food biosecurity and low stress levels during the first few days of treatment, which may not be practical or possible. Recently, (**Santini *et al.*, 2010**), suggested

that *Bifidobacterium longum* PCB 133, possesses high probiotic properties and marked anti-campylobacter activities both in vivo and vitro, and is an excellent candidate as a feed additive for poultry for reduction of food –borne *Campylobacteria* in humans. **Higgins *et al.*, (2007)**, suggested that macrophages are directly or indirectly involved in the diminution of *Salmonella* colonisation caused by the administration of probiotics.

2.2.2.7 Evaluating probiotic effects on immune response:

Kabir *et al.*, (2004), evaluated the dynamics of probiotics on immune response of broilers and they reported significantly higher antibody production ($p < 0.01$) in experimental birds as compared to control ones. They also demonstrated that the differences in the weight of spleen and bursa of probiotics and conventional fed broilers could be attributed to different level of antibody production in response to SRBC. Similarly, **(Khaksefidi and Ghoorchi, 2006)**, reported that the antibody titer in the 50 mg /kg probiotic supplemented group was significantly higher at 5 and 10 days of postimmunization (PI) compared to control, when SRBC was injected at 7 and 14 days of age. In addition, **(Haghighi *et al.*, 2005)**, demonstrated that administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. On the other hand, **(Dalloul *et al.*, 2005)**, examined the effects of feeding a *Lactobacillus*-based probiotic on the intestinal immune responses of broiler chickens over the course of an *E. acervulina* infection and they demonstrated that the probiotic continued to afford some measure of protection through immune modulation despite a fairly overwhelming dose of *E. acervulina*. They also suggested a positive impact of the probiotic in stimulating some of the early immune responses against *E. acervulina*, as characterized by early IFN- γ and IL-2 secretions, resulting in improved local immune defenses against coccidiosis. **(Brisbin *et al.*, 2008)**, investigated spatial and temporal expression of immune system genes in chicken cecal tonsil and spleen mononuclear cells in response to

structural constituents of *L. acidophilus* and they found that cecal tonsil cells responded more rapidly than spleen cells to the bacterial stimuli, with the most potent stimulus for cecal tonsil cells being DNA and for splenocytes being the bacterial cell wall components. They also discovered that in both splenocytes and cecal tonsil cells, STAT2 and STAT4 genes were highly induced and the expression of STAT 2, STAT4, IL-18, MyD88, IFN – alpha, and IFN – gamma genes were up – regulated in cecal tonsil cells after treatment with *L. acidophilus* DNA. (**Higgins *et al.*, 2007**), suggesting that, probiotics have the ability to modulate the innate immunity of broilers. However, it has been shown that all probiotic organisms do not act to induce the same immunological functions in the gastrointestinal tract and that proper strain selection or probiotic product with the desirable probiotic strains will affect the outcome of treatment (**Maassen *et al.*, 1998**). Simultaneously, several investigators demonstrated the potential effect of probiotics on immunomodulation (**Matsuzaki and Chin, 2000; Zulkifli *et al.*, 2000; Dalloul *et al.*, 2005; Haghghi *et al.*, 2005 ; Mathivanan and Kalaiarasi, 2007; Nayebpor *et al.*, 2007; Apata, 2008**). On the other hand, (**Midilli *et al.*, 2008**), showed the ineffectiveness of additive supplementation of probiotics on systemic IgG.

2.2.3 The effect of dietary probiotic on the performance and carcass characteristics of broilers:

Odefemi, (2016), investigated on the effect of antibiotics, probiotics and prebiotics as feed additives in broiler diets on performance and carcass characteristics. The treatments were assigned into 5 dietary treatments containing 0.01% antibiotics, 0.06% probiotics, 0.1% probiotics and 0.2% prebiotics while the first treatment which served as control diet not include any additives. The results showed that, the birds fed with probiotics had the highest weight gain (1218.15g and 1163.68g), highest drumstick%, and high feed intake. No significant differences were observed between the various

treatment groups in feed conversion ratio, dressing %, breast, thigh %, liver and heart %.

Idoui and Karam, (2016), reported on the effects of autochthonous lactobacillus plantarum feeding on growth performances, carcass traits, serum composition and faecal microflora of broiler chickens. The broiler chickens were assigned to two treatments, all birds were fed with commercial diet but drinking water of the experimental group was supplemented by probiotic *Lb. plantarum* and each ml of contained 65×10^{11} cfu. The results showed a significant positive effect ($p < 0.05$) of probiotic on body weight, feed intake and feed conversion ratio. Also there were significant differences between groups in gizzard% while no significant differences in liver and heart% between groups. It was concluded that autochthonous probiotic improved growth and feed efficiency in broiler chickens and consider the improvements in carcass traits.

Pourakbari *et al.*, (2016), investigated the effects of probiotic levels on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. Five treatments were used in this experiment: Control, and the same control diet supplemented with 0.005%, 0.01%, 0.015% and 0.02% probiotics. The results indicated that the probiotics in feed at 0.02% or higher levels of supplementation improved body weight gain (+12%) and feed conversion rate (-5%) compared with the control. There were no effects on carcass traits (breast, drumstick% and liver%).

Mokhtari *et al.*, (2015), studies the efficiency of different growth promoters on the productive performance and carcass yield of broiler chickens. The treatments were allocated into six groups: group 1. Control diet (without any promoter), group 2. Control diet + antibiotic, group 3. Control diet + probiotic, group 4. Control diet + prebiotic, group 5. Control diet + phytobiotic and group 6. Control diet + synbiotic. The results indicated that there were no significant differences between treatment groups in body weight gain

($p > 0.05$), but all of them had beneficial effect compared to control. Lowest feed conversion ratio was observed in probiotic group and caused more efficient feed intake. Treatments vs. control increased carcass significantly but the difference between treatments was not significant. Breast and thigh percentage were not affected by treatments and there were no significant difference between treatment and control group. According to our results, probiotic and symbiotic appeared to be superior compared to other growth promoters.

Zhang and Kim, (2014), investigated on the effects of multistrain probiotics supplementation in broilers. The treatments were allotted in to four groups: 1. An antibiotic –free diet (control-). 2. (Control +) 5 mg/kg of avilamycin. 3. Control + 1×10^5 cfu of multistrain probiotics /kg of diet (p_1) and 4. Control + 2×10^5 cfu of multistrain probiotics /kg of diet (p_2). The results indicated that birds fed with p_1 and p_2 diets had greater body weight gain and better feed conversion ratio than the birds fed with control diet. No significant differences were observed in feed intake and mortality rate among treatments throughout the experimental period.

EL-Hammady *et al.*, (2014), evaluated that, the effect of a probiotic as alternative to antibiotics growth promoters for broiler chicks. The ration used in the first group without supplements (control) while those of 2-5 treatment groups used the basal diets supplemented with antibiotic Neomycin (20mg/kg diet), probiotic (1g/kg diet), probiotic (1.5g/kg diet), and probiotic (2g/kg diet). The results obtained that, the birds fed ration supplemented with antibiotic had significantly ($P < 0.05$) heavier final body weight (BW) and higher body weight gain (BWG) than the birds fed with basal diet supplemented with different levels of probiotics or control diet. However, birds received 1g and 1.5g probiotic/kg diets had significantly higher BW and BWG, and better feed conversion ratio (FCR) than those fed with probiotic diet 2g/kg and the control diet. No significant differences were observed among

the groups in percentage of carcass and body organ percentage (gizzard, liver and heart). The total mortality rate of birds in group 3 (1g probiotic/kg deit) was lower than those of the other groups.

Bai *et al.*, (2013), evaluated that, the effects of a probiotic product incorporating *Lactobacillus fermentum* and *Saccharomyces cerevisiae* on the growth performance and intestinal immune status in broiler chickens. The treatments were assigned in to 4 dietary treatments, containing basal diet (NC), and the basal diets supplemented with an antibiotic (100mg of chlortetracycline/kg of diet PC), 0.1% or 0.2% probiotic prouduct (containing 1×10^7 cfu/g of *Lactobacillus fermentum* JS and 2×10^6 cfu/g of *Saccharomyces cerevisiae*). The results showed a significant positive effect ($P < 0.05$) of probiotic on average daily gain (ADG) and feed efficiency compared with NC, and were similar to the PC group during 1 to 21 days. However, there were no significant differences in growth performance of broilers during 22 to 42 days among different dietary treatments. No significant effect of dietary treatment were observed on body weight (BW) at 42 d. There was no difference ($p > 0.05$) in the above parameters of broilers performance in starter, grower, and overall periods among PC, 0.1% and 0.2% probiotic treatments.

Alloui *et al.*, (2012), reported that, the effect of probiotic feed additives on broiler chickens health and performance. Bacterial probiotic used in this experience is a *Pediococcus acidilactici*. The broiler chickens were assigned into two experimental group treatment: (10^9 cfu/kg of feed of *Pediococcus acidilactici* MA 18/5M) and control. The results indicated that, the administration of *Pediococcus acidilactici* affected positively the growth performance of broilers (2586.43 vs. 2252.79 grams $p \leq 0.01$) and feed conversion ratio (2.00 vs. 2.5). There were no significant differences between groups in carcass dressing, breast meat and thigh percent. Mortality was almost similar in both groups (6.56 vs. 6.51).

Dizaji *et al.*, (2012), evaluated that, the effects of dietary supplementations of prebiotics, probiotics, synbiotics and acidifiers on growth performance and organs weights of broiler chickens. The chickens were randomly assigned to one of five dietary treatments for six weeks. The dietary treatments as follows: 1. Control (basal diets). 2. Basal diets supplemented with prebiotic (1kg of Active MOS/ton). 3. Basal diets supplemented with probiotic (150/100/50g of Protexin/ton of the starter, grower and final diets respectively). 4. Basal diets supplemented with synbiotic (1kg of Amax4x/ton). 5. Basal diets supplemented with acidifier (2liter Globacid/ton). At the end of the experiment the results indicated that, broilers supplemented with prebiotic, synbiotic and acidifier had higher body weight in compared of control group ($p < 0.05$). However, there was no significant differences ($p > 0.05$) between probiotic and control groups in body weight. Feed conversion ratio decreased significantly ($p < 0.05$) in synbiotic and acidifier groups compare the control group. However, there were no significant ($p > 0.05$) differences in FCR of broiler chicks in prebiotic and probiotic groups compared with control group. No significant ($p > 0.05$) differences between groups in feed intake, gizzard and liver %.

Kral *et al.*, (2012), investigated on the effect of probiotics on the performance of broiler chickens. The broiler chickens were divided into two dietary group, control group were fed with standard feed mixture and experimental group fed with probiotics mixed with feed mixture. The results showed that, no significant ($p > 0.05$) differences in body weight of broilers among the groups were observed from initial age to the 4th weeks. From the 5th to finally part of feeding experiment was significant ($p < 0.05$) differences in body weight of final fattening broiler chickens. Control group obtained higher body weight (1689.6g) than experimental group (1360.6g) at the end of experiment.

Ohimain and Ofongo, (2012), conducted an experiment to study the effect of probiotic and prebiotic feed supplementation on chicken health and

microflora: The study found that, dietary supplements containing probiotic, prebiotic and enzymes are able to enhance performance while protecting the chickens from microbial infection.

Aliakbarpour *et al.*, (2012), evaluated the effect of commercial monostrain and multistrain probiotics in diets on growth performance, intestinal morphology and mucin gene (MUC2) expression in broiler chicks. The treatments were allocated in three experimental groups as follows: control - without supplement, control diets Supplemented with *Bacillus subtilis* (BS) at level 1000mg/kg, and control diets supplemented with Lactic acid bacteria (LAB) at level 50 mg/kg. The results showed a significant ($p < 0.05$) differences in growth performance, birds fed with probiotics had higher final body weight, body weight gain, and better (FCR) compared with control birds. No significance ($p > 0.05$) differences in feed intake between control group and probiotic groups. Also no significant differences ($p > 0.05$) in growth performance were observed in birds fed different types of probiotic supplemented diets.

Liu *et al.*, (2012), investigated on the effects of *Bacillus licheniformis* on growth performance and meat quality of broilers. Three treatments were used: i) control, ii) basal diet supplemented with 1ml of *B. licheniformis* per chick in feed water per day. and iii) basal diets supplemented with 2ml of *B. licheniformis* per chick in feed water per day. The results showed that significantly increased body weight in grower chickens ($p < 0.05$), and significantly improved the feed conversion in 3 to 6 and 0 to 6 wk feeding period compared with the control group ($p < 0.05$). Further more, improvement in sensory attributes was observed in broilers fed with the probiotic. In conclusion, *B. licheniformis* treatments resulted in a significant increase ($p < 0.05$) in broiler productivity based on an index taking into account daily weight gain and feed conversion rate. Overall, the study indicated that *B. licheniformis* can be used as a growth promoter and meat quality enhancer in

broiler chicks. Administration of both 1ml and 2ml of *B. licheniformis* preparation had no effect on mortality.

Shabani, *et al.*, (2012), reported on the effect of probiotics on carcass and internal organs of broilers. In this study, three kinds of commercial probiotics were used to maximize broiler chickens performance. chickens were divided into four treatment groups: 1- control group (with out probiotics), 2- experimental group containing protexin, 3- experimental group containing primalac, and 4- experimental group containing calcipatine. The results revealed that the treatments had significant ($p < 0.05$) effects in full carcass weight and empty carcass weight. However, the chicken broilers fed with protexin, resulted in the most favorable carcass weight while broilers fed with ratios of premalac and calciporin were ranked second and third, and broilers in control group were ranked fourth. Internal organs means were resulted that, no significant effect ($p > 0.05$) on gizzard% between treatment groups.

Ashayerizadeh *et al.*, (2011), reported on the effects of antibiotic, probiotic, prebiotic and mixture of probiotic and prebiotic as dietary growth promoter on growth indices and serum biochemical parameters of broiler chickens. Five dietary treatments were used as follows: control- basal diet, basal control diet with antibiotic (Flavomycin, 650 g/ton), probiotic (primalac, 900g/ton), prebiotic (Biolex-MB, 2000g/ton) and mixture of probiotic (900g/ton) plus prebiotic (2000g/ton) synbiotic. Specific growth rate (SGR) and growth efficiency (GE) were highest in birds under prebiotic and synbiotic treatments in starter and total rearing period, respectively. The results suggested that, the mixture of probiotic and prebiotic could be effective as antibiotic to improve the performance of broiler chickens.

Lee *et al.*, (2010), investigated on the effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. In this work chickens fed with a diet supplemented with *Bacillus* spp. as direct-fed microbials (DFM). Two treatments were used:

control group and experimental group supplemented with 1.5×10^5 cfu/g of DFM a commercial product incorporating 3 DFM, or a non supplemented diet. Direct-fed microbials did not significantly modify body weight gain (BWG).

Mountzouris *et al.*, (2010), reported that, the effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulin, and cecal microflora composition. Five bacterial spp. Probiotic was used in broilers nutrition. The treatments assigned into 5 dietary treatments as follows: No addition negative control, 10^8 cfu probiotic/kg of diet (p_1), 10^9 cfu probiotic/kg of diet (p_2), 10^{10} cfu probiotic/kg of diet (p_3), and 2.5mg of Avilamycin/kg of diet positive control. The results showed that, the birds fed with (p_1) had the highest body weight (BW) and body weight gain (BWG) (2.343, 2.293 g) compared with p_2 (2.213, 2.163 g), negative control (2.215-2.165g) and p_3 (2.217, 2.167 g), and with positive control (2.280, 2.230 g) being intermediate and not different from p_1 . Overall feed conversion ratio values were similar and significantly better for p_1 (1.80) and positive control (1.80) compared with p_2 (1.87), negative control (1.89), and p_3 (1.92). There were no significant differences in feed intake (FI) between treatments during the experimental period.

Zhou *et al.*, (2010), evaluated that, the effect of dietary probiotic, *Bacillus coagulans* ZJU0616, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. The treatments segregated into 4 dietary treatment groups, control group were fed basal diet without any probiotic and other groups were fed the diets that consisted of 3 probiotic levels at initial concentrations of 1.0×10^6 cfu g^{-1} (T_1), 2.0×10^6 cfu g^{-1} (T_2) and 5.0×10^6 cfu g^{-1} (T_3). The results showed that, the lowest final body weight and daily body weight gain were found in control group and there were no significant differences among probiotic treatments. Significantly lower feed conversion ratio and higher survival rate were observed in T_2 and T_3 than that of the

control. Finally the addition of *Bacillus coagulans* to broiler feed, improved growth performance, FCR and meat quality of Guangxi yellow.

Eckert *et al.*, (2010), evaluated that, body weight gain and FCR were improved in response to *Lactobacillus*-based probiotics. Similarly, **Zhu *et al.*, (2009)**, reported that *Lactobacillus salivarius* improved body weight gain and FCR of broilers. **O'Dea *et al.*, (2006)**, examined probiotic mixtures (*Lactobacillus acidophilus*, *Lactobacillus bifidus*, and *Streptococcus faecalis*) using different regimes and concluded that weight gain improved significantly ($p < 0.05$) in broilers fed the supplemented diet. Accumulated evidence suggests that inactivated probiotics could have similar beneficial effects to those of live probiotics. **Huang *et al.*, (2004)**, investigated that inactivated probiotics, after disruption with a high pressure homogeniser, have beneficial effects on the productivity of broiler chicks when used at a certain concentration. They also found that body weight gain was improved with disrupted, cobalt-enriched Lactic acid bacteria (*L. acidophilus* and *L. casei*) and Fungal mycelium (*S. acidophilum*), when sprayed into mash basal diet. **Zhou *et al.*, (2010)**, found that *Bacillus coagulans* ZJU0616, improved growth performance, FCR, and meat quality of Guangxi Yellow chickens. **Hassanein and Soliman, (2010)**, found that supplementing with a live yeast culture of *Saccharomyces cerevisiae* at the level of 0.4% and 0.8% improved FCR in white leghorn birds. **Panda *et al.*, (2008)**, reported that dietary preparation of *L. sporogenes* at 100 mg (6×10^8 spore) per kg of diet, significantly enhanced feed efficiency in white leghorn breeders, which was ascribed to the beneficial effects of probiotic feeding on digestion and utilisation of nutrients. In the same study, no positive effect of this probiotic was recorded on body weight gain and feed intake. **Zhu *et al.*, (2009)**, described that the degree of probiotics effect depends upon species, bacterial strain, application method, bird's age, overall hygiene condition on farm and environmental factors.

Opalinski *et al.*, (2007), evaluated the effect of a probiotic (*Bacillus subtilis*, strain DSM17299) in broiler diets on feed intake, weight gain, and feed conversion ratio. Four treatments were applied: T₁: negative control (NC) basal diet without growth promoter; T₂: NC+*Bacillus subtilis* (8×10^5 cfu_s/gfeed); T₃: NC+*Bacillus subtilis* (3×10^5 cfu_s/gfeed) and T₄: positive control (PC) Avilamycin anticoccidial from 1 to 35 days of age. The results indicated that there was an increase of antibiotic-free diet intake as compared to the diets with growth promoters ($p < 0.05$), but there was no difference, however, as compared to the diets with probiotic as a growth promoter ($p > 0.05$). The use of growth promoters did not improve weight gain. There was a marked improvement in the feed conversion ratio of broilers fed the diet with antibiotics and of broilers fed the diet with *B. subtilis*. It is concluded that the probiotic *Bacillus subtilis* can be used as a growth promoter in broiler diets.

Kabir₁ *et al.*, (2005), evaluated that the effects of probiotics on the sensory characteristics and microbiological quality of dressed broiler meat and reported that supplementation of probiotics in broiler ration improved the meat quality both at prefreezing and postfreezing storage. **Mahajan *et al.*, (2000)**, stated that the scores for the sensory attributes of the meat balls appearance, texture, juiciness and overall acceptability were significantly ($p < 0.001$) higher and those for flavour were lower in the probiotic (Lacto-Sacc) fed group. On the other hand, **Loddi *et al.*, (2000)**, reported that neither probiotic nor antibiotic affected sensory characteristics (intensity of aroma, strange aroma, flavour, strange flavour, tenderness, juiciness, acceptability, characteristic colour and overall aspects) of breast and leg meats. On the other hand, **Zhang *et al.*, (2005)**, conducted an experiment to investigate the effects of *Saccharomyces cerevisiae* (Sc) cell components on the meat quality and they reported that meat tenderness could be improved by the whole yeast (WY) or *Saccharomyces cerevisiae* extract (YE).

Abdel-Raheem *et al.*, (2005), evaluated that, the effect of prebiotic, probiotic and symbiotic supplementation on intestinal microflora and histomorphology of broilers. Treatment groups were as follows: 1. Basal diet (control); 2. Basal diet plus mannanoligosaccharide (MOS) at levels of 2 g/kg of the starter diets and 0.5 g/kg of grower diets. 3. Basal diet plus probiotic (3g/kg diet, *Saccharomyces cerevisiae*); and 4. Basal diet plus the combination of pre and probiotics (synbiotic). The results showed that, the birds fed with probiotic and synbiotic had the highest final body weight (BW), body weight gain (BWG) and better feed conversion efficiency compared with the control and prebiotic groups.

Kabir *et al.*, (2004), indicated that probiotic supplementation can have positive effects on the beneficial impact on poultry performance. The results showed that the live weight gain and carcass yield were significantly ($p < 0.05$) higher in experimental birds as compared to control ones at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated and non vaccinated birds. This result is in agreement with many investigators: (**Jin *et al.*, 1998; Kalavathy *et al.*, 2003; Islam *et al.*, 2004; and Ashayerizadeh *et al.*, 2009**), who demonstrated increased live weight gain in probiotic fed birds. On the other hand, (**Lan *et al.*, 2003**), found higher ($p < 0.05$) weight gain in broiler subjected to two probiotic species. **Huang *et al.*, (2004)**, demonstrated that in activated probiotics, disrupted by a high pressure homogenizer, have positive effects on the producing performance of broiler chickens used at certain concentrations. In addition, (**Torres-Rodriguez *et al.*, 2007**), reported that administration of the selected probiotic (FM-B11) to turkeys increased the average daily gain and market body weight (BW) representing an economic alternative to improve turkey production. However, (**Karaoglu and Durdag, 2005**), used *Saccharomyces cerevisiae* as a dietary probiotic to assess performance and found no overall weight gain difference. **Mahajan *et al.*, (1999)**, recorded in their study that mean values of giblets, hot dress weight

and dressing percentage were significantly ($p < 0.05$) higher for probiotic (Lacto - Sacc) fed broilers.

Panda *et al.*, (2003), reported that the inclusion of *L. sporogens* (100mg/kg) resulted in an increased body weight and improved FCR in commercial broilers. In another study, (**Mohan *et al.*, 1996; Choudhari *et al.*, 2008**), conducted the addition of probiotic (*L. acidophilus* and *S. faecium*) to broiler feed significantly improved the growth rate. **Choudhari *et al.*, (2008)**, evaluated that, the inclusion of live yeast culture of *S. cerevisiae* along with *L. acidophilus* and *S. faecium* (1kg/ton) resulted in an improved weight gain and FCR of broilers. **Balevi *et al.*, (2001)**, found that supplementation of the diet with commercial probiotic (protexin) TM at 500g/ton resulted in an improved feed intake, body weight gain and FCR of broilers.

Mead, (2000), described field experiences with competitive exclusion usage for control of salmonella in poultry and clearly states that it is possible to control pathogen infection without supratherapeutic antibiotic application, which was incompatible with probiotics. In field trials with market turkeys, we have demonstrated that *Lactobacillus reuteri* improved weight gain at 120 days of age by 4.8% (**Casas *et al.*, 1998**). In ovo *Lactobacillus reuteri*-treated broiler chickens given *S. typhimurium* challenge, body weight improved by 206g at 40 days of age and mortality was reduced by 32% (**Edens *et al.*, 1997_a**). **Lan *et al.*, (2003)**, reported that broiler chickens given *Lactobacillus agilis* JCM1048 and *Lactobacillus salavarius* subsp. *salicinius* JCM 1230 significantly increased weight gain by 10.7%. Use of *Bacillus subtilis* (calsporin; calpis corporation, Tokyo, Japan) did not improve body weight (calsporin 2416 g vs. control 2407g) at 42 days of age, but feed conversion ratio was improved (calsporin 1.74 vs. control 1.77). **Fritts *et al.*, (2000)**, have shown that calsporin will improve broiler body weight gain, feed conversion and reduced mortality.

CHAPTER THREE

MATERIALS AND METHODS

This experiment was conducted during winter season from (15th of January to 18th February 2016). The ambient temperature averaged (12-30c) appendix (1), during the experimental period (5 weeks).

3.1 Experimental chicks:

A total number of 200 day-old commercial unsexed broilers of Arbor Acres strain were purchased from local commercial hatchery (Mico) and transported to Damazin poultry farm, General Administration of Animal Resources and Fisheries. The chicks were adapted to the premises and fed for (5 days) before start of the experiment. At the end of adaptation period, all chicks were weighted with an average initial weight of (125 gm). The chicks were then allotted randomly into 5 experimental groups A, B, C, D and E, with 5 replicates each of 8 chicks (5x5x8) in a complete randomized design (CRD), feed and water provided *ad libitum* throughout the experimental period. Chicks were bought vaccinated against Marek' disease, and against Newcastle (ND) and Infectious Bronchitis disease (IBD) in hatchery by (ND +IB) spray day one, and inactivated ND injection day one. On farm vaccinated against Gamboro disease by (D78) at 12 days of age. The dosage was repeated at 21 and 28 days of age for ND BY (Clone 30) and (IBD) by (IBDO78) respectively. Soluble multivitamin compounds (Pantominovit - pantex Holland B.V. 5525 ZG Duized- Holland) provided three days before and after vaccination to guard stress.

3.2 Housing:

An open system poultry house was used. The house was constructed on concrete floor with corrugated metal sheat roof and solid brick western-eastern. The house dimensions (length, width and height) were 15x6.5x3.5

meters respectively. Experiments 25 pens (1x1m) were prepared using wire mesh partitions and then were cleaned washed and disinfected by formalin and white phenol solution. Before start the experiment a layer of wood shairy (5cm) thick was laid on the floor as littler material. Each pen was provided with one feeder (5kg) and drinker (2.5lit.) which were adjusted to the progressive growth of chicks. Light was provided approximately 24 hours, natural light during the day and artificial light during the night (60 watt) all through the experimental period.

3.3 Experimental ration:

The commercial bacterial probiotic product (Dexflor- PR) was used in this experiment, it is the feed additive based on different standard strains of *Bacillus cereus* var. *toyoi* in minimum concentration of 100 million organisms per gram and absorbed on avegetal support. The product Dexflor-PR was purchased from Hadir international Co. LTD Khartoum Sudan. Manufactured by SAMU MEDIAA CO..LTD. (KOREA). Lot No: 100898, Mfg. date: 2015.12.15, Exp. Date: 2017.12.14. The chicks were divided into 5 dietary treatments, the first group A, fed on basal diet without feed additives (negative control), the second group B, fed on basal diet with an antibiotic (Neomycin 20mg/kg) as positive control, the other groups C, D and E were fed on basal diet supplemented with bacterial probiotic (*Bacillus cereus* var. *Toyoi*) at levels 1, 2 and 3 gm/kg respectively. The basal diet was formulated to meet the nutrient requirement of broiler chicks according to NRC (1994).

The ingredients percent composition and the calculated chemical analysis of the experiment diet were presented in table (1 and 2). Experiment diets were fed for 5 weeks.

Table 1. The ingredients percent composition of experimental diets (6-35 days) containing graded levels of dietary bacterial probiotic

Ingredients %	Diets				
	A	B	C	D	E
Sorghum	66.55	66.55	66.55	66.55	66.55
Ground nut cake	24.30	24.30	24.30	24.30	24.30
Lime – stone	0.80	0.80	0.80	0.80	0.80
Concentrate	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	0.58	0.58	0.58	0.58	0.58
Salt	0.20	0.20	0.20	0.20	0.20
Vegetable oil	1.50	1.50	1.50	1.50	1.50
Antitoxins	0.20	0.20	0.20	0.20	0.20
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.27	0.27	0.27	0.27	0.27
Coccidiostatic	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100
Antibiotic (Neomycin) Mg/Kg	-	20	-	-	-
Bacterial probiotic gm/kg	-	-	1	2	3

Broiler concentrate ME 2.122 kcal/kg , crude protein 40%, crude fiber 1.5%, Lysine 1.5 %, lysine 13.5%, methionine 5.9%, met- +cystin 6.25%, calcium 6.8%, phosphours av. 4.6%, phosphorus tot 3% sodium 1.5%, vitamin A 250.000IU/kg, vitamin E 800 ppM , vitamin k3 60 ppM, vitamin B1 40 ppM, vitamin B2 100 ppM, B6 50 ppM, vitamin B12 300 ppM, vitamin C 400 ppM, biotin 2000 ppM, folic acid 30 ppM, choline chloride 30000 ppM, Betain 3000ppM,iron (fe) 1.000 ppM, cooper 300 ppM, zinc 1000 ppM, manganese 1600ppM, Iodine 20 ppM, selenium 5 ppM, cobalt 12 ppM, 16 phytase 1500 FYT antioxidant added.

Table 2. Calculated chemical analysis of experimental basal diet

Components %	Basal diet
Dry matter	94.85
Crude protein	22.70
Crude fiber	04.35
Ether Extract	03.35
Ash	04.65
Nitrogen Free Extract	59.80
Calcium	01.06
Available phosphorous	00.50
Lysine	01.33
Methionine	00.60
ME (Kcal/kg)	3117

Calculated according to (Ellis, 1981; kuku Bulletin)

3.4 Data collected:

3.4.1 Performance data:

Average body weight, weight gain, feed consumption (gm), and feed conversion ratio (FCR) for each group were determined weekly throughout experimental period. Health of the experimental stock was closely observed.

3.4.2 Slaughter procedure and data:

At the end of the experimental period (5 weeks) birds were fasted overnight with only water allowed. Five birds of similar live body weight were selected randomly from each treatment group and weighted individually before slaughter by severing the right and left carotid and jugular vessels, trachea and esophagus. After bleeding they were scalded in hot water, hand plucked and washed. Head was removed close to skull, feet and shanks were removed at the hock joint. Evisceration was accomplished by posterior ventral cut to completely remove the visceral organs, (heart, liver and gizzard) and then were separated weighted individually and were expressed as a percentage of live weight. The hot carcass were weighted to calculate the dressing percentage. The carcass was then divided in to wright and left sides by mid sawing along the vertebral column and each side was weighted. The left side was divided into three commercial cuts breast, thigh and drumstick, each cut was weighted separately, and were expressed as percentage of the carcass weight. Then they deboned, the meat and bone were weighted separately, and were expressed as percentage of their cuts. The meat was frozen and stored for further analysis.

3.4.3 The taste panel:

Frozen deboned breast, thigh and drumstick cuts were thawed at 5-7c before cooking for sensory evaluation. The meat was trapped in aluminum foil placed in roast pan and cooked at 176.7c in conventional preheated electrical

oven to about 80c internal muscles temperature, the cooked meat was allowed to cool to room temperature for about 10 minutes. The samples were kept warm until served. Trained panelists were instructed to eat crackers drink water between samples evaluated. Following recommended procedure (**Hawrysh *et al*; 1980**), the sensory panel evaluated the chops for tenderness, flavor, color and juiciness using an eight point scale (Appendix 2).

3.5 Experimental Design and Statistical Data Analysis

Completely randomized design (CRD) was used in this experiment, the data was analyzed by using the statistix 10 trial according to (statistix 2013), the analysis of variance (one-way ANOVA) was used to compare between the groups. All values were presented as means and standard error. The significantly set up ($p \leq 0.05$).

CHAPTER FOUR

RESULTS

Response of broiler chicks to dietary bacterial probiotic (*Bacillus cereus* var. Toyoi) commercial products (Dex flor-PR)

4.1 Performance:

The effect of feeding different levels of dietary bacterial probiotic for 5 weeks on performance of broiler chicks is shown in table (3). The results indicated that, the chicks of groups B, C, D and E obtained significantly ($p \leq 0.05$) higher weight gain than that of group A and the chicks of groups D and E obtained significantly ($p \leq 0.05$) higher weight gain than that of groups B and C, whereas no significant differences ($p \geq 0.05$) were observed between groups B and C in weight gain throughout the experimental period.

No significant ($p \geq 0.05$) differences were observed between groups A, B, C, D and E in feed consumption. However, the chicks in groups D and E consumed more feed than that chicks in groups A, B and C during the experimental period.

The chicks of groups B, C, D, and E had significantly ($p \leq 0.05$) better feed conversion ratio (FCR) than that of group A, and the chicks of groups D and E had significantly ($p \leq 0.05$) better (FCR) than that of groups B and C, whereas no significant differences ($p \geq 0.05$) were observed between groups B and C in feed conversion ratio throughout the experimental period.

No mortalities were recorded in all treatment groups throughout the experimental period.

Table 3. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on final body weight (gm), body weight gain (gm), feed intake (gm) and feed conversion ratio

Items	A	B	C	D	E	SE±	Lsd0.05
Initial body weight (gm)	125	125	125	125	125		
Final body weight (gm)	1815 ^d	1900 ^c	1940 ^c	2124 ^b	2377 ^a	11.909	S
Body weight gain (gm)	1690 ^d	1775 ^c	1815 ^c	1999 ^b	2252 ^a	11.909	S
Feed intake (gm)	3540 ^a	3530 ^a	3538 ^a	3560 ^a	3570 ^a	32.324	NS
Feed conversion ratio (FCR)	2.09 ^d	1.99 ^c	1.95 ^c	1.78 ^b	1.58 ^a	0.0192	S

Any two mean values having same superscript within rows are not significantly different ($P \leq 0.05$).

SE± = Standard error.

Key:

A = Control (-) without additive.

B = Control (+) with antibiotic.

C = Bacterial probiotic 1gm/Kg.

D = Bacterial probiotic 2gm/Kg.

E = Bacterial probiotic 3gm/Kg.

Figure 1. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on final body weight (gm), body weight gain (gm), feed intake (gm)

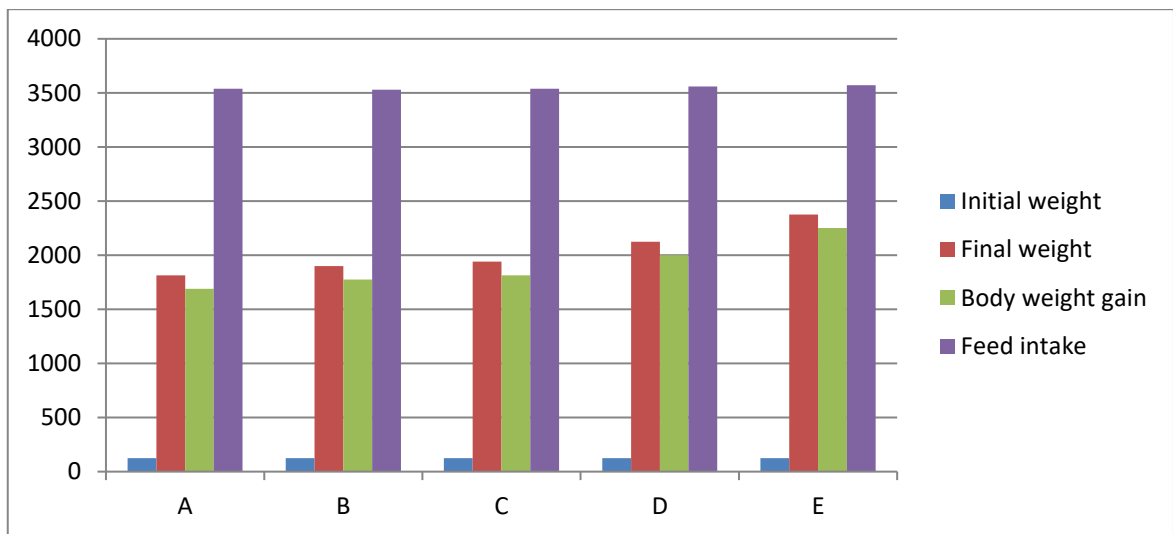
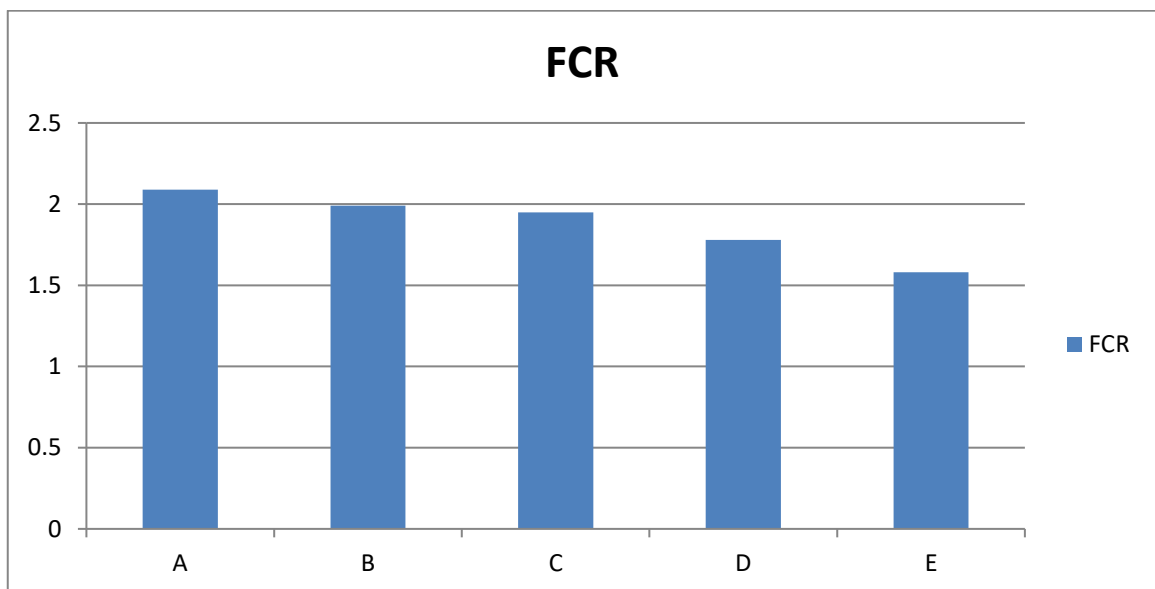


Figure 2. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on feed conversion ratio



Carcass measurement

4.2.1 Carcass and non carcass yield

As shown in table (4), the results indicated that the chicks of groups D and E obtain significantly ($p \leq 0.05$) higher carcass dressing percentage than that of group A, while no significant differences ($p \geq 0.05$) were observed between groups B, C, D and E, and also no significant differences ($p \geq 0.05$) were observed between groups A, B and C in carcass dressing percentage.

The results deals with giblets (liver, heart and gizzard) indicated that, no significant differences ($p \geq 0.05$) among the all treatment groups.

4.2.2 Commercial cuts

Commercial cuts breast, thigh and drumstick percentages are given in table (5), the results indicated that, the chicks of groups C, D, and E obtained significant ($p \leq 0.05$) higher breast, thigh and drumstick percentages than that of groups A and B, and the chicks of groups D and E obtained significantly ($p \leq 0.05$) higher breast, thigh and drumstick percentages than that of group C. The chicks of group E obtained significantly ($p < 0.05$) higher percent of commercial cuts compared with all groups, whereas no significant differences ($p \geq 0.05$) were observed between groups A and B in breast, thigh and drumstick percentages.

The treatment group values of meat expressed as percentages from total weight of selected commercial cuts was given in table (6) the results showed that, the chicks of groups C, D, and E obtained significantly ($p \leq 0.05$) higher breast, thigh and drumstick meat percentages than that of groups A and B, and the chicks of groups D and E obtained significantly ($p \leq 0.05$) higher breast, thigh and drumstick meat percentages than that of group C. The chicks of group E obtained significantly higher percent of meat values compared with all groups, whereas no significant differences ($p \geq 0.05$) were observed between groups A and B in breast, thigh and drumstick meat percentages.

Table 4. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on dressing (%), gizzard (%), liver (%) and heart (%)

Items	A	B	C	D	E	SE±	Lsd0.05
Dressing (%)	69.97 ^b	70.1 ^{ab}	70.1 ^{ab}	70.17 ^a	70.25 ^a	0.0566	S
Gizzard (%)	1.76 ^a	1.77 ^a	1.77 ^a	1.75 ^a	1.76 ^a	0.0324	NS
Liver (%)	3.02 ^a	3.06 ^a	2.98 ^a	2.98 ^a	2.99 ^a	0.1171	NS
Heart (%)	0.73 ^a	0.74 ^a	0.73 ^a	0.73 ^a	0.73 ^a	0.0165	NS

Any two mean values having same superscript within rows are not significantly different ($P \leq 0.05$).

SE± = Standard error.

Key:

A = Control (-) without additive.

B = Control (+) with antibiotic.

C = Bacterial probiotic 1gm/Kg.

D = Bacterial probiotic 2gm/Kg.

E = Bacterial probiotic 3gm/Kg.

Figure 3. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on gizzard (%), liver (%) and heart (%)

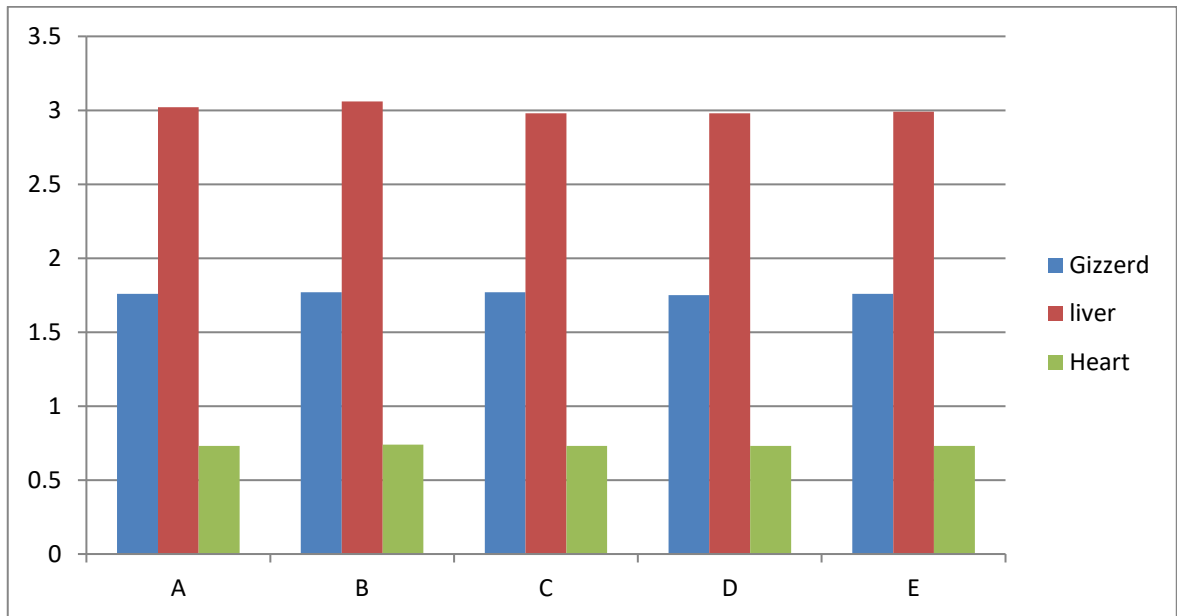


Figure 4. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on dressing (%)

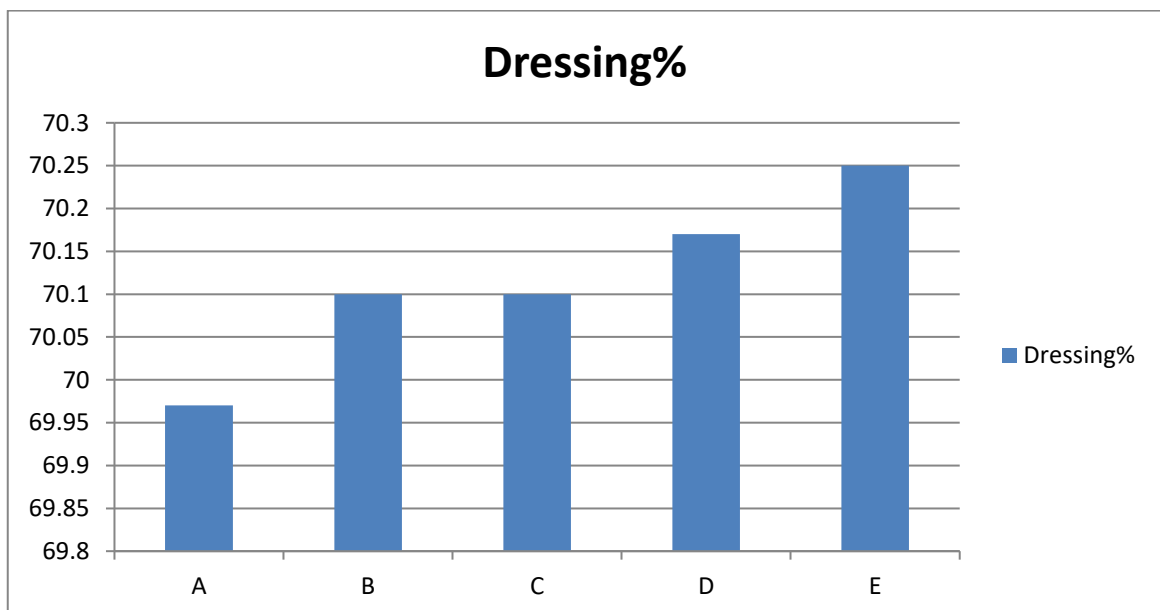


Table 5. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on breast (%), thigh (%) and drumstick (%)

Items	A	B	C	D	E	SE _±	Lsd0.05
Breast (%)	17.18 ^d	17.49 ^d	18.54 ^c	19.0 ^b	20.1 ^a	0.1825	S
Thigh (%)	13.55 ^d	13.63 ^d	14.04 ^c	14.6 ^b	15.01 ^a	0.0972	S
Drumstick (%)	6.8 ^d	6.85 ^d	7.09 ^c	7.54 ^b	8.07 ^a	0.0569	S

Any two mean values having same superscript within rows are not significantly different ($P \leq 0.05$).

SE_± = Standard error.

Key:

A = Control (-) without additive.

B = Control (+) with antibiotic.

C = Bacterial probiotic 1gm/Kg.

D = Bacterial probiotic 2gm/Kg.

E = Bacterial probiotic 3gm/Kg

Figure 5. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on breast (%), thigh (%) and drumstick (%)

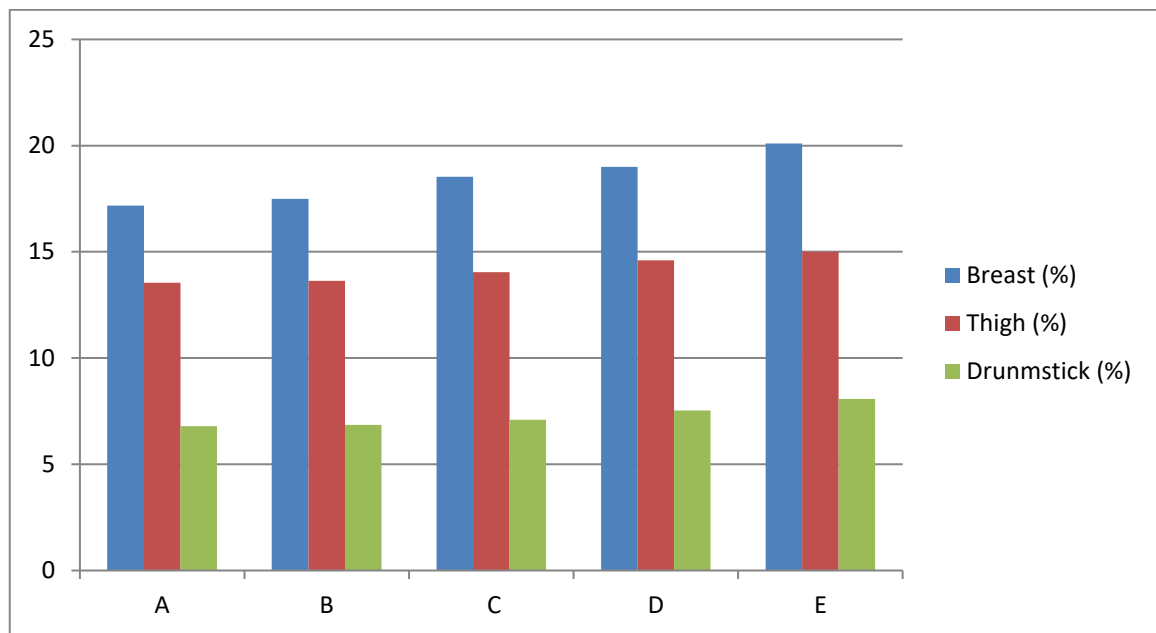


Table 6. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on breast meat (%), thigh meat (%) and drumstick meat (%)

Items	A	B	C	D	E	SE _±	Lsd0.05
Breast meat (%)	87.32 ^d	87.43 ^d	88.33 ^c	89.39 ^b	90.06 ^a	0.3553	S
Thigh meat (%)	77.80 ^d	77.83 ^d	78.55 ^c	79.35 ^b	80.04 ^a	0.0641	S
Drumstick meat (%)	77.88 ^d	77.85 ^d	78.53 ^c	79.33 ^b	80.07 ^a	0.0713	S

Any two mean values having same superscript within rows are not significantly different ($P \leq 0.05$).

SE_± = Standard error.

Key:

A = Control (-) without additive.

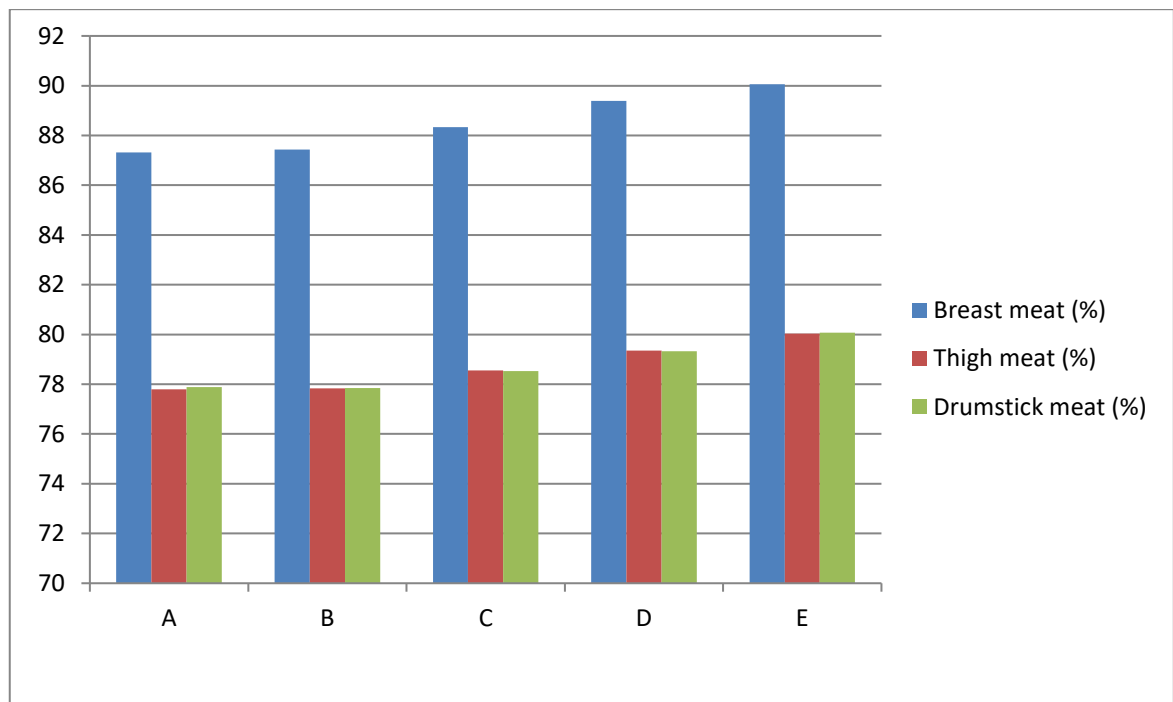
B = Control (+) with antibiotic.

C = Bacterial probiotic 1gm/Kg.

D = Bacterial probiotic 2gm/Kg.

E = Bacterial probiotic 3gm/Kg

Figure 6. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on breast meat (%), thigh meat (%) and drumstick meat (%)



4.3 Panel test (subjective meat attributes)

The effect of dietary treatments on subjective meat attributes was shown in table (7). The average subjective meat quality score value of color, tenderness, juiciness and flavor of breast, thigh and drumstick did not differ significantly ($p \geq 0.05$) among the dietary treatments and score given for all attributes are above moderate acceptability level.

4.4 Economic appraisal

The total cost, returns and profitability ratio per head of broiler chicks fed different levels of bacterial probiotic (*Bacillus cereus* var. Toyoi) for 5 weeks are shown in table (8). Chicks purchase, management and feed cost value were the major input considered. The total selling values of meat is the total revenues obtained. The result of economical evaluation indicated that, the dietary groups B, C, D and E gained more net profit than that of group A. But the value of profitability ratio (1.23) of group E (3 gm /kg, Bacterial probiotic) was the highest of the tested groups.

Table 7. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. Toyoi) on quality attributes

Items	A	B	C	D	E	SE+	Lsd0.05
Color	6.00 ^a	6.00 ^a	6.10 ^a	6.10 ^a	6.10 ^a	0.1095	NS
Tenderness	5.98 ^a	6.00 ^a	6.03 ^a	6.05 ^a	6.06 ^a	0.1669	NS
Flavor	6.06 ^a	6.07 ^a	6.07 ^a	6.14 ^a	6.15 ^a	0.0930	NS
Juiciness	6.00 ^a	6.00 ^a	6.10 ^a	6.10 ^a	6.15 ^a	0.1703	NS

Any two mean values having same superscript within rows are not significantly different ($P \leq 0.05$).

SE_± = Standard error.

Key:

A = Control (-) without additive.

B = Control (+) with antibiotic.

C = Bacterial probiotic 1gm/Kg.

D = Bacterial probiotic 2gm/Kg.

E = Bacterial probiotic 3gm/Kg

Figure 7. Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on quality attributes

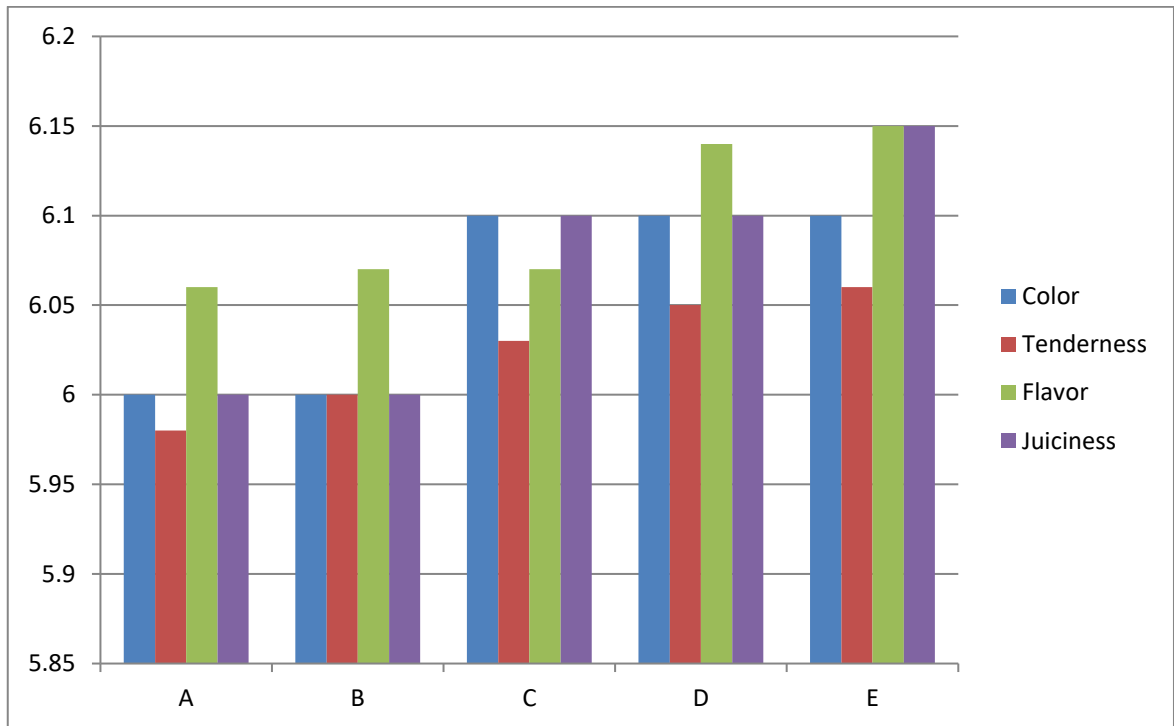


Table 8. Total cost, returns and profitability ratio per head of broiler chicks fed different amounts of bacterial probiotic (*Bacillus cereus* var.*Toyoi*) for 5 weeks

Items	A	B	C	D	E
Cost					
Chicks purchase	6.500	6.500	6.500	6.500	6.500
Feed cost	12.390	12.555	12.631	12.958	13.245
Management	3.000	3.000	3.000	3.000	3.000
Total cost	21.890	22.055	22.131	22.458	22.745
Revenues					
Average carcass weight	1.270	1.332	1.360	1.490	1.670
Price /Kg	33.000	33.000	33.000	33.000	33.000
Total revenues	41.910	43.956	44.880	49.170	55.110
Total cost	21.890	22.055	22.131	22.458	22.745
Net profit /bird	20.02	21.901	22.749	26.712	32.365
Net profit /Kg/meat	15.763	16.442	16.727	17.928	19.380
Profitability ratio /Kg meat	1	1.04	1.06	1.14	1.23

** Total cost calculated according to February 2016.

** At Current (2016) price of meat33 (SDG) kg.

CHAPTER FIVE

DISCUSSION

In modern poultry production, different types of growth promoters were used (probiotic, prebiotic, synbiotic and phytogenic) (**Dhama *et al.*, 2014**). It has been reported recently that utilization of probiotics in animal nutrition is of economic and health benefits (**Azza *et al.*, 2012**).

A probiotic was defined as alive microbial feed supplemented that beneficially effects the host animal by improving its microbial intestinal balance, digestive function, intestinal environment, immune system, and broiler health (**Fuller, 1989**).

This experiment was conducted to evaluate the response of broiler chicks fed graded levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) commercial products (Dexflor-PR) as natural growth promoter alternative to antibiotic on performance and carcass characteristics. The bacterial probiotic (BP) was added to the basal diet at levels 1, 2, and 3 gm/kg diet, whereas the basal diet which received no probiotic additive was served as control diet. In this study the apparent health of experimental stock was good throughout the experimental period. The general behavior of the stock also was good. The ambient temperature during the experimental period fell within the thermoneutral zone has extracted no heat on the experimental period.

In the present study the results indicated that, no mortalities were recorded among the different treatment groups throughout the experimental period. This may be due to the hygienic situation of the experimental. In this study birds were kept in clean disinfected environment of following all hygiene regulations program. And also may be due to the ability of dietary (BP) to reduce enteric disease infection, through stimulating of the immune system by increase the production of immunoglobulin spacially IgA (Immunoglobulin

A) it is an antibody that plays a critical role in immune function in the mucous membranes and stimulates phagocytic activity (**Sanders, 1999 and Matsuzaki et al, 1998**). Moreover, the probiotic could be suppressed pathogenic bacteria in intestinal tract by preventing from attaching to the epithelium, effectively blocking all receptor sites (**Fuller, 1975**). This result was supported by the findings of (**Higgins et al., 2007**), who found that, the addition of probiotic to the broiler diets was more effective in reducing Salmonella colonization and reduced mortality rate. Similar results obtained by (**EL-Hammady et al., 2014**), who reported that, the addition of probiotics to the broiler diets had significant effect ($p < 0.05$) on mortality rate. On the other hand, the results were in contrast with the findings of (**Alloui et al., 2012; Zhang and Kim, 2014**), who found that, inclusion of *Pediococcus acidilactici* as a probiotic in broiler diets had no significant effect ($p > 0.05$) on mortality rate.

The results of this study revealed that, inclusion of dietary (BP) at different levels had no significant effects ($p > 0.05$) on feed intake among treatment groups throughout the experimental period. This result was agreed with the findings of (**Zhang and Kim, 2014; Dizaji et al., 2012; Aliakbarpour et al., 2012**), who found that, the inclusion of protexin and *Bacillus subtilis* as a probiotic had no significant effect ($p > 0.05$) on feed intake of broilers. Similarly (**Mountzouris et al., 2010; Zhu et al., 2009; Miles et al., 2006**), observed that, no significant differences in feed intake of broilers fed on *Lactobacillus salivarius* as a probiotic. This results contrary to the findings of (**Odefemi, 2016; Idoui and Karam, 2016; Panda et al., 2003**), who reported that, supplementation of the diet with *Lactobacillus acidophilus* and *Streptococcus faecium* as a probiotic, significantly ($p < 0.05$) improved the feed intake of broilers.

In this study the addition of dietary (BP) at different levels in broiler diets, improved significantly ($p < 0.05$) the body weight gain (BWG) compared to

negative control group (NC). The levels of inclusion 2 and 3 gm/kg dietary (BP) had higher significantly ($p < 0.05$) BWG compared to the Neomycin antibiotic and 1 gm/kg dietary (BP) groups. Whereas the chicks fed with highest level of dietary (BP) 3gm/kg obtained significantly ($p < 0.05$) higher BWG than those groups fed with Neomycin, 1 and 2 gm/kg dietary (BP).

The improvement in (BWG) by the addition of probiotic may be due to beneficial effects of probiotics by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria. Probiotics are alive micro-organisms that claim to be beneficial to animals and maintain a balance of microflora in the digestive tract (**Goldin, 1998**). Once probiotics established in the gut, produce substances with bactericidal or bacteriostatic properties (bacteriocine) such as lactoferrin, lysozyme, as well as several organic acids (**Fuller, 1989**). Also produce volatile fatty acids (VFAs) as a part of their natural breakdown and metabolism of nutrients in the gut digesta. These substances have a detrimental impact on pathogenic bacteria by lowering the pH below that essential for the survival and inhibit the growth of pathogenic, such as *E. coli* and *Salmonella spp.* (**Fuller, 1989; Pascual et al., 1999; Yoruk et al., 2004; Choudhari et al., 2008**), then increase the population of useful microflora in the gut and promote a better flora balance (**Kabir et al., 2004**). This may lead to better capacity for absorption of available nutrients (**Roosbeh et al., 2012**). Furthermore, the effect of probiotics on reduction of pathogenic bacteria could reduce the breakdown of proteins to nitrogen. In this way the utilization of proteins (amino acids) was improved, particularly from food that does not contain them in optimum quantities (**Mikulec et al., 1999**). Another effect of probiotics is through the competition for adhesion sites on the intestinal epithelium, thus preventing colonies of pathogenic bacteria forming (**Guillot, 2003; O'Dea et al., 2006 Revollo et al., 2006**). This competitive exclusion of harmful bacteria is achieved through colonisation of favourable sites of adhesion such as the

intestinal villous and colonic crypts, or excretion of the mucins from goblet cells which inhibits the adherence enteropathogenic bacteria (**Chichlowski et al., 2007**). Also supplementation of probiotics to broiler diets creation of a microecology that is hostile to other bacteria species, elimination of available receptor sites. In addition, the competition for energy and essential nutrients between probiotic and other bacteria may result in suppression of pathogenic species and prevent implantation in the gut, then modify the intestinal milieu (**Kabir et al., 2004 and Santin et al., 2001**). The improvement of the gastrointestinal ecosystem by addition of probiotics improved intestinal environment, integrity of the intestinal mucosal barrier, digestive and immune function of intestine and broiler health (**Mountzouris et al., 2010**). Another beneficial effect of probiotics is lowering the activities of the intestinal and facial bacteria enzymes (formation of the toxin in the body), by attaching themselves along the chicken intestine, thus preventing colonisation of the bacteria with toxicant-promoting enzymes (**Jin et al., 2000**). Besides, probiotics are responsible for protection against toxins produced by pathogenic micro-organisms, and subsequently improve animal health and growth performance (**Fuller, 1989**). Finally, each of the above mentioned reasons may lead to better growth response of broiler chicks.

The results of this study were consistent with the findings of (**Idoui and Karam, 2016; Pourakbari et al., 2016; Odefemi, 2016**), who found that, the administration of *Lactobacillus plantarum* as a probiotic in broiler diets, had significant positive effect ($p < 0.05$) on (BWG) and improved growth performance. Similarly, the beneficial effects of probiotic on (BWG) of broilers were reported by several researchers (**Zhang and Kim, 2014; Aliakbarpour et al., 2012; Eckert et al., 2010; Zhou et al., 2010**), who mentioned that, the birds fed with probiotic (*Bacillus subtilis and Lactobacillus based*) diets had significantly ($p < 0.05$) higher (BWG) compared with (NC). Also the results were in line with the findings of

(Ohimain and Ofongo, 2012), who stated that, dietary supplements containing probiotics are able to enhance performance while protecting the chickens from microbial infection. Like – wise several researchers observed that, the addition of *Bacillus licheniformis*, protexin and primalac at different levels to broiler diets, increased significantly ($p < 0.05$) BWG (**Mokhtari et al., 2015; Liu et al., 2012; Hassanein and Soliman, 2010; and Shabani et al., 2012**). On the other hand, many researchers indicated that, there were no significant effect ($p > 0.05$) on (BWG) of broilers fed dietary BP (*Bacillus subtilis*) (**EL-Hammady et al., 2014; Karaoglu and Durdag, 2005; Opalinski et al., 2007; Lee et al., 2010**).

Concerning of feed conversion ratio (FCR) in the present study, the results showed that, supplementation of dietary BP at various levels in broiler diets, improved significantly ($p < 0.05$) FCR compared to NC. The inclusion level of 2 and 3 gm /kg dietary (BP) had better significantly ($p < 0.05$) FCR compared to the Neomycin and 1 gm/kg dietary (BP) groups. Whereas the chicks fed with highest level of dietary (BP) 3gm/kg had obtained significantly ($p < 0.05$) better FCR than those groups fed on Neomycin, 1 and 2 gm /kg dietary (BP).

The improvement in FCR by the addition of probiotic may be due to alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, toxin neutralization, enhancement of digestion and utilization of nutrients, and immunity stimulation (**Yeo and Kim, 1997**). Therefore, the major outcomes from using probiotics include improvement in growth, reduction in mortality, and improvement in feed conversion efficiency (**Yeo and Kim, 1997**). Similar results were obtained by (**Alloui et al., 2012; Zhou et al., 2010; Panda et al., 2008**), who reported that, administration of *Pediococcus acidilactici* and *Lactobacillus sporogenes* as a probiotic improved significantly ($p < 0.05$) FCR of broilers. Like-wise several researchers

observed that, the inclusion of (*L. sporogens*, *L. acidofillus* and *S. faecium*) as probiotics to broiler feed, resulted in an improved FCR (**Zhu *et al.*, 2009; Choudhari *et al.*, 2008; Abdel-Raheem *et al.*, 2005; Panda *et al.*, 2003**). In contrast several studies showed that, there were no significant effect ($p>0.05$) on FCR of broilers fed dietary probiotics (protexin, *L. fermentum*) (**Dizaji *et al.*, 2012; Odefemi, 2016; Bai *et al.*, 2013**).

The results of the present study showed that, the carcass dressing percentage was significantly ($p<0.05$) affected by supplementation of dietary (BP). The results were in line with the findings of (**Mahajan *et al.*, 1999**), who found that, mean values of dressing percentage were significantly ($p<0.05$) higher for probiotic (*Lacto – Sacc*) fed broilers. In contrast, several studies obtained by (**Odefemi, 2016; Alloui *et al.*, 2012**), who observed that, there were no significant differences ($p>0.05$) between groups in carcass dressing percentage for probiotic fed broilers.

In this study, the results illustrated that, no significant differences ($p>0.05$) were observed between all treatment groups in giblets percentage (gizzard, liver and heart). This results were in line with the findings of (**EL-Hammady *et al.*, 2014**), who found that, the gizzard, liver, and heart percentage were not affected significantly ($p>0.05$) by the dietary probiotics. This results were partially consistent with the findings of (**Idoui and Karam, 2016; Odefemi, 2016**), who found that, no significant differences ($p>0.05$) were observed between the various treatment groups in liver and heart percentage of broilers fed with dietary probiotics. Also (**Pourakbari *et al.*, 2016**), found that, there were no effects on liver percent of broilers fed with dietary probiotics (*L. plantarum*). This results were disagreed partially with those obtained by (**Idoui and Karam, 2016**), who reported that, the groups fed on probiotics had a higher percent of gizzard compared with (NC) group.

The results of the present study showed that, the addition of dietary (BP) at the different levels were increased significantly ($p<0.05$) the percentage of

commercial cuts (breast, thigh, and drumstick) compared to the (NC) group. The inclusion level of dietary (BP) 3gm/kg had significantly ($p < 0.05$) higher breast, thigh and drumstick percentage compared to Neomycin, 1 and 2 gm/kg dietary (BP). Whereas, no significant differences ($p > 0.05$) between (NC) and Neomycin groups in the percentage of commercial cuts. This results were partially consistent with the findings of (**Mehr *et al.*, 2007**), who reported that, birds fed with higher level of probiotic had obtained higher percent of breast compared with (NC). Also, (**Odefemi, 2016**), found that birds fed with probiotics had higher percent of drumstick compared to (NC). On the other hand, the results were in contrast partially with the findings of (**Pourakbari *et al.*, 2016**), who observed that, there were no significant effect on breast and drumstick percentage of broilers fed probiotics diets. Also, (**Odefemi, 2016; and Mokhtari *et al.*, 2015**), reported that, no significant differences ($p > 0.05$) were observed between various treatment groups in breast and thigh percentage of broilers fed probiotics.

In this study the results showed that, (breast, thigh, drumstick meat %) were increased significantly ($p > 0.05$) in broilers fed dietary BP compared to (NC). The inclusion level of dietary BP 3gm/kg had significantly ($p < 0.05$) higher percentage of (breast, thigh, and drumstick meat) compared to Neomycin, 1 and 2 gm/kg dietary (BP). Whereas, no significant differences ($p > 0.05$) between Neomycin and (NC) groups in meat percent of commercial cuts. This results were contrary with the findings of (**Alloui *et al.*, 2012**), who stated that, the administration of *Pediococcus acidilactici* as a probiotic had no effect on the breast and thigh meat percentage.

No significant differences ($p > 0.05$) were observed among all treatment groups in the subjective meat quality attributes (color, flavor, juiciness, and tenderness) and all scores being above moderate values in the present study. This results were supported by the findings of (**Loddi *et al.*, 2000**), who reported that, neither probiotic nor antibiotic affected the subjective meat

quality attributes (flavor, color, juiciness, and tenderness). The results were disagreed with that obtained by (**Kabir et al., 2005; Liu et al., 2012**), who found that, administration of *Bacillus licheniformis* as probiotics in broiler diets, improved significantly ($p < 0.05$) meat quality and sensory attributes (flavour, tenderness, juiciness, and colour). Also the results were contrary with the findings of (**Zhang et al., 2005; Mahajan et al., 2000**), who indicated that, supplementation of probiotics (Lacto-Sacc) in broiler diets had significant effects ($p < 0.05$) on sensory parameters.

In this study the results showed that, application of dietary (BP) had significant effect ($p < 0.05$) on performance and carcass characteristics of broilers. However, the results cited in literature are highly variable about the degree of improvement in productive performance and carcass characteristics of broilers obtained by dietary probiotic as growth promoters. This may be attributed to the variation efficiency of this natural feed additives which depends on several factors, such as microbial species, bacterial strain (single or multi strain), viability, administration level, application method, frequency of application, bird strain, bird age, overall diet, overall farm hygiene status and environmental stress factors (**Patterson and Burkholder, 2003; Choudhari et al., 2008; Mountzouris et al., 2010**).

The results of economical evaluation of experimental diets, showed that the addition of dietary (BP) at various levels to the diet of broiler was economically more profitable compared to (NC). This may be due to the highest return of the weight gains recorded by chicks fed these feed additives without affected feed intake significantly. The value of profitability ratio (1.23) of group E (3gm/kg dietary BP) was the highest of the tested groups. This results were in line with the finding of (**Elfaki, 2015; Mohamed, 2015**), who indicated that, supplementation of probiotics in broiler diets had economically more profitable compare to (NC).

CONCLUSION AND RECOMMENDATIONS

Conclusion:

- In conclusion, the results of this study showed that all levels of bacterial probiotic (BP) added to the diet as natural feed additives significantly improved the body weight gain and feed conversion ratio without any effect on feed intake of the broiler chicks.
- The inclusion level of dietary (BP) 3 gm/kg had significantly ($p < 0.05$) recorded the best performance of broiler chicks.
- The results of the present study indicated that, no mortalities were recorded in all treatment groups throughout the experimental period.
- Adding of dietary (BP) at all inclusion levels in the broiler diets had significant effect on carcass dressing percentage, commercial cuts and their percentage of separable tissue compared to negative control (NC).
- Inclusion of different levels of dietary (BP) in broiler diets made no change in giblets percentage and the subjective meat quality attributes.
- Using of dietary (BP) at all inclusion levels in broiler diets economically is profitable.

Recommendations:

Practical implications:

- Based on the results of this study, dietary (BP) could be considered as potential growth promoters that may replace the antibiotic in broiler diets without any adverse effect.
- All levels of dietary (BP) added to broiler diet in this study were recommended economic – wise, but the level of dietary (BP) 3 gm/kg was more profitable.

Suggestion for future research:

- More trails are needed to clarify the effects of dietary (BP) product as natural feed additives on performance, carcass yield and meat quality, digestive system development, immune system, intestinal micro flora and blood constituent of poultry with regard to various management condition, including different stress factors, species and strain of bacteria, optimal dietary (BP) application levels, dietary ingredients and nutrients contents.
- Further experiments are needed to test the synergistic effect of dietary (BP) to prove additive or other wise.
- Finding of this study piont to the possibility of using (BP) as natural feed additives in layers as well as testing for egg production and quality.
- The future research also should be focus on the use of other natural feed additive such as herbs and spices, essential oils extracted from aromatic plants, enzymes prebiotic, synbiotic and organic acid in poultry production.

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Appendices

Appendix 1. Weekly minimum and maximum experimental temperature during the 15th January to 18th February. Temperature (°C) 2016

Weeks	Minimum	Maximum
1	13	32
2	12	28
3	10	26
4	12	30
5	13	34
Average temperature	12	30

Appendix -2

Card used for judgment of subjective meat quality attributes sensory evaluation. Evaluate these sample for tenderness, flavor, color and juiciness, for each sample, use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample, if you have any question please ask, thanks for your cooperation

Name:

Date:

Tenderness	Flavor	Color	Juiciness
8-Extremely tender	8-Extremely intense	8-Extremely desirable	8-Extremely juicy
7-Very tender	7-Very intense	7-Very desirable	7-Very juicy
6-Moderately	6-Moderately intense	6-Moderately	6-Moderately juicy
5-Slightly tender	5-Slightly intense	5-Slightly desirable	5-Slightly juicy
4-Slightly tough	4-Slightly bland	4-Slightly undesirable	4-Slightly dry
3-Moderately tough	3-Moderately bland	3-Moderately undesirable	3-Moderately dry
2-Very tough	2-Very bland	2-Very undesirable	2-Very dry
1-Extremely tough	1-Extremely bland	1-Extremely undesirable	1-Extremely dry

Serial	Sample code	Tenderness	Flavor	Color	Juiciness	Comments
A	1					
B	2					
C	3					
D	4					
E	5					

Appendix -3

Arbor Acres Plus Broiler *As-Hatched Performance* 9

Day	Body Weight (g) ¹	Daily Gain (g)	Average Daily Gain/Week (g)	Daily Intake (g)	Cumulative Intake (g) ²	FCR ³
0	42					
1	57	15		14	14	0.246
2	72	15		17	31	0.433
3	89	17		20	51	0.577
4	109	20		24	75	0.688
5	131	22		27	102	0.776
6	156	25		31	132	0.846
7	185	28	20.40	35	167	0.903
8	216	31		39	206	0.951
9	251	35		43	249	0.991
10	289	38		48	296	1.026
11	330	41		53	349	1.057
12	375	45		58	407	1.085
13	423	48		63	470	1.111
14	474	51	41.37	69	539	1.136
15	529	55		74	613	1.159
16	587	58		80	694	1.181
17	648	61		86	780	1.203
18	713	64		92	872	1.224
19	780	67		99	971	1.245
20	850	70		105	1076	1.266
21	923	73	64.06	111	1187	1.286
22	998	75		117	1304	1.306
23	1076	78		123	1427	1.326
24	1156	80		129	1556	1.346
25	1238	82		135	1691	1.366
26	1322	84		141	1832	1.386
27	1408	86		147	1979	1.406
28	1495	87	81.74	152	2131	1.426
29	1584	89		158	2289	1.446
30	1674	90		163	2452	1.465
31	1764	91		168	2621	1.485
32	1856	92		173	2794	1.505
33	1949	93		178	2972	1.525
34	2042	93		183	3155	1.545
35	2136	94	91.52	187	3342	1.565
36	2230	94		191	3533	1.585
37	2324	94		195	3728	1.605
38	2418	94		199	3928	1.624
39	2512	94		203	4131	1.644
40	2606	94		206	4337	1.664
41	2699	94		210	4547	1.684
42	2793	93	93.86	213	4759	1.704
43	2885	93		216	4975	1.724
44	2978	92		218	5193	1.744
45	3069	91		221	5414	1.764
46	3160	91		223	5637	1.784
47	3250	90		225	5863	1.804
48	3339	89		227	6090	1.824
49	3427	88	90.58	229	6319	1.844
50	3514	87		231	6550	1.864
51	3600	86		232	6782	1.884
52	3684	85		233	7015	1.904
53	3768	83		234	7250	1.924
54	3850	82		235	7485	1.944
55	3931	81		236	7721	1.964
56	4010	79	83.32	236	7957	1.984
57	4088	78		237	8194	2.004
58	4164	76		237	8431	2.025
59	4239	75		237	8667	2.045
60	4312	73		236	8903	2.065
61	4384	71		236	9139	2.085
62	4453	70		235	9374	2.105
63	4521	68	73.03	234	9608	2.125
64	4587	66		233	9841	2.145
65	4652	64		232	10072	2.165
66	4714	62		230	10302	2.185
67	4774	60		228	10530	2.206
68	4833	58		226	10757	2.226
69	4889	57		224	10981	2.246
70	4944	55	60.39	222	11203	2.266

¹On-barn body weight (i.e., feed present in intestinal tract).

²Feed consumption per living bird.

³FCR includes initial body weight at placement and does not account for mortality.

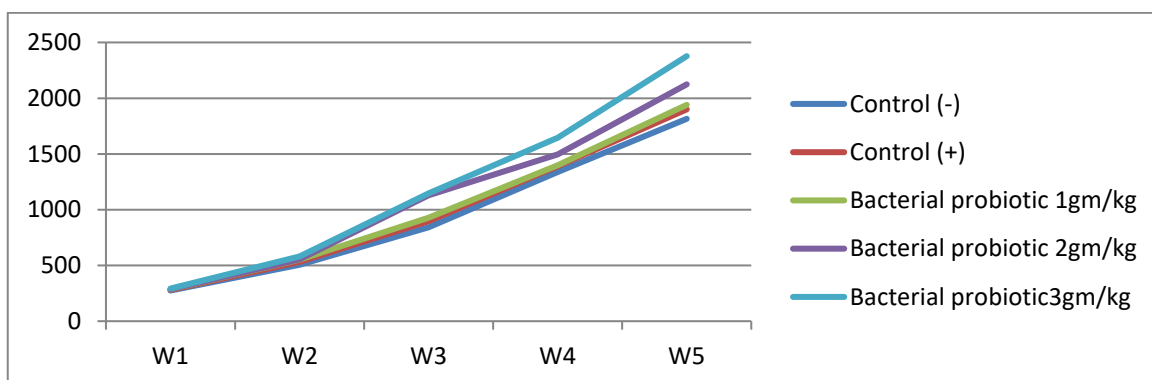
Appendix-4

Table: Effect of adding different levels of bacterial probiotic (*Bacillus cereus*. Var. Toyoi) on final body weight (gm) b/w

Items	W1	W2	W3	W4	W5
(A) Control (-)	275a	506b	841c	1339d	1815d
(B)Control(+) Antibiotic	280a	531b	897b	1389c	1900c
(C)Bacterial probiotic 1gm/kg	285a	551b	927b	1400c	1940c
(D)Bacterial probiotic 2gm/kg	287a	554a	1130a	1495b	2124b
(E)Bacterial probiotic 3gm/kg	293a	581a	1145a	1645a	2377a

Any tow mean values having same superscript within rows are not significantly different ($p < 0.05$)

Figure: Effect of adding different levels of bacterial probiotic (*Bacillus cereus*. Var. Toyoi) on final body weight (gm) b/w



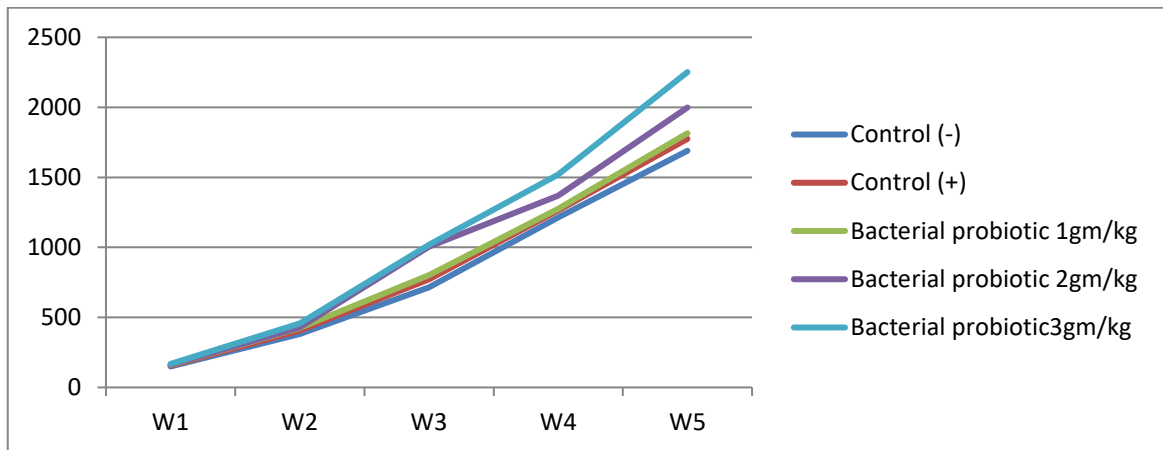
Appendix-5

Table: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on body weight gain (gm) b/w

Items	W1	W2	W3	W4	W5
(A)Control(-)	150a	381b	716c	1214d	1690d
(B)Control(+)	155a	406b	772b	1264c	1775c
(C)Bacterial probiotic 1gm/kg	160a	426ab	802b	1275c	1815c
(D)Bacterial probiotic 2gm/kg	162a	429a	1005a	1370b	1999b
(E)Bacterial probiotic 3gm/kg	168a	456a	1020a	1520a	2252a

Any two mean values having same superscript within rows are not significantly different ($p < 0.05$)

Figure: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on body weight gain (gm) b/w



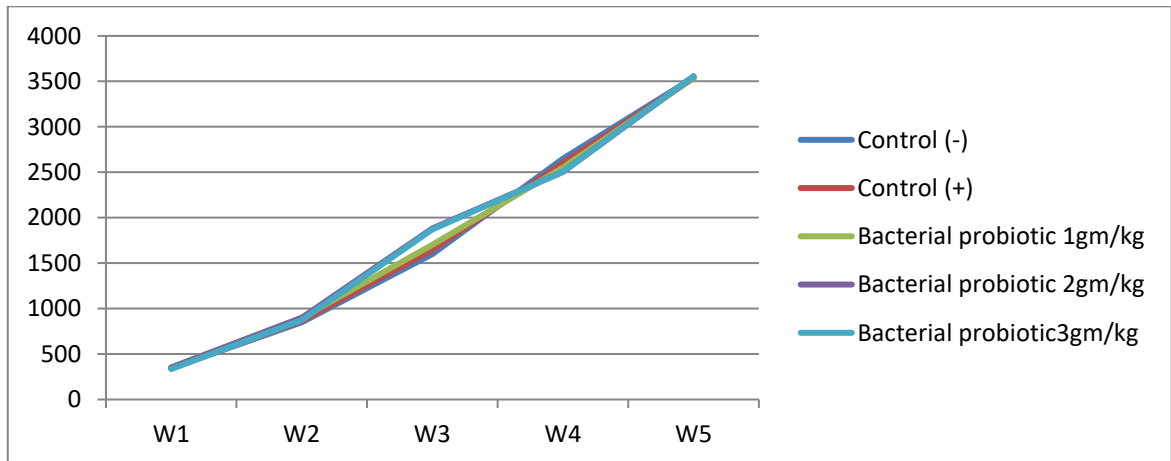
Appendix-6

Table: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on feed intake (gm) b/w

Items	W1	W2	W3	W4	W5
(A)Control(-)	345a	850a	1600a	2650a	3540a
(B)Control(+) Antibiotic	345a	865a	1650a	2600a	3536a
(C)Bacterial probiotic 1gm/kg	355a	900a	1700a	2550a	3538a
(D)Bacterial probiotic 2gm/kg	350a	900a	1880a	2503a	3555a
(E)Bacterial probiotic 3gm/kg	335a	875a	1870a	2505a	3557a

Any tow mean values having same superscript within rows are not significantly different ($p < 0.05$)

Figure: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyoi*) on feed intake (gm) b/w



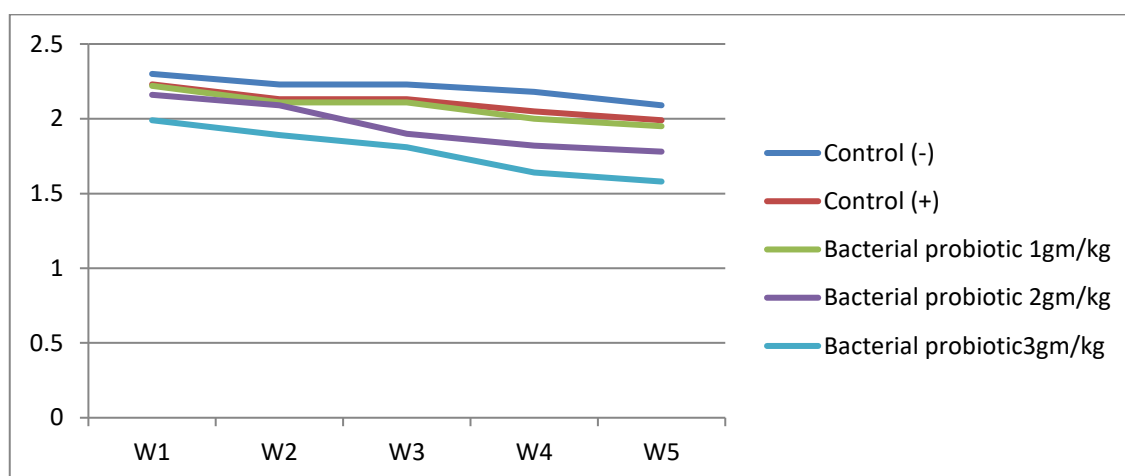
Appendix-7

Table: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyo*) on feed conversion ratio (FCR)

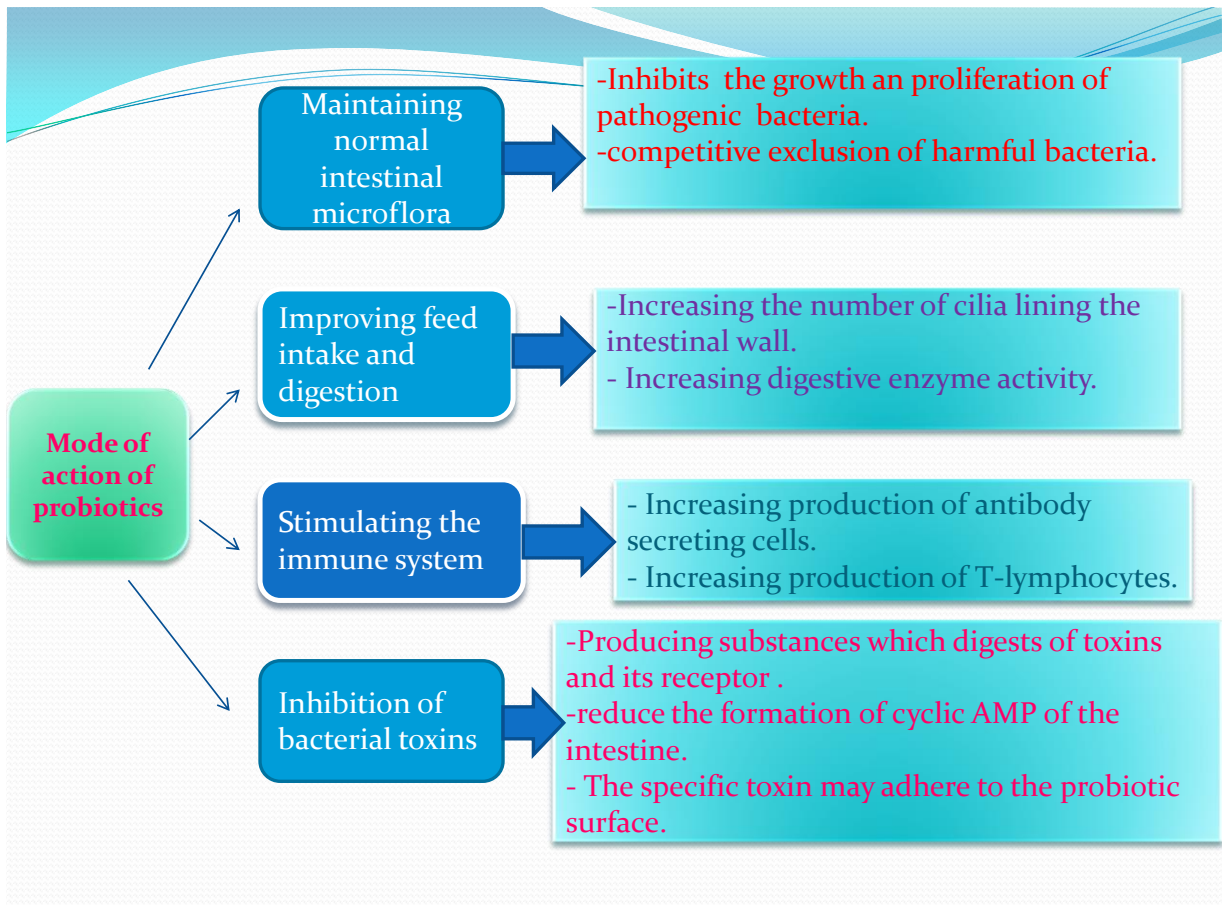
Items	W1	W2	W3	W4	W5
(A)Control(-)	2.30 ^b	2.23 ^c	2.23 ^c	2.18 ^d	2.09 ^d
(B)Control(+)	2.23 ^b	2.13 ^b	2.13 ^b	2.05 ^c	1.99 ^c
(C)Bacterial probiotic 1gm/kg	2.22 ^b	2.11 ^b	2.11 ^b	2.00 ^c	1.95 ^c
(D)Bacterial probiotic 2gm/kg	2.16 ^b	2.09 ^b	1.90 ^a	1.82 ^b	1.78 ^b
(E)Bacterial probiotic 3gm/kg	1.99 ^a	1.89 ^a	1.81 ^a	1.64 ^a	1.58 ^a

Any two mean values having same superscript within rows are not significantly different ($p < 0.05$)

Figure: Effect of adding different levels of bacterial probiotic (*Bacillus cereus* var. *Toyo*) on feed conversion ratio (FCR)



Mode of action of probiotic



Appendix-9

Classification of *Bacillus cereus* var. *Toyoi*

Scientific classification	
Domain:	Bacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Bacillaceae
Genus:	<i>Bacillus</i>
Species:	<i>B. cereus</i>

Variety

Toyoi

Appendix -10



Birds during adaptation period



Birds in the 5th week (at the end of the experiment period)



Birds in different stages of growth



Sample of carcass weight

