Sudan University of Science and Technology,

College of Graduate Studies

Response of Broiler Chicks to Various Levels of Dietary Probiotic, Prebiotic and Synbiotic as Natural Growth Promoters

إستجابة الدجاج اللاحم للعلائق المحتوية على مستويات مختلفة من البروبايوتك، البريبايوتك والساينوبايوتك محفزاتاً طبيعية للنمو

A Thesis Submitted in Fulfillment for the Requirements of the Sudan University of Science and Technology for the Degree of **Doctor of Philosophy**

(Animal Production)

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April 2022

Sudan University of Science & Technology

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جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية الدراسات العليا

Ref: SUST/ CGS/AI1

Approval Page

(To be completed after the college council approval)

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Thesis title: Response of broiler chicks to various Levels of dietary Probiotic, Prebiotic and Synbiotic as natural growth promoters.

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صدق الله العظيم

سورة الواقعة الآية 21

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.

With my love

IGBAL

ACKNOWLEDGEMENTS

I would firstly like to thank **My God** for giving me patience and support to complete these studies and make it a reality.

I wish to show my appreciation to **My Supervisor Prof. Dr. Kamal Abdelbagi Mohammed**, Department of Animal Production, College of Agricultural Studies, Sudan University of Sciences and Technology, for his guidance, help and for revising the text and giving valuable advices, and close attention and patient through this work and had never preserved his efforts to help, I am so grateful for him.

I would like to express my deep gratitude to **My Co- Supervisor Prof. Dr. Mukhtar Ahmed Mukhtar**, Department of Animal Production, College of Agricultural Studies, Sudan University of Sciences and Technology, for his unlimited help, who gave me the golden opportunity to do this fruitful research and help me to publish my research article in World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS).

My thanks extend to **Staff and workers** of Animal production Department, College of Agricultural Studies, Sudan University of Science and Technology, for their assistance.

Especially thank to **My Friends** who helped me in this work.

And I cannot fail to thank all **My Family**, for their unlimited help and support during my research study, especial thanks to **My Son Mohamed and My Dear Daughter Sara** for their unlimited help.

I am thankful to all those who helped me and whose name could not be mentioned here.

ABSTRACT

Three experiments were conducted to evaluate the response of broiler chicks to diet containing various levels of dietary commercial natural products which are Bacterial Probiotic Biogen.S (BPB), Prebiotic Y-MOS (PYM) and Synbiotic Biogen.S + Y-MOS 1:1 (SBYM) as natural growth promoters. Experiment parameters covered growth performance, carcass characteristics, serum attributes and economic appraisal of broilers. The experimental design used was complete randomize design (CRD). A total of 288, five days old, 170g initial weight, unsexed Cobb-500 strain broiler chicks were used. The chicks were divided into 3 experimental groups of 96 birds in each experiment, and randomly assigned to 4 treatment diets with three replicates, each of eight chicks (3x4x3x8). The first group A fed on basal diet without feed additives as control diet, the other groups B, C, and D were fed on basal diet supplemented with one of tested products, in each experiment at graded levels of (0.5, 1.0 and 1.5g/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 recorded no mortalities throughout the experimental period. The application of dietary BPB, PYM and SBYM at all inclusion levels improved significantly ($p \leq 0.05$) the broilers performance compared to control without any effect on feed intake of broilers. The results also, reveal that, the addition of dietary BPB, PYM and SBYM in broiler diets significantly ($p \leq 0.05$) affect carcass dressing percentages. The results showed no significant differences ($p \geq 0.05$) among all treatment groups in giblets percentages (gizzard, liver and heart) and non-carcass components except intestine length, the inclusion levels 1.0 and 1.5g/kg of dietary PYM and SBYM had recorded significantly ($p \leq 0.05$) the longest means values of an intestine as compared to the inclusion level 0.5g/kg and

control. Also, the addition of dietary BPB, PYM and SBYM in broiler diets recorded no significant differences ($p \geq 0.05$) in percentages of commercial cuts and their meat (breast, thigh and drumstick) and the subjective and objective meat quality attributes, the same trend for serum metabolites (total protein, albumin, creatinine, uric acid, urea, cholesterol, HDL, LDL, triglyceride and glucose), serum enzymes (AST and ALP) and serum minerals (Ca and P) of broilers, except the dietary SBYM at all inclusion levels recorded significantly higher means values of serum minerals (Ca and P) compared to control.

The results of interaction between dietary Probiotic, Prebiotic, Synbiotic and their levels recorded significant improvement in body weight, body weight gain and FCR with increasing inclusion level compared to control diet, whereas, Synbiotic treatment obtained the best performance followed by Prebiotic, and then Probiotic, and the level 1.5g/kg recorded the best level followed by 1.0g/kg, and then 0.5g/kg.

The results of economical evaluation of experimental diets showed that, the addition of dietary BPB, PYM and SBYM at all inclusion levels are economically profitable compared to control, although the level 1.5g/kg of all tested products was more profitable (1.54, 1.67 and 1.73 respectively). The results of comparative between treatments Probiotic, Prebiotic and Synbiotic in profitability ratio showed that, Synbiotic treatment was more profitable followed by Prebiotic, and then Probiotic. According to the results of these studies, dietary Probiotic, Prebiotic and Synbiotic could be considered as potential natural growth promoters without any adverse effect, and can be used as replacement for antibiotics in broiler diets.

ARABIC ABSTRACT

الملخص

تم إجراء ثلاثة تجارب لتقييم مدى إستجابة الدجاج اللاحم للعلائق المحتوية على مستويات مختلفة من المنتجات الطبيعية التجارية والتي شملت ال Probiotic المعزز الحيوي الباكتيري (Biogen.S)، ال Prebiotic البادئ الحيوي (Y-MOS) وال Synbiotic المتوافق الحيوي الذي يتكون من مزيجي ال Probiotic (Biogen.S) وال Prebiotic (Y-MOS) بنسبة 1:1 كمحفزات طبيعية للنمو. شملت قياسات التجارب الأداء الإنتاجي، خصائص الذبيحة، خواص مصل الدم والتقييم الإقتصادي للدجاج اللاحم. صممت كل تجربة بإستخدام النظام العشوائي الكامل (CRD). تم إستخدام (288) كتكوت عمر خمسة أيام بمتوسط وزن إبتدائي (170) جرام من سلالة C0bb-500 غير مجنسة. تم تقسيم الكتاكيت عشوائيا الى ثلاثة مجموعات تجريبية بكل مجموعة 96 كتكوت (لكل تجربة). وقسمت كل تجربة الى أربعة معاملات تغذوية إحتوت كل معاملة تلاتة مكررات وبكل مكرر 8 كتاكيت (3x4x3x8). المجموعة الأولى (A) تمت تغذيتها على عليقة أساسية بدون أي إضافة علفية. أما المجموعات الآخرى (B, C and D) تمت تغذيتها على العليقة الأساسية مع أحد المنتجات التجريبية لكل تجربة بمستويات متدرجة (0.5، 1.0و1.5جرام لكل كيلو جرام علف على التوالي). تم تكوين العليقة الأساسية وفقآ للإحتياجات الغذائية للدجاج اللاحم طبقآ لمجلس بحوث التغذية 1994.

لم تسجل النتائج أي حالات نفوق خلال فترة التجربة لكل المعاملات. أظهرت النتائج أن إضافة ال Probiotic (Biogen,S)، ال Prebiotic (Y-MOS) و ال Synbiotic (Y-MOS + Biogen.S) بكل مستوياتها أدت الى تحسن معنوي عند مستوى معنوية 5% في الأداء الإنتاجي للدجاج اللاحم مقارنة مع العليقة القياسية بينما لا يوجد أي تأثيرمعنوي في العليقة المستهلكة بين كل المجموعات التجريبية. أوضحت النتائج أيضآ أن كل الإضافات أثرت معنويآ على نسبة التصافي. أظهرت النتائج أنه لا توجد فروق معنوية بين المجموعات في نسب الأعضاء الحيوية القانصة، الكبد، القلب والمكونات الغير مأكولة من الذبيحة ما عدا في طول الإمعاء، نجد أن مستويات الإضافة (1.0 و1.5 جرام لكل كيلوجرام علف) لإضافة ال Prebiotic وال Synbiotic سجلت معنويآ أطول قيم لمتوسطات الإمعاء مقارنة بمستوى الإضافة 0.5 جرام لكل كيلو جرام و مقارنة بالعليقة القياسية.

أيضا لا توجد فروق معنوية عند مستوى معنوية 5% في نسب القطعيات التجارية (الصدر، الفخذ والساق)، اللحم المشفى وصفات جودة اللحم الإنطباعية النوعية والموضوعية، وكانت نتائج التحليل لمكونات مصل الدم

في نفس الاتجاه إذ لا توجد فروق معنوية بين المعاملات في (مستوى البروتين الكلي، الألبيومين، الكيراتنين، حمض اليوريك، اليوريا، الكلسترول العام، الكلسترول المرتفع والمنخفض، ثلاثي الجلسرايد والجلكوز)، كذلك إنزيمات المصل (AST and ALP) ومعادن المصل (الكالسيوم والفسفور)، ما عدا إضافة ال Synbiotic (Y-MOS + Biogen,S) بكل مستوياتها سجلت معنوياً أعلى قيم متوسطات للمعادن (Ca and P) في مصل الدم مقارنة بالمجموعة القياسية.

سجلت نتائج التفاعل والمقارنة بين العلائق المحتوية على ال Probiotic ، ال Prebiotic وال Synbiotic بمستوياتها وجود تحسن معنوي في وزن الجسم، الوزن المكتسب ومعدل التحويل الغذائي مع زيادة مستوى الدمج مقارنة بالعليقة القياسية، بينما حصلت معاملة ال Synbiotic على أفضل أداء إنتاجي تليها معاملة ال Prebiotic ثم معاملة ال Probiotic، والمستوى 1.5 هو الأفضل يليه 1.0 ثم 0.5 جرام لكل كيلو جرام علف.

أظهرت نتائج التقييم الإقتصادي للعلائق التجريبية أن إضافة ال Probiotic، ال Prebiotic وال Synbiotic بكل مستوياتها مربحة إقتصاديا مقارنة مع العليقة القياسية، ولكن مستوى الإضافة 1.5 جرام لكل كيلو جرام علف لجميع الإضافات المختبرة كان الأكثر ربحية (1.54، 1.67، 1.73 على التوالي). كما أظهرت نتائج المقارنة بين المعاملات في الربحية النسبية أن معاملة ال Synbiotic كانت الأكثر ربحية تليها معاملة ال Prebiotic ثم معاملة ال Probiotic .

إستناداً لنتائج هذه التجارب إتضح أن إضافة ال Probiotic، ال Prebiotic وال Synbiotic يمكن أن تعتبر محفزات طبيعية للنمو دون أي تأثير سلبي، وعليه فإنه يمكن إستخدامها كبديل للمضادات الحيوية في علائق الدجاج اللاحم.

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LIST OF ABBREVIATIOS

No.	Abbreviation	Meaning
1-	**ADG**	Average Daily Gain
2-	**AGP**	Antibiotic Growth Promoters
3-	**ALP**	Alkaline phosphatase
4-	**AMP**	2-Amino-2-Methyl-1-Propanol
5-	**AOAC**	Association of Official Analytical Chemists
6-	**AST**	Aspartate Amino Transferase
7-	**BPB**	Bacterial Probiotic Biogen.S
8-	**BS**	Bacillus subtilis
9-	**BWG**	Body Weight Gain
10-	**CA**	Crude Ash
11-	**CAMP**	Cyclic Adenosine Mono-phosphate
12-	**CD**	Cluster of Differentiation
13-	**CE**	Competitive Exclusion
14-	**CF**	Crude Fat
15-	**CFU**	Colony Forming Unit
16-	**COS**	Chitosan oligo-saccharides
17-	**CP**	Crude Protein
18-	**CRD**	Complete Randomize Design
19-	**CV**	Coefficient of variation
20-	**DFM**	Direct Fed Microbial
21-	**DNA**	Deoxyribonucleic Acid
22-	**DP**	Degrees of Polymerization
23-	**E. coli**	Escherichia coli
24-	**EFAs**	Essential Fatty Acids
25-	**EOs**	Essential Oils

26-	**EU**	European Union
27-	**FAO**	Food and Agriculture Organization
28-	**FBW**	Final Body Weight
29-	**FCR**	Feed Conversion Ratio
30-	**FI**	Feed Intake
31-	**FOS**	Fructo-oligo-saccharides
32-	**GALT**	Gut-associated Lymphoid Tissue
33-	**GE**	Growth Efficiency
34-	**GGMO**	Galacto-glucomannan oligo-saccharides
35-	**GGMO-AX**	Galacto-glucomannan oligosaccharides-arabinoxylan
36-	**GIT**	Gastro-intestinal Tract
37-	**GOS**	Glacto-oligo-saccharides
38-	**GOT**	Glutamyl Oxaloacetic Transaminase
39-	**HDL**	High Density Lipoproteins
40-	**HSD**	Honest significant difference
41-	**IBD**	Infechious Bronchitis Disease
42-	**IFN-gamma**	Interferon-gamma
43-	**IgA**	Immunoglobulin A
44-	**IgG**	Immunoglobulin G
45-	**IgM**	Immunoglobulin M
46-	**ISAPP**	International Scientific Association for Probiotics and Prebiotics
47-	**LAB**	Lactobacillus
48-	**LDL**	Low Density Lipoproteins
49-	**LSD**	Least Significant Difference
50-	**MDH**	Malate Dehydrogenase
51-	**MOS**	Mannan-oligo-saccharides
52-	**NC**	Negative Control
53-	**ND**	Newcastle Disease

54-	**NFE**	Nitrogen Free Extraction
55-	**NLRs**	Nod-like Receptors
56-	**NRC**	Nutritional Research Council
57-	**NS**	No significant
58-	**NSP**	Non-starch Polysaccharide
59-	**OF**	Oligo-fructose
60-	**PAMPs**	Pathogen-associated Molecular Patterns
61-	**PC**	Positive Control
62-	**PH**	Potential Hydrogen
63-	**PRRs**	Pattern Recognition Receptors
64-	**PYM**	Prebiotic Y-MOS
65-	**RNA**	Ribonucleic Acid
66-	**SBYM**	Synbiotic Biogen.S + Y-MOS
67-	**Sc**	Saccharomyces cerevisiae
68-	**SCFAs**	Short-Chain Fatty Acids
69-	**SE**	Standard Error
70-	**SGR**	Specific Growth Rate
71-	**TEO**	Thyme Essential Oil
72-	**TLC**	Total Lactobacillus Count
73-	**TLRs**	Toll-like Receptors
74-	**TVC**	Total Viable Count
75-	**VFAs**	Volatile Fatty Acids
76-	**VLDL**	Very Low- Density Lipoproteins
77-	**WHO**	World Health Organization
78-	**WY**	Whole Yeast
79-	**XOS**	Xylo-oligo-saccharides
80-	**YE**	Yeast Extract

CHAPTER ONE

INTRODUCTION

Poultry industry is under increasing pressure to produce high quantity and quality products for consumers. Commercial poultry production ranks among the highest source of animal protein and the increase in the size of the poultry industry has been faster than other food-producing animal industries (Cengiz *et al*., 2015). 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 improvement of health and performance of birds (Abudabos *et al*., 2017 and Khan *et al*., 2016). 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 (Holmes *et al*., 2016). Since January 2006 the uses of antibiotic as growth promoter was prohibited by the European Union and have been banned or limited in many countries (Abou-Zeid *et al*., 2015 and Attia *et al*., 2014). Currently, many parts of the world are experimenting alternative natural feed additives that be used to elevate the problems associated with the withdrawal of antibiotics from feeds, Alleman, (2013), such as herbs, spices, various plant extracts, antioxidants, enzymes, probiotics, prebiotics and synbiotic (Ricke, 2018; Abo Omar *et al*., 2016 and Karadas *et al*., 2016). Probiotics have become more popular in the world of dietary supplements and feed additives within the poultry industry, acting as antibiotic substitutes (Krysiak *et al*., 2021). A probiotic has been defined as alive microbial feed supplement which beneficially affects the host animal by improving its intestinal balance, (Majidi-Mosleh *et al*., 2017). Probiotics have shown promise as an alternative to in-feed antibiotics in reducing enteric diseases and eliminating subsequent contamination of poultry products (Krysiak *et al*., 2021 and Al-Shawi

et al., 2020). The most important advantage of probiotic is that it doesn't have any residues in animal products (Rowghani *et al*., 2007). The common probiotic used as feed supplements are the live beneficial bacteria and yeast (Patterson and Burkholder, 2003). They are two main mechanisms that have been proposed to explain how probiotic products work; (1) Nutritional effect. (2) Health effects. 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, bird age, overall diet, overall farm hygiene and environmental stress factors (Mountzouris *et al.*, 2010).

Preboitics, are non-digestible feed ingredients, beneficially affect the host by selectively stimulating the growth and /or activity of one limited number of bacteria in colon and improving the host health (Butel *et al*., 2016). Prebiotics are striking products because they are non-viable, not affected by temperature and variation in moisture like the live micro-organisms probiotic (Hutkins *et al*., 2016). The prebiotics significantly improved body weight, weight gain and feed efficiency, in broilers (Yadav *et al*., 2016). Fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS) and inulin are the major prebiotics; they have shown beneficial effects in performance, gut health and immunity (Huang *et al.*, 2015). The main prebiotic functions are immune-modulation, changes in intestinal microbiota, inhibition of carcinogenesis, nutrient absorption effects and pathogen inhibition by their mechanisms of action (Hamasalim, 2012 and Venter, 2007).

Synbiotics are a combination of probiotics and prebiotics; they display a synergistic relationship that positively affects the host by facilitating the implantation and survival of probiotic micro-organisms in the gastro-intestinal tract (Naghi *et al*., 2017 and Nihar *et al*., 2016). The use of synbiotics in the poultry

industry was based on the irability to balance the gut environment and its microbiota, Dhama *et al*., (2011), by providing substrates for bacterial fermentation, generating antibacterial substances, competing for nutrients and modulating immune responses, Rooks and Garrett, (2016), competing with pathogens for adhesion receptors on the intestinal epithelium, Adil and Magray, (2012), and improves the growth of broilers (Mookiah *et al*., 2014). Synbiotics, when compared with probiotics or prebiotic alone, have beneficial effects on broiler growth performance, intestinal microflora population, cecal volatile fatty acid concentration and intestinal histo-morphological parameters (Hamasalim, 2016 and Bai *et al.*, 2013). Application of Synbiotics can cause concentration of organic acids, reduce cholesterol level and change the population of beneficial poultry intestinal bacteria (Liong *et al*., 2006). Also, Synbiotics have affected on stomach and intestines extent and cause better glucose absorption in poultry (Awad *et al*., 2008).

Therefore, this work has the objectives to assess the effects of graded levels of dietary probiotic, prebiotic and synbiotic commercial products as natural growth promoter's alternative to antibiotics on the performance, carcass characteristics and blood parameters 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. In the past decades, a variety of feed accretive had been employed in poultry diet. These feed accretive led to an improved rendition and effective utilization of feed in poultry birds (Chand *et al*., 2016a; Shah *et al*., 2016; Xing *et al*., 2017 and Saeed *et al*., 2017a,b). 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 (Fadlalla *et al*., 2010 and Abouelfetouh *et al*., 2012). In some intestances 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). Routinely being utilized in accretive of feed as: emulsifiers, antimicrobials, antioxidants, biological products, herbs, pH control agent's binders and enzymes as well (Siyal *et al.*, 2017; Tareen *et al*., 2017 and Saeed *et al*., 2017 c,e). 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) Probiotics, which can be used to influence the intestinal microflora; (3) Enzymes, which under certain condition, to improve the digestibility of specific nutrients; (4) Worming drugs, which are periodically added tofeed 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) Anticoccidials, which are routinely used in broiler feeds and also (usually at lower levels) in diets for rearing replacement pullets; (7) Antifungal, have been used to prevent growth of harmful molds and fungi in feeds or in the digestive tract of the chicken; (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 (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 rapidity the growth and accordingly increase the body size and weight of animals (Biovet, 2005).Growth promoting is not the only use for feed additives but they have used also for stabilizing the beneficial gut microflora by for estalling beneficial microorganisms (Abudabos *et al*., 2017). 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 spoken through better appetite, improved feed conversion, regulating the intestinal micro-flora, stimulation of the immune system and increased animation, etc. (Peric *et al*., 2009).

2.2.1 Antibiotics:

Antibiotics represent a group of chemicals compounds produced biologically by certain plants or micro-organism, 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 bactericidal, and those that slow the growth of bacteria are referred to as bacteria-static, and at the effective levels, are not toxic to chickens or other host animals (Parks *et al.,*2000). 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- range 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 sometimes used in usual 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 world wide in human and animal infectious diseases (Earss, 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 signal 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 sub-therapeutic 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 (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 improve availability or absorption of certain nutrient (Roozbeh *et al*., 2012); (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 favor the growth nutrients-synthesizing microbes or inhibit that of nutrient destroying microorganism; (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 (Miles *et al*., 2006).

2.2.1.2 Ban of antibiotics:

Due to the appearance of residues and resistant strains of bacteria, the use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance (Yoshimura *et al*., 2000). In the last few years, antibiotics that are used in animal feed as growth promoters have been under severe attention, since they pose a potential threat to consumers by generating resistance in the host against the bacteria (Sultan *et al*., 2015). However, the first to suggest that the use of sub-therapeutic levels of antibiotics for growth promotion and disease prevention was reported by Swan committee (1969), this could increase the risk of bacteria acquiring resistance to specific antibiotics (Nasir and Grashorn, 2006). The resistant bacteria present in the gut flora can multiply to higher or lower degree at the time of contact with the antibiotic, while susceptible bacteriaare suppressed in growth or destroyed. Suppression of antibiotics, sensitive bacteria created an opportunity for colonization by resistant bacteria derived from external sources. Frequent use of anti-biotics not only conducive to the formation also fortification of resistance in bacteria (Dankowiakowska and Marek, 2013).

Continued use of antibiotics to promote growth of poultry and other food animals might result in antimicrobial resistance of pathogenic bacteria in humans early as the 1950s. The first report of resistant bacteria in food animals fed an antibiotic reported by Starr and Reynolds (1951), who reported on the resistant bacteria in turkeys after they had been fed streptomycin, may have been the authors

mentioned the possibility of spread of resistant *Salmonella* from poultry to humans, and the possibility of cause disease in the turkeys. Resistant bacteria in poultry have been characterized 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 and Kemmett *et al*., 2013). These transferred, resistant strains can cause infection in young broiler chicks (Kemmett *et al*., 2014). Antibiotic-susceptible strains caused Colibacillosis in young chicks, so the frequency of infections with resistant strain is not known. The report of Huijsdens *et al*., (2006), involved *Staphylococcus aureus*, and the others involved *Salmonella*. A currently ongoing outbreak of multi-drug-resistant *Salmonella heidelberg* infections has been linked to poultry meat from Foster farms in California (CDCP, 2013). Silbergeld *et al*., (2008), have summarized 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, emphasized 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 antibiotics 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). Conclusively, the European Union had banned the supplementation of growth promoting antibiotics in the animal diet since 2006 (Khan *et al*., 2016).

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 to boost the health and production performanceof poultry birds (Babazadeh *et al*., 2011 and Vahdatpour *et al*., 2011). Herbs and spices, essential oils extracted from aromatic plants, enzymes, hormones, organic acid, probiotics, prebiotics, and synbiotics all shown promising beneficial effects on the broiler performance and intestinal health (Arsène *et al*., 2021; Buyarov *et al*., 2020 and Khochamit *et al*., 2020). Several alternatives to growth –promoting antimicrobials have been investigated in recent years (Huyghebaert *et al*., 2011). In modern poultry production, different types of new and safer alternatives growth promoters were used (Alzueta *et al*., 2010):

Enzymes: defined as substances that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process (Kuhne, 1878). The application of feed enzymes to poultry diets for the enhancement of nutrient availability had been reported since 1926. Earlier, the research conducted on feed enzymes in poultry nutrition focused on non-starch poly saccharide (NSP) degrading enzymes, especially β-glucanase and xylanase, in diets containing wheat, barley and rye (Choct, 2006). The use of unconventional feed stuff for poultry production is however limited due to their fibrousness and inability of birds to possess the cellulase enzyme that can digest the fiber (Adebiyi

et al., 2010). The effect of dietary enzyme on the animal is influenced by the type and concentration of the undesirable carbohydrate present in the feed stuff and the age and class of the livestock and poultry that consume it. Enzymes that appear to be beneficial for non-ruminant animals are the xylanases, or more specifically the endo-xylanases for the feed which containrye, triticale and wheat and the β-glucanases or cellulases for those which contain oats and barley (Marquardt *et al*., 1996). Phytase, in addition to the above mentioned enzymes, is an enzyme, which increases the availability of phosphorus from phytate, a bound form of phosphate found in cereals and other plant material (Marquardt *et al*., 1996). It has become available for use in the feed industry and may assist in reducing phosphorus requirements in non-ruminant animals and therefore it can solve problems associated with environmental pollution.The improvements obtained by adding enzymes to the diet of poultry depends on many factors, including the type and amount of cereal in the diet; the level of anti-nutritive factor in the cereal, which can vary within a given cereal; the spectrum and concentration of enzymes used; the type of bird and their age (young bird tend to respond better to enzymes than older birds). Enzyme supplementation to field bean diets has been shown to be effective in improving chick performance (Castanon and Marquardt, 1989). Four main reasons for using enzymes in animal feed: i) to breakdown anti-nutritional factors that are present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous digestive enzymes, can interfere with the normal digestion, causing poor performance and digestive upsets (Sheppy, 2003). ii) To increase the availability of the proteins, starches, and minerals that are either enclosed within the fiber-rich cell wall and, therefore, not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (e.g, phosphorus as phytic acid). iii) To break down specific chemical bonds in raw materials that are not usually broken

down by animal's own enzymes, thus releasing more nutrients. iv) To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be in adequate (Sheppy, 2003).

Phytogenic: defined as a group of natural growth promoters derived from herbs, spices or other plants (Dhama *et al*., 2014). Feed additives of plant origin have gained a great interest in the poultry industry as they are safer, with wide dose range and so exceptional adverse effects (Alzawqari *et al*., 2016 and Abudabos *et al*., 2016). Recently, many experiments had shown a number of significant effects on growth parameters; immune response and gut health status in birds fed on diets contain phytogens (Saeed *et al*., 2015; El-Hack *et al*., 2016 and Saeed *et al*., 2017f, g, h). Spices, plant extracts and herbs received increasing attentions. Essential oils (EOs) are found to have antibacterial ability, and also exhibit antioxidant, anti-inflammatory, anticarcinogenic, digestion stimulating, and hypolipidemic activities (Viuda-Martos *et al*., 2010). The EOs is mixture of fragrant and volatile compounds, which are usually originated from plant, and are named with the aromatic characteristics considering the origin of plant (Oyen and Dung, 1999). The term 'essential' was proposed by Paracelsus in his theory of 'quinta essentia', and described that this quintessence could be an effective element for medical use (Oyen and Dung, 1999). But, the term 'volatile oil' had been proposed in medieval pharmacy (Hay and Waterman, 1993). The use of EOs in enhancing productivity may give promising effects as growth and health promoter. The chemical composition and concentration of EOs are variable. Thus, EOs as single or mixture can be used as growth promoters in broiler production (Kirsti *et al*., 2010). Many studies have shown positive effects of dietary EOs on performance of broilers, carcass quality and quality traits of meat Bampidis *et al*., (2005), and improve immune system (Mathivanan *et al*., 2007). The antimicrobial properties of the

diverse chemical compounds present in EOs are not the result of one specific mode of action, but a cumulative effect on many different targets in various parts of the cell (Burt, 2004). It has been reported that their effectiveness might depend on pH, chemical structure, concentration or the individual bioactive compound, along with the population and types of affected micro-organisms. The antimicrobial mechanisms include different activities, such as membrane disruption by terpenoids and phenolics, metal chelation by phenols and flavonoids, and effect on genetic material by coumarin and alkaloids that are thought to inhibit growth of microorganisms (Cowan, 1999). In many cases, the antimicrobial activity results through a complex interaction between different classes of compounds such as aldehydes, ketones, phenols, esters, alcohols, hydrocarbons or ethers found in the EOs. It was reported that EOs having phenols or aldehydes, for example, cinnamaldehyde, thymol, carvacrol, citral or eugenol as major components could show considerable antibacterial activity (Dormans and Deans, 2000), they also had anti-inflammatory, antioxidant activity, and anti-diarrheal (Botsoglou *et al*., 2004), and also enhance digestibility by facilitating nitrogen absorption and stimulating endogenous enzyme activity (Gill, 2001). Factors that may affect the quality of EOs in plants include soil type, climate, genetics, use of chemical (fertilizers), harvesting, age of the plant, chemo-type, and method of extraction.

Probiotics: defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Majidi-Mosleh *et al*., 2017).

Prebiotic: defined as non-digestible food ingredients that induce the growth or activity of beneficial micro-organismin of the gut, thus confer a beneficial physiological effect on the host (Sobolewska *et al*., 2017and Bindels *et al*., 2015).

Synbiotic: defined as a combination of probiotics and prebiotics which reveals a synergistic relationship that positively affects the host by facilitating the

implantation and survival of probiotic micro-organisms in the gastro-intestinal tract (Naghi *et al*., 2017 and Nihar *et al*., 2016).

Those strategies have paying attention on preventing the proliferation of pathogenic bacteria, modulating beneficial gut microflora and improving the health, immune status and performance (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 (Kamlesh *et al*., 2011), has been defined as "alive microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Majidi-Mosleh *et al*., 2017). Probiotics, sometimes used inter change ably with the term direct fed microbial (DFM), are gaining acceptance as potential alternatives to antibiotics to improve production efficiency (Lee *et al*., 2010). Probiotic is defined as mono or mixed cultures of "live microorganisms which, when administered in adequate amounts confer a health benefit on the host"(Hamasalim, 2015). The definition is very broad provided a basis for the use of numerous bacteria and yeast for the enhancement of health and well-being in host animals. 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*., 2010c). Unlike antibiotics, the probiotics are living organisms and their mode of action relies on replication and survival in the gastro intestinal tracts (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 multi-strains, 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 (Rowghani *et al*., 2007). More accurately, probiotics are live microorganisms of nonpathogenic and nontoxic in nature, which when administered through the digestive path, are favorable to the host's health and improved the immune system, then reducing enteric diseases and eliminating subsequent contamination of poultry products (Krysiak *et al*., 2021 and Al-Shawi *et al*., 2020). 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, Lactococcus, Enterococcus, Bifidobacterium, Escherchia, Streptococcus* and *Lactobacillus*. Several fungal genera, which include *Asperigillus, Oryzae, Saccaromyces cerevisiae* and *Saccaromyces cidophilum*, have also been reported as probiotics (Awad *et al*., 2010 and Ferreira *et al*., 2011). More lately there has been an importance in the use of live yeast cultures as probiotics, such yeast cultures are usually dried from *Sccharomyces species*, in particular, *Sccharomyces cerevisiae*, (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 (Gillot and Ruckebuch, 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 probiotic supplement depends upon what it contains, a good probiotic should have the following criteria (Mohit *et al*., 2012):

*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 have survival ability and multiply quickly in the conditions within the poultry gut (Choudhari *et al*., 2008).

* The culture should not have any side effects. It should be neithertoxicnor pathogenic to the host (Patterson and Burkholder, 2003; Choudhari *et al*., 2008).

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

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

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

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

2.2.2.3 Beneficial effects of probiotics:

A growing body of scientific research supports the role of probiotics as effective alternative to use of antibiotic growth promoters in animal nutrition (Hume, 2011). More recently, beneficial effects of probiotics on: i) Broiler performance (Abudabos *et al*., 2017; Sohail *et al*., 2015), ii) Nutrient digestibility, iii) Modulation of intestinal microflora and improve feed efficiency (Latorre *et al*., 2017; Song *et al*., 2014), iv) Pathogens inhibition (Mountzouris *et al*., 2007). v) Immune modulation and gut mucosal immunity (Bai *et al*., 2013), vi) 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) Intestinal microbial effects,maintaining normal intestinal microflora and prevent pathogenic bacteria such as Salmonella from colonizing in the gut through the mechanism of competitive exclusion and antagonism, support and improve digestive process and improve performance (Abudabos *et al*., 2013). (ii) Metabolic effects, altering metabolism by increasing digestive enzyme activity and supply nutrients, decreasing bacteria enzyme activity and ammonia production (Yoon *et al*., 2004). (iii) Improving feed intake and digestion (Awad *et al*., 2006). (iv) Immuno-modulation strengthens innate immunity and improves resistance to allergies (Apata, 2008). (v) Therapeutic effects, prevention of urogenital infection, synthesis of vitamins (B_2, B_6, B_{12}) and prevention of diarrhea.

The beneficial effects of probiotics are mediated by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria. Later volatile fatty acids (VFAs) were found to be evenly effective in the suppression of pathogenic gut flora and essential fatty acids (EFAs) (linolenic acid and alpha linolenic acid) can be increased via supplementation with probiotics (Yi *et al*, 2014). Probiotics produce VFAs and organic acids as a part of their natural breakdown and metabolism of nutrients in the gut digesta (Majidi-Mosleh *et al*., 2017). 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 proficient via lowering the pH through the production of VFAs which inhibit the growth of harmful bacteria, the bacterial cell takes up un-dissociated fatty acid, which, by ionizing fatty acid inside the bacterial cell, there is a change in the intracellular pH leading to the death of bacteria Khan and Iqbal, (2016), and promote growth performance of broilers (Quaisrani *et al*, 2015). Another mechanism is through the competition for

adhesion sites on the intestinal epithelium, thus preventing colonies of pathogenic bacteria forming (Abudabos *et al*., 2013). This 'competitive exclusion' of harmful bacteria is achieved through colonization of favorable 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 entro-pathogenic bacteria (Chichlowski *et al*., 2007). The colonization of the caecal wall in the chicken and their competitive exclusion effect by lactic acid bacteria has been explained by (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 implicated in producing beneficial impacts on the host's body is the stimulation of the immune system (Bermudez-Brito *et al*., 2012). Mechanisms of action of probiotics to modulate immune system mostly depend on the strains of bacteria or microorganisms used (Huang *et al*., 2004), probiotic preparation method, routes of administration and environment where birds are raised (Ajuwon, 2015). 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, natural killer cells, antibody production, expression of pro- and anti- inflammatory cytokines, interleukins, IFN- gamma, respiratory burst in macrophages and delayed type hypersensitivity reactions (Musa *et al*., 2009). Another mode of action of probiotics is lowering the activities of the intestinal and faecal B-glucuronidase and B-glucosidase-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 colonization of the bacteria with toxicant-promoting enzymes (Jin *et al*., 2000). Additionally, lyzozyme produced by Bifidobacteria, has been recorded to alter the pathogenic activities of bacteria,

reduce antibiotic-induced side-effects, inhibits mammary and liver tumours and in union with oligo-fructose decrease 1, 2-dimethylhydrazine induced carcinogenesis (Chichlowski *et al*., 2007).

Competition for nutrients in the gut, especially carbohydrate, is well recognized (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). Probiotics has also inhibition bacterial toxins 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 (Brandao *et al*., 1998). Secondly, bactrial probiotic reduce the formation of cyclic AMP (cAMP) of the intestine cholera and *E. coli* toxins catalyze the activation of adenyle cyclase causing a rise in cAMP that triggers active secretion of bicarbonate and chloride in crypt cells and inhibits water absorption in the villus resulting in diarrhea. *S. boulardii* was established 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 like lihood 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 localize in the animal gut (Vandenbergh, 1993). This class of small antimicrobial molecules, referred to as bacteriocins, defensins and cathelicidines, act to combat the pathogenic bacteria or impede their colonization. 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

dilapidation, has been shown to inhibit the binding of *Shigella* and *Salmonella* to the intestinal epithelial cells (Chichlowski *et al*., 2007).

Application 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 (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 recorded 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 bacterial cultures, yeast cells, 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 recorded that yeast products affect nutrient digestibility and intestinal mucosal of development (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, reduction the number of

Gram-negative bacteria, suppression the growth of intestinal pathogens, Latorre *et al.*, (2017), 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), improvement in feed conversion efficiency and reduction in mortality (Kumprecht and Zobac, 1998). These results are consistent with previous experiment of Tortuero and Fernandez, (1995), who observed improved feed conversion efficiency with the application of probiotic to the diet.

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

Kabir *et al*., (2005) attempted to evaluate the effect of probiotics with view to clearing bacterial infections and regulating intestinal flora by determining the total lactobacillus count (TLC) and total viable count (TVC) of the crop and cecum samples of probiotics and usual fed groups at the 6^{th} week of age. Their result exposed competitive antagonism, also evidenced that probiotic organisms inhibited some non-beneficial pathogens by occupying intestinal wall space. They also confirmed that broilers fed with probiotics had a trend to display pronounced intestinal histological changes such as active thrust in cell mitosis and increased nuclear size of cells than the controls. This results of histological changes prop up the findings of Samanya and Yamauchi, (2002). and they illustrated that birds who were fed dietary *B. subtilis var. natto* for 28 days had a tendency to display greater growth performance and pronounced intestinal histology's, 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), recorded 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), confirmed that, *L. salivarius* 3d strain reduced the number of *Clostridium perfringens* and *Salmonella enteritidis* 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*. Lately Mountzouris *et al*., (2007) and Higgins *et al*., (2007) demonstrated that, probiotic species belonging to *Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Bacillus, Aspergillus, Candida*, and *Saccharomyces* have a probable 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 latest 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 *Campylobacter spp.* and *Salmonella* (Cox and Pavic, 2009). Callaway *et al*., (2008), affirmed that, the 'link between human *Salmonella* and host animals are most clear in poultry' and those raw eggs and undercooked poultry are considered to be hazardous. Eggs have been implicated as vehicles in numerous out breaks 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 Gastro-intestinal tract (GIT) 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 strains 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, Micrococcus spp., Clostridium perfringens, Klebsiella spp., Staphylococcus spp.* and *Campylobacter spp.* in layers. When poultry meat and eggs were recognized as a vehicle for human *Salmonella*, supplimentation of probiotics as a tool for preventing this disease was actively explored. Cox and Pavic, (2009), recorded that, increased numbers of *Bifido-bacterium spp.* A and *Lactobacillus* associated with reduced *Salmonella spp.* prevalence. Starvic (1987), treated newly hatched chicks with different strains of bacteria belonging to *Lactobacillus, Streptococcus spp., Escherichia* and *Bacteroides,* and observed an increased inhibition of *Salmonella spp.* colonization. Pascual *et al*., (1999), stated that, a single strain of *Lactobacillus salivarius* was able alone to eliminating *Salmonella enteritidis* from the gut of one day old chicks. The immunological properties of probiotics have been extensively studies, indicated that certain *Lactobacilli augment* systemic and mucosal immunity against entero-pathogens, 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 entero-pathogens (Pascual *et al*., 1999).

Probiotics have been added 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 fructo-oligosaccharides, lactose and mannose on the extent of inhibition of *C. jejuni* was explored. These compounds were found to enhance the effectiveness of probiotics. Lately, (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 Paeni-bacillus-polymyxa) substantially reduced *C. jejuni* colonization in live birds. Cox and Pavic, (2009) recorded that, competitive exclusion through probiotics may give the best tool to rule out *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 Campylo-bacteria in humans. Higgins *et al*., (2007), suggested that macrophages are directly or indirectly involved in the decrease of *Salmonella* colonization caused by the application of probiotics.

2.2.2.7 Evaluating probiotic effects on immune response:

Kabir *et al*., (2004), investigated on the dynamics of probiotics on immune response of broilers and they recorded significantly higher antibody production ($p \leq 0.01$) in experimental birds as compared to control ones. They also, illustrated 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. 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. In addition, Haghighi *et al*., (2005), illustrated that, application 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 give 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*, 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. Higgins *et al*., (2007), were 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 immune-modulation (Nayebpor *et al*., 2007; Apata, 2008). On the other hand, Midilli *et al*., (2008), showed the uselessness of additive supplementation of probiotics on systemic IgG.

2.2.2.8 The effect of dietary probiotic on the performance, carcass characteristics and blood parameters of broiler chicks:

Krysiak *et al*., (2021), investigated that, probiotics have become more popular in the world of dietary supplements and feed additives within the poultry industry, acting as antibiotic substitutes. Above all, probiotics are universal feed additives that can be used in conjunction with other additives to promote improved performance and health. Their positive effects can be observed directly in the gastrointestinal tract and indirectly in immune-modulation of the poultry immune system. Nutritional effects seen in flocks given probiotics include increased daily increments, improved feed conversion ratio (FCR), and quality of meat. This suggests producers can improve production results through the use of probiotics. In addition to these production effects, bird immunity is improved by allowing the organism to better protect itself against pathogens and stress. The lack of accuracy in the formulation of non-European preparations needs to be further developed due to unknown interactions between probiotic bacteria strains as well as their metabolites. The versatility of probiotics and the fact that the bacteria used in their production are an integral part of animal digestive tracts make them safe feed additives. Despite restrictions from the European Union, probiotics have potential to improve production and health within the poultry industry and beyond. The following article will review the use of probiotic in poultry production.

Probiotic have a positive effect on overall carcass weight, with abdominal fat reduced, leading to improved poultry carcass quality. Increased carcass yield was

noted in chickens, regardless of sex (Hidayat *et al*., 2016). This represents an important economic side. The presence of probiotic feed additives results in increased absorption of nutrients, including amino acids needed to construct tissues resulting in increased carcass weight (Hidayat *et al*., 2016 and Aziz *et al*., 2020). According to Duskaev *et al*., (2020), probiotics also have a positive effect on raising the amount of chemical elements in the liver (Ca, K, Mg, Mn, Si, and Zn), chicken breast muscles (Ca, Na, Co, Cu, Fe, Mn, Ni, and Zn) and blood biochemical. In this study, the primary parts of the carcass considered were the heart, liver, thighs, breast, back, and neck, whose weight was not significantly increased (Hidayat *et al*., 2016).

Al-Shawi *et al*., (2020), evaluated that, the effect of probiotics on animal growth and development, immune response, and productivity. Several benefits have been associated with the use of probiotics in farm animals, such as improved growth and feed efficiency, reduced mortality, and enhanced product quality. It can be concluded that, the use of probiotics improvegut microbiota composition, immune response, nutrient digestibility and absorption, feed intake, body weight, FCR and meat quality and their sensory properties in broilers. Therefore, future research should focus on finding more effective probiotic strains for the desired use and identifying the optimum dose, administration time, delivery method, and mechanism of action for each strain/host.

Idoui and Karam, (2016), reported on the effects of autochthonous lactobacillus plantarum feeding on growth performances, carcass traits, serum composition and faecal micro-flora of broiler chickens. The broiler chickens were assigned to tow 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 $65x10^{11}$ cfu. The results showed a significant positive effect ($p<0.05$) of probiotic on body weight, feed intake and feed conversion ratio. Also, there was a

significant difference between groups in gizzard percent, while no significant differences between groups in liver, heart and intestine weights percentage. For serum metabolite there was a significant difference between probiotic and control group in cholesterol and triglycerides, probiotic reduce them. While the serum glucose elevated in experimental groups as compared to control group. It was concluded that autochthonous probiotic improved growth and feed efficiency in broiler chickens and consider the improvements in carcass traits. In addition, this probiotic has a cholesterol and triglycerides-depressing effect in the serum and plays a positive effect on gut micro flora of broiler chickens.

Pourakbari *et al*., (2016), investigated on the effects of probiotic levels on growth performance, carcass traits, blood parameters, cecal micro biota, 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 among treatments (breast, drumsticks and liver), but the relative weights of drumstick and wings showed increasing and decreasing linear responses, respectively, to probiotic supplementation levels. Nevertheless, carcass weights showed appositive linear trend with increasing probiotic supplementations, except for abdominal fat was higher in the control treatment and did not show linear trend. Blood serum glucose and albumin contents linearly increased with increasing probiotic supplementations (increasing blood glucose and albumin contents indicated a better digestion and absorption of nutrients in supplemented treatments). Triglycerides and cholesterol contents were lower in probiotic supplementations treatments.

Mohamed, (2014), investigated that, the effect of dairy supplementation of probiotic (*Lactobacillus acidophilus*) on economic and productive efficiency of

broilers. A total number of 450 birds, consists of three breeds (Hubbard, Ross and Cobb) of broilers were used in this research (150 birds for each breed). Two groups of each breed (75) were used, where on treated with probiotic (*L. acidophilus* 1g/kg rations) and control not treated. The different productive and economic measures are applied. The results showed significant effect ($p \leq 0.05$) of probiotic on final body weight, body weight gain and net profit compared to control which given diet without any feed additives. In addition there was no significant difference ($p \geq 0.05$) among all groups of breeds in dressing percentage and abdominal fat percent of broiler chicks. The improvement which occurred in values of net profit of treated groups may be attributed to improvement which occurred in body weight, body weight gain, feed conversion ratio, stimulation of birds immunity and reduction of mortality rate of broiler chicks. Finally we concluded that the probiotic (*L. acidophilus*) play important role in improving the economic and productive efficiency of poultry farm although it constitutes small cost portion from the total or variable costs of poultry production.

Zhang and Kim, (2014), investigated on the effects of multi-strain probiotics supplementation in broilers. The treatments were allotted in to four groups: 1.An antibiotic –free diet (control-). 2. (Control +) 5mg/kg of avilamycin. 3. Control $+1x10^5$ cfu of multi-strain probiotics /kg of diet (p_1) and 4. Control $+2x10^5$cfu of multi-strain 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 ($P<0.05$) heaver final body weight (FBW) 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 BWand 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 weights (gizzard, liver and heart) as well as the lengths of intestines. The abdominal fat percentage in G1 and G4 was decreased compared to the other groups. The total mortality rate of birds in group 3(1g probiotic/kg diet) 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 product (containing $1x10^7$cfu/g of *Lactobacillus fermentum JS* and $2x10^6$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 effects 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*., (2013), reported on the effect of probiotic feed additives on broiler chickens health and performance. Bacterial probiotic used in this experience is a *Pedio-coccus-acidilactici*. The broiler chickens were assigned in to two experimental group's treatment: (10^9cfu/kg of feed of *Pedio-coccus-acidilactici* MA 18/5M) and control. The results indicated that, the administration of *Pedio-coccus-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 ($p \geq 0.01$) between groups in carcass dressing, breast meat and thigh percent. Mortality was almost similar in both groups (6.56 vs. 6.51).

Kral *et al*., (2012), investigated on the effect of probiotics on the performance of broiler chickens. The broiler chickens were divided into two dietary groups, control group were fed with standard feed mixture and experimental group fed with probiotics mixed with feed mixture. The results showed that, no significant ($p \geq 0.05$) differences in body weight of broilers among the groups were observed from initial age to the 4^{th}weeks. From the $5^{th}$to finally part of feeding experiment was significant ($p \leq 0.05$) 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.

Aliakbarpour *et al*., (2012), evaluated that, the effect of commercial mono-strain and multi-strain 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 ($p \leq 0.05$) 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 ($p \geq 0.05$) 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 ($p<0.05$). Furthermore, 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 poultry. 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 (without 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, liver and lungs% between treatment groups. On the other hand, the results recoded that, there were significant ($p\leq0.05$) effect on head and neck percent of broiler chicks.

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 $1.5x10^5$cfu/g of DFM a commercial product incorporating 3 DFM, or a non-supplemented diet. Direct- fed microbials didnot 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 immunoglobuline, and cecal microflora composition. Five bacterial spp. Probiotic was used in broilers nutrition. The treatments assigned into 5 dietray treatments as follows: No addition negative control, 10^8cfu probiotic/kg of diet (p_1), 10^9cfu 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 coagulants* 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 a basal diet without any probiotic and other groups were fed the diets that consisted of 3 probiotic levels at initial concentrations of $1.0x10^6cfu/g^{-1}(T_1)$, $2.0x10^6cfu/g^{-1}(T_2)$ and $5.0x10^6cfu/g^{-1}(T_3)$. 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. For meat chemical analysis, no significant ($p\geq0.05$) differences were found in the contents of moisture and crude ash among treatment groups, although, probiotic supplementation showed an increasing trend in CP (T-1, 23.77 ± 1.60%; T-2, 24.24 ± 1.39%; and T-3, 24.11 ± 2.04%, respectively) compared with the control (23.19 ± 1.35%). However, there was no significant difference ($P\geq0.05$) in CP content among treatments groups. The crude fat percentage in chicken breast ranged from 5.85 to 6.56%. Relative lower crude fat percentage was observed in T-3 in this study. However, there were also, no significant differences ($P\geq0.05$) among the T-1, T-2, T-3, and control groups.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 *Sterptococcus faecalis*) using different regimes and concluded that weight gain improved significantly ($p\leq0.05$) 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 homogenizer, 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 onto 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 cerevisae* 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 (6x108spore) 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 utilization 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 that, 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: T_1: negative control (NC) basal diet without growth promoter; T_2: NC+*Bacillus subtilis* ($8x10^5 cfu_s$/gfeed); T_3: NC+*Bacillus subtilis* ($3x10^5 cfu_s$/gfeed) and T_4: positive control (PC) *Avilamycin anti-coccidial* 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 ($p \leq 0.05$), but there was no difference, however, as compared to the diets with probiotic as a growth promoter ($p \geq 0.05$). 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's concluded

that the probiotic *Bacillus subtilis* can be used as a growth promoter in broiler diets.

Kabir *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 refreezing 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 (p60.001) higher and those for flavor 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, flavor, strange flavor, tenderness, juiciness, acceptability, characteristic color 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 yeast (*Saccharomyces cerevisiae)* extract (YE).

Abdel-Raheem *et al*., (2005), evaluated that, the effect of prebiotic, probiotic and synbiotic supplementation on intestinal microflora and histo-morphology of broilers. Treatment groups were as follows: 1. Basal diet (control); 2.Basal diet plus mannan-oligosaccharide (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 cervisiae)*; and 4. Basal diet plus the combination of prebiotic 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 ($p \leq 0.05$) higher in experimental birds as compared to control ones at all levels during the period of 2^{nd}, 4th, 5th and 6^{th}weaks of age, both in vaccinated and no vaccinated birds.This result is in agreement with many investigators, Islam *et al*., (2004) and Ashayerizadeh *et al*., (2009), who demonstrated that, increased live weight gain in probiotic fed birds. On the other hand, Lan *et al.,* (2003), found higher ($p \leq 0.05$) 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 adietary 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 ($p \leq 0.05$) 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) and Choudhari *et al*., (2008), conducted the addition of probiotic (*L. acidofillus* 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. acidophillus* 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) TMat 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 sup-therapeutic 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 an ovo *Lactobacillus reuteri*-treated broiler chickens given *S. typhymurium 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 subtilus* (calsporin; calpiscorporation,Tokyo, Japan) did not improve body weight (calsporin 2416 g vs. control 2407g) at 42 days of age, but feed conversion ratio was improved (calsprin 1.74 vs. control 1.77). Fritts *et al*., (2000), have shown that, calsporin will improve broiler body weight gain and feed conversion. Body weight gain, feed conversion and reduced mortality are characteristics of performance that ultimately dictate whether managerial and company practices will be altered for acceptance of a new way of managing poultry.

2.2.3 Prebiotic

2.2.3.1 Definition of prebiotics

The recent use of prebiotics has been well documented. The term “prebiotic” was first coined by Gibson and Roberfroid in 1995 and defined as “a non-digestible substances ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the

colon"(FAO; WHO, 2006; Das *et al*., 2012), and thus improves host health (Butel *et al*., 2016). Sobolewska *et al*., (2017) defined the prebiotics as non-digestible components of feed derived from sugars, including raffinose, galacto-oligosaccharides and *B*-glucans. Recently, the prebiotic is defined as "a non-digestible compound that, through its meta-bolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host" (Bindels *et al*., 2015). Over the years, further findings have led to several suggested modifications of the definition such as the addition of the term "selectively fermentable" (Langen and Dieleman, 2009). Gibson *et al*., (2004), revised the definition and defined a prebiotic as a selectively fermented ingredient that allows specific changes in the composition and/or activity in the intestinal microbiota that confers benefits upon the host's well-being and health. Other modification of the definition of prebiotic is the term "nonviable"Hutkins *et al*., (2016) and Pineiro *et al*., (2008), defined a Prebiotic as attractive products because they are non-viable, not affected by temperature and variation in moisture like the live micro-organisms (probiotic). More recently, an-expert consensus from the International Scientific Association for Probiotics and Prebiotics (ISAPP), defined prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson *et al*., 2018). Gibson and Roberfroid, (1995), demonstrated that the intake of prebiotics could regulate specific gastrointestinal tract (GIT) microorganisms to alter the microbiome.

2.2.3.2 Sources and Types of prebiotics:

Wodzimierz *et al*., (2005) and Van Loo *et al*., (1995) detailed several natural sources of prebiotics including certain fruits like bananas, garlic, onions, tomato, wheat, and asparagus, typically including fiber and oligo-saccharides (Charalampopulos and Rastall, 2009). Prebiotic oligosaccharide can be produced in three different ways: by extraction from plant materials Eckert *et al*., (2010), like

fructo-oligosaccharides (FOS), inulin (fructans), mannan-oligosaccharides (MOS), glacto-oligosaccharides (GOS) and xylo-oligosaccharides (XOS),by microbiological synthesis or enzymatic synthesis (Fermacto) which is dried from mycelium of *Aspergillus spp*. Roberfroid, (2000), and by enzymatic hydrolysis of polysaccharides (Fructans), or produced by microorganism, have been administered recently in broiler diets. Fructans are classified into three distinct types: the inulin group, the levan group, and the branched group. Firstly, the inulin group, also known as fructo-oligosaccharides (FOS) can be divided into different categories based on degrees of polymerization (DP): Inulin, normally extracted from chicory roots (*Cichorium intybus L*.), consists of a DP of 3 to 60, and Oligofructose (OF), which can be generated by partial hydrolysis of inulin, enzymatic conversion of sucrose, or lactose, contains a DP of 2 to 10 (Ritsema and Smeekens, 2003 and Rossi *et al*., 2005). FOS (inulin) are linear polymers of β-(2-1)-linked fructosyl units, terminated by one glucose residue and are not digested in the upper gut of avian species (Roberfroid *et al*., 2007). Secondly, the levan group is another group of fructans, which are mostly linked by β-(2-6) fructosyl-fructose bonds. Lastly, fructans which belong to the branched group contain both β-(2-1) fructosyl-fructose and β-(2-6) fructosyl-fructose bonds in fair amounts (Zhao *et al*., 2013). It is the β-glycosidic bond in fructans that resists their breakdown by digestive enzymes in poultry and enhances the population of beneficial bacteria, such as *Bifido-bacteria* and *Lactobacilli*, and suppresses levels of pathogenic bacteria, such as *Clostridium pefringens* and *E. coli*, in the intestine of broilers (Ricke, 2015; Kim *et al*., 2011 and Xu *et al*., 2003). Mannan oligosaccharides (MOS) are mannose-based oligomers linked together by β-(1-4) glycosidic bonds, found in cell wall of *Saccharomyces* yeast (Teng and Kim, 2018; Pourabedin and Zhao, 2015). Most of the mannan-oligosaccharide (MOS) products are derived from yeast cell walls *Saccharomyces cerevisiae*, Esecel *et al*., (2012), and are rich

in manno-proteins 12.5%, mannan 30%, and glucan 30% (Baurhoo *et al*., 2009 and Yang *et al*., 2009). Mannan oligosaccharides are known for their ability to bind pathogenic bacteria, which possess type-1 fimbriae, such as *E. coli* and *Salmonella*s pecies (Spring *et al*., 2000). By blocking bacterial lectin, MOS could reduce colonization and attachment of these pathogens in the intestine of animals and thus reduce the adverse effects of microflora and metabolites. Previous studies indicated that, supplementation of MOS from 0.08 to 0.5% could alter cecal microbial community composition by increasing total anaerobic bacteria, *Lactobacillus* and *Bifido-bacterium*, and decreasing *Salmonella, E. coli*, Clostridium perfringens, and Campylobacter (Corrigan *et al*., 2015 and Fernandez *et al*., 2002). Galacto-oligosaccharides (GOS) are synthetic prebiotics Jung *et al*., (2008), with galactose (1–4 or 1–6) β-linkages, are normally produced from lactose by the enzyme lactase with high galacto-syltrans-ferase activity (Alles *et al*., 999). In ovo injection of GOS could increase body weight of broilers 34 days after hatching (Pruszynska *et al*., 2015). Park *et al*., (2017), reported that, GOS treatment exhibited higher levels of Alistipes genus, Lactobacillus intestinalis, and Faecalibacterium prausnitzii in the ceca of broilers compared with the control group. Xylo-oligosaccharides are oligosaccharides, which consist of xylose sugar units with β-(1-4) linkages (De Maesschalck *et al*., 2015; Aachary and Prapulla, 2008). Xylan, the main component of cereal fiber such as corn cobs, straws, hulls, and bran are the raw resources for XOS production (Mussatto and Mancilha, 2007). Xylan could be degraded to XOS by xylanase of fungi, steam, or diluted solutions of mineral acid (Mussatto and Mancilha, 2007). Similar to other prebiotics, XOS could improve growth performance, increase the intestinal villus height, increase the proportion of Lactobacillus, and enhance the levels of acetate, butyrate, and lactate in the ceca of broilers (De Maesschalck *et al*., 2015 and Zhenping *et al*., 2013). It was suggested that, XOS would improve humoral

immunity in poultry. An increase in antibody titers against avian influenza H5N1 was observed in broilers by XOS addition (Zhenping *et al*., 2013). Other potential oligosaccharides used in chickens are Chitosan oligosaccharides (COS), Galacto-glucomannan oligo-saccharides (GGMO) and galacto-glucomannan oligosaccharides-arabinoxylan (GGMO-AX), and lactose (Hajati and Rezaei, 2010). Chitosan oligosaccharides (COS) areextracted from chitin, and contain 2–10 sugar units of N- acetyl glucosamine with (1–4) β-linkages. It has been reported that, the supplementation of COS in broiler diets could modulate immune responses and enhance nutrient digestibility and feed efficiency. Huang *et al*., (2007) indicated that, chicken with COS supplementation had higher weight of bursa of Fabricius and thymus, higher IgG, IgA, and IgM in serum and higher antibody titers against Newcastle disease vaccines. Galacto-glucomannan oligo-saccharides (GGMO) and galacto-glucomannan oligo-saccharides-arabinoxylan (GGMO-AX) are novel prebiotics extracted and processed from the wood chips of soft wood trees (Capek *et al*., 2000). These oligo-saccharides (GGMO and GGMO-AX) are consisting of mannose, glucose, and galactose monomers. Several commercial prebiotics are prepared from yeast cells including cell walls and fermentation products (Ding *et al*., 2014; Santin *et al*., 2001). Other compounds that show prebiotics-like effects include *Saccharomyces cerevisiae* fermentation products or yeast culture (Roto *et al*., 2015). The prebiotics have been shown to improve body weight and feed efficiency, significantly in broilers (Eckert *et al*., 2010). Prebiotics are non-viable, not affected by temperature and variation in moisture like the live micro-organismsprobiotic, prebiotics have more advantages than probiotics, while probiotics are intended to bring beneficial microbes to the gut, prebiotics are thought to selectively stimulate the beneficial microbes that already live there (Yang *et al*., 2009).

2.2.3.3 Characteristics of effective prebiotics:

Ideal characteristics of prebiotics were described by (Patterson and Burkholder, 2003):

(1) prebiotics should not be hydrolyzed by animal gastrointestinal enzymes and being resistant to acidic pH, (2) prebiotics can not be absorbed directly by cells in the gastro-intestinal tracks (GIT), (3) prebiotics selectively enrich one or limited numbers of beneficial bacteria and stimulate the growth, (4) prebiotics alter the intestinal microbiota and their activities, and (5) prebiotics ameliorate luminal or systemic immunity against pathogen invasion and modulate host defense system.

2.2.3.4 Beneficial effects of prebiotics:

Growth performance is the general and direct indicator in poultry as it involves feed utilization and overall effectiveness of poultry production (Ajuwon, 2015). Some of the major prebiotics that have shown beneficial effects on:

(1) Performance of broilers, improved body weight gain, feed conversion ratio therefore, improved the overall growth rate of broilers and carcass weight (Lu *et al*., 2012).

(2) Modulation of intestinal micro-flora, reduced *Salmonella* infection, Donalson *et al*., (2008), and increased the growth of beneficial bacteria in the GIT, such as Lactobacillus (LAB), Bacteroides and Bifido-bacterium, Bozkurt *et al*., (2014); Kim *et al*., (2011) and Johnson *et al*., (2015), and low intestinal pH (Fernandez *et al*., 2002).

(3)Increase amylase production in the GIT, improved villi height and crypt depth, and improve nutrient digestability (Huang *et al*., 2015 and Hanning, 2012).

(4) Influence the immunity and increased intestinal immune function (Kim *et al*., 2011; Bozkurt *et al*., 2014 and Huang *et al*., 2015).

(5) Reduced mortality rate (Cao *et al*., 2005).

2.2.3.5 Mode of action of prebiotics:

Major Prebiotics mechanisms of action include modulation of gut microbiota by selectively regulating beneficial groups of bacteria by providing food for them Hajati and Rezaei, (2010), and by reducing undesired intestinal colonization of bacteria, thus improving the integrity of gut mucosa (Iji and Tivey, 1998). The ecosystem of the gut is composed of three crucial elements: (1) microbial community, (2) intestinal epithelial cells, and (3) immune system pathogenic (Lavelle *et al*., 2010). Prebiotics are not digested or absorbed in the upper Gastro-intestinal-tract (GIT) and instead provide food source for host beneficial bacteria such as Lactobacillus (LAB) and Bifidobacteria in the lower GIT (Torok, *et al*., 2011 and Park, *et al*., 2017). This eventually excludes the attachment of pathogens including Salmonella by providing binding sites for bacteria to be flushed out of the digestive tract Charalampopoulos and Rastall, (2009), and promotes microbiota in the gut (Durant, *et al*., 2000). Some sugars are able to block the binding of pathogens to the mucosa. For example, mannan-oligo-saccharide (MOS) is able to bind to mannose-specific lectin of gram-negative pathogens that express Type-1 fimbriae such as E. coli resulting in their excretion from the intestine (Thomas *et al*., 2004). MOS are commonly derived from yeast and the outer cell of yeast. MOS are found to modulate the immune system improve the growth of the intestinal mucosa layer and microbiota diversity, Pourabedin *et al*., (2014), and eliminate pathogens from intestinal tract (Fernandez *et al*., 2002).Galacto-oligo-saccharides (GOS) have been shown to increase certain beneficial bacteria such as LAB, Bifidobacteria or their fermentation products (Macfarlane *et al*., 2008). Generally, prebiotics can be fermented by health-promoting bacteria in the intestine, producing lactic acid, short-chain fatty acid (SCFA), or some antibacterial substances, such as bacteriocine against pathogenic species (Bogusl *et al*., 2012). These products may not only benefit the intestinal microbial structure

but also improve the integrity of intestinal epithelial cells, which further increase the absorption of nutrients and enhance the growth performance of animals (Lan *et al*., 2005). Production of short chain fatty acids (SCFA), mainly butyrate, propionate and acetate as a part of fermentation process, is one of the main mechanisms of prebiotics (Pourabedin *et al*., 2015). SCFA lower the pH of gut lumen and provide energy to epithelial cells. This modulates the inflammation and regulates the metabolic functions, Pourabedin *et al*., (2015), then improved growth performance or antioxidant capacity, as they are covered extensively in (Dhama *et al*., 2014 and Yadav *et al*., 2016). Intestinal microbiota are influenced by various factors, including diet, gender, background genotype, housing environment, litter, and also age of birds (Pourabedin and Zhao, 2015). These factors can alter the abundance of dominant bacterial phyla and families in each part of the intestine. Application of prebiotics in diets could establish a healthy microbial community in the intestine of young broilers by enhancing the abundance of Lactobacilli and Bifido-bacteria and reducing the titers of Coliform (Chee *et al*., 2010). Furthermore, the modulation of intestinal microbiota is associated with immune responses. On the one hand, inhibiting pathogen colonization by prebiotics can decrease detrimental molecules produced by pathogenic bacteria, which have been known as exogenous signals (Tizard, 2013). These signals are also called pathogen-associated molecular patterns (PAMPs). The PAMPs can be recognized by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and Nod-like receptors (NLRs), which are expressed on the surface of sentinel cells (Tizard, 2013). Once PRRs recognize PAMPs, sentinel cells, such as epithelial cells, macrophages, mast cells, and dendritic cells, are activated, producing cytokines for the regulation of further innate immune responses. On the other hand, prebiotics can act as non-pathogenic antigens themselves. They can be recognized by receptors of immune cells, which consequently modulate host immunity

beneficially. Various prebiotics are composed of diverse sugar units. Therefore, each prebiotics may influence the animal's differently.

2.2.3.6 Evaluating prebiotics effects on growth performance and immune response:

Growth performance is the general and direct indicator in poultry as it involves feed utilization and overall effectiveness of poultry production (Ajuwon, 2015).The major prebiotics that have shown beneficial effects in performance, gut health and immunity arefructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS) and inulin (Huang *et al*., 2015; Kim *et al*., 2011). Replacement of antibiotics as growth promoters (AGP) with prebiotics or probiotics to observe the effect mainly in growth is the major reason for the researches. Supplementation of MOS and FOS in broilers is found to be associated with improved body weight gain (BWG), feed conversion ratio (FCR) and carcass weight (Baurhoo *et al*., 2009 and Sims *et al*., 2004). Improving broiler performance by dietary beta-glucans and MOS has been found to be associated with the improvement of innate immune function (Bozkurt *et al*., 2014). Also, production of short chain fatty acids (SCFA) is the reason behind better growth performance as this increases the partition of nutrients into other tissues of body (Lu *et al*., 2012; Ajuwon, 2015). The improvement of growth performance in chickens by prebiotics is affected by many factors. Prebiotics may increase short chain fatty acids (SCFAs) which are directly absorbed in the hind gut and used as an energy source in tissues (Chapman *et al*., 1994). Performance, egg cholesterol and gut microflora were improved by addition of inulin in laying hens diet (Shang *et al*., 2010). Improvement in egg shell and bone quality that increased the overall mineral metabolism due to inulin or oligofructose was also observed (Swiatkiewicz and Arczewska-Wlosek, 2012). Prebiotics like MOS, FOS and inulin were found to modulate the immune responses in the gut-associated lymphoid tissue (GALT) of chickens like cecal

tonsil, enhanced antibody titers of plasma IgM and IgG, cecum IgA levels, mucin mRNA expression and also enhanced intestinal immune functions (Janardhana *et al*., 2009 and Huang *et al*., 2015). Prebiotic treated group (both MOS and FOS) had similar performance to an (AGP) treated group with better (GALT) immunity in chickens (Janardhana *et al*., 2009). Prebiotic-mediated immunological changes may in part be due to direct interaction between prebiotics and gut immune cells as well as due to an indirect action of prebiotics via preferential colonization of beneficial microbes and microbial products that interact with immune cells (Janardhana *et al*., 2009).

2.2.3.7 Evaluating prebiotic effects on the intestinal microbiota and intestinal morphology:

In a study by Huang *et al*., (2015), dietary inulin supplemented at 5–10 g/kg had better effects on a starter phase (0–21 d) in both feed intake (FI) and intestinal IL-6, IgA, CD8, CD4 lymphocytes, and did not have any effect on d 42 broiler chicks. Length of time for adaptation and the exposure of Gastro-intestinal-tract (GIT) microbes to the supplemented fructo-oligosaccharides (FOS) plays major role in producing positive effect due to FOS. When FOS was added for a longer duration, it produced better results with villi height and crypt depth of intestine (Hanning *et al*., 2012). It is presumed that increased villi height is associated with the increased absorption of feed due to increased surface area transporting more feed nutrients (Amat *et al*., 1996). Feeding mannan-oligosaccharides (MOS) and lignin in poultry has resulted in low pH, high production of short chain fatty acids (SCFAs) like butyric acids and healthy gut, particularly increased villi height (Baurhoo *et al*., 2009). Astudy with (MOS) showed improved intestinal development as well as a healthy microbial community in broilers (Baurhoo *et al*., 2009). Prebiotics beneficially interact with animal's physiology by selectively stimulating favorable microbiota in the intestinal system (Macfarlane *et al*., 2008). Abundance of LAB

and Bifido-bacteria in chicken gut has been associated with theprebiotics supplementation, mainly MOS, FOS and inulin type fructans (Zhao *et al*., 2013 and Kim *et al*., 2011). Microbial flora such as LAB and Bifidobacterium sp. supports the defense system of animal against invading pathogens by stimulating (GIT) immune response (Mead, 2000). According to Seifert and Watzl, (2007), prebiotics such as inulin and oligo-fructans can modulate immune system directly. However, it is not clear if prebiotics directly affect the pathogen or host in a microbiota-independent manner. Oligosaccharides like beta-glucans stimulate the performance by enhancing phagocytosis and proliferating monocytes and macrophages (Novak and Vetvicka, 2008). Prebiotics compete for the sugar receptors thus preventing adhesion of pathogens like *Salmonella* and *E. coli* (Iji and Tivey, 1998). MOS have receptor properties for fimbriae of *E. coli* and *Salmonella* that leads to elimination of such pathogens with the flow of digesta instead binding mucosal receptor (Fernandez *et al*., 2002). Studies have showed an increase in Bifido-bacteria and LAB count and decrease in *Salmonella*, *E. coli* and *Clostridium perfringes* numbers in broilers fed MOS, FOS, fructan and lignin supplemented diets (Zhao *et al*., 2013; Baurhoo *et al*., 2009 and Macfarlane *et al*., 2008). The population of *Clostridium* and *E. coli* decreased with 0.25% FOS and 0.05% MOS supplementation whereas, LAB diversity increased in ileum by these two prebiotics (Kim *et al*., 2011). MOS have been reported to promote LAB growth contributing to overall microbial diversity in the contents of chicken cecum (Pourabedin *et al*., 2014). Feeding lignin or MOS increased cecal population of LAB and Bifido-bacteria whereas reduced E. coli in cecum of broilers (Baurhoo *et al*., 2009). The reason behind this might be the competitive exclusion (CE) where LAB and Bifido-bacteria competed against *E. coli*. On the other hand, bacteriocin produced by LAB and organic acids produced by *Bifido-bacteria* might suppress the colonization of pathogenic bacteria. The increase in intestinal microbial

diversity is believed to have positive effects on gut and overall host health (Janczyk *et al*., 2009). Due to the low pH created by SCFAs, pathogens like *Salmonella* and *Campylobacter* are reduced from the gut. Fermentation products such as SCFAs increased after prebiotic supplementation as a result of oligosaccharide fermentation by resident microbiota (Macfarlane *et al*., 2008). SCFAs such as acetate, propionate, butyrate etc. modify the bacterial ecosystem by lowering the pH that becomes intolerant to pathogens. Due to low pH of the cecum, prebiotics have been shown to inhibit pathogens growth and stimulate the growth of beneficial bacteria like Bifido-bacterium and LAB, and the process is the most effective in cecum (Cummings *et al*., 2001). The overall integrity of gut is also improved due to the production of SCFAs (Alloui *et al*., 2013). Stimulation of immune system includes increase in antibodies like secretory IgA and activation of phagocytic cells (Macfarlane *et al*., 2008). Thus, production of SCFAs and reduction of gut pH are key mechanisms of prebiotics in order to limit pathogen colonization and maintain optimal growth performance and health in poultry.

2.2.3.8 The effect of dietary prebiotic on the performance, carcass characteristics and blood parameters of broilers:

Al-Baadani, *et al*., (2016), investigated on the effects of dietary inclusion of probiotics, prebiotics and synbiotics on intestinal histological changes in broiler chickens. Two hundred and forty newly hatched male broilers (Ross 308) were equally distributed into six treatments: negative control group: un-supplemented; positive control group: supplemented; neoxyval-fed group: 0.5 g/kg diet (antibiotic); GalliPro-fed group: 0.6 g/kg diet (probiotic); Techno-Mos-fed group: 0.75 g/kg diet (prebiotic) and synbiotic-fed group, for 35 days. The results showed that the length and surface area of intestine were lower in the positive control, whereas, length and surface area of intestine of all feed additive treatments were higher, compared with the control and antibiotics.

Odefemi, (2016), evaluated that, 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 (1218.15 and 1163.68g), high feed intake, highest drumstick, back and head%. No significant differences were observed between the various treatment groups in feed conversion ratio, dressing, breast, thigh, wings, neck, liver and heart% of broiler chicks.

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 in to 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 synbiotic appeared to be superior compared to other growth promoters.

Dizaji *et al.*, (2012), reported on 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- Contol (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 and body weight gain 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 and body weight gain. 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 boiler chicks in prebiotic and probiotic groups compared with control group. No significant ($p>0.05$) differences between groups in feed intake, gizzard and liver%.

Ohimain and Ofongo, (2012), conducted an experiment to study the effect of probiotic and prebiotic feed supplementation on chicken health and microflora: A Review. The study found that, dietary supplements containing probiotic, prebiotic and enzymes are able to enhance performance while protecting the chickens from microbial infection.

Amouei *et al*., (2021), investigated on the effect of thyme (Thymus vulgaris L.) essential oil (TEO) or increasing inclusion of a prebiotic (TechnoMOS®) on growth performance and carcass characteristics of Ross 308 broilers, 400 one-day-old male broilers (43.5 g, as mean of body weight) were placed in 20 pens (2.0×1.0 m, with a floor area of 0.10 m2 per bird) in groups of 20, and each pen cage was assigned to a specific dietary treatment (four replicates per each one). The dietary treatments included basic diet (no additive; CTR), basic diet including 0.025%, 0.075%, or 0.125% of TechnoMOS® (MOS025, MOS075, and MOS125, respectively), or basic diet including 0.075% thyme extract (TEO.075). All dietary treatments were offered from the beginning of the study until the end of the trial.

There were no effects of MOS or TEO on carcass characteristics. No significant effects of treatment on weight gain were obtained on a week-by-week basis; however, CTR birds gained less weight during the grower phase and overall compared with MOS birds. The same contrast for feed intake revealed that CTR birds had greater feed intake than MOS birds during both the grower phase and overall (492.18 g and 486.35 g, respectively). In conclusion, treated groups showed an improved feed conversion ratio.The main effect, of both MOS and TEO, revealed better weight gain, feed intake, and feed conversion ratio but no improvements on carcass traits compared with control group. Eventhough average weight gainand feed intake have been influenced by treatments, no differences in feed conversion ratio had been found between treatments. Moreover, at similar dose level, MOS and TEO treatments did not show differences. Probably adifferent dosage should be used according mainly to the week of life, in order to reach a greater effect from a performance but also economic stand point.

2.2.4 Synbiotic:

2.2.4.1 Definition of Synbiotic:

Synbiotics are a combination of Probiotics and Prebiotics; they exhibit a synergistic relationship that positively affects the host by facilitating the implantation and survival of probiotic micro-organisms in the gastrointestinal tract (Ashraf *et al*., 2013; Naghi *et al*., 2017 and Nihar *et al*., 2016). The use of Synbiotics in the poultry industry is based on their ability to balance the gut environment and its microbiota (Dhama *et al*., 2011) by providing substrates for bacterial fermentation, generating antibacterial substances, competing for nutrients, modulating immune responses (Rooks and Garrett, 2016), competing with pathogens for adhesion receptors on the intestinal epithelium (Adil and Magray, 2012) and improves the growth of broilers (Mookiah *et al*., 2014). Beneficial effects of synbiotics, when compared with probiotics alone, on broiler growth

performance, intestinal microflora population, cecal volatile fatty acid concentration and intestinal histo-morphological parameters have been reported previously (Awad *et al*., 2009 and Bai *et al.,* 2013).

2.2.4.2 Mode of action of Synbiotic:

It has been suggested that a combination of a probiotic and a prebiotic, i.e. Synbiotics, might be more effect than either a probiotic or prebiotic alone (Bengmark, 2005; DeVrese and Schrezenmeir, 2008). Furthermore, synbiotic is a mixture of probiotic and prebiotic which beneficially affect the host by improving the survival and the implantation of live microorganisms dietary supplements in the gastrointestinal tract, and thus improving host health (Harish and Varghese, 2006). Mode of action of synbiotic are the mixture of mode of action of probiotic and prebiotic which give more beneficial effects on broilers performance.

2.2.4.3 The effect of dietary Synbiotic on the performance, carcass characteristics and blood parameters of broilers:

Ashayerizadeh *et al*., (2011), reported on the effect of Antibiotic, Probiotic, Prebiotic and mixture of Probiotic and Prebiotic as dietary growth promoters on growth indices and serum biochemical parameters of broilers. 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) pluse 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. At 42 day of age, HDL and LDL levels were not affected by dietary treatments, while the synbiotic and probiotic supplemented groups had lower cholesterol and triglycerides concentrations as compared with those of control and antibiotic supplemented groups respectively. The results

suggested that, the mixture of probiotic and prebiotic could be effective as antibiotic to improve the performance of broiler chickens.

Sarangi *et al*., (2016), investigated that, the effect of dietary supplementation of Prebiotic, Probiotic, and Synbiotic on growth performance and carcass characteristics of broiler chickens. A total of 360 1-day-old Vencobb broiler chickens of either sex were randomly assigned to four dietary treatments each consisting of three replicates and each replicate having 30 birds for 6 weeks. The dietary treatments were (1) control group with basal diet, (2) basal diet supplemented with Prebiotic (at 400 g/tonne of starter as well as finisher ration), (3) basal diet supplemented with Probiotic (at 100 g/tonne of starter ration and 50 g/tonne of finisher ration), and (4) basal diet supplemented with Synbiotic (at 500 g/tonne of starter as well as finisher ration). The birds were provided with *ad-libitum* feed and drinking water during the entire experimental period. The results recorded the highest body weight observed in a synbiotic group, which was non-significantly ($p>0.05$) higher than the control group. Prebiotic and probiotic groups showed lower body weight than synbiotic and control groups. A total feed intake did not show any significant ($p>0.05$) difference between experimental groups. There were no significant ($p>0.05$) differences in feed conversion ratio of broiler chickens in prebiotic, probiotic, and synbiotic groups as compared with control group. There was no significant ($p>0.05$) difference in the carcass traits with respect to dressing percentage, carcass percentage, heart weight, liver weight and gizzard weight, wing percentage, breast percentage, back percentage, thigh percentage, and drumstick percentage in Cobb broilers under study. They concluded that, the growth performance and percentage of carcass yield did not show any significant increase by the dietary inclusion of prebiotic, probiotic, and synbiotic compared with unsupplemented control in a commercial broiler chicken.

Wang *et al*., (2018), evaluated that, the effects of micro-encapsulated probiotics and prebiotics in broilers. A total of 108 one-day-old male Arbor Acres broilers were randomly divided into 3 groups (CON: basal diet; MEP: basal diet + compound microecologic products; ANT: basal diet + antibiotics), and there were 6 replicates per group and 6 birds per replicate. Compared with CON, diets supplemented with MEP or ANT significantly increased average daily gain and serum immunoglobulin M level at day 21, and serum total antioxidant capacity (T-AOC) level at day 42. Compared with CON and ANT groups, birds in MEP group had greater serum T-AOC, immunoglobulin A, interleukin-2 (IL-2) levels, and caecal Lactobacilli counts at day 21, and had greater serum IL-2, interleukin-6 levels, and caecal Lactobacilli counts at day 42. In conclusion, compound microecologic products had beneficial effects on body weight gain, serum immune function, and caecal Lactobacillus counts in broilers, which can be recommended as alternative to antibiotics

Hamasalim, (2016), investigated on the effect of Synbiotic as feed additives relating to animal health and performance, in early cases, probiotic as mono or mixed beneficial live microorganism was used as feed additive that plays a significant role in several health conditions and performances. In another way, the scientists use some ingredients indigestible with carbohydrates origin, especially oligosaccharides as a source of energy for beneficial micro-organisms in the body which were called prebiotic, and it is indigestible fermented food substrates that stimulate the growth, composition and activity of microorganisms in gastrointestinal and improve host. Most of the scientists urged to use all the above in such way that have more benefits in animal health and performance which were therefore called synbiotic, that was a combination between probiotic and prebiotic which beneficially had significant effects on the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract,

and thus improving animal health and performance. So, it was proposed that the synbiotic in this research increased beneficial microorganisms in the gastrointestinal tract and improved intestinal architect, and then promoted intestine environment. Consequently, it can improve blood indices, and especially decrease bad cholesterol (Low-density lipoprotein), decrease harmful microorganisms and toxins. However, it can also improve ingredient product, increase mineral absorption and nutrient. In conclusion, it can improve animal health and performance.

CHAPTER THREE

MATERIAL AND METHODS

Three experiments testing three types of natural feed additives were carried out at experimental farm of Department of Animal Production, College of Agricultural Studies, Sudan University of Science and Technology, in Shambat Khartoum North. One day old commercial unsexed broilers of Cobb-500 strain was obtained from Dajin Breeder Company Mico–Sudan. These experiments were conducted during winter season from (19 January to 23 February 2019). The ambient temperature average 20–26℃, **appendix (1)** during the experimental period for 5 weeks.

3.1 Experiment (1) Response of broiler chicks to graded levels of Bacterial Probiotic Biogen.S (BPB):

3.1.1 Experimental chicks:

A total number of 96, one day old were adapted to the premises and feed for (5days) before the start of experimental period. At the end of adaptation period, all chicks were weighed with an average initial weight of 170g. The chicks were then allotted randomly into four experimental groups A, B, C and D with three replicates each of eight chicken arrangement (4x3x8) in a complete randomized design (CRD), feed and water provided *ad libitum* throughout the experimental period. Chicks were bought vaccinated against Newcastle disease (ND) and against Infechious Bronchitis disease (IBD) in hatchery by (ND+IB) spray day one, inactivated ND injection and Gumbobest injection day one. On the farm the chicks vaccinated against Gumboro disease by Bur 706-France at (11) days of age, and against Newcastle disease by Avinew–France at (18) days of age. The dosage was repeated at (22) and (28) days of age for Gumboro disease by Bur (706–France) and for ND by Avinew–France respectively. Soluble multi-vitamin compounds

(pantominovite – pantex-Holland and B.V. 5525 ZG Duizel-Holland), provided three days before and after vaccination in order to guard stress.

3.1.2 Housing:

The experimental chicks were kept in semi closed house with direction east –west. The dimensions of house were 25 m. length, 8.8 m. width and 3.05 m height. The roof ceiling was made of trapezoid corrugated aluminum sheet and was insulated of (100mm) glass wool with thermal conductivity of (0.04 w/m^2). The Northern and Southern sides of the house were built from red blocks raised high to the level of 0.69 m. the house was equipped with adjustable side wall curtains to control the flow of air into the house. The top and bottom of the curtain opening was equipped with a curtain rod to minimized draft when fully closed. The floor was concrete.

Mechanical ventilation system was used in the house to generate on one direction air flow to provide the required levels of uniformity of air distribution over wide range of climatic condition. Two exhaust fan (fan diameter 1.29 with air 44500 m^2/ h). Two exhaust fan Positioned in the middle of the western side wall of the house to maintain negative pressure inside the house as a result of negative pressure outside air flows into the house through inlet opening with cellulose pad besides maintaining the desired temperature and ventilations inside also an outlet on the roof was required to exit surplus heat, gases, moisture and supply fresh air

Cooling system was evaporative cooling panel comportment, the cooling pad banks dimensions were (4 m. long × 1.4 m. length × 0.15 width) and that of air inlet valve was 0.45 m. the cooling pad was situated of the at two sides, north and south direction at the rear of the poultry house.

Cooling pad was made of specially impregnated cellulose paper of wait ability, arranged in self-supporting structure that guaranteed long life without sagging or deterioration.

The other integral components provided with each pad cooling bank were pump, polyester, water tank capacity (1000 liters). For storage of water which was continuously supplied from main tap water under control of flouter which was put in the tank also there was one horse power electrical motor for pumping water from the tank to the top of pad cooling banks.

The house was provided with piping system for supply and return of water, the cooling and humidification of outside air is obtained by evaporation of very fine water particles. Due to negative pressure maintained by the exhaust fans air flow through the pad and then through special air inlet to the house. Special geometry of the pads enables the air to pass through small opening or flutes in turbulent state thus creating ideal condition for maximum evaporation and consequently maximum cooling to take place as a result of the layer contact area between water and air (excess water is returned to the bank where it is pumped to the top edge of the pad for re-circulation. Inside the house the temperature was maintained at 27-30℃ throughout the experimental period.

36 experimental replicates (1.5 × 1 m.) were prepared using wire mesh portioned and then were cleaned washed and disinfected by white phenol solution and formalin. Allayer of wood shairy (5cm) was laid on the floor as littler material before starting the experiments. Each replicate was provided by (5 kg) rounded feeder and (2.5 lit.) baby drinker which were adjusted to the progressive growth of chicks. The light program was 24 hours light from 1-3 days and 23 hours day for the rest period.

3.1.3 Experimental ration:

The commercial bacterial probiotic product (Biogen.S- soluble powder) was used in this experiment; it is the feed additive which contains *Bacillus subtillus natto* not less than $1x10^{11}$ CFU with Dextrose q.s. in each kg of product. The product Biogen.s was purchased from Melody Pharma CO. Ltd Khartoum Sudan,

Manufactured by SAMU MEDIAA CO. LTD. (KOREA). Lot No.: 101001, Mfg. date: 06.02.2018, Exp. date: 05.02.2020 **Appendix (2)**. The chicks were divided into four dietary treatments, the first group A, fed on basal diet without feed additives (control), the other groups B, C and D were fed on basal diet supplemented with bacterial probiotic (*Bacillus subtillus natto*) at levels 0.5, 1.0 and 1.5g/kg respectively. The basal diet was formulated to meet the nutrient requirements of broiler chicks according to Nutritional Research Council (NRC, 1994).

The ingredients percent composition, the calculated chemical analysis and approximate chemical analysis of the experimental diet were presented in table (1), (2) and (3), experimental diets were fed for 5 weeks.

Table (1) Composition of experimental diets used in the Probiotic experiment

Ingredients%	**A**	**B**	**C**	**D**
Dura	64.29	64.29	64.29	64.29
Ground nut Cake	12	12	12	12
Sesame Cake	17	17	17	17
Concentrate*	5	5	5	5
Dicalcium phosphate.	0.62	0.62	0.62	0.62
Oyster shell	0.49	0.49	0.49	0.49
Salt	0.25	0.25	0.25	0.25
Methionine	0.11	0.11	0.11	0.11
Lysine	0.24	0.24	0.24	0.24
Total	100	100	100	100
Feed additive: Probiotic g/kg	-	0.5	1.0	1.5

*Nutrient composition of the broiler concentrate used in the diet formulation, and the vitamin-mineral provided: ME 2122 kcal/kg, crude protein 40%, crud fiber 1.5, calcium 6.8%, phosphorus av. 4.6%, phosphorus tot. 3%, lysine 1.5%, methionine 5.6%, methionine + systin 6.25%, Sodium 2.60%, vitamin A: 200.000I.U/Kg, vit. E: 500mg/Kg, vit. B1: 40mg/Kg, vit. B2: 100 mg/Kg, vit. B6: 50mg/Kg, vit. B12: 300mg/Kg, vit. C 400mg/kg, Biotin: 1000mg/kg, Nicotinicacid: 600mg/kg, Folicacid: 30mg/kg, vit. K30mg/kg, pantothenic acid: 150mg/kg; choline chloride: 30000mg/kg, copper 200mg/kg, iodine 15mg/kg, Cobalt: 12mg/kg, selenium: 5mg/kg, manganese: 1200mg, zinc: 800mg/kg, iron1000mg/kg, B.H.T.:900mg/kg, Salinomycin-Na: 1.200, phytase: 16 and 1500 FYT antioxidant added.

Table (2) Calculated chemical analysis of the experimental control diet

Ingredients	A	B	C	D
Dry matter	94.00	94.00	94.00	94.00
Crude protein	22.80	22.80	22.80	22.80
Crude fiber	4.10	4.10	4.10	4.10
Lysine	1.39	1.39	1.39	1.39
Methionine	0.60	0.60	0.60	0.60
Calcium	1.18	1.18	1.18	1.18
Phosphor	0.77	0.77	0.77	0.77
Nitrogen free extract	58.86	58.86	58.86	58.86
ME/ Kcal	3111	3111	3111	3111

Calculated according to (Ellis, 1981; Kuku Bulletin)

Table (3) Chemical analysis of the experimental control diet

Components	%
Dray matter	94.00
Crude protein	23.19
Crude fiber	4.35
Ether Extract	3.00
Ash	4.60

(Kuku Research Center Laboratory)

3.1.4 Data collected:

3.1.4.1 Performance data:

Feed intake, body weight and average body weight gain were recorded weekly by pen, and feed conversion ratio FCR (g:g) was calculated throughout experimental period. Health of the experimental stock was closely observed.

Parameters studied of growth performance traits:

The body weight of each bird was recorded on a weekly basis and weight gain was calculated by using the following formula:

Body weight gain (g) = Final body weight (g) – Initial body weight (g).

Feed intake (g) was recorded on a daily basis by using the follow formula:

Feed Intake (g) = Feed offered (g) – Feed residue (g).

Weekly feed conversion ratio was calculated as follows:

Feed conversion ratio =Feed intake (g)/ Weight gain (g)

3.1.4.2 Slaughter procedure and data:

At the end of the experimental period (5weeks) birds were fasted overnight with only water allowed. Three birds of similar live body weight were selected randomly from each treatment group and weighed 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 closed 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, gizzard, abdominal fat and intestine), all non carcass components were expressed as percentage of live body weight, and then the hot carcass was separated weighed individually and were expressed as a percentage of live weight to calculate the dressing percentage.

3.1.4.3 Carcass data:

The hot carcass was prepared for analysis by removal of the skin and neck near to the body and each was weighed separately. The carcass was then divided in to right and left sides by mid sawing along the vertebral column and each side was weighed. The left side was divided in to three commercial cuts, breast, thigh, and drumstick, each cut was weighed separately, and was expressed as percentage of the carcass weight. Then they were deboned, the meat and bone were weighed separately, and were expressed as percentage of their cuts. The meat was frozen and stored for further analysis

3.1.4.4 Blood serum profile:

Blood samples were pulled from wings veins by syringe. Serum prepared from the blood analyzed for concentration of metabolites total protein, albumin, cholesterol, cholesterol HDL, cholesterol LDL, triglycerides, glucose, urea, uric acid, creatinine, enzyme activities ALP, AST and minerals (Ca and P).

3.2 Experiment (2) response of broiler chicks to graded levels of Prebiotic Y-MOS (PYM):

3.2.1 Experimental chicks:

The same planned in experiment one, preventive health program were the same in the first experiment.

3.2.2 Housing:

The house was as the same as described in the first experiment.

3.2.3 Experimental ration:

The commercial prebiotic product (Y-MOS) was used in this experiment as feed additive is the remaining product after hydrolysis of bakery yeast (*Saccharomyces cerevisiae*) and after separation of the cell content by centrifugation. Besides the yeast cell wall, Y-MOS also, contains fractions of the yeast cell content. Y-MOS originated only from yeast (*Saccharomyces cerevisiae*). The product Y-MOS was

purchased from Khayrat Elnile Co. Ltd. Khartoum, manufactured by Nutrex Co. Ltd Belgium. Mfg. date: 05.10.2018, Exp date: 04.10.2020 **Appendix (3)**. The chicks were divided into four dietary treatments, the first group A, fed on basal diet without feed additives (control), the other groups B, C, and D were fed on basal diet supplemented with prebiotic (Y-MOS) at levels 0.5, 1.0, and 1.5g/kg respectively. The ingredients percent composition of the prebiotic experimental diet was presented in table (4).

Table (4) Composition of experimental diets used in the prebiotic experiment

Ingredients%	**A**	**B**	**C**	**D**
Dura	64.29	64.29	64.29	6429
Ground nut Cake	12	12	12	12
Sesame Cake	17	17	17	17
Concentrate*	5	5	5	5
Dicalcium phosphate.	0.62	0.62	0.62	0.62
Oyster shell	0.49	0.49	0.49	0.49
Salt	0.25	0.25	0.25	0.25
Methionine	0.11	0.11	0.11	0.11
Lysine%	0.24	0.24	0.24	0.24
Total	100	100	100	100
Feed additive: Prebiotic g/kg	-	0.5	1.0	1.5

*Nutrient composition of the broiler concentrate used in the diet formulation, and the vitamin-mineral provided: ME 2122 kcal/kg, crude protein 40%, crud fiber 1.5, calcium 6.8%, phosphorus av. 4.6%, phosphorus tot. 3%, lysine 1.5%, methionine 5.6%, methionine + systin 6.25%, Sodium 2.60%, vitamin A: 200.000I.U/Kg, vit. E: 500mg/Kg, vit. B1: 40mg/Kg, vit. B2: 100 mg/Kg, vit. B6: 50mg/Kg, vit. B12: 300mg/Kg, vit. C 400mg/kg, Biotin: 1000mg/kg, Nicotinicacid: 600mg/kg, Folicacid: 30mg/kg, vit. K30mg/kg, pantothenic acid: 150mg/kg; choline chloride: 30000mg/kg, copper 200mg/kg, iodine 15mg/kg, Cobalt: 12mg/kg, selenium: 5mg/kg, manganese: 1200mg, zinc: 800mg/kg, iron1000mg/kg, B.H.T.:900mg/kg, Salinomycin-Na: 1.200, phytase: 16 and 1500 FYT antioxidant added.

3.2.4 Data to be collected:

Data collected were the same as described in the first experiment in aspects of chick performance, slaughter and carcass data, blood serum, enzyme activities, metabolic indicator and minerals.

3.3 Experiment (3) response of broiler chicks to graded levels of dietary Synbiotic Biogen.S + Y-MOS- 1:1 (SBYM):

3.3.1 Experimental chicks:

The same planned in experiment one, preventive health program were the same in the first experiment.

3.3. 2 Housing:

The house was in as the same as described in the first experiment

3.3.3 Experimental ration:

Synbiotic used in this experiment is the combination of probiotic Biogen.S (used in experiment 1) and prebiotic Y-MOS (used in experiment 2) 1:1.The chicks were divided into four dietary treatments, the first group A, fed on basal diet without feed additives (control), the other groups B, C, and D were fed on basal diet supplemented with synbiotic (Biogen.S + Y-MOS 1:1) at levels 0.5, 1.0 and 1.5g/kg respectively. The ingredients percent composition, of the synbiotic experimental diet was presented in table (5).

Table (5) Composition of experimental diets used in the Synbiotic experiment

Ingredients%	**A**	**B**	**C**	**D**
Dura	64.29	64.29	64.29	64.29
Ground nut Cake	12	12	12	12
Sesame Cake	17	17	17	17
Concentrate*	5	5	5	5
Dicalcium phosphate.	0.62	0.62	0.62	0.62
Oyster shell	0.49	0.49	0.49	0.49
Salt	0.25	0.25	0.25	0.25
Methionine	0.11	0.11	0.11	0.11
Lysine	0.24	0.24	o.24	0.24
Total	100	100	100	100
Feed additive: Synbiotic g/kg	-	0.5	1.0	1.5

*Nutrient composition of the broiler concentrate used in the diet formulation, and the vitamin-mineral provided: ME 2122 kcal/kg, crude protein 40%, crud fiber 1.5, calcium 6.8%, phosphorus av. 4.6%, phosphorus tot. 3%, lysine 1.5%, methionine 5.6%, methionine + systin 6.25%, Sodium 2.60%, vitamin A: 200.000I.U/Kg, vit. E: 500mg/Kg, vit. B1: 40mg/Kg, vit. B2: 100 mg/Kg, vit. B6: 50mg/Kg, vit. B12: 300mg/Kg, vit. C 400mg/kg, Biotin: 1000mg/kg, Nicotinicacid: 600mg/kg, Folicacid: 30mg/kg, vit. K30mg/kg, pantothenic acid: 150mg/kg; choline chloride: 30000mg/kg, copper 200mg/kg, iodine 15mg/kg, Cobalt: 12mg/kg, selenium: 5mg/kg, manganese: 1200mg, zinc: 800mg/kg, iron1000mg/kg, B.H.T.:900mg/kg, Salinomycin-Na: 1.200, phytase: 16 and 1500 FYT antioxidant added.

3.3.4 Data to be collected:

Data collected were the same as described in the first experiment in aspects of chicken performance, slaughter and carcass data, blood serum, enzyme activities, metabolic indicator and minerals.

3.5 Methods of analysis:

3.5.1 Method used for meat quality assessment:

3.5.1.1 Subjective meat quality attributes:

3.5.1.1.1 The taste panel:

Frozen deboned breast drumstick and thigh cuts of the right side were thawed at 5-7 ℃ before cooking for sensory evaluation. The meat was trapped in aluminum foil, placed in roast pan and cooked at 180.7℃ in the conventional preheated electrical oven to about 80 ℃ internal muscle temperature. The cooked meat was allowed to cool to room temperature in about 10 minutes. The samples were kept warm until served. Semi 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 (4)**.

3.5.2 Chemical methods:

3. 5.2.1 Serum determination:

Venous unheparition blood samples obtained at the end of experiment from chicks were centrifuged at 3000 r. p. m. for 5 minutes and serum were stored at -20C until analyzed in the National Public Health Laboratory Chemical Pathology (STAK), using Biosystem A 25, which made in Germany. Quality system certified according to EN ISO 13485 and EN ISO 9001 standards.

Procedure of system:

Full automated biochemical analyzer.

Well prepared sample and reagent.

Well calibrated and controlled analyzer.

Insert sample and code number.

Select tests and click on the position in the bottom.

Better to use test tube rather than cubs.

Clicks on accept and then click start.

Analyze by batch not by individual sample.

For result click on current state (result) then print.

Reagents preparation:

Reagents are provided ready to use for measurements of serum samples, kits provided by BioSystems S.A. Costa Brava, 30.08030 Barcelona (Spain).

3.5.2.1.1 Total protein:

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry.

Composition:

A. Reagent, Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.

Corrosive (C): R34: Causes burns. S26-45: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately.

S. Protein Standard. Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology. USA).

Storage:

Reagent (A): Store at 15-30°C.

Protein standard (S): Store at 2-8C, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 545 nm.

-Standard: Presence of particulate material, turbidity.

3.5.2.1.2 Albumin (Bromocresol Green):

Principle of the method:

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measued by spectrophotometry.

Composition:

Reagent. 5 x 50 mL. Acetate buffer 100 mmol/L, bromocresol green 0.27 mmol/L, detergent, pH 4.1.

Storage:

Reagent (A): Store at 2-8°C.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in “Assay parameters”.

3.5.2.1.3 Creatinine (Alkaline Picrate):

Principle of the method:

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interferences.

Composition:

Reagent. 5 x 50 mL. Sodium hydroxide 0.2 mol/L, detergent.

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

Reagent. 5 x 50 mL. Picric acid 25 mmol/L.

Storage:

Store at 2-8°C.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).

3.5.2.1.4 Uric Acid (Uricase/Peroxidase):

Principle of the method:

Uric acid in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DCFS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Composition:

Reagent. 10 x 50 mL. Phosphate 100 mmol/L, detergent 1. g/L, dichloro-Phenol-sulfonate 4mmol/L, uricase > 0.12 U/ml, ascorbate oxidase > 5 U/mL, Peroxidase> 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.8.

Storage:

Store at 2-8°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in “Assay parameters”.

3.5.2.1.5 Urea /Bun-UV (Urease/Glutamate Dehydrogenase):

Principle of the method:

Urea in the sample consumes, by means of the coupled reactions described below, NADH that can be measured by spectrophotometry.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow[\text{Glutamate}]{\text{dehydrogenase}} \text{Glutamate} + NAD^+$$

Composition:

Reagent: 5 x 40 mL Tris 100 mmol/L, 2-oxoglutarate 5.6 mmol/L, urease > 140 U/mL, glutamate dehydrogenase > 140 U/mL, ethyl-eneglicol 220 g/L, sodium azide 0.95, pH 8.0.

Warning: H302: Harmful if swallowed. P301 + P312: If Swallowed: Call a Poison Center or doctor/physician if you feel unwell. P330: Rinse mouth.

Reagent: 5 x 10 mL, NADH 1.5 mmol/L, sodium azide 9.5 g/L.

Warning: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301 + P312: If Swallowed: Call a Poison Center or doctor/physician f you feel unwell. P330: Rinse mouth.

Storage:

Store at 2-8°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, and absorbance of the blank lower the limit indicated in “Assay parameters”.

3.5.2.1.6 Glucose (Glucose Oxidase/Peroxidase):

Principle of the method:

Glucose in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

$Glucose + {}^{1}/_{2}O_2 + H_2O \xrightarrow{glucose\ oxidase} Gluconate + H_2O_2$

$2H_2O_2 + phenol + 4\text{-}Amino\text{-}antipyrine \xrightarrow{peroxidase} Quinoneimine + 4H_2O$

Composition:

Reagent 10 x 50 mL. Phosphate 100 mmol/L, phenol 5 mmol/L, glucose oxidase > 10 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine o.4 mmol/L, pH 7.5.

Storage:

Store at 2-8°C.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in "Assay parameters".

3.5.2.1.7 Cholesterol – Cholesterol Oxidase/Peroxidase:

Principle of the method:

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

$Cholesterol\ ester + H_2O \xrightarrow{Chol.esterase} Cholestero1 + fatty\ acid$

$Cholesterol + {}^{1}/_{2}O_2 + H_2O \xrightarrow{Chol.oxidase} Cholestenone + H_2O_2$

$2H_2O_2 + 4\text{-}Amino\ antipyrine + phenol \xrightarrow{Peroxidase} Quinoneimine + 4\ H_2O$

Composition:

Reagent. 10 x 50 ml. Pipes 35 mmol/L, sodium cholate 0.1 U/ml, peroxidase > 0.8U/ml, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

Storage:

Store at 2-8℃.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagent: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in “Assay parameters”.

3.5.2.1.8 Cholesterol HDL:

Principle of the method:

The cholesterol from low density lipoproteins (LDL), very low- density lipoproteins (VLDL) and chylomicrons is broken down by the cholesterol oxidase in an enzymatic accelerated non-color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from high density lipoproteins (HDL) in the sample. The HDL cholesterol is then spectro-photo-metrically measured by means of the coupled reactions described below.

Cholesterol ester $+H_2O \xrightarrow{\text{Chol.esterase}}$ Cholestero1+ fatty acid

Cholesterol $+{}^1/_2O_2 + H_2O \xrightarrow{\text{Chol.oxidase}}$ Cholestenone$+H_2O_2$

$2H_2O_2$ + 4-Aminoantipyrine + DSBmT $\xrightarrow{\text{peroxidase}}$ Quinoneimine + 4 H_2O

Contents and Composition:

Reagent. 3 x 20 mL. Goods buffer, cholesterol oxidase < 1 U/mL, peroxidase < 1 U/mL, N, N-bis (4-sulfobutyl)-m-toluidine (DSBmT) 1 mmol/L, accelerator 1 mmol/L.

Reagent. 1 x 20 mL. Goods buffer, cholesterol esterase < 1.5 U/mL, 4-aminoantipyrine 1mmol/L, ascorbate oxidase < 3.0 KU/L, detergent.

Storage:

Store at 2-8℃.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

3.5.2.1.9 Cholesterol LDL:

Principle of the method:

A specific detergent solubilizes the cholesterol from high density lipoproteins (HDL), very low- density lipoprotein (VLDL) and chylomicrons. The cholesterol esters are broken down by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low density lipoproteins (LDL) in the sample. The LDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below.

Cholesterol ester $+H_2O \xrightarrow{\text{Chol.esterase}}$ Cholestero1+ fatty acid

Cholesterol $+{}^1/_2O_2 + H_2O \xrightarrow{\text{Chol.oxidase}}$ Cholestenone$+H_2O_2$

$2H_2O_2$ + 4-Aminoantipyrine + DSBmTP $\xrightarrow{\text{peroxidase}}$ Quinoneimine + 4 H_2O

Contents and Composition:

Reagent. 3 x 20 mL. MES buffer > 30 mmol/L, cholesterol esterase < 1.5 U/mL, cholesterol oxidase <1.5 U/mL, 4-aminoantipyrine 0.5mmol/L, ascorbate oxidase < 3.0 U/L, peroxidase > 1 U/mL, detergent, pH 6.3.

Reagent. 1 x 20 mL. MES buffer > 30 mmol/L, 1mmol/L, detergent, pH 6.3.

Storage:

Store at 2-8℃.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

3.5.2.1.10 Triglycerides (Glycerol Phosphate oxidase/peroxidase):

Principle of the method:

Triglycerides in the sample originate, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

Triglycerides + H_2O —lipase→ Glycerol + Fatty acids

Glycerol + ATP —glycerol kinase→ Glycerol -3- P + ADP

Glycerol – 3- P + O_2 —G-3-P-oxidase→ Dihydroxyacetone-p + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine+ 4- Chlorophenol —peroxidase→ Quinoneimine + $4H_2O$

Composition:

Reagent: 10 x 50 mL. Pipes 45 mmol/L, magnesium chloride 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0

Storage:

Store at 2-8°C.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in “Assay parameters”.

3.5.2.1.11 Aspartate Amino Transferase - Glutamyl Oxaloacetic Transaminase (AST/GOT):

Principle of the method:

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH), coupled reacti.

Aspartate + 2-Oxoglutarate —AST→ Oxalacetate + Glutamate

Oxalacetate + NADH + H^+ —MDH→ Malate + NAD^+

Composition:

Reagent: 5 x 40 mL. Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, pH 7.8.

WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+338: IF IN

EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

Reagent: 5 x 10 ml. NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

Storage:

Store at 2-8℃.

Reagents are stable until the expiry date show on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, and absorbance of the blank lower the limit indicated in “Assay parameters”.

3.5.2.1.12 Alkaline phosphatase (ALP) – AMP 2-Amino -2- Methyl -1-

Propanol Buffer:

Principle of the method:

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol

(AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm.

4-Nitrophenylphosphate +AMP $\xrightarrow{\text{ALP}}$ AMP– phosphate+ 4-Nitrophenol

Composition:

A.Reagent: 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxy-ethyl-ethyl-enediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.

B. Reagent 4-Nitrphenylphosphate 60 mmol/L.

Storage:

Store at 2-8℃.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

3.5.2.1.13 Calcium – Arsenazo (Arsenazo III):

Principle of the method:

Calcium in the sample reacts with arsenazo III forming a coloured complex that can be measured by spectrophotometry.

Composition:

Reagent 10 x 50 ml. Arsenazo III 0.2 mmol/L, imidazole 75 mmol/L.

Storage:

Store at 2-8℃.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity.

3.5.2.1.14 Phosphorus (Phosphomolybdate/UV):

Principle of the method:

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming phosphomolybdate complex that can be measured by spectrophotometry.

Contents and composition:

Reagent: 4 x 60 mL. Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L.

DANGER: H314: Causes severe skin burns and protective gloves/protective clothing/eye protection/face protection. P303 +361+P353: IF ON SKIN (O hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

Reagent: 2 x 50 mL Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303 +361+P353: IF ON SKIN (O hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

Storage:

Store at 15-30℃.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.500 a 340 nm.

3.5.2.2 Meat chemical analysis:

The Approximate chemical analysis of meat samples was carried out at Animal Production Research, Animal nutrition Laboratory Kuku according to (AOAC 1995) methods. Crude protein was determined using kjeldhal method, and crude fat was measured using the Soxhlet Auto Extraction.

3.5.2.2.1 Determination of moisture and dry matter:

Principle:

Moisture as removed from the samples by heating at 105℃ in a force – draught oven for 3 hour or overnight.

Calculation:

$$\% \text{ moisture} = \frac{(\text{WT of original sample + dish}) - (\text{dried sample + dish})}{(\text{WT of original sample “5 gm”})} \times 100$$

Or

% moisture= 100 - % dry matter

$$\% \text{ dry matter} = \frac{(\text{WT of dried sample + dish}) - (\text{WT of dish})}{(\text{WT of original sample “5 gm”})} \times 100$$

3.5.2.2.2 Determination of Ash and organic matter

Principle:

The sample is ignited at 500-550 ℃ to burn off all organic material. The inorganic material which does not volatilize at that temperature is called ash. The difference between sample and ash gives the organic matter.

Calculation:

$$\% \text{ Ash} = \frac{(\text{WT. of Ash + dish}) - (\text{WT. of dish})}{(\text{WT. of original sample})} \times 100$$

% organic matter = 100 - % Ash.

Nitrogen free Extraction (N.F.E).

%N.F. E= (100- (Moist + Ash + Crude fat + crud protein + Crude fiber).

3.5.2.2.3 The determination of Crude Fat (soxhlet)

Principle:

The sample is extracted with petroleum spirit, the solvent is distilled off and the extract dried and weighed.

Reagent:

Petroleum spirit, boiling point (60-80 ℃).

Calculation:

%Crude fat = (WT. of flask +oil - WT. of flask)× 100

WT. of original sample (2.5)

3.5.2.2.4 Determination of total nitrogen (crude protein)

Principle:

Total nitrogen is determined using the kjeldhal method. Organic nitrogen is converted in to ammonium ions by digestion with concentrated sulphuric acid in the presence of a catalyst such as a mixture of copper sulphate with selenium.

As the digestion proceeds, some of sulphuric acid is reduced to sulphur dioxide which in turn reduces the nitrogenous material to ammonia. The ammonia combines with sulphuric acid to form ammonium sulphate. Amonia is liberated by boiling with sodium hydroxide, steam distilled in to boric acid plus indicator and determined by titration

Reagent:

ConcentrateSulphuric acid.

Catalyst (Copper sulphat+selenium).

Sodium hydroxide solution 50%.

Standard solutionof ammonium sulphate.

Standard acid 0.01 N -HCL.

Boric acid+ bromocresol green/methyl red indicator solution.

Calculation:

$$\text{Titrate - \% CP} = \frac{\text{Blank}}{\text{Standard} - \text{Blank}} \times \frac{75\text{ ml}}{3\text{ml}} \times \frac{1}{0.5\text{ g}} \times 6.25 \times \frac{1}{1000} \times 100$$

3.6 Statistical analysis:

All data were analyzed as one way-ANOVA completely randomized design (CRD) using Statistix10 trial according to **Statistix, 2013.** Means were compared using Tukey‘s Honestly Significant Difference (HSD) multiple range test. Performance data of three experiments were analyzed as two way-ANOVA factorial design for determene the interaction between treatments and their levels. All values were presented as means ± standard error of mean, the significantly set up ($p<0.05$), frequency distributions were set and treatment means were compared for significance at the level of probability 5%.

CHAPTER FOUR

RESULTS

4.1 Experiment one: Response of broiler chicks to graded levels of dietary Bacterial Probiotic Biogen.s (BPB).

4.1.1 Performance:

The effect of feeding graded levels of dietary bacterial probiotic Biogen.S (BPB) on performance of broiler chicks, was tabulated in table (6) and figures (1-2). Firstly for feed intake, the results recorded that, the effect of treatments on feed consumption was not significant ($p \geq 0.05$) among the all treated groups with BPB supplementation at levels of (0.0 .0.5, 1.0 and 1.5g/kg) throughout the experimental period. However, the chicks fed on 1.5g/kg BPB recorded numerically the higher mean value of feed intake (3436g), followed by control diet (3428g) and 0.5g/kg BPB (3421g) then 1.0g/kg BPB (3414g) as the lowest feed intake.

Results of final body weight, illustrated that, the chicks fed on all levels of BPB (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) higher means for final body weight (2090, 2219g and 2315g respectively) as compared to chicks fed on control diet (1926g), also, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) heaviest mean of final body weight (2315g) than those chicks fed on 0.5g/kg BPB and chicks fed on control diet (2090g and 1926g respectively), with an increasing estimated by about 10.77% and 20.20% respectively. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5 and 1.0g/kg BPB (2090g and 2219g respectively), also, between groups of chicks fed on 1.0 and 1.5g/kg BPB (2219g) and (2315g) in final body weight of broilers throughout the experimental period.

Application of graded levels of BPB significantly ($p \leq 0.05$) affected body weight gain, the results revealed that, chicks fed on all levels of BPB (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) the higher means for body weight gain (1922, 2048g and 2143g respectively) as compared to the chicks fed on control diet (1757g), also, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) heaviest mean of body weight gain (2143g) as compared to those chicks fed on 0.5g/kg BPB and control diet (1922g and 1757g respectively), with an increasing estimated by about 11.50% and 21.97% respectively. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5 and 1.0g/kg BPB (1922g and 2048g respectively), also, between groups of chicks fed on 1.0 and 1.5g/kg BPB (2048g and 2143g respectively) in body weight gain of broiler chicks throughout the experimental period.

Finally, the results concerning feed conversion ratio (FCR) revealed that, the chicks fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) better FCR (1.78, 1.67g:g and 1.60g:g respectively) as compared to the chicks fed on control diet (1.95g:g), also, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) the best FCR (1.60g:g) as compared to the chicks fed on 0.5g/kg BPB (1.78g:g), whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 1.0 and 1.5g/kg BPB (1.67g:g and 1.60g:g), also, between groups of chicks fed on 0.5 and 1.0g/kg BPB (1.78g:g and 1.67g:g) in FCR of broilers throughout the experimental period. No mortalities were recorded in all treatment groups throughout the experimental period.

Table (6) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on performance of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Feed intake (g)	3428 ±117.38	3421 ±70.06	3414 ±21.70	3436 ±71.33	3.94	NS
Initial weight (g)	169 ±1.15	168 ±1.15	171 ±1.15	172 ±1.15	1.14	NS
Final body weight (g)	1926^{c} ±56.86	2090^{b} ±15.37	2219^{ab} ±8.14	2315^{a} ±15.88	2.48	S
Body gain (g)	1757^{c} ±56.86	1922^{b} ±15.37	2048^{ab} ±8.14	2143^{a} ±15.88	2.71	S
FCR (g:g)	1.95^{c} ±0.05	1.78^{b} ±0.03	1.67^{ab} ±0.03	1.60^{a} ±0.04	3.32	S

Values are mean ± SE Mean.

^{a,b,c}Any two means values having same superscripts within row are not significantly different ($p \geq 0.05$) according to (DMRT).

C.V: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

DMRT: Duncan multiple range test

Figure (1) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on body weight, body weight gain and feed intake (g) of broilers

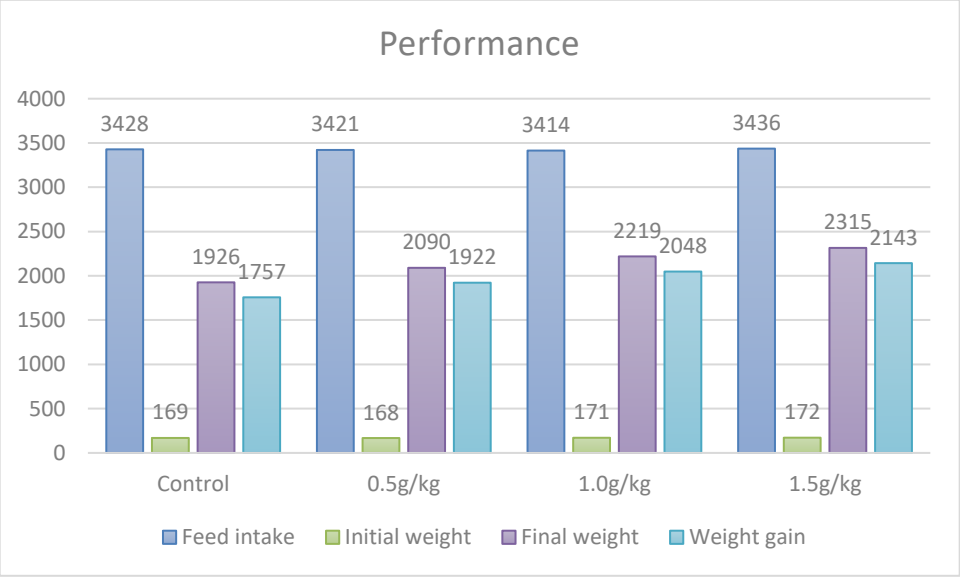
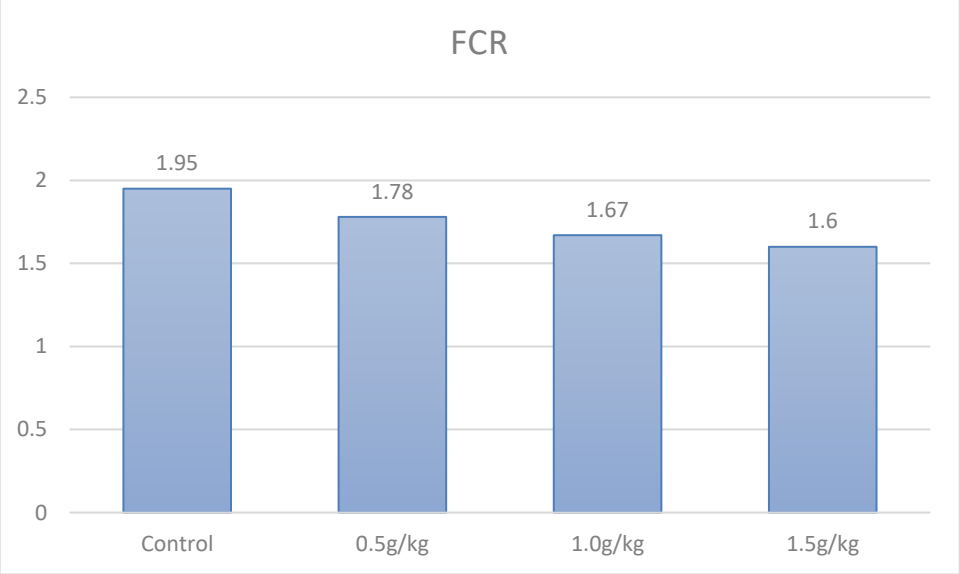


Figure (2) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on feed conversion ratio (FCR) of broilers



4.1.2 Carcass measurements:

4.1.2.1 Percentages of carcass dressing and giblets:

As shown in table (7) and figures (3), application of graded levels of BPB significantly ($p \leq 0.05$) affected carcass dressing percentage of broiler chicks. The results indicated that, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) better carcass dressing percentage (71.83%) as compared to chicks fed on control diet (70.31%). Whereas, no significant differences ($p \geq 0.05$) were observed between groups of the chicks fed on all levels of BPB supplementations 0.5, 1.0 and 1.5g/kg (71.36, 71.40% and 71.83% respectively), also, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5, 1.0g/kg BPB and group of chicks fed on control diet (71.36, 71.40% and 70.31% respectively) in carcass dressing percentage of broiler chicks. All percentages values of carcass dressing were in normal range of broilers **appendix (6).**

The results deal with giblets percentages (gizzard, liver and heart) recorded, no significant differences ($p \geq 0.05$) among the all treatments groups. However, the chicks fed on 1.5g/kg BPB had obtained numerically higher percentage value of gizzard (1.61%) as compared to the chicks fed on 0.5g/kg BPB 1.55%, 1.0g/kg BPB 1.55% and control diet (1.54%), also, the same trend for liver and heart percentages values. All percentages values of giblets (gizzard, liver and heart) were in normal range of broiler chicks according to (Oladimeji *et al*., 2020 and Karthika *et al*., 2019).

Table (7) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on percentages of dressing, gizzard, liver and heart of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Dressing%	70.31^{b} ±0.33	71.36^{ab} ±0.31	71.40^{ab} ±0.30	71.83^{a} ±0.08	0.67	S
Gizzard%	1.54 ±0.10	1.55 ±0.07	1.55 ±0.12	1.61 ±0.06	9.81	NS
Liver%	2.05 ±0.20	2.05 ±0.08	2.07 ±0.17	2.13 ±0.21	14.42	NS
Heart%	0.51 ±0.01	0.50 ±0.04	0.53 ±0.01	0.57 ±0.01	6.58	NS

Values are mean ± SE Mean

[a,b]Any two means values having same superscripts within row are not significantly different P≤0.05).

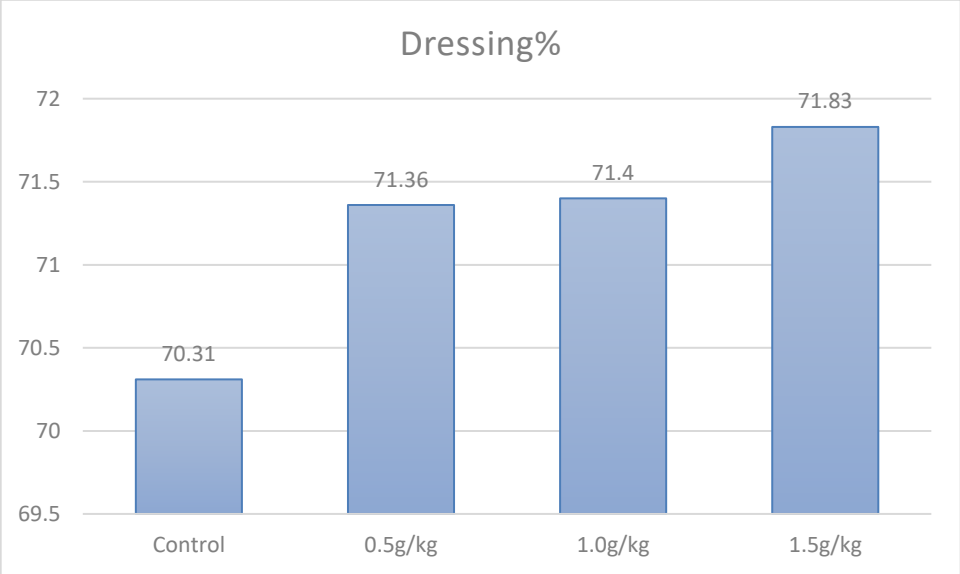
C.V: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference (p≤0.05).

NS: No significant difference (p≥ 0.05).

Figure (3) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on dressing percentage of broilers



4.1.2.2 Percentages of back, wings and neck:

The effect of feeding graded levels of BPB for 5 weeks on percentages of back, wings and neck of broiler chicks, was given in table (8). The results showed, no significant differences ($p \geq 0.05$) were observed between all tested groups of BPB supplementations (0.5, 1.0 and 1.5g/kg) and control group in percentages of back, wings, neck of broiler chicks. However, the chicks fed on 1.0 and 1.5g/kg BPB had obtained numerically higher percentages values of back, wings and neck (20.18, 10.61, 5.43% and 20.57, 10.84, 5.44% respectively) as compared to chicks fed on control diet and chicks fed on 0.5g/kg BPB (19.69, 10.47, 5.19% and 19.81, 10.55, 5.38% respectively). Although, all percentage values of back, wings, neck with in normal range of broilers according to (Oladimeji *et al*., 2020).

Table (8) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on percentages of back, wings and neck of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Back%	19.69 ±0.84	19.81 ±0.26	20.18 ±0.07	20.57 ±0.82	4.13	NS
Wing%	10.47 ±0.39	10.55 ±0.05	10.61 ±0.04	10.84 ±0.24	3.76	NS
Neck%	5.19 ±0.28	5.38 ±0.05	5.43 ±0.07	5.44 ±0.09	2.97	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.2.3 Non carcass components

The effect of treatments on non- carcass components of broiler chicks fed graded levels of BPB for 5 weeks, was shown in table (9). The results indicated, no significant differences ($p \geq 0.05$) observed between all tested groups of BPB (0.5, 1.0 and 1.5g/kg) and control group in percentages of head, legs, lungs, kidney and abdominal fat of broiler chicks. However, chicks fed on 1.5g/kg BPB had obtained numerically higher percentage values of non- carcass components which mentioned above (2.60, 4.19, 0.72% and 0.48% respectively), except abdominal fat recorded numerically the lowest percentage value (0.79%) as compared to the others tested groups. Although, all percentage values of non-carcass components within normal range of broilers according to (Oladimeji *et al*., 2020)

Data collected for an intestine revealed that, no significant differences ($p \geq 0.05$) were observed between all tested groups in length of an intestine (cm) and percentage of an intestine weights of broiler chicks. However, the group of chicks fed on 1.5g/kg BPB obtained numerically the heaviest weight percent of an intestine (3.86%), followed by group of chicks fed on 1.0g/kg BPB 3.84% and 0.5g/kg BPB 3.83% then control diet 3.80%, also, that group 1.5g/kg BPB obtained numerically the longest intestine (189cm) as compared to chicks fed on 1.0g/kg BPB (186cm), chicks fed on 0.5g/kg BPB (186cm) and chicks fed on control diet (182cm). Although, all percentages weights of an intestine and intestine length were in normal range of broilers according to (Oladimeji *et al*., 2020 and Kokoszyński *et al*., 2017).

Table (9) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on percentages and length of non- carcass components of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Head%	2.55 ±0.07	2.56 ±0.06	2.56 ±0.03	2.60 ±0.01	3.39	NS
Legs%	4.11 ±0.25	4.14 ±0.05	4.17 ±0.05	4.19 ±0.04	5.45	NS
Lungs%	0.72 ±0.0	0.71 ±0.03	0.72 ±0.02	0.73 ±0.01	8.47	NS
Kidney%	0.41 ±0.02	0.42 ±0.02	0.46 ±0.03	0.48 ±0.02	8.03	NS
Abdominal fat%	1.07 ±0.11	1.01 ±0.12	0.82 ±0.10	0.79 ±0.11	20.93	NS
Intestine weight%	3.80 ±0.23	3.83 ±0.13	3.84 ±0.16	3.86 ±0.40	7.56	NS
Intestine length (cm)	182 ±1.45	186 ±2.08	186 ±4.33	189 ±4.16	3.03	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.2.4 Commercial cuts:

The results of the percentages of commercial cuts (breast, thigh and drumstick) were presented in table (10), the results showed, no significant differences ($p \geq 0.05$) between all tested groups in percentages of breast, thigh and drumstick of broiler chicks fed graded levels of BPB for 5 weeks. However, the chicks fed on 1.5g/kg BPB had obtained numerically the highest percents of breast, thigh and drumstick (40.33, 15.87% and 12.60% respectively) as compared to chicks fed on 1.0g/kg BPB (39.64, 15.47% and 12.20%), chicks fed on 0.5g/kg BPB (39.35, 15.12% and 12.03%) and chicks fed on control diet (39.22, 15.07% and 11.73%). However, all percentages values of commercial cuts were within normal range for broiler chicks according to Cobb 500 broiler yield performance **appendix (6)** and according to (Oladimeji *et al*., 2020 and Soares *et al*., 2017).

Table (10) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on breast, thigh and drumstick% of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Breast%	39.22 ±0.51	39.35 ±0.01	39.64 ±0.03	40.33 ±0.10	1.13	NS
Thigh%	15.07 ±0.40	15.12 ±0.15	15.47 ±0.15	15.87 ±0.07	2.59	NS
Drumstick %	11.73 ±0.74	12.03 ±0.03	12.20 ±0.10	12.60 ±0.07	5.33	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$)

4.1.2.5 Meat of commercial cuts:

The treatment groups values for meat expressed as percentages from total weight of selected commercial cuts (breast, thigh and drumstick) was given in table (11). For breast meat, the results indicated that, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) the highest meat percent of breast (87.02%) as compared to the chicks fed on control diet (85.15%). Whereas, no significant differences ($p \geq 0.05$) were observed between groups fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg) 86.25%, 86.29% and 87.02% respectively, also, no significant differences ($p \geq 0.05$) were observed between groups of the chicks fed on 0.5, 1.0g/kg BPB and the chicks fed on control diet (86.25, 86.29% and 85.15% respectively) in meat percent of breast of broiler chicks.

The results deal with meat percent of thigh and drumstick revealed, no significant differences ($p \geq 0.05$) between all tested groups. However, chicks fed on 1.5g/kg BPB had obtained numerically higher meat percent for thigh and drumstick (85.89% and 75.46% respectively) as compared to chicks fed on 1.0g/kg BPB (85.14% and 75.07% respectively), chicks fed on 0.5g/kg BPB (85.10% and 75.03% respectively) and chicks fed on control diet (84.95% and 74.53% respectively). However, all percentages values of meat of commercial cuts, within normal range of broiler chicks **appendix (6).**

Table (11) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on breast, thigh and drumstick meat%

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Breast meat %	85.15^{b} ±0.31	86.25^{ab} ±0.54	86.29^{ab} ±0.36	87.02^{a} ±0.19	0.75	S
Thigh meat %	84.95 ±0.05	85.10 ±0.10	85.14 ±0.26	85.89 ±0.31	0.43	NS
Drumstick meat %	74.53 ±1.31	75.03 ±0.73	75.07 ±0.66	75.46 ±1.08	2.27	NS

Values are mean ± SE Mean

[a,b]Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.3 Meat quality parameters:

4.1.3.1 Panel taste (subjective meat attributes):

The effect of graded levels of (BPB) on subjective meat attributes of broiler chicks, were shown in table (12). The results revealed that, no significant differences($p \geq 0.05$)were shown among the all dietary treatments on the average subjective meat quality score values of colour, tenderness, juiciness and flavour using an eight-point scale, and score given for all attributes were above moderate acceptability level **appendix (4).** However, the chicks fed on 1.0 and 1.5g/kg (BPB) had obtained numerically the best moderately desirable colour (6.65 and 6.67 respectively), best tender (6.55 and 6.55 respectively), best juicy (6.55 and 6.58 respectively) and best intense flavour (6.61 and 6.63 respectively) as compared to the other tested groups of broilers.

Table (12) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on meat quality attributes

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Tenderness	6.50 ±0.12	6.53 ±0.17	6.55 ±0.17	6.55 ±0.17	4.26	NS
Flavor	6.55 ±0.17	6.59 ±0.17	6.61 ±0.17	6.63 ±0.17	4.55	NS
Juiciness	6.50 ±0.06	6.53 ±0.12	6.55 ±0.12	6.58 ±0.06	2.42	NS
Color	6.40 ±0.12	6.63 ±0.17	6.65 ±0.17	6.67 ±0.17	4.23	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.3.2 Meat chemical analysis (objective meat attributes):

The results of meat chemical analysis of broiler chicks fed graded levels of BPB for 5 weeks were illustrated in table (13). The results recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of meat chemical composition (dry matter, moisture, protein, ash and ether extract). However, the groups of chicks fed on 1.0 and1.5g/kg BPB recorded numerically higher percentages values of moisture (74.40% and 74.35% respectively), protein (22.58% and 22.61% respectively), ash (1.26% and 1.33% respectively) and ether extract (1.35% and 1.37% respectively), on the other hand, those groups on 1.0 and 1.5g/kg BPB obtained numerically the lowest percentage values of dry matter (25.60% and 25.65% respectively) of meat chemical analysis as compared to chicks fed on 0.5g/kg BPB and chicks fed on control diet (26.07% and 25.77% respectively). However, all percentages values of meat chemical analysis within normal range of broilers according to (Snezana *et al*., 2010).

Table (13) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on meat chemical composition of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Dry matter%	25.77 ±0.61	26.07 ±0.15	25.60 ±0.23	25.65 ±0.35	2.51	NS
Moisture%	74.23 ±0.61	73.93 ±0.17	74.40 ±0.23	74.35 ±0.18	0.81	NS
Protein%	22.58 ±0.06	22.60 ±0.03	22.58 ±0.05	22.61 ±0.02	0.32	NS
Ash%	1.20 ±0.03	1.20 ±0.12	1.26 ±0.03	1.33 ±0.01	8.69	NS
Ether extract %	1.34 ±0.43	1.35 ±0.03	1.35 ±0.03	1.37 ±0.01	4.19	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.4 Serum parameters:

4.1.4.1 Serum metabolites:

The effect of graded levels of BPB on serum metabolites of broiler chicks was shown in table (14). The results illustrated that, no significant ($p \geq 0.05$) differences were observe between control group and groups of BPB supplementations (0.5, 1.0 and 1.5g/kg) in serum total protein, albumin, creatinine, uric acid, urea, and glucose. Also, there was no significant ($p \geq 0.05$) treatments effect on cholesterol, HDL cholesterol, LDL cholesterol and triglycerides of broiler chicks. However, the chicks fed on 1.5g/kg BPB recorded numerically the higher values of total protein and glucose (4.25g/dl and 221.00mg/dl respectively) as compared to the other tested groups. At the same time (simultaneously) this group (1.5g/kg BPB), recorded the lowest values of albumin, creatinine, uric acid and urea (2.15g/dl, 2.10mg/dl, 3.41mg/dl and 6.85mg/dl respectively), also, the same trend for cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (122.00, 128.30, 21.00mg/dl and 42.00mg/dl respectively) as compared to the other tested groups. Although, all values of serum metabolites mentioned above were in normal range of serum profile for broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

Table (14) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on serum metabolites of broiler chicks

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
Total protein (g/dl)	3.95 ±0.03	4.05 ±0.09	4.10 ±0.12	4.25 ±0.14	4.37	NS
Albumin (g/dl)	2.25 ±0.03	2.25 ±0.03	2.20 ±0.00	2.15 ±0.06	2.75	NS
Creatinine (mg/dl)	2.13 ±0.00	2.11 ±0.00	2.11 ±0.00	2.10 ±0.00	0.01	NS
Uric acid (mg/dl)	3.45 ±0.03	3.43 ±0.03	3.40 ±0.06	3.41 ±0.06	2.33	NS
Urea (mg/dl)	7.00 ±0.00	6.90 ±0.06	6.85 ±0.03	6.85 ±0.03	0.89	NS
Cholesterol (mg/dl)	124.50 ±0.29	122.30 ±0.33	122.00 ±0.25	122.00 ±0.25	0.87	NS
HDL Cholesterol (mg/dl)	130.50 ±0.29	129.00 ±0.87	128.50 ±0.20	128.30 ±0.33	0.65	NS
LDL Cholesterol (mg/dl)	21.50 ±0.01	21.50 ±0.00	21.10 ±0.00	21.00 ±0.00	0.05	NS
Triglyceride(mg/dl)	43.50 ±0.29	42.50 ±0.29	42.00 ±0.58	42.00 ±0.58	1.85	NS
Glucose (mg/dl)	220.50 ±0.29	220.00 ±0.58	219.00 ±0.58	221.00 ±0.78	2.30	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.4.2 Serum enzymes activity and Serum electrolytes:

As shown in table (15), the results indicated that, no significant ($p \geq 0.05$) effect on serum enzymes activities values (Aspartate amino-transferase AST and Alkaline phosphatase ALP) were observed between all tested groups of broiler chicks fed graded levels of BPB for 5 weeks. However, the chicks fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg) had recorded numerically the lowest values of serum enzymes AST and ALP (37.85, 37.80, 37.80 iu/L and 174.95, 174.90, 174.85 iu/L respectively) as compared to control group (38.95 iu/L and 175.00 iu/L respectively). While all values of serum enzymes activities of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

Data collected for serum minerals revealed that, no significant ($p \geq 0.05$) differences were observed between the chicks fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg) and the chicks fed on control diet in serum electrolytes values (Calcium Ca and Phosphorus P). However, the chicks fed on all levels of BPB recorded the highest values of minerals Ca and P (8.37, 8.35, 8.40 mg/dl and 8.80, 8.85, 8.85 mg/dl respectively) as compared to control group (8.15mg/dl and 8.75 mg/dl respectively).While all values of serum electrolytes values of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Adeyemo *et al*., 2018).

Table (15) Effect of graded levels of Bacterial Probiotic Biogen.s (BPB) on serum enzymes and serum minerals of broilers

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB	CV%	HSD 0.05
AST (iu/L)	38.95 ±0.03	37.85 ±0.03	37.80 ±0.06	37.80 ±0.06	0.16	NS
ALP (iu/L)	175.00 ±0.00	174.95 ±0.03	174.90 ±0.00	174.85 ±0.03	0.02	NS
Ca (mg/dl)	8.15 ±0.03	8.37 ±0.12	8.35 ±0.03	8.40 ±0.12	1.79	NS
P (mg/dl)	8.75 ±0.02	8.80 ±0.00	8.85 ±0.03	8.85 ±0.03	0.49	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.5 Economic appraisal:

Appraisal of total cost, revenues and profitability ratio per head of broiler chicks fed on graded levels of dietary bacterial probiotic Biogen.S BPB for 5 weeks, demonstrated in table (16) and figure (4). Chicks purchase, management and feed cost values were the major input considered. The total selling values of meat is the total revenues obtained. The results of economical evaluation indicated that, the chicks fed on BPB with all levels (0.5, 1.0 and 1.5g/kg) had gained more net profit/bird (100.16, 103.82 and 114.36 respectively) as compared to those chicks fed on control diet (74.09), also, recorded highest values of profitability ratio/bird (1.35, 1.40 and 1.54 respectively) as compared to chicks fed on control diet (1), but the group of chicks fed on 1.5g/kg BPB was the highest of the tested groups (1.54). For net profit/kg meat, the results noticed that, chicks fed on BPB with all levels (0.5, 1.0 and 1.5g/kg) had gained more net profit/kg meat (60.96, 61.65 and 63.64 respectively) as compared to chicks fed on control diet (53.77), also, recorded the highest values of profitability ratio/kg meat (1.13, 1.15 and 1.18 respectively) as compared to chicks fed on control diet (1), but the group of chicks fed on 1.5g/kg BPB was the highest of the tested groups (1.18).

Table (16) Total cost, returns and profitability ratio per head of broiler chicks fed graded levels of Bacterial Probiotic Biogen.s (BPB) for 5 weeks

Items	Control 0.0g/kg	0.5g/kg BPB	1.0g/kg BPB	1.5g/kg BPB
Cost				
Chicks purchase	19	19	19	19
Feed cost	37.71	38.14	38.58	39.34
Electricity and management	7	7	7	7
Total	63.71	64.14	64.58	65.34
Revenues				
Average weight of carcass	1.378	1.643	1.684	1.797
Price/kg of bird	100	100	100	100
Total	137.80	164.30	168.40	179.70
Profits				
Total revenues	137.80	164.30	168.40	179.70
Total cost	63.71	64.14	64.58	65.34
Net profit/bird	74.09	100.16	103.82	114.36
Net profit/kg meat	53.77	60.96	61.65	63.64
Profitability ratio/bird	1	1.35	1.40	1.54
Profitability ratio/kg meat	1	1.13	1.15	1.18

The total cost was calculated according to January 2019.

Price/kg was 100 SDG according to February 2019.

Figure (4) Effect of graded levels of probiotic Biogen.s (BPB) on profitability ratio/kg meat of broilers

4.2 Experiment two: Response of broiler chicks to graded levels of dietary Prebiotic Y-MOS (PYM).

4.2.1 Performance:

The effect of feeding graded levels of dietary Prebiotic Y-MOS (PYM) on performance of broiler chicks for 5 weeks was illustrated in table (17) and figures (5-6). Firstly for feed consumption, the results indicated that no significant differences ($p \geq 0.05$) were observed between control group and groups supplemented with PYM at levels (0.5, 1.0 and 1.5g/kg) in feed consumption of broilers throughout the experimental period. However, chicks fed on control diet consumed numerically more feed (3428g) followed by chicks fed on 1.5g/kg PYM (3420g) and 1.0g/kg PYM (3381g) then 0.5g/kg PYM (3381g), with an increasing estimated by about 0.23%, 1.39% and 1.39% respectively.

According to final body weight, the results revealed that, chicks fed on all levels of PYM supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) higher means (2108, 2204g and 2383g respectively) as compared to chicks fed on control diet (1926g). Also, the chicks fed on 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) heaviest mean of final body weight (2383g) as compared to chicks fed on 0.5, 1.0g/kg PYM and control diet (2108, 2204g and 1926g respectively), with an increasing estimated by about 13.05%, 8.12% and 23.73% respectively, whereas, no significant differences ($p \geq 0.05$) were observed between chicks fed on 0.5 and 1.0g/kg PYM (2108g and 2204g respectively) in final body weight of broilers, throughout the experimental period.

Application of graded levels of PYM significantly ($p \leq 0.05$) affected body weight gain, the results indicated that, chicks fed on diets supplemented with graded levels of PYM (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) higher means values (1940, 2032g and 2212g respectively) as compared to chicks fed on control diet (1757g), also, chicks fed on 1.5g/kg PYM had obtained significantly ($p \leq 0.05$)

higher mean value of body weight gain (2212g) as compared to those chicks fed on 0.5, 1.0g/kg PYM and control diets (1940, 2032g and 1757g respectively), with an increasing estimated by about 14.02%, 8.86% and 25.90% respectively. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5 and 1.0g/kg PYM (1940g and 2032g respectively) in body weight gain of broilers throughout the experimental period.

Finally the results deal with feed conversion ratio (FCR), showed that, chicks fed on diets supplemented with graded levels of PYM (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) better FCR (1.74, 1.66g:g and 1.55g:g respectively) as compared to the chicks fed on control diet (1.95g:g), whereas, no significant differences ($p \geq 0.05$) were observed between chicks fed on 0.5 and 1.0g/kg PYM (1.74g:g and 1.66g:g respectively), also, between chicks fed on 1.0 and 1.5g/kg PYM (1.66g:g and 1.55g:g respectively) in FCR of broilers. No mortalities were recorded in all treatment groups throughout the experimental period.

Table (17) Effect of graded levels of Prebiotic Y-MOS (PYM) on performance of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	C.V%	HSD 0.05
Feed intake (g)	3428 ±117.38	3381 ±8.02	3381 ±16.80	3420 ±15.93	3.05	NS
Initial weight (g)	169 ±1.15	168 ±1.15	172 ±1.15	171 ±1.15	1.18	NS
Final body weight (g)	1926^{c} ±56.86	2108^{b} ±4.04	2204^{b} ±18.46	2383^{a} ±15.28	2.47	S
Body weight gain (g)	1757^{c} ±56.86	1940^{b} ±4.04	2032^{b} ±18.46	2212^{a} ±15.28	2.73	S
FCR (g:g)	1.95^{c} ±0.05	1.74^{b} ±0.03	1.66^{ab} ±0.01	1.55^{a} ±0.01	2.59	S

Values are mean ± SE Mean

^{a,b,c}Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$) according to (DMRT).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

Figure (5) Effect of graded levels of prebiotic Y-MOS (PYM) on final body weight, body weight gain and feed intake (g) of broilers

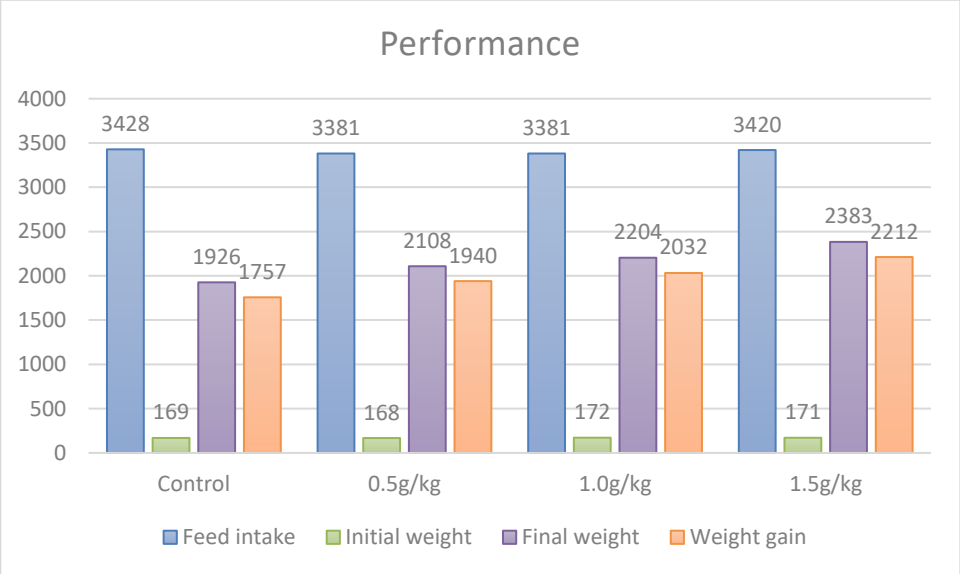
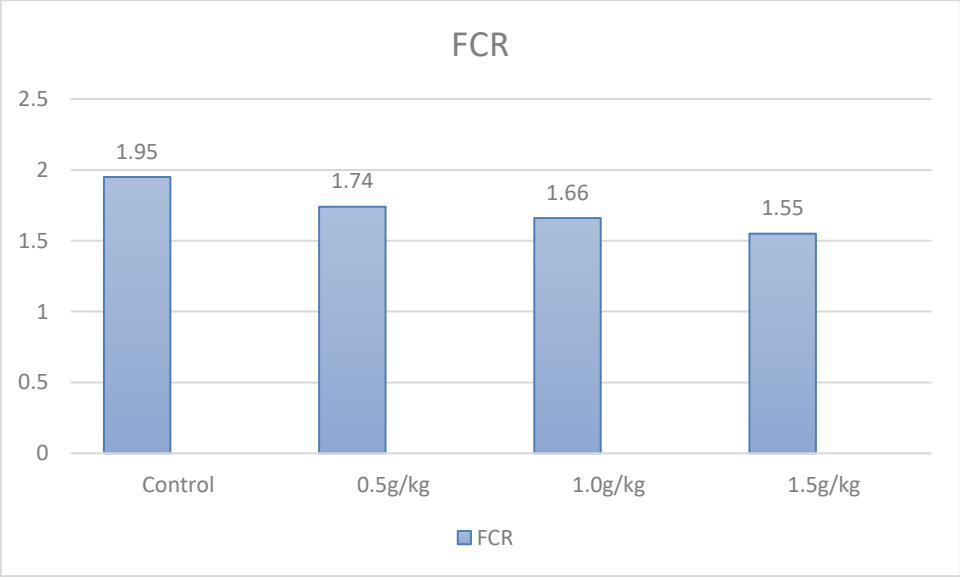


Figure (6) Effect of graded levels of Prebiotic Y-MOS (PYM) on feed conversion ratio (FCR) of broilers



4.2.2 Carcass measurements:

4.2.2.1Carcass dressing and giblets percentages:

As shown in table (18) and figure (7), treatments effect on percent of carcass dressing were significantly ($p \leq 0.05$) different. The results revealed that, chicks fed on 1.0 and 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) higher means values of carcass dressing percentage (71.48% and 71.42% respectively) as compared to chicks fed on control diet (70.22%), while no significant differences ($p \geq 0.05$)were observed between all levels of PYM supplementations (71.34%,71.48% and 71.42% respectively), also, between groups of chicks fed on 0.5g/kg PYM and control group (71.34% and 70.22%) in carcass dressing percentage of broilers.

The results concerning giblets percentages values (gizzard, liver and heart) recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups PYM supplementations 0.5, 1.0, 1.5g/kg and control group in percentages of gizzard, liver and heart. However, chicks fed on 1.5g/kg PYM obtained higher percentage value of gizzard (1.72), followed by chicks fed on 0.5g/kg PYM 1.59% and 1.0g/kg PYM 1.56% then control 1.55%, also, the same trend for liver and heart percentage values was recorded. All percentages values of carcass dressing and giblets (gizzard, liver and heart) were in normal range of broilers **appendix (6)** and according to (Oladimeji *et al*., 2020 and Karthika *et al*., 2019).

Table (18) Effect of graded levels of Prebiotic Y-MOS (PYM) on percentages of dressing, gizzard, liver and heart of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	CV%	HSD 0.05
Dressing%	70.22[b] ±0.24	71.34[ab] ±0.27	71.42[a] ±0.22	71.48[a] ±0.27	0.61	S
Gizzard %	1.55 ±0.10	1.59 ±0.03	1.56 ±0.12	1.72 ±0.03	8.77	NS
Liver %	2.05 ±0.20	2.05 ±0.17	2.10 ±0.02	2.11 ±0.11	11.88	NS
Heart %	0.51 ±0.01	0.54 ±0.01	0.52 ±0.01	0.56 ±0.04	7.81	NS

Values are mean ± SE Mean

[a,b]Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$).

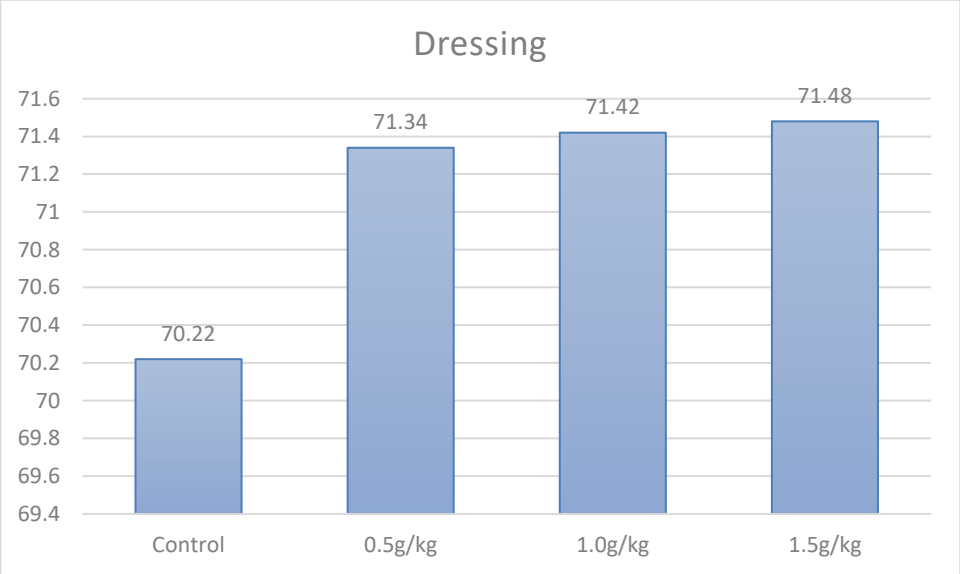
CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

Figure (7) Effect of graded levels of Prebiotic Y-MOS (PYM) on carcass dressing percentage of broilers



4.2.2.2 Percentages of back, wings and neck:

The effect of graded levels of PYM for 5 weeks onpercentages of back, wings and neck of broiler chicks was shown in table (19). The results revealed that, no significant differences ($p \geq 0.05$) were observed between all tested groups of PYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in percentages of back, wings and neck of broiler chicks. However, chicks fed on 1.5g/kg PYM had obtained numerically higher percentage values of back, wings and neck (20.66, 11.12 and 5.33% respectively) as compared to chicks fed on 1.0g/kg PYM (20.31, 10.47% and 5.30% respectively) and chicks fed on 0.5g/kg PYM (20.25, 10.47% and 5.25% respectively) then chicks fed on control diet (19.69, 10.31% and 5.19%). Although all percentages values of back, wings and neck with in normal range of broilers according to (Oladimeji *et al*., 2020).

Table (19) Effect of graded levels of Prebiotic Y-MOS (PYM) on percentages of back wings and neck of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	CV%	HSD 0.05
Back%	19.69 ±0.84	20.25 ±0.31	20.31 ±0.04	20.66 ±0.07	3.84	NS
Wing%	10.47 ±0.39	10.31 ±0.35	10.47 ±0.15	11.12 ±0.23	4.85	NS
Neck%	5.19 ±0.28	5.25 ±0.03	5.30 ±0.07	5.32 ±0.09	4.96	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of variation.

HSD: Honest significant difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.2.2.3 Non carcass components:

The effect of treatments on non- carcass components of broiler chicks fed graded levels of PYM for 5 weeks, was given in table (20). The results recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of head, legs, lung, kidney, intestine weight and abdominal fat of broiler chicks. However, the chicks fed on 1.5g/kg PYM had obtained numerically higher percentages values of non- carcass components which mentioned above (2.59, 4.28, 0.74, 0.46% and 3.89% respectively), except abdominal fat recorded numerically the lowest percentage value (0.96%) as compared to all tested groups. However, all percentages values of non-carcass components within normal range of broilers according to (Oladimeji *et al*., 2020).

On the other hand, the significant differences ($p \leq 0.05$) were found in length of an intestine (cm), the results indicated that, the chicks fed on 1.0 and 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) longest means values of an intestine (197cm and 202cm respectively) as compared to chicks fed on control diet (182cm). Whereas, no significant differences ($p \geq 0.05$) were observed between groups of the chicks fed on 0.5g/kg PYM and the chicks fed on control diet (188cm and 182cm respectively), also, between chicks fed on 0.5 and 1.0g/kg PYM (188cm and 197cm respectively). Although, all intestine length were in normal range of broilers according to (Oladimeji *et al*., 2020 and Kokoszyński *et al*., 2017).

Table (20) Effect of graded levels of Prebiotic Y-MOS (PYM) on percentages and length of non- carcass components of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	CV%	HSD 0.05
Head%	2.55 ±0.07	2.55 ±0.02	2.57 ±0.05	2.59 ±0.05	3.47	NS
Legs%	4.11 ±0.25	4.24 ±0.01	4.26 ±0.16	4.28 ±0.02	6.07	NS
Lungs%	0.73 ±0.05	0.70 ±0.02	0.73 ±0.02	0.74 ±0.02	7.32	NS
Kidneys%	0.41 ±0.02	0.44 ±0.02	0.44 ±0.01	0.46 ±0.02	6.90	NS
Abdominal fat%	1.07 ±0.11	1.03 ±0.17	0.99 ±0.06	0.96 ±0.20	4.67	NS
Intestine weight%	3.80 ±0.22	3.83 ±0.37	3.87 ±0.04	3.89 ±0.02	9.79	NS
Intestine length (cm)	182^{c} ±1.45	188^{bc} ±4.00	197^{ab} ±2.19	202^{a} ±1.15	2.22	S

Values are mean ± SE Mean

^{a,b,c}Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.2.4 Commercial cuts:

The effect of adding graded levels of PYM on percentages of commercial cuts (breast, thigh and drumstick%) of broiler chicks for 5 weeks, were given in table (21), the results revealed that, no significant differences ($p \geq 0.05$) were observed between all tested groups of PYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in percentages of commercial cuts (breast, thigh and drumstick%). However, the chicks fed on 1.5g/kg PYM had obtained numerically heaviest percentages values of breast, thigh and drumstick (40.12, 15.88% and 12.93% respectively) as compared to chicks fed on 1.0g/kg PYM (40.03, 15.77% and 12.78% respectively) and chicks fed on 0.5g/kg PYM (39.62, 15.47% and 12.15% respectively) then chicks fed on control diet (39.22, 15.07% and 11.73% respectively) of broiler chicks. However, all percentages values of commercial cuts, within normal range of broilers **appendix (6)** and according to (Oladimeji *et al*., 2020 and Soares *et al*., 2017).

Table (21) Effect of graded levels of Prebiotic Y-MOS (PYM) on breast, thigh and drumstick% of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	C.V%	HSD 0.05
Breast%	39.22 ±0.51	39.62 ±0.71	40.03 ±0.52	40.12 ±0.42	2.40	NS
Thigh%	15.07 ±0.40	15.47 ±0.31	15.77 ±0.04	15.88 ±0.35	3.53	NS
Drumstick %	11.73 ±0.74	12.15 ±0.54	12.78 ±0.40	12.93 ±0.32	7.35	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.1.2.5 Meat of commercial cuts:

The treatment groups values of meat expressed as percentages from total weights of selected commercial cuts was showed in table (22), the results indicated that, the meat percent of commercial cuts were not significantly affected by application of graded levels of PYM, however, chicks fed on 1.5g/kg PYM had obtained numerically heaviest meat percent of breast, thigh and drumstick (86.60, 85.93% and 75.87% respectively), followed by chicks fed on 1.0g/kg PYM (86.42, 85.66% and 75.56% respectively) and chicks fed on 0.5g/kg PYM (85.69, 85.22% and 75.06% respectively) then chicks fed on control diet (85.15, 84.78% and 74.53% respectively) of broiler chicks. Nevertheless, all percentages values of meat of commercial cuts (breast, thigh and drumstick) within normal range of broilers.

Table (22) Effect of graded levels of Prebiotic Y-MOS (PYM) on breast, thigh and drumstick meat % of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	C.V%	HSD 0.05
Breast meat %	85.15 ±0.32	85.69 ±1.17	86.42 ±0.36	86.60 ±0.73	1.47	NS
Thigh meat %	84.78 ±0.22	85.22 ±0.17	85.66 ±0.19	85.93 ±0.48	0.59	NS
Drumstick meat %	74.53 ±1.31	75.06 ±0.59	75.56 ±1.13	75.87 ±0.01	2.11	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.3 Meat quality parameters:

4.2.3.1 Panel taste (subjective meat attributes):

The effect of graded levels of PYM on subjective meat attributes of broiler chicks, were presented in table (23). The results indicated that, no significant differences ($p \geq 0.05$) among the all dietary treatments of broiler chicks in the average subjective meat quality score value of colour, tenderness, juiciness and flavour using an eight-point scale, and score given for all attributes are above moderate acceptability level **appendix (4**). However, the chick fed on 1.0 and 1.5g/kg PYM had obtained numerically the best desirable colour (6.63 and 6.65 respectively), best tender (6.54 and 6.56 respectively), best juicy (6.55 and 6.57 respectively) and best intense flavour (6.60 and6.62 respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet.

Table (23) Effect of graded levels of Prebiotic Y-MOS (PYM) on meat quality attributes of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	CV%	HSD 0.05
Tenderness	6.50 ±0.12	6.52 ±0.17	6.54 ±0.12	6.56 ±0.12	3.51	NS
Juiciness	6.50 ±0.06	6.55 ±0.12	6.55 ±0.17	6.57 ±0.12	3.24	NS
Flavor	6.55 ±0.17	6.58 ±0.12	6.60 ±0.12	6.62 ±0.12	3.48	NS
Color	6.40 ±0.12	6.61 ±17	6.63 ±0.12	6.65 ±0.12	3.49	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.3.2 Meat chemical composition (objective meat attributes)

The results of meat chemical analysis of broiler chicks fed graded levels of PYM were demonstrated in table (24). The results recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of moisture, dry matter, protein, ash and ether extract of meat chemical composition of broiler chicks. However, chicks fed on 1.0 and 1.5g/kg PYM recorded numerically the highest moisture percentages (74.87% and 74.90% respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet (74.60% and 74.23% respectively), also, obtained numerically the highest values of protein percent (22.62% and 22.64% respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet (22.60% and 22.58% respectively), highest values of ash percent (1.25% and 1.30% respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet (1.23% and 1.20% respectively), and highest values of ether extract percent (1.38 and 1.38% respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet (1.35% and 1.32% respectively) of broiler chicks. On the other hand, chicks fed on 1.0 and 1.5g/kg PYM recorded numerically the lowest values of dry matter percentages (25.13% and 25.10% respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet (25.40% and 25.77% respectively) of broiler chicks.Although, all percentages values of meat chemical analysis, within normal range of broilers according to (Snezana *et al*., 2010).

Table (24) Effect of graded levels of Prebiotic Y-MOS (PYM) on meat chemical composition of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	C.V%	HSD 0.05
Dry matter%	25.77 ±0.61	25.40 ±0.12	25.13 ±0.09	25.10 ±0.23	2.25	NS
Moisture%	74.23 ±0.61	74.60 ±0.12	74.87 ±0.09	74.90 ±0.23	0.77	NS
Protein%	22.58 ±0.06	22.60 ±0.12	22.62 ±0.12	22.64 ±0.17	0.94	NS
Ash%	1.20 ±0.03	1.23 ±0.02	1.25 ±0.01	1.30 ±0.09	6.51	NS
Ether extract %	1.32 ±0.01	1.35 ±0.02	1.38 ±0.02	1.38 ±0.04	3.48	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.4 Serum parameters:

4.2.4.1 Serum metabolites:

The effect of graded levels of PYM on serum metabolites of broiler chicks was shown in table (25). The results illustrated that, no significant ($p \geq 0.05$) differences were observe between all groups of PYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in serum total protein, albumin, creatinine, uric acid, urea, and glucose of broiler chicks. Also, there were no significant ($p \geq 0.05$) treatments effect on cholesterol, HDL cholesterol, LDL cholesterol and triglycerides of broiler chicks. However, the chicks fed on 1.0 and 1.5g/kg PYM recorded numerically the highest means values of total protein and glucose (4.25, 4.25g/dl and 220.60, 220.70mg/dl respectively) as compared to the chicks fed on 0.5g/kg PYM and chicks fed on control diet (4.20, 3.95g/dl and 220.00, 220.50mg/dl respectively). At the same time (simultaneously) this groups recorded the lowest values of albumin (2.35g/dl and 2.30g/dl respectively), creatinine (2.10mg/dl and 2.09mg/dl), uric acid (3.35mg/dl and 3.30mg/dl respectively) and urea (6.85mg/dl and 6.85mg/dl respectively). Also, the same trend for cholesterol (123.10mg/dl and 123.00mg/dl respectively), for HDL cholesterol (128.50mg/dl and 128.20mg/dl respectively), LDL cholesterol (21.50mg/dl and 21.30mg/dl respectively) and for triglycerides (42.10mg/dl and 42.00mg/dl respectively) as compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet. Although all values of serum metabolites mentioned above were in normal range of serum profile of broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

Table (25) Effect of graded levels of Prebiotic Y-MOS (PYM) on serum metabolites of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	C.V%	HSD 0.05
Protein (g/dl)	3.95 ±0.03	4.20 ±0.14	4.25 ±0.00	4.25 ±0.14	4.29	NS
Albumin (g/dl)	2.45 ±0.09	2.45 ±0.09	2.35 ±0.03	2.30 ±0.00	4.52	NS
Creatinine (mg/dl)	2.12 ±0.00	2.11 ±0.00	2.10 ±0.00	2.09 ±0.00	0.00	NS
Uric acid (mg/dl)	3.45 ±0.03	3.40 ±0.06	3.35 ±0.09	3.30 ±0.09	3.54	NS
Urea (mg/dl)	6.90 ±0.00	6.90 ±0.00	6.85 ±0.03	6.85 ±0.03	0.51	NS
Cholesterol (mg/dl)	124.50 ±0.29	123.50 ±0.00	123.10 ±0.29	123.00 ±1.15	0.86	NS
Cholesterol HDL (mg/dl)	129.33 ±0.33	129.00 ±0.29	128.50 ±0.00	128.20 ±0.29	0.35	NS
Cholesterol LDL (mg/dl)	22.50 ±0.29	22.00 ±0.00	21.50 ±0.29	21.30 ±0.29	1.98	NS
Triglyceride(mg/dl)	43.50 ±0.29	42.50 ±0.58	42.10 ±0.32	42.00 ±0.11	1.44	NS
Glucose (mg/dl)	220.50 ±0.29	220.00 ±0.00	220.60 ±0.87	220.70 ±0.58	0.43	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.4.2 Serum enzymes activity and serum electrolytes:

As shown in table (26), the results indicated that, no significant ($p \geq 0.05$) effect on serum enzymes activities values Aspartate amino-transferase (AST) and Alkaline phosphatase (ALP) were observed between all tested groups of broiler chicks fed graded levels of PYM for 5 weeks. However, the groups of chicks fed on PYM supplementations (0.5, 1.0, and 1.5g/kg) recorded numerically the lowest means values of serum enzymes AST and ALP (38.85, 38.80, 38.75 iu/L and 174.93, 174.90, 174.88 iu/L respectively) as compared to control group (38.90 iu/L and 174.95 iu/L). All values of serum enzymes activities of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

The results deal with serum minerals Calcium (Ca) and Phosphorus (P) recorded that, no significant differences ($p \geq 0.05$) were observed between control group and all levels of PYM supplemented groups. However, the chicks fed on all levels of PYM supplementations (0.5, 1.0, and 1.5g/kg), had obtained numerically highest values of Ca and P (8.20, 8.25, 8.30 mg/dl and 8.85, 8.90, 8.90 mg/dl respectively) as compared to control group (8.15 mg/dl and 8.75 mg/dl). All values of serum minerals of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Adeyemo *et al*., 2018).

Table (26) Effect of graded levels of Prebiotic Y-MOS (PYM) on serum enzymes activity and serum minerals of broilers

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM	CV%	HSD 0.05
AST (iu/L)	38.90 ±0.00	38.85 ±0.00	38.80 ±0.00	38.75 ±0.00	0.01	NS
ALP (iu/L)	174.95 ±0.00	174.93 ±0.00	174.90 ±0.03	174.88 ±0.00	0.01	NS
Ca (mg/dl)	8.15 ±0.03	8.20 ±0.06	8.25 ±0.03	8.30 ±0.00	0.74	NS
P (mg/dl)	8.75 ±0.03	8.85 ±0.03	8.90 ±0.58	8.90 ±0.00	5.66	NS

Values are mean ± SE Mean

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

CV: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

4.2.5 Economic appraisal:

Appraisal of total cost, revenues and profitability ratio per head of broiler chicks fed on graded levels of dietary Prebiotic Y-MOS (PYM) for 5 weeks, shown in table (27) and figures (8). Chicks purchase, management and feed cost values were the major input considered. The total selling values of meat is the total revenues obtained. The results of economical evaluation indicated that, chicks fed on PYM with all levels (0.5, 1.0 and 1.5g/kg) had gained more net profit/bird (97.10, 105.89 and 123.58 respectively) as compared to chicks fed on control diet (74.09), also, recorded highest values of profitability ratio/bird (1.31, 1.43 and 1.67 respectively) as compared to chicks fed on control diet (1), but the group of chicks fed on 1.5g/kg PYM was the highest of the tested groups (1.67). For net profit/kg meat, the results noticed that, chicks fed on PYM with all levels (0.5, 1.0 and 1.5g/kg) had gained more net profit/kg meat (60.39, 62.25 and 65.59 respectively) as compared to chicks fed on control diet (53.77), also, recorded highest values of profitability ratio/kg meat (1.12, 1.16 and 1.22 respectively) as compared to chicks fed on control diet (1), but the group of chicks fed on 1.5g/kg PYM was the highest of the tested groups (1.22).

Table (27) Total cost, returns and profitability ratio per head of broilers fed graded levels of Prebiotic Y-MOS (PYM) for 5 weeks

Items	Control 0.0g/kg	0.5g/kg PYM	1.0g/kg PYM	1.5g/kg PYM
Cost				
Chicks purchase	19	19	19	19
Feed cost	37.71	37.70	38.21	38.82
Electricity and management	7	7	7	7
Total	63.71	63.70	64.21	64.82
Revenues				
Average weight of carcass	1.378	1.608	1.701	1.884
Price/kg of bird	100	100	100	100
Total	137.80	160.80	170.10	188.40
Profits				
Total revenues	137.80	160.80	170.10	188.40
Total cost	63.71	63.70	64.21	64.82
Net profit/bird	74.09	97.10	105.89	123.58
Net profit/kg meat	53.77	60.39	62.25	65.59
Profitability ratio/bird	1	1.31	1.43	1.67
Profitability ratio/kg meat	1	1.12	1.16	1.22

The total cost was calculated according to January 2019.

Price/kg meat was 100 SDG according to February 2019.

Figure (8) Effect of graded levels of Prebiotic Y-MOS (PYM) on profitability ratio/kg meat of broilers

4.3 Experiment three: Response of broiler chicks to graded levels of dietary Synbiotic Biogen.S + Y-MOS (SBYM)

4.3.1 Performance:

The results of the performance of broiler chicks fed on diets containing graded levels of synbiotic Biogen.S + Y-MOS (SBYM) for 5 weeks, were demonstrated in table (28) and figures (9-10). Firstly for feed intake the results recorded that, the effect of treatments on the feed consumption was not significant ($p \geq 0.05$) among the all supplemented groups of SBYM at levels (0.5, 1.0 and 1.5g/kg) and control group. However, the chicks fed on control diet obtained the insignificantly higher mean value of this parameter (3428g) as compared to 0.5g/kg SBYM (3383g), 1.0g/kg SBYM (3382g) and 1.5g/kg SBYM (3390g), with an increasing estimated by about 1.33%, 1.36% and 1.12% respectively.

For final body weight the results revealed that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) higher means values of final body weight (2129, 2215g and 2386g respectively) as compared to chicks fed on control diets (1926g), also, the chicks fed on 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) higher mean value of final body weight (2386g) than those chicks fed on 0.5, 1.0g/kg SBYM and chicks fed on control diet (2129, 2215g and 1926g respectively), with an increasing estimated by about 12.07%, 7.72% and 23.88% respectively. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5 and 1.0g/kg (SBYM) (2129g and 2215g respectively) in final body weight of broilers throughout the experimental period.

Application of graded levels of SBYM significantly ($p \leq 0.05$) affected body weight gain, the results indicated that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) higher means values of body weight gain (1957, 2047g and 2215g respectively) as

compared to chicks fed on control diet (1757g), also, chicks fed on 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) higher mean value of body weight gain (2215g) as compared to those chicks fed on 0.5, 1.0g/kg SBYM and control diet (1957, 2047g and 1757g respectively), with an increasing estimated by about 13.18%, 8.21% and 26.07% respectively. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 0.5 and 1.0g/kg SBYM (1957g and 2047g respectively) in body weight gain of broilers throughout the experimental period.

The results deal with feed conversion ratio (FCR) shows that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) better FCR (1.73, 1.65g:g and 1.53g:g respectively) as compared to chicks fed on control diet (1.95g:g), also, chicks fed on 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) better FCR (1.53g:g) as compared to chicks fed on 0.5g/kg SBYM (1.73g:g), whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on 1.0 and 1.5g/kg SBYM (1.65g:g and 1.53g:g respectively), also, between groups of chicks fed on 0.5 and 1.0g/kg SBYM (1.73g:g and 1.65g:g respectively) in FCR of broilers. No mortalities were recorded in all treatment groups throughout the experimental period.

Table (28) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on performance of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Feed intake (g)	3428 ±117.38	3383 ±10.17	3382 ±17.67	3390 ±21.22	3.09	NS
Initial weight (g)	169 ±1.15	172 ±1.15	168 ±1.15	171 ±1.15	1.18	NS
Final body weight (g)	1926^{c} ±56.86	2129^{b} ±28.84	2215^{b} ±24.97	2386^{a} ±8.19	2.74	S
Body weight gain (g)	1757^{c} ±56.86	1957^{b} ±28.84	2047^{b} ±24.97	2215^{a} ±8.19	2.99	S
FCR (g:g)	1.95^{c} ±0.05	1.73^{b} ±0.02	1.65^{ab} ±0.02	1.53^{a} ±0.02	2.90	S

Values are mean ± SE Mean.

^{a,b,c}Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$) according to (DMRT).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No Significant difference ($p \geq 0.05$).

Figure (9) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on body weight, body weight gain and feed intake (g) of broilers

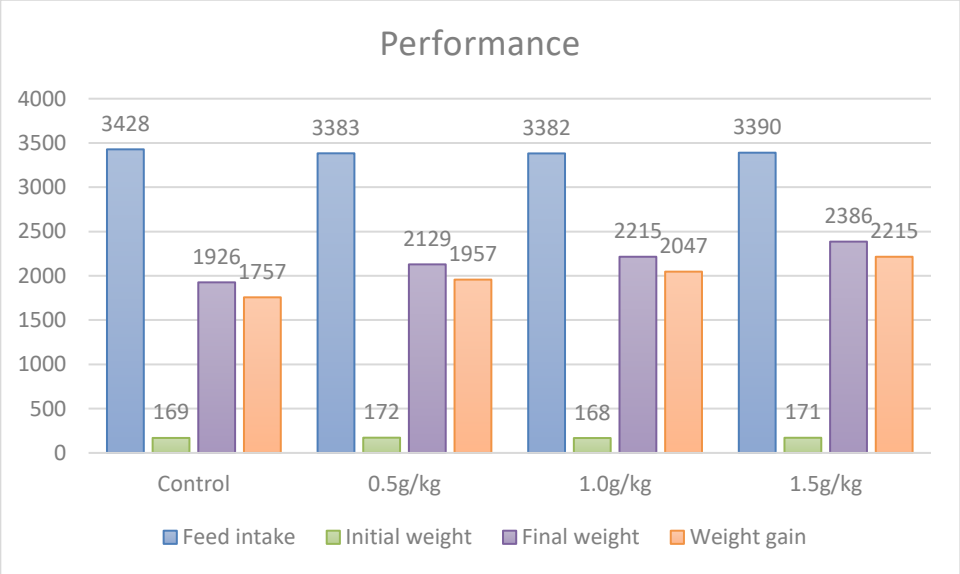
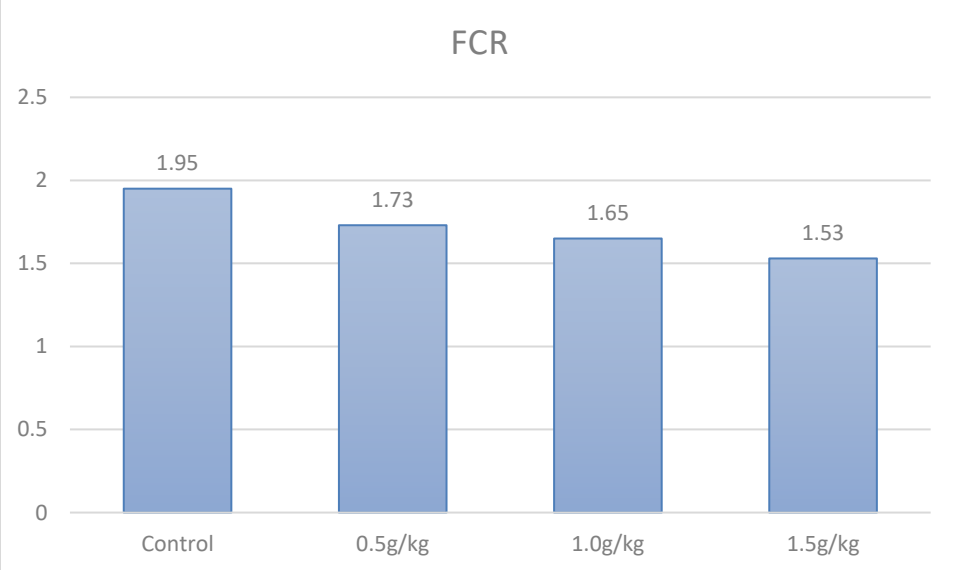


Figure (10) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on feed conversion ratio of broilers



4.3.2 Carcass measurement:

4.3.2.1 Carcass dressing and giblets percentage:

The results concerning dressing and giblets percentages were given in table (29) and figures (11). The results shows that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) highest percentages values of dressing (71.35, 71.39% and 71.48% respectively) as compared to the chicks fed on control diet (70.31%), whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg) in dressing percentages of broiler chicks.

The results of giblets percentages values (gizzard, liver and heart) were showed that, no significant differences ($p \geq 0.05$) among the all treatment groups of SBYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in giblets percentages of gizzard, liver and heart. However, the chicks fed on 1.5g/kg SBYM had obtained numerically highest percentage value of gizzard (1.63%) as compared to chicks fed on 0.5, 1.0g/kg SBYM and chicks fed on control diet (1.60, 1.60% and 1.55% respectively), also, the same trend for liver and heart percentages values. All percentages values of carcass dressing and giblets (gizzard, liver and heart) were in normal range of broilers **appendix (6)** and according to (Oladimeji *et al*., 2020 and Karthika *et al*., 2019).

Table (29) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on percentage of dressing, gizzard, liver and heart of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Dressing%	70.31[b] ±0.33	71.35[a] ±0.01	71.39[a] ±0.08	71.48[a] ±0.10	0.43	S
Gizzard%	1.55 ±0.10	1.60 ±0.07	1.60 ±0.11	1.63 ±0.20	4.02	NS
Liver%	2.05 ±0.20	2.05 ±0.05	2.06 ±0.05	2.08 ±0.06	9.18	NS
Heart%	0.51 ±0.01	0.53 ±0.02	0.54 ±0.02	0.56 ±0.03	5.38	NS

Values are mean ± SE Mean.

[a,b]Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$).

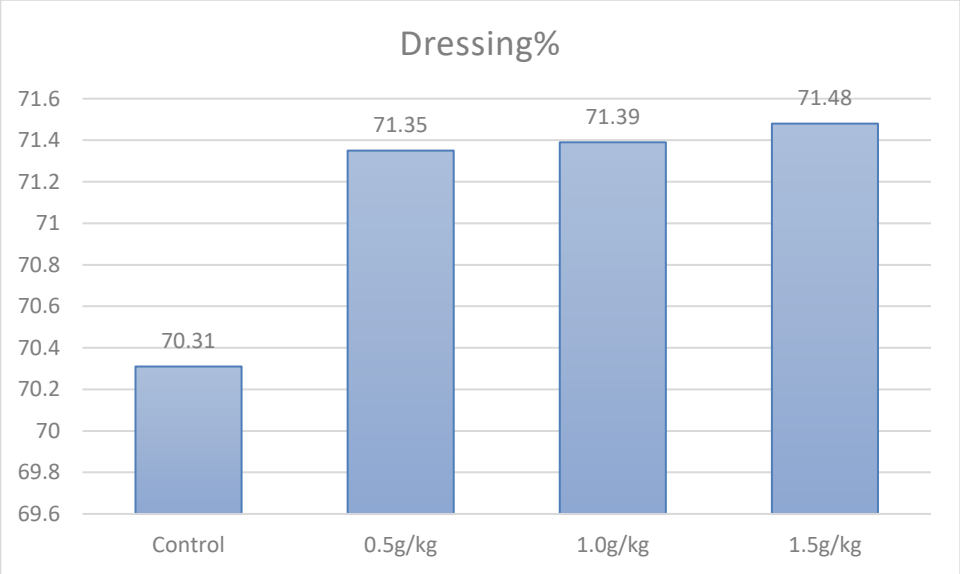
C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

Figure (11) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on carcass dressing percentage of broilers



4.3.2.2 Percentages of back, wings and neck:

The results of the effect of treatments on percentages of back, wings and neck were illustrated in table (30). The results indicated that, no significant differences ($p \geq 0.05$) among the all supplemented groups of SBYM at (0.5, 1.0 and 1.5g/kg) and control group. However, chicks fed on 1.5g/kg SBYM had obtained numerically highest percentages values of back, wings and neck (20.91, 10.78% and 5.26% respectively) as compared to chicks fed on 1.0g/kg SBYM (20.80, 10.71% and 5.17% respectively) and chicks fed on 0.5g/kg SBYM (20.50, 10.60% and 5.07% respectively) then chicks fed on control diet (19.69, 10.47% and 5.04% respectively) of broiler chicks. Although, all percentages values of back, wings and neck with in normal range of broilers according to (Oladimeji *et al*., 2020).

Table (30) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on percentages of back, wings and neck of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Back%	19.69 ±0.84	20.50 ±0.16	20.80 ±0.07	20.91 ±0.23	3.75	NS
Wing%	10.47 ±0.39	10.60 ±0.06	10.71 ±0.24	10.78 ±0.24	4.23	NS
Neck%	5.04 ±0.16	5.07 ±0.14	5.17 ±0.28	5.26 ±0.05	5.94	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.2.3 Percentages of non-carcass components:

The effect of treatments on non- carcass components of broiler chicks fed on graded levels of (SBYM) for 5 weeks, was shown in table (31). The results recorded that, no significant differences ($p \geq 0.05$) were observed between groups of SBYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in percentages of head, legs, lung, kidney, intestine weights and abdominal fat of broiler chicks. However, the chicks fed on 1.5g/kg SBYM had obtained higher percentages values of non- carcass components which mentioned above (2.63, 4.37, 0.74, 0.46% and 3.87% respectively), except abdominal fat recorded numerically the lowest percentage value (0.86%) as compared to chicks fed on control diet (1.07%) and chicks fed on 0.5, 1.0g/kg SBYM (0.91% and 0.90% respectively) of broiler chicks. However, all percentages values of non-carcass components which mentioned above, within normal range of broilers according to (Oladimeji *et al*., 2020).

Meanwhile, the significant differences($p \leq 0.05$) were found in the length of an intestine (cm), the results indicated that, the chicks fed on 1.0 and 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) longest intestine (198cm and 202cm respectively) as compared to those chicks fed on 0.5g/kg SBYM (188cm) and chicks fed on control diet (182cm), whereas, no significant differences ($p \geq 0.05$) were observed between the chicks fed on 1.0 and 1.5g/kg SBYM (198cm and 202cm respectively), also, no significant differences ($p \geq 0.05$) between the chicks fed on 0.5g/kg SBYM and chicks fed on control diet (188cm and 182cm respectively) in length of an intestine (cm) of broilers. Nevertheless, all intestine length was in normal range of broilers according to (Oladimeji *et al*., 2020 and Kokoszyński *et al*., 2017).

Table (31) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on percentages and length of non- carcass components of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Head%	2.55 ±0.07	2.57 ±0.04	2.58 ±0.01	2.63 ±0.04	3.14	NS
Legs%	4.11 ±0.25	4.29 ±0.09	4.35 ±0.09	4.37 ±0.03	5.70	NS
Lung%	0.72 ±0.05	0.73 ±0.01	0.73 ±0.01	0.74 ±0.04	6.72	NS
Kidney%	0.41 ±0.02	0.43 ±0.02	0.44 ±0.02	0.46 ±0.03	8.59	NS
Abdominal fat%	1.07 ±0.11	0.91 ±0.15	0.90 ±0.05	0.86 ±0.03	8.05	NS
Intestine weight%	3.80 ±0.22	3.84 ±0.17	3.87 ±0.04	3.87 ±0.03	6.47	NS
Intestine length (cm)	182^{b} ±1.45	188^{b} ±0.58	198^{a} ±0.58	202^{a} ±2.00	1.17	S

Values are mean ± SE Mean.

^{a,b}Any two means values having same superscripts within rows are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.2.4 Commercial cuts:

The effect of treatments on commercial cuts (breast, thigh and drumstick) of broiler chicks fed graded levels of SBYM for 5 weeks, was tabulated in table (32). The results illustrated that, application of graded levels of SBYM did not significantly affect commercial cuts (breast, thigh and drumstick), however, the chicks fed on 1.5g/kg SBYM had obtained numerically highest percentages values of breast, thigh and drumstick (40.32, 15.50 and 12.57 respectively) as compared to chicks fed on 1.0g/kg SBYM (40.30, 15.42% and 12.22% respectively) and chicks fed on 0.5g/kg SBYM (40.25, 15.38% and 12.51 respectively) then chicks fed on control diet (39.22, 15.07% and 11.735 respectively). However, all percentages values of commercial cuts (breast, thigh and drumstick), within normal range of broilers **appendix (6)** and according to (Oladimeji *et al*., 2020 and Soares *et al*., 2017).

Table (32) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on breast, thigh and drumstick% of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Breast%	39.22 ±0.51	40.25 ±0.26	40.30 ±0.20	40.32 ±0.05	1.31	NS
Thigh%	15.07 ±0.40	15.38 ±0.26	15.42 ±0.06	15.50 ±0.15	2.87	NS
Drumstick %	11.73 ±0.74	12.51 ±0.12	12.55 ±0.02	12.57 ±0.05	5.26	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.1.2.5 Meat of commercial cuts:

The treatment groups values of meat expressed as percentages from total weights of selected commercial cuts was given in table (33), the results indicated that, the meat percent of commercial cuts were not significantly affected by application of graded levels of SBYM, however, the chicks fed on 1.5g/kg SBYM had obtained numerically heaviest meat percent of breast, thigh and drumstick (86.21, 85.97% and 76.10% respectively) as compared to chicks fed on 1.0g/kg SBYM (86.16, 85.89% and 75.97% respectively) and chicks fed on 0.5g/kg SBYM (86.06, 85.88% and 75.59% respectively) then chicks fed on control diet (85.15, 84.98% and 74.53% respectively) of broiler chicks. Nevertheless, all percentages values of meat of commercial cuts, within normal range of broilers.

Table (33) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on breast, thigh and drumstick meat% of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Breast meat %	85.15 ±0.31	86.06 ±0.76	86.16 ±0.83	86.21 ±0.04	1.18	NS
Thigh meat %	84.98 ±0.02	85.88 ±0.22	85.89 ±0.41	85.97 ±0.56	0.74	NS
Drumstick meat%	74.53 ±1.32	75.59 ±0.78	75.97 ±0.71	76.10 ±0.47	2.01	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.3 Meat quality parameters:

4.3.3.1 Panel taste (subjective meat attributes):

The effect of graded levels of dietary SBYM on subjective meat attributes of broiler chicks, were presented in table (34). The results illustrated that, no significant differences ($p \geq 0.05$) among the all dietary treatments of broiler chicks on the average subjective meat quality score value of colour, tenderness, juiciness and flavour using an eight-point scale, and score given for all attributes are above moderate acceptability level **appendix (4)**. However, chick fed on 1.0 and 1.5g/kg SBYM had obtained numerically the best desirable colour (6.65 and 6.68 respectively), best tender (6.56 and 6.59 respectively), best juicy (6.55 and 6.57 respectively) and best intense flavour (6.59 and 6.62 respectively) as compared to chicks fed on 0.5g/kg SBYM and chicks fed on control diet.

Table (34) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on meat quality attributes of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Tenderness	6.50 ±0.12	6.54 ±0.16	6.56 ±0.15	6.59 ±0.15	2.02	NS
Juiciness	6.50 ±0.06	6.53 ±0.05	6.55 ±0.05	6.57 ±0.05	1.53	NS
Flavor	6.55 ±0.17	6.57 ±0.16	6.59 ±0.16	6.62 ±0.12	2.63	NS
Color	6.40 ±0.12	6.63 ±0.16	6.65 ±0.16	6.68 ±0.15	2.01	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.3.2 Meat chemical analysis (objective meat attributes):

The results of meat chemical analysis of broiler chicks fed graded levels of SBYM for 5 weeks were illustrated in table (35). The results recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups of SBYM supplementations (0.5, 1.0 and 1.5g/kg) and control group in percentages of meat chemical composition (dry matter, moisture, protein, ash and ether extract). However, the chicks fed on 1.5g/kg SBYM recorded numerically highest percentage value of moisture (74.50%) as compared to chicks fed on 1.0, 0.5g/kg SBYM and chicks fed on control diet (74.25, 74.10% and 74.23% respectively), also, highest protein (22.65%) as compared to chicks fed on 1.0, 0.5g/kg SBYM and chicks fed on control diet (22.60, 22.63% and 22.58% respectively), then highest ash (1.34%) as compared to chicks fed on 1.0, 0.5g/kg SBYM and chicks fed on control diet (1.23, 1.25% and 1.20% respectively), finally highest ether extract (1.38%) as compared to chicks fed on 1.0, 0.5g/kg SBYM and chicks fed on control diet (1.36, 1.34% and 1.32% respectively).

On the other hand, chicks fed on 1.5g/kg SBYM recorded numerically the lowest percentages values of dry matter (25.50%) as compared to chicks fed on 1.0, 0.5g/kg SBYM and chicks fed on control diet (25.75, 25.90% and 25.77% respectively) of broiler chicks.Although, all percentages values of meat chemical analysis, within normal range of broilers according to (Snezana *et al*., 2010).

Table (35) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on meat chemical composition of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Dry matter%	25.77 ±0.61	25.90 ±0.25	25.75 ±0.24	25.50 ±0.13	2.36	NS
Moisture%	74.23 ±0.61	74.10 ±0.25	74.25 ±0.24	74.50 ±0.13	0.83	NS
Protein%	22.58 ±0.06	22.63 ±0.02	22.60 ±0.12	22.65 ±0.03	0.52	NS
Ash%	1.20 ±0.03	1.25 ±0.01	1.23 ±0.12	1.34 ±0.02	8.45	NS
Ether extract %	1.32 ±0.01	1.34 ±0.01	1.36 ±0.02	1.38 ±0.04	3.17	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.4 Serum parameters:

4.3.4.1 Serum metabolites:

The effect of graded levels of dietary SBYM on serum metabolites of broiler chicks was shown in table (36). The results revealed that, no significant ($p \geq 0.05$) differences were observed between all groups of SBYM) supplementations (0.5, 1.0 and 1.5g/kg) and control group in serum total protein, albumin, creatinine, uric acid, urea, and glucose. Also, there were no significant ($p \geq 0.05$) treatments effect on cholesterol, HDL cholesterol, LDL cholesterol and triglycerides of broiler chicks. However, chicks fed on 1.5g/kg SBYM recorded numerically the highest values of serum total protein and glucose (4.35g/dl and 221.00mg/dl respectively) as compared to the groups of chicks fed on control diet and chicks fed on 0.5, 1.0g/kg SBYM (4.25, 4.25, 4.30g/dl and 220.50, 220.00, 220.02mg/dl respectively). At the same time (simultaneously) this group (1.5g/kg SBYM) recorded numerically the lowest values of albumin, creatinine, uric acid and urea (2.15g/dl, 2.10mg/dl, 3.40mg/dl and 6.85mg/dl respectively), also, the same trend for cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (123.50, 129.00, 22.00 mg/dl and 42.00mg/dl respectively) as compared to the other tested groups. Although all values of serum metabolites mentioned above were in normal range of serum profile of broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

Table (36) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on serum metabolites of broilers

Items	0.0g/kg Control	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
Protein (g/dl)	4.25 ±0.03	4.25 ±0.03	4.30 ±0.06	4.35 ±0.14	3.25	NS
Albumin (g/dl)	2.30 ±0.00	2.25 ±0.09	2.25 ±0.03	2.15 ±0.03	3.75	NS
Creatinine (mg/dl)	2.13 ±0.00	2.12 ±0.00	2.12 ±0.00	2.10 ±0.00	0.00	NS
Uric acid (mg/dl)	3.45 ±0.03	3.43 ±0.02	3.42 ±0.05	3.40 ±0.06	1.70	NS
Urea (mg/dl)	7.00 ±0.00	6.90 ±0.00	6.90 ±0.00	6.85 ±0.00	0.00	NS
Cholesterol (mg/dl)	124.50 ±0.29	124.00 ±0.00	123.60 ±0.29	123.50 ±0.29	0.35	NS
HDL Cholesterol (mg/dl)	130.50 ±0.29	129.50 ±0.29	129.50 ±0.29	129.00 ±0.58	0.51	NS
LDL Cholesterol (mg/dl)	22.50 ±0.29	22.00 ±0.00	22.04 ±0.00	22.00 ±0.58	2.53	NS
Triglyceride(mg/dl)	43.50 ±0.29	42.50 ±0.29	42.20 ±0.58	42.00 ±0.00	1.44	NS
Glucose (mg/dl)	220.50 ±0.29	220.00 ±0.29	220.02 ±0.29	221.00 ±0.58	2.29	NS

Values are mean ± SE Mean.

Any means values having no superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.4.2 Srum enzymes and minerals:

As shown in table (37), the results indicated that, Aspartate amino-transferase (AST) and Alkaline phosphatase (ALP) enzymes were not significantly ($p \geq 0.05$) affected by application of graded levels of SBYM, however, the chicks fed on control diet obtained numerically highest means values of AST and ALP enzymes (38.95 iu/L and 175.00 iu/L respectively) as compared to chicks fed on all levels of SBYM supplementations, chicks fed on 0.5g/kg (37.90 iu/L and 174.90 iu/L respectively), chicks fed on 1.0g/kg (37.85 iu/L and 174.85 iu/L respectively) and chicks fed on 1.5g/kg (37.85iu/L and 174.85 iu/L respectively). Although, all values of serum enzymes activities (AST and ALP) of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Odunitan-Wayas *et al*., 2018).

On the other hand, the results concerning serum minerals Calcium (Ca) and Phosphorus (P) recorded that, application of graded levels of SBYM significantly affected Ca and P values. The results revealed that, application of SBYM with all levels (0.5, 1.0 and 1.5g/kg) recorded, a significantly ($p \leq 0.05$) higher means values of Ca and P (8.25, 8.30, 8.30mg/dl and 8.80, 8.85, 8.85mg/dl respectively) as compared to control group (8.15mg/dl and 8.75mg/dl), whereas, no significant differences ($p \geq 0.05$) were observed between all levels of SBYM supplementations (0.5, 1.0, and 1.5g/kg) in values of Ca and P of broiler chicks. Although, all values of serum serum minerals (Ca and P) of all tested groups, were in normal range of serum profile of broilers **appendix (8)** and according to (Adeyemo *et al*., 2018).

Table (37) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on serum enzymes and serum minerals of broilers

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM	C.V%	HSD 0.05
AST (iu/L)	38.95 ±0.03	37.90 ±0.58	37.85 ±0.03	37.85 ±0.03	1.32	NS
ALP (iu/L)	175.00 ±0.00	174.90 ±0.00	174.85 ±0.03	174.85 ±0.03	0.02	NS
Ca (mg/dl)	8.15^{b} ±0.03	8.25^{a} ±0.03	8.30^{a} ±0.00	8.30^{a} ±0.00	0.43	S
P (mg/dl)	8.75^{b} ±0.03	8.80^{a} ±0.00	8.85^{a} ±0.03	8.85^{a} ±0.03	0.49	S

Values are mean ± SE Mean.

^{a,b}Any two means values having same superscripts within row are not significantly different ($P \leq 0.05$).

C.V: Coefficient of Variation.

HSD: Honest Significant Difference.

S: Significant difference ($p \leq 0.05$).

NS: No significant difference ($p \geq 0.05$).

4.3.5 Economic appraisal:

Appraisal of total cost, revenues and profitability ratio per head of broiler chicks fed on graded levels of synbiotic Biogen.S + Y-MOS (SBYM) for 5 weeks given in table (38) and figure (12). Chicks purchase, management and feed cost values were the major input considered. The total selling values of meat is the total revenues obtained. The results of economical evaluation revealed that, the chicks fed on dietary SBYM supplementations with all levels (0.5, 1.0 and 1.5g/kg) gained more net profit/bird (104.38, 117.78 and 127.91 respectively), as compared to those chicks fed on control diet (74.09), also, recorded highest values of profitability ratio/bird (1.41, 1.60 and 1.73 respectively), but the group of chicks fed on 1.5g/kg SBTM was the highest of the tested groups (1.73). For net profit/kg meat, the results noticed that, chicks fed on SBYM with all levels (0.5, 1.0 and 1.5g/kg) had gained more net profit/kg meat (62.09, 64.71 and 66.45 respectively) as compared to chicks fed on control diet (53.77), also, recorded highest values of profitability ratio/kg meat (1.16, 1.20 and 1.24 respectively) as compared to chicks fed on control diet (1), but the group of chicks fed on 1.5g/kg SBYM was the highest of the tested groups (1.22).

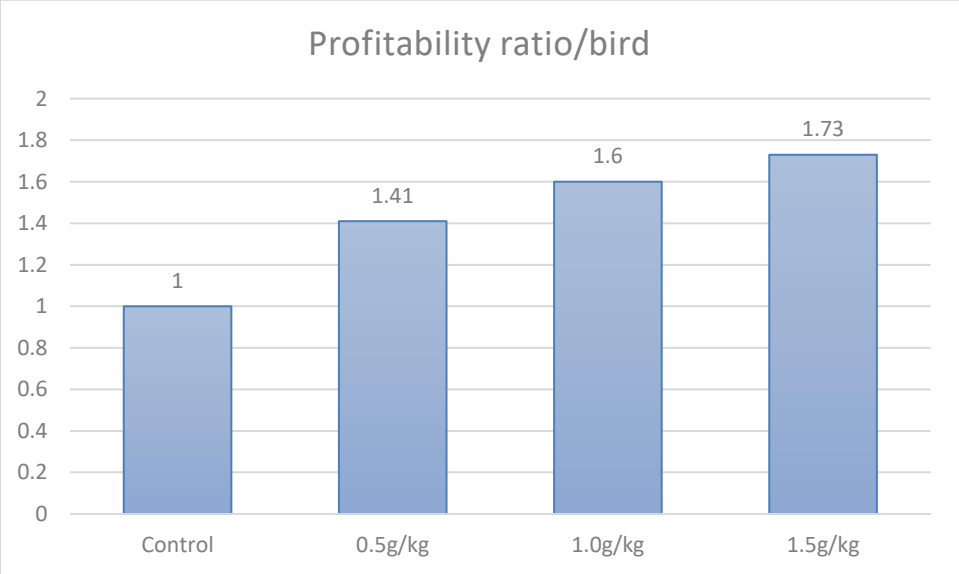
Table (38) Total cost, returns and profitability ratio per head of broilers fed graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) for 5 weeks

Items	Control 0.0g/kg	0.5g/kg SBYM	1.0g/kg SBYM	1.5g/kg SBYM
Cost				
Chicks purchase	19	19	19	19
Feed cost	37.71	37.72	38.22	38.59
Electricity and management	7	7	7	7
Total	63.71	63.72	64.22	64.59
Revenues				
Average weight of carcass	1.378	1.681	1.820	1.925
Price/kg of bird	100	100	100	100
Total	137.80	168.10	182.00	192.50
Profits				
Total revenues	137.80	168.10	182.00	192.50
Total cost	63.71	63.72	64.22	64.59
Net profit/bird	74.09	104.38	117.78	127.91
Net profit/kgmeat	53.77	62.09	64.71	66.45
Profitability ratio/bird	1	1.41	1.60	1.73
Profitability ratio/kg meat	1	1.16	1.20	1.24

The total cost was calculated according to January 2019.

Price/kg meat was 100 SDG according to February 2019.

Figure (12) Effect of graded levels of Synbiotic Biogen.S + Y-MOS (SBYM) on profitability ratio/bird of broilers



4.4 Effect of Probiotic, Prebiotic, Synbiotic and their levels on performance and their interactive action

As shown in table (39) and figures (13, 14, 15 and 16), the results of interaction between Probiotic, Prebiotic, Synbiotic and their levels indicated that, there are significant improvement ($p \leq 0.05$) in body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) in probiotic, prebiotic or synbiotic at all inclusion levels as compared to control diet, whereas, no significant ($p \geq 0.05$) effect on feed intake. The best improvement was obtained by synbiotic treatment, followed by prebiotic then probiotic, the best level 1.5g/kg followed by 1.0g/kg then 0.5g/kg. Whereas, the best numerical values of BW, BWG and FCR was achieved with synbiotic treatment when added at level 1.5g/kg (2375.33g, 2190.33g and 1.56g:g respectively).

Table (39) Effect of Probiotic, Prebiotic, Synbiotic and their Levels on performance and their interactive action of broilers

Treatments		FI	BW	BWG	FCR
Control		3428	1926^{c}	1757^{c}	1.95^{c}
Experiments Effect					
1	Probiotic	3423.89	2222.78_{b}	2037.78_{b}	1.68^{b}
2	Prebiotic	3394.22	2246.44_{ab}	2061.56_{ab}	1.65^{ab}
3	Synbiotic	3385.22	2258.56_{a}	2073.56_{a}	1.64^{a}
	SEM	21.02	9.94	10.11	0.01
	p–value	0.41	0.06	0.06	0.02
	Sig	NS	*	*	*
Levels Effect					
1	0.5 g/Kg	3395.22	2124.89_{c}	1939.89_{c}	1.75^{a}
2	1.0 g/Kg	3392.44	2227.56_{b}	2042.67_{b}	1.66^{b}
3	1.5 g/Kg	3415.68	2375.33_{a}	2190.33_{a}	1.56^{c}
	SEM	21.02	9.94	10.11	0.01
	p–value	0.70	0.00	0.00	0.00
	Sig	NS	**	**	**
Experiments X Levels (Interaction)					
	SEM	36.41	17.22	17.52	0.02
	p–value	0.99	0.17	0.19	0.63
	Sig	NS	NS	NS	NS

a-b: Means in a column and main effect with no common superscript differ significantly (P≤0.05).
*: Significant with (P≤0.05). NS: Not significant.

FI: Feed intake. BW: Body weight. BWG: Body weight gain. FCR: Feed conversion ratio.

Figure (13) Effect of Probiotic, Prebiotic, Synbiotic and their Levels on feed intake and their interactive action of broilers

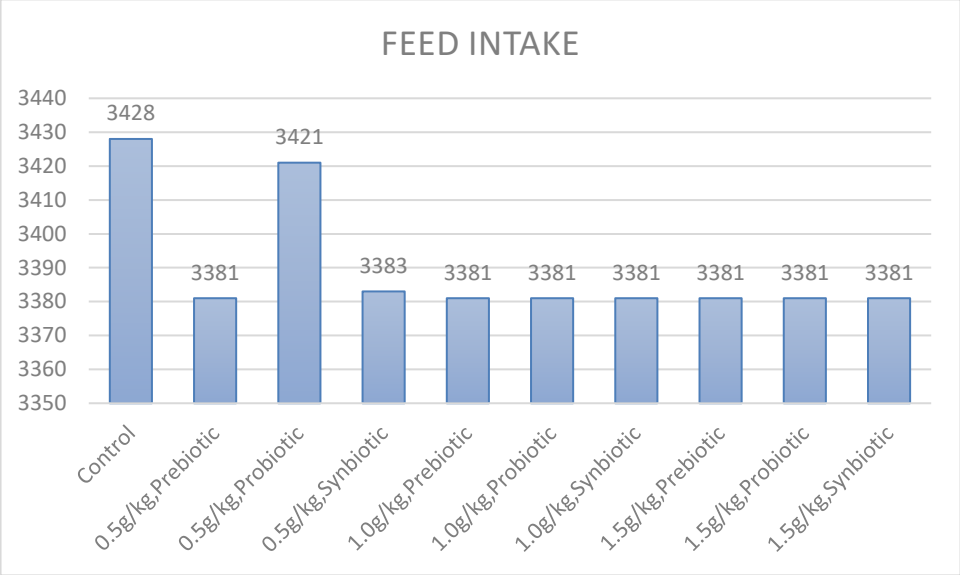


Figure (14) Effect of Probiotic, Prebiotic, Synbiotic and their Levels on body weight and their interactive action of broilers

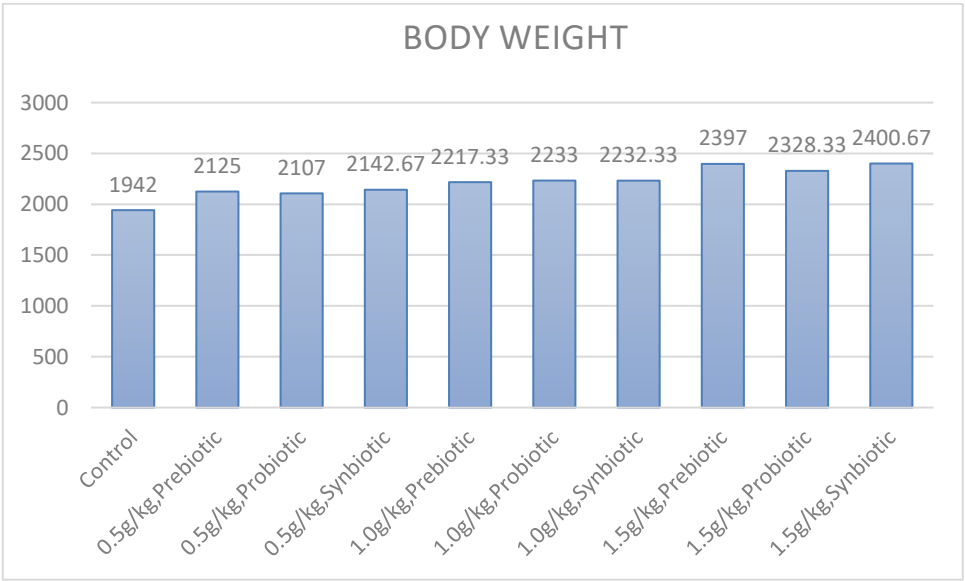


Figure (15) Effect of Probiotic, Prebiotic, Synbiotic and their Levels on body weight gain and their interactive action of broilers

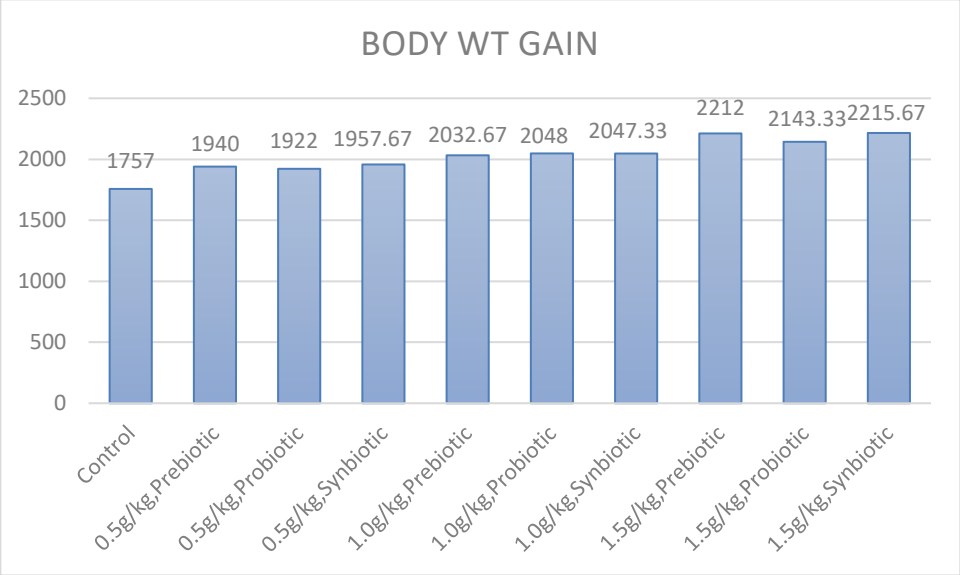
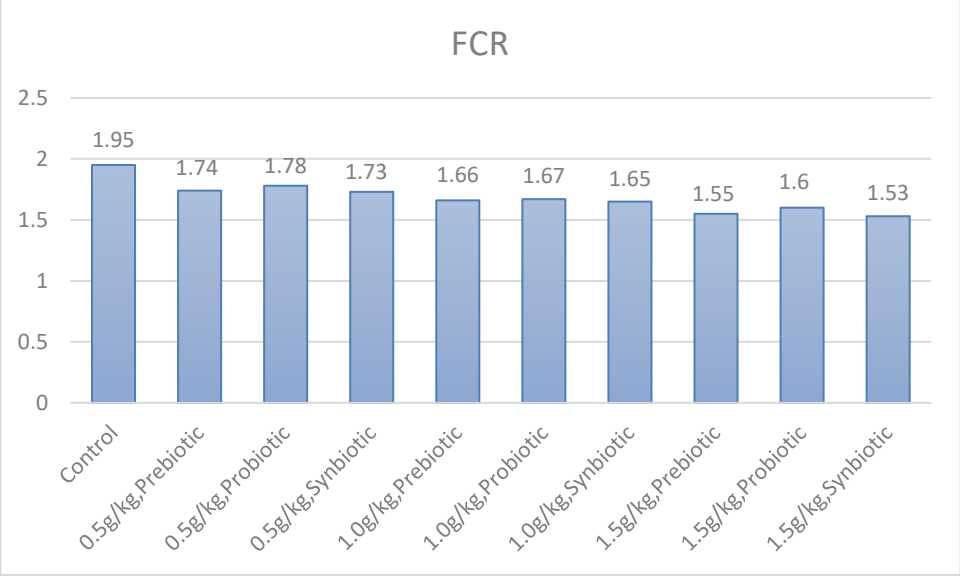


Figure (16) Effect of Probiotic, Prebiotic, Synbiotic and Levels on feed conversion ratio (FCR) and their interactive action of broilers



4.5 Comparative between treatments in: Total cost, returns and profitability ratio per head of broilers fed graded levels of Synbiotic (SBYM), Prebiotic (PYM) and Bacterial Probiotic (BPB)

The results of comparative between all products in total cost, returns and profitability ratio of broilers presented in table (40), the results revealed that, the addition of Synbiotic at all levels (0.5, 1.0 and 1.5g/kg) were obtained the best profitability ratio (1.41, 1.60 and 1.73 respectively), followed by prebiotic at all levels (1.31, 1.43 and 1.67 respectively) then probiotic at all levels (1.35, 1.40 and 1.54 respectively). Whereas, the level 1.5g/kg for all products (Synbiotic, Prebiotic and Probiotic) recorded the best profitability ratio (1.73, 1.67 and 1.54 respectively) compared with other levels. But the level 1.5g/kg of Synbiotic was the best one, had gained more net profit/bird.

Table (40) Comparative between treatments in: Total cost, returns and profitability ratio per head of broilers fed graded levels of Synbiotic (SBYM), Prebiotic (PYM) and Bacterial Probiotic (BPB) for 5 weeks

Products	Items	Control	0.5g/kg	1.0g/kg	1.5g/kg
Synbiotic Biogen.S + Y-MOS (SBYM)	Total cost	63.71	63.72	64.22	64.59
	Revenue	137.80	168.10	182.00	192.50
	Net profits	74.09	104.38	117.78	127.91
	Profitability ratio/bird	1	1.41	1.60	1.73
Prebiotic Y-MOS	Total cost	63.71	63.70	64.21	64.82
	Revenue	137.80	160.80	170.10	188.40
	Net profits	74.09	97.10	105.89	123.58
	Profitability ratio/bird	1	1.31	1.43	1.67
Bacterial Probiotic Biogen.S	Total cost	63.71	64.14	64.58	65.34
	Revenue	137.80	164.30	168.40	179.70
	Net profits	74.09	100.16	103.82	114.36
	Profitability ratio/bird	1	1.35	1.40	1.54

The total cost was calculated according to January 2019.

Price/kg meat was 100 SDG according to February 2019.

CHAPTER FIVE

DISCUSSION

These experiments were conducted to evaluate the response of broiler chicks to graded levels of dietary probiotic, prebiotic and synbiotic as natural growth promoters, and their effect on performance, carcass characteristics and serum parameters. In these studies the apparent health of experimental stock was good; the general behavior of the stock also was good throughout the experimental period. The ambient temperature during the experimental period fell within the thermos-neutral zone has extracted no heat on the experimental period.Results obtained for broiler chicks supplemented with graded levels of (probiotic, prebiotic and synbiotic), showed no mortality throughout the experimental period due to treatments, this might be due to the good hygiene conditions, also may be due to the ability of dietary probiotic, prebiotic and synbiotic to reduce enteric disease infection, through immune-modulation of the poultry immune system and enhanced antibody titers of plasma IgM and IgG and reduction mortality rate of broiler chicks (Krysiak *et al*., 2021; Al-Shawi *et al*., 2020 and Huang *et al*., 2015). The results were consistent with the finding of Al-Shawi *et al*., (2020); Talebi, *et al*., (2015); EL-Hammady *et al*., (2014) and Babazadeh *et al*., (2011), who reported that, application of probiotic, prebiotic and synbiotic on broiler diets recorded no mortalities between all tested groups.

5. 1 Response of broiler chicks fed graded levels of dietary Bacterial Probiotic Biogen.s (BPB).

The Bacterial Probiotic Biogen.S (BPB) was added to the basal diet at levels 0.5, 1.0, and 1.5 g/kg BPB, whereas, the basal diet which received no BPB additive was served as control diet. The results of this study indicated that, no significant differences ($p \geq 0.05$) were observed among the all treatment groups in feed

consumption of broiler chicks. However, the chicks fed on 1.5g/kg BPB recorded numerically the highest value of feed intake, while the chicks fed on 1.0g/kg BPB recorded numerically the lowest value. This result was in accordance with the findings of Zhang and Kim, (2014), whom found that, the inclusion of protexin and *Bacillus subtilis* as a probiotic did not affected significantly ($p \geq 0.05$) feed intake of broilers. These results contrary to the findings of Al-Shawi *et al*., (2020); Melkamu, (2019) and Ahmed *et al*., (2017), who reported that, supplementation of the diet with *Lacto-bacillus-acidofillus* and *Streptococcus faecium* as probiotics; significantly ($p \leq 0.05$) improved the feed intake of broilers.

In this study the addition of graded levels of BPB in broiler diets, improved significantly ($p \leq 0.05$) the final body weight (FBW) and body weight gain (BWG), the results illustrated that, the chicks fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) the heaviest means values of FBW and BWG as compared to chicks fed on control diet, also, the chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) the heaviest means of FBW and BWG as compared to chicks fed on 0.5g/kg BPB.

The improvement in FBW andBWG) by the addition of BPB may be due to beneficial effects of probiotic by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria and increase the population of useful microflora in the intestine, this may lead to better capacity for absorption of available nutrients (Roozbeh *et al*., 2012). The positive effect of probiotics can be observed directly in the gastrointestinal tract, nutritional effects seen in flocks given probiotics include increased daily increments, improved feed conversion ratio (FCR), and quality of meat (Krysiak *et al*., 2021). Probiotics produce organic acids and volatile fatty acids VFAs (Majidi-Mosleh *et al*., 2017). These substances lowering the pH and inhibit the growth of pathogenic, such as *E. coli* and *Salmonella spp*. (Yi *et al*, 2014 and Choudhari *et al*., 2008). Another

effect of probiotic is through the competition for adhesion sites on the intestinal epithelium, thus preventing colonies of pathogenic bacteria forming (Abudabos *et al*., 2013 and Ferket, 2011). These results were consistent with the findings of Krysiak *et al*., (2021); Al-Shawi *et al*., (2020); Khabirov *et al*., (2020); Melkamu, (2019); Kunová, (2019) and Haščík *et al*., (2019), who found that, the administration of *Lactobacillus plantarum*and *Bacillus licheniformis* as a probiotic in broiler diets, had significant positive effect ($p \leq 0.05$) on FBW and BWG, and improved growth performance. Similarly, the beneficial effects of probiotic on FBW and BWG of broilers were reported by several researchers Mahfuz *et al*., (2017); Ahmed *et al*., (2017); Idoui and Karam, (2016); Pourakbari *et al*., (2016); Odefemi, (2016) and Mokhtari *et al*., (2015), who mentioned that, chicks fed on probiotic (*Bacillus subtilis and Lactobacillus based*) diets had obtained significantly ($p \leq 0.05$) the heaviest means of FBW and BWG as compared to control. On the other hand, the results were disagreed with the findings of many researchers, who indicated there were no significant effect ($p \geq 0.05$) on BW and BWG of broilers fed on dietary bacterial probiotic *Bacillus subtilis*and Protexin (EL-Hammady *et al*., 2014; Dizaji *et al.*, 2012; EL-Banna *et al.*, 2010 and Lee *et al*., 2010).

Concerning feed conversion ratio (FCR) in the present study, the results showed that, the chicks fed on all levels of BPB supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) better FCR as compared to chicks fed on control diet, also, chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) better FCR as compared to chicks fed on 0.5g/kg BPB throughout the experimental period. The improvement in FCR by the addition of probiotic may be due to alteration in intestinal flora and improving microbial balance, suppression growth of intestinal pathogens, toxin neutralization, enhancement of digestion, utilization of nutrients and immunity stimulation (Yeo and Kim, 1997). Therefore, the major

outcomes from using probiotic include improvement in growth, reduction in mortality, and improvement in feed conversion efficiency (Yeo and Kim, 1997). Similar results were obtained by Krysiak *et al*., (2021); Al-Shawi *et al*., (2020); Matarneh, (2020) and Khabirov *et al*., (2020), who reported that, administration of *Pedio-coccus-acidilactici* and *Lactobacillus sporogenes* as a probiotic, improved significantly ($p \leq 0.05$) FCR of broilers. Like-wise several researchers observed the inclusion of (*L. sporogens, L. acidofillus and S. faecium*) as probiotics to broiler feed, resulted in an improved FCR (Haščík *et al*., 2019; Ahmed *et al*., 2017; Mohamed, 2014 and EL-Hammady *et al*., 2014). In contrast several studies showed that, there were no significant effects ($p \geq 0.05$) 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 displayed that, the carcass dressing percentages of broiler chicks was significantly ($p<0.05$) affected by supplementations of BPB. The results indicated that, chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) better carcass dressing percent as compared to control group, whereas, no significant differences ($p \geq 0.05$) were observed between groups of chicks fed on all levels of BPB supplementations. These results were in line with the findings of Hidayat *et al*., (2016); Aziz *et al*., (2020) and Mahajan *et al*., (1999), who found that, mean values of dressing percentages were significantly ($p \leq 0.05$) higher for probiotic (*Lacto – Sacc)* fed broilers. In contrast, several studies obtained by Haščík *et al*., (2019); Odefemi, (2016); Pourakbari *et al*., (2016); Mohamed, *et al*., (2015); EL-Hammady *et al*., (2014) and Mohamed, (2014), observed that, there were no significant differences ($p \geq 0.05$) between groups in carcass dressing percentages for probiotic fed broilers. In addition, the results were disagreed with the findings of Ahmed *et al*., (2017), who found that, the higher level of BPB recorded the lowest value of dressing percentage compared to other tested groups.

In this study the results deal with giblets percentages (gizzard, liver and heart) recorded that, no significant differences ($p \geq 0.05$) among the all treatments groups. These results were in line with the findings of Haščík *et al*., (2019) and EL-Hammady *et al*., (2014), found that, giblets (gizzard, liver, and heart percentages) were not affected significantly ($p \geq 0.05$) by the dietary probiotics. The results were partially consistent with the findings of Krysiak *et al*., (2021); Ahmed *et al*., (2017) and Hidayat *et al*., (2016), which show that, heart and liver percents not affected significantly ($p \geq 0.05$) by the dietary probiotic. The results were partially consistent with the findings of Mohamed, *et al*., (2015), who found that, the gizzard and heart percentages were not affected significantly ($p \geq 0.05$) by the dietary probiotic. Also, the results were partially consistent with the findings of Idoui and Karam, (2016); Odefemi, (2016) and Dizaji *et al*., (2012), found that, no significant ($p > 0.05$) effect on gizzard and liver percents of broilers fed probiotics. Also, Pourakbari *et al*., (2016) found that, there were no effects on liver percent of broilers fed probiotics (*L. plantarum*). These results were disagreed partially with those obtained by Idoui and Karam, (2016), reported that, the groups fed on probiotics had a higher percentage of gizzard compared with control group. Similarly, (Mohamed *et al*., 2015), observed that, there were significant differences ($p \leq 0.05$) in liver percent of broilers fed probiotics. The results were partially disagreed with the findings of (Ahmed *et al*., 2017), found that, the control group had a higher percentage of gizzard compared with probiotic groups.

In the present study the results recorded that, no significant differences ($p \geq 0.05$) were observed between all tested groups in back, wings, neck percentages of broiler chicks. However, the chicks fed on 1.0 and 1.5g/kg BPB had obtained numerically the highest percentages values. This result was in accordance with the findings of Ahmed *et al*., (2017) and Odefemi, (2016), who found that, no significant differences ($p \geq 0.05$) in percentages of wings and neck of broilers chicks

fed probiotic. The results were consistent with the findings of Krysiak *et al*., (2021) and Hidayat *et al*., (2016), found addition of probiotic not affect significantly back and neck percentages. On the other hand, the result was partially disagreed with the finding of (Odefemi, 2016), found that, the birds fed with probiotic had the highest back percentage compared to the other tested groups. These results were partially disagreed with those obtained by Pourakbari *et al*., (2016), which show that, there were significant effects on wings percent of broilers fed probiotic.

In the present study, the results proved that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of head, legs, lungs, kidney and abdominal fat of broiler chicks. However, chicks fed on 1.5g/kg BPB had obtained numerically the lowest percentage value of abdominal fat as compared to the others tested groups. The result of lungs was in agreement with the findings of Odefemi, (2016) and Shabani *et al*., (2012), who found that, no significant differences ($p \geq 0.05$) in percentages of lungs of broilers fed on probiotic. For head, this result was disagreed with the findings of Odefemi, (2016) and Shabani *et al*., (2012), reported that, the birds fed with probiotic had the highest mean percentage of head as compared to control group. For abdominal fat, this result in agreement with the findings of Haščík *et al*., (2019); Mohamed, *et al*., (2015); Mohamed, (2014) and Mehr *et al*; (2007), who found that, no significant difference ($p \geq 0.05$) among all groups in abdominal fat percentage of broilers fed probiotic. On the other hand, this result was disagreed with the findings of Krysiak *et al*., (2021); Pourakbari *et al*., (2016) and EL-Hammady *et al*., (2014), recorded that birds fed on diet supplemented with probiotic had significantly ($p \leq 0.05$) less abdominal fat compared to control diet.

In the present study no significant differences ($p \geq 0.05$) were observed between all tested groups in weights percentage of an intestine and length (cm) of broilers.

However, the group of chicks fed on 1.5g/kg BPB had obtained numerically the longest intestine as compared to other tested groups. This result was agreed with the findings of Idoui and Karam, (2016), who found that, no significant differences between groups in percentage weights of intestine. For the length of intestine, this result was in agreement with the findings of EL-Hammady *et al*., (2014), who found that, supplementation of probiotic did not affect the lengths of intestines. On the other hand, this results were disagreed with the findings of Jayaraman *et al*., (2013), who found that, *B. subtilis* in broiler diets increase the length of intestine when compared with the control, in the same line with the findings of Al-Baadani *et al*., (2016), who found that, length of intestine of probiotic feed additive, were longer compared with the control.

The results exposed that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of commercial cuts (breast, thigh and drumstick) of broilers. However, the chicks fed on 1.5g/kg BPB had obtained numerically the heaviest means percents of breast, thigh and drumstick as compared to the other tested groups. These results were consistent with the findings of Ahmed *et al*., (2017), reported that, no significant effect on breast, thigh and drumstick percentages of broilers fed probiotics diets. These results were partially consistent with the findings of Pourakbari *et al*., (2016), observed that, no significant effect on breast and drumstick percentages of broilers fed probiotics diets. Also, Krysiak *et al*., (2021); Hidayat *et al*., (2016); Haščík *et al*., (2019); Odefemi, (2016); and Mokhtari *et al*., (2015), reported that, no significant differences ($p \geq 0.05$) were observed between various treatment groups in breast and thigh percents of broilers fed probiotic. On the other hand, the results were in contrast partially with the findings of (Mehr *et al*., 2007) who reported that, birds fed with higher level of probiotic had obtained significantly higher percent of breast compared with control. Also, Odefemi, (2016) and Pourakbari *et al*., (2016),

found that, birds fed with probiotics had obtained significantly highest percent of drumstick as compared to control.

The results of the present study showed chicks fed on 1.5g/kg BPB had obtained significantly ($p \leq 0.05$) higher meat percent of breast as compared to chicks fed on control diet, whereas, no significant differences ($p \geq 0.05$) between supplemented groups. The results deal with meat percent of thigh and drumstick revealed that, no significant differences ($p \geq 0.05$) between all tested groups of broiler chicks.These results were consistent with the findings of Ahmed *et al*., (2017), found no significant effect on meat percentages of breast, thigh and drumstick of broilers fed probiotics diets. These results were partially consistent with the findings of Alloui, *et al*., (2012), who stated administration of *Pedio-coccus-acidilactici* as a probiotic had no effect on the thigh meat percentage. On the other hand, the results were in contrast with the findings of Alloui *et al*., (2012), who stated administration of *Pedio-coccus-acidilactici* as a probiotic had no effect on the breast meat percent.

In the present study the results illustrated that, no significant differences ($p \geq 0.05$) were shown among the all dietary treatments on the average subjective meat quality score values of colour, tenderness, juiciness and flavour using an eight-points scale, and score given for all attributes are above moderate acceptability level.These results were supported by the findings of Ahmed *et al*., (2017) and Loddi *et al*., (2000), reported that, probiotic was not affected subjective meat quality attributes. The results were disagreed with that obtained by Krysiak *et al*., (2021); Al-Shawi *et al*., (2020); Matarneh, (2020); and Liu *et al*., (2012), found that, administration of *Bacillus licheniformis* as probiotics in broiler diets was improved significantly ($p \leq 0.05$) meat quality and sensory attributes. Also the results were contrary with the findings of Zhang *et al*., (2005); Mahajan *et al*., (2000), indicated that, supplementation of probiotics (Lacto-Sacc) in broiler diets had significant effects ($p \leq 0.05$) on sensory parameters. The results were partially

disagreed with the findings of Cramer *et al*., (2018), who found that, addition of probiotic improved tenderness of meat quality.

The results of the present study flagged that, no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of meat chemical composition (dry matter, moisture, protein, ash and ether extract). However, the groups of the chicks fed on 1.0 and 1.5g/kg BPB recorded numerically higher percentages values of moisture, protein, ash and ether extract, and lower percentages values of dry matter as compared to the chicks fed on 0.5g/kg BPB and control diet. This result was reinforced by the findings of Ahmed *et al*., (2017) and Zhou *et al*., (2010), recorded that, no significant ($p \geq 0.05$) differences were found in the contents of moisture, crude ash and crude protein and crude fat (ether extract) among treatment groups.

In this study the results illustrated that, no significant ($p \geq 0.05$) differences were observe between all tested groups in serum metabolites (total protein, albumin, creatinine, uric acid, urea, and glucose). Also, there was no significant ($p \geq 0.05$) treatments effect on cholesterol, cholesterol HDL, cholesterol LDL and triglycerides of broiler chicks. However, the chicks fed on 1.5g/kg BPB recorded numerically the highest values of total protein and glucose as compared to the other tested groups. At the same time (simultaneously) this group1.5g/kg BPB recorded the lowest values of albumin, creatinine, uric acid and urea, also the same trend for cholesterol, cholesterol HDL, cholesterol LDL and triglycerides as compared to the other tested groups. These results were in line with the results obtained by Khabirov *et al*., (2020), recorded that, addition of probiotic in broiler diets did not significantly affect serum protein, glucose and albumin. Also, the results were in line with the findings of Mohamed *et al*., (2015); Yalcin *et al*., (2013)(and Pouraziz *et al*., 2013), found that, no significant effect on total protein and triglycerides between probiotic and control group. Similarly the results were in

agreement with the findings of Ahmadi, (2011), observed that, the low levels of cholesterol synthesis in broiler chickens treated with probiotics. The results were disagreed with those obtained by Idoui and Karam, (2016); Pourakbari *et al*., (2016); Alloui *et al*., (2012) and Ashayerizadeh *et al*., (2011), who reported that, probiotic significantly reduced cholesterol and triglycerides, while the serum glucose values were elevated. The result was in agreement with the findings of Ashayerizadeh *et al*., (2011), who found that, HDL and LDL levels were not affected by dietary probiotic. On the other hand, the result was disagreed with those obtained by Pourakbari *et al*., (2016), found that, addition of probiotic significantly affected the serum HDL and LDL. For uric acid the result was in line with the result obtained by Pourakbari *et al*., (2016), found that, addition of probiotic did not affected the serum uric acid. For albumin the result was disagreed with the findings of (Mohamed *et al*., (2015) and Pourakbari *et al*., (2016), who found that, addition of probiotic significantly affected the serum albumin.

A reduction in the serum cholesterol level may be due to the ability of probiotic to de-conjugate with bile acids, enzymatically increasing their rate of excretion and the use of cholesterol to synthesize new bile led to the reduction of serum cholesterol level (Lye *et al*., 2009). Supplementation of *Bacillus subtillus* to the ration of broilers, in addition to reducing the carcass fat, reduces the triglycerides concentration in the serum due to an increase in the population of lactic acid (Santose *et al*., 1995).

In this study, the results indicated that, no significant ($p \geq 0.05$) effect on serum enzyme AST and ALP between all tested groups. However, the chicks fed on all levels of BPB supplementations had recorded numerically the lowest values of serum enzymes AST and ALP as compared to control. In addition, the results revealed that, no significant ($p \geq 0.05$) differences between all tested groups in serum electrolytes values Ca and P. However, the chicks fed on all levels of BPB

recorded numerically the highest means values of minerals Ca and P as compared to control. The results were in line with the findings of Khabirov *et al*., (2020), found that, addition of probiotic in broiler diets did not affect serum enzyme AST. The results of serum minerals Ca were in agreement of the findings of Pourakbari *et al*., (2016), who found that, addition of probiotic did not affected the serum minerals Ca. The results of serum enzymes ALP and serum minerals P were disagreed with the findings of Khabirov *et al*., (2020) and Pourakbari *et al*., (2016), who found that, application of probiotic significantly affected ALP enzyme and phosphorus (P). On the other hand, Duskaev *et al*., (2020), reported tha probiotics have a positive effect on increasing the amount of chemical elements in the liver, chicken breast and blood biochemical. Also, the results were disagreed with the findings of Khabirov *et al*., (2020), found addition of probiotic in broiler diets significantly affected serum mineral (Ca).

The results cited in literature are highly variable about the degree of improvement in productive performance, carcass characteristics and serum analysis of broilers obtained by dietary probiotic as natural 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 (Choudhari *et al*., 2008 and Mountzouris *et al*., 2010).

The results of economical evaluation indicated that, chicks fed on dietary BPB with all levels had gained more net profit/bird and highest value of profitability ratio/bird compared to control diet.However, the group of chicks fed on 1.5g/kg BPB was the highest of the tested groups (1.54). The improvement which occurred in values of net profit of treated groups may be attributed to improvement which occurred in body weight, body weight gain, feed conversion ratio, stimulation of

birds immunity and reduction of mortality rate of broiler chicks (Mohamed, 2014). These results were in line with the finding of Krysiak *et al*., (2021); Hidayat *et al*., (2016); Mohamed, (2015) and Mohamed, (2014), who indicated that, supplementation of probiotics in broiler diets had economically more profitable as compared to control group.

5. 2 Response of broiler chicks to graded levels of dietary Prebiotic Y-MOS (PYM).

The dietary Prebiotic Y-MOS (PYM) was added to the basal diet at levels 0.5, 1.0, and 1.5 g/kg PYM, whereas, the basal diet which received no PYM additive was served as control diet. In the present study the results indicated no significant differences ($p \geq 0.05$) were observed among the all treatment groups in feed consumption of broiler chicks throughout the experimental period. However, chicks fed on control diet consumed numerically more feed compared to the additive groups (0.5, 1.0 and 1.5g/kg) PYM with an increasing estimated by about 1.39%, 1.39% and 0.23% respectively. The probable reason of these results might be that, the supplementations of growth promoters like prebiotics in broiler diets enhance the biological functions of the beneficial microbes in the gut of the host birds and increase the nutrient absorption thereby decreasing the feed intake (Sara *et al*., 2016). These results were consistent with the findings of Abdel-Hafeez *et al*., (2017); Sarangi *et al*., (2016) and Dizaji *et al*., (2012), who found that, there were no significant ($p>0.05$) differences between all tested groups in feed intake of broiler chicks fed on prebiotic. Also, the results were in line with the findings of Wang *et al*., (2016) and Abudabos *et al*., (2015), who found that, addition of mannan-oligo-saccharide (MOS) to diet of broiler chicks did not affected feed intake. On the other hand, the results were disagreed with the findings of many researchers Bednarczyk *et al*., (2016); Nikpiran *et al*., (2014) and Babazadeh *et al*., (2011), who found that, adding MOS to broiler diets had gave higher ($p \leq 0.05$) feed

intake as compared to control diet. In addition, the results were disagreed with the findings of Altaf *et al*., (2019), who found addition of prebiotic significantly increased feed intake compared to control.

In this study the results revealed that, chicks fed on all levels of PYM supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) the heaviest means values of FBW as compared to chicks fed on control diet. However, chicks fed on 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) heaviest mean value of FBW as compared to chicks fed on 0.5, 1.0g/kg PYM and control diet, with an increasing estimated by about 12.8%, 8.12% and 23.43% respectively. These results were supported by the findings of Irina, (2021); Chayatid *et al*., (2019); Tavaniello *et al*., (2018) and Abudabos *et al*., (2015), who found that, adding MOS to diet of broiler chicks had obtained higher ($p \leq 0.05$) body weight as compared to control diet. Also, the results were consistent with the findings of Nikpiran *et al*., (2014); Dizaji *et al*., (2012) and Babazadeh *et al*., (2011), who found that, inclusion of prebiotic had improved body weight and growth performance of broilers. On the other hand, the results were disagreed with the finding of many researchers Altaf *et al*., (2019); Ameni *et al*., (2019); Sarangi *et al*., (2016) and Wang *et al*., (2016), who found, there were no significant effect ($p \geq 0.05$) on body weight of broilers fed on prebiotic MOS and inulin compared to control.

In this study the results illustrated that, chicks fed on all levels of PYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) the heaviest means values of body weight gain as compared to chicks fed on control diet. The higher level of application 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) the heaviest mean value of body weight gain compared to those chicks fed on 0.5, 1.0g/kg PYM and control diet, with an increasing estimated by about 14.02%, 8.86% and 25.90% respectively. This result was in accordance with the

findings of Bednarczyk *et al*., (2016) and Abudabos *et al*., (2015), who found that, adding MOS to diet of broiler chicks had obtained higher ($p \leq 0.05$) body weight gain as compared to control diet. Also, the results were consistent with the findings of Abdel-Hafeez *et al*., (2017) and Dizaji *et al.*, (2012), who found that, inclusion of prebiotic had improved body weight gain of broilers. On the other hand, the results were disagreed with the findings of many researchers Ameni *et al*., (2019); Sarangi *et al*., (2016) and Wang *et al*., (2016), who indicated that, there were no significant effect ($p \geq 0.05$) on body weight gain of broilers fed on prebiotic.

The improvement in FBW and BWG by the addition of PYM may be due to beneficial effects of prebiotic by their mechanism of action include modulation of intestinal micro-flora by selectively regulating beneficial groups of bacteria by providing food for them (Hajati and Rezaei, 2010), and increased the growth of reported beneficial bacteria such as Lactobacillus (LAB), Bacteroides and Bifido-bacterium (Bozkurt *et al*., 2014 and Johnson *et al*., 2015), and low intestinal pH (Fernandez *et al*., 2002). Prebiotic increase amylase production in the gastro-intestinal tract (GIT), improved villi height and crypt depth, and improve nutrient digestibility (Huang *et al*., 2015 and Hanning, 2012), this eventually excludes the attachment of pathogens and promotes micro-biota in the gut. Some sugars MOS are able to block the binding of pathogens to the mucosa (Thomas *et al*., 2004). One of the main mechanisms of prebiotics is production of short chain fatty acids SCFAs (Pourabedin *et al*., 2015). This modulates the inflammation and regulates the metabolic functions (Pourabedin *et al*., 2015). Finally, each of the above mentioned reasons may lead to better growth response of broiler chicks.

In this study, the results deal with feed conversion ratio (FCR), recorded that, chicks fed on all levels of PYM supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) better FCR compared to the chicks fed on control. However, chicks fed on 1.5g/kg PYM obtained the best mean value of FCR. The

improvement in FCR by the addition of prebiotic may be due to alteration in intestinal flora and improving microbial balance. Generally, prebiotics can be fermented by health-promoting bacteria in the intestine, producing lactic acid, and SCFAs against pathogenic species (Bogusl *et al*., 2012). SCFAs lower the pH of gut lumen and provide energy to epithelial cells, this modulates the inflammation and regulates the metabolic functions and increase the absorption of nutrients (Pourabedin *et al*., 2015), then improved growth performance or antioxidant capacity, as they are covered extensively by (Dhama *et al*., 2014; and Yadav *et al*., 2016). These results were in agreement with the findings of Chayatid *et al*., (2019); Altaf *et al*., (2019); Bednarczyk *et al*., (2016) and Abudabos *et al*., (2015), who found that, adding MOS to diet of broiler chicks had gave better FCR as compared to control diet. On the other hand, the results disagreed with the findings of Abdel-Hafeez *et al*., (2017; Sarangi *et al*., (2016); Wang *et al*., (2016) and Dizaji *et al*., (2012), who found that, addition of prebiotic to broiler diet did not improved FCR.

In the present study chicks fed on 1.0 and 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) the heaviest means values of carcass dressing percent compared to chicks fed on control diet, while no significant differences ($p \geq 0.05$)were observed between all levels of PYM supplementations, also, between groups of chicks fed on 0.5g/kg PYM and control group. The results were consistent with the findings of Irina, (2021) and Mokhtari *et al*., (2015), who found that, prebiotic additive significantly ($p \leq 0.05$) increased carcass dressing percentage compared to control. On the other hand, the results were in contrast with the findings of Tavaniello *et al*., (2018); Abdel-Hafeez *et al*., (2017); Sarangi *et al*., (2016); Abudabos *et al*., (2015) and Wang *et al*., (2016), who found that, there was no significant ($p > 0.05$) difference between all tested groups in the carcass traits with respect to dressing percentage of broiler chicks fed on prebiotic.

In this study the results recorded no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of gizzard, liver and heart. However, chicks fed on 1.5g/kg PYM obtained numerically higher percents values of gizzard, liver and heart. The results were in line with the findings of (Sarangi *et al*., (2016) and Odefemi, (2016), who found that, there was no significant ($p>0.05$) difference between all tested groups in percentages of heart, liver and gizzard of broiler chicks fed on prebiotic. The results were partially consistent with the findings of Dizaji *et al.*, (2012), who found that, no significant effect ($p \geq 0.05$) on gizzard and liver percentages of broiler chicks fed preboitic diet. Also, the results were partially consistent with the findings of Abdel-Hafeez *et al*., (2017) and Abudabos *et al*., (2015), who found that, no significant effect ($p \geq 0.05$) on liver percentages of broiler chicks fed preboitic diet. On the other hand, the results were partially in contrast with the findings of Abdel-Hafeez *et al*., (2017), who found that, application of prebiotic on broiler diets significantly ($p \leq 0.05$) affects heart percentage.

In the present study the results revealed no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of back, wings and neck of broiler chicks. However, chicks fed on 1.5g/kg PYM had obtained numerically higher means values of back, wings and neck percentages. This result was supported by the findings of Sarangi *et al*., (2016), who found that, there was no significant ($p>0.05$) difference between all tested groups in percentages of back, wings, and neck of broiler chicks fed on prebiotic. The results were partially consistent with the findings of Odefemi, (2016), who found there was no significant ($p>0.05$) difference between all tested groups in percentages of wings and neck of broiler chicks fed on prebiotic. Also, the results were partially consistent with the findings of Wang *et al*., (2016), who recorded that, there was no significant ($p>0.05$) difference between all tested groups in percentages of

wings of broilers fed on prebiotic. On the other hand, the results were partially in contrast with the findings of Odefemi, (2016), who found that, addition of prebiotic significantly affected back percentage of broilers.

In this study the results recorded no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of head, legs, lung, kidney, intestine weights and abdominal fat of broiler chicks. However, the chicks fed on 1.5g/kg PYM had obtained numerically the lowest percentage value of abdominal fat as compared to all tested groups. The results were in agreement with the findings of Abudabos *et al*., (2015), who found that, no significant differences ($p \geq 0.05$) between tested groups in abdominal fat of broilers fed prebiotic. Also, the results were partially consistent with the findings of Odefemi, (2016), who found that, no significant differences ($p \geq 0.05$) between tested groups in lung percentage of broilers fed prebiotic. The results were partially disagreed with the findings of Odefemi, (2016), who found that, application of prebiotic significantly ($p \leq 0.05$) affected head percentage of broilers.

In this study the significant differences ($p \leq 0.05$) were found in length of an intestine (cm), chicks fed on 1.0 and 1.5g/kg PYM had obtained significantly ($p \leq 0.05$) longest means values of an intestine compared to chicks fed on control diet. Whereas, no significant differences ($p \geq 0.05$) were observed between groups of the chicks fed on 0.5g/kg PYM and chicks fed on control diet. Improvement of an intestine length may be due to benefit of MOS which commonly derived from yeast and the outer cell of yeast. MOS are found to modulate and improve the growth of the intestinal mucosa layer and microbiota diversity (Pourabedin *et al*., 2014), MOS also improve the integrity of intestinal epithelial cells (Lan *et al*., 2005). Prebiotic increase amylase production in the GIT, improved villi height and crypt depth, and improve nutrient digestibility (Huang *et al*., 2015 and Hanning, 2012). These results were in the same line with the findings of Al-Baadani *et al*.,

(2016), who found that, length of an intestine of chicks fed on prebiotic were higher, compared with chicks fed on the control diet.

In the present study the results revealed no significant differences ($p \geq 0.05$) were observed between all tested groups in percentages of commercial cuts and their meat (breast, thigh and drumstick %). However, chicks fed on higher level of additive 1.5g/kg PYM had obtained numerically heaviest percentages values and meat percentages of breast, thigh and drumstick compared to the other tested groups of broilers. These results were in agreement with the findings of Sarangi *et al*., (2016) and Wang *et al*., (2016), recorded that, there was no significant ($p > 0.05$) difference between all tested groups in percentages of breast, thigh and drumstick of broiler chicks fed on prebiotic. The results were partially consistent with the findings of Odefemi, (2016) and Abudabos *et al*., (2015), who found addition of prebiotic did not affect breast percentage of broiler chicks. Also, the results were partially consistent with the findings of Odefemi, (2016) and Mokhtari *et al*., (2015), who found that, there were no significant differences ($p \geq 0.05$) in breast and thigh percentages of broiler chicks. In contrast the results were partially disagreed with the findings of Odefemi, (2016), who found application of prebiotic significantly ($p \leq 0.05$) affected drumstick percent of broiler chicks. Also, the results were partially disagreed with the findings of Tavaniello *et al*., (2018), found application of prebiotic significantly ($p \leq 0.05$) affected breast percent of broiler chicks.

In the present study the results indicated that, no significant differences ($p \geq 0.05$) among the all dietary treatments of broiler chicks in the average subjective meat quality. However, the chick fed on 1.0 and 1.5g/kg PYM had obtained numerically the best desirable colour, tender, juicy and intense flavour compared to the other tested groups. The results were partially consistent with the findings of Wang *et al*., (2016), who found no significant ($p > 0.05$) difference between all tested groups

in the average subjective meat quality score value of tenderness of broiler chicks fed on prebiotic. In contrast the results were disagreed with the findings of Irina, (2021) and Tavaniello *et al*., (2018), found that, adding prebiotic in broiler diet has a positive effect on the meat quality traits.

In this study the results recorded no significant differences ($p \geq 0.05$)were observed between all tested groups in percentages of moisture, dry matter, protein, ash and ether extract of meat chemical composition of broiler chicks. However, chicks fed on 1.0 and 1.5g/kg PYM recorded numerically the highest percentages values of moisture, protein, ash and ether extract, and recorded numerically the lowest percentages values of dry matteras compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet. This result was consistent with the findings of Fritts and Waldroup, (2003), who found that, Prebiotics used in a mixture for fattening chickens does not affect the quality of meat chemical composition expressed. Also, the results were partially consistent with the findings of Irina, (2021), recorded that, adding prebiotic in broiler diets increase protein percent in meat chemical analysis compared to control but not significant. In contrast the results were disagreed with the findings of Irina, (2021), recorded that, adding prebiotic in broiler diets decrease the level of fat in meat chemical analysis compared to control but not significant.

In the present study the results illustrated that, no significant ($p \geq 0.05$) differences were observe between all tested groups in serum total protein, albumin, creatinine, uric acid, urea, and glucose of broiler chicks. This results were partially consistent with the findings of Abdel-Hafeez *et al*., (2017), who found there were no significant difference in serum total protein, albumin and glucose with prebiotic supplementation in broilers diets. Also, there were no significant ($p \geq 0.05$) treatments effect on cholesterol, HDL, LDL and triglycerides of broiler chicks. However, chicks fed on 1.0 and 1.5g/kg PYM recorded numerically the lowest

values of cholesterol, HDL, LDL and triglycerides compared to chicks fed on 0.5g/kg PYM and chicks fed on control diet. These results were partially consistent with the findings of Ashayerizadeh *et al*., (2011), who recorded that; application of prebiotic did not affected serum HDL and LDL of broiler chicks. In addition, the results were consistent with the findings of Abdel-Hafeez *et al*., (2017), who found addition of prebiotic to broiler diets did not affect serum cholesterol.

In this study the results indicated that, no significant ($p \geq 0.05$) effect on serum enzymes activities values Aspartate amino-transferase (AST) and Alkaline phosphatase (ALP) were observed between all tested groups of broiler chicks. However, the groups of chicks fed on PYM supplementations (0.5, 1.0, and 1.5g/kg) recorded numerically the lowest means values of serum enzymes AST and ALP compared to control group. The results were in agreement with the findings of Babazadeh *et al*., (2011), who found that, application of prebiotic on broiler diets recorded no significant ($p \geq 0.05$) effects on AST and ALP enzymes between all tested groups.

The results deal with serum minerals Calcium (Ca) and Phosphorus (P) recorded that, no significant differences ($p \geq 0.05$) were observed between control group and PYM supplemented groups. However, the chicks fed on all levels of PYM supplementations had obtained numerically the highest values of Ca and P compared to control group. The result was consistent with the findings of Sohail *et al*., (2011), found that, MOS may improve the absorption of serum trace minerals.

There are wide variation in the results cited in literature concerning with the response of broiler chicks fed on prebiotic supplemented in diets. This may be attributed to the variation efficiency of this natural feed additives which depends on several factors, such as type of prebiotic, administration level, application method, frequency of application, bird strain, bird age, overall diet, overall farm hygiene status and environmental stress factors (Mountzouris *et al*., 2010).

In this study the results of economical evaluation indicated that, chicks fed on PYM with all levels had gained more net profit/bird and highest values of profitability ratio/bird compared to chicks fed on control diet. However, the group of chicks fed on 1.5g/kg PYM was the highest of the tested groups (1.67). The improvement which occurred in values of net profit of treated groups may be attributed to improvement which occurred in body weight, body weight gain, feed conversion ratio, stimulation of birds immunity and reduction of mortality rate of broiler chicks (Mohamed, 2014). These results were in agreement with the findings of Abdel-Hafeez *et al*., (2017), who found that, supplementation of prebiotics in broiler diets had economic return and more profitable as compared to control group.

5. 3 Response of broiler chicks to graded levels of dietary Synbiotic Biogen.S + Y-MOS (SBYM)

Dietary Synbiotic Biogen.S + Y-MOS (SBYM) was added to the basal diet at levels 0.5, 1.0, and 1.5 g/kg SBYM, whereas, the basal diet which received no SBYM additive was served as control diet. In the present study the results for feed intake recorded that, the effect of treatments on the feed consumption was not significant ($p \geq 0.05$) among the all treatment groups. However, the chicks fed on control diet obtained the insignificantly higher mean value of this parameter as compared to 0.5, 1.0 and 1.5g/kg SBYM, with an increasing estimated by about 1.33%, 1.36% and 1.12% respectively. The probable reason of these results might be that, the supplementations of growth promoters like prebiotics in broiler diets enhance the biological functions of the beneficial microbes in the gut of the host birds and increase the nutrient absorption thereby decreasing the feed intake (Sara *et al*., 2016).

These results were consistent with the findings of Wang *et al*., (2018); Abdel-Hafeez *et al*., (2017); Balamuralikrishnan *et al*., (2017) and Wang *et al*., (2016),

who found that, there were no significant ($p>0.05$) differences between all tested groups in feed intake of broilers fed on synbiotic. Also, the results were in line with the findings of Abudabos *et al*., (2015) and Marwa, (2015), who observed that, addition of dietary synbiotic to broiler diets did not affects feed intake. On the other hand, the results were disagreed with the findings of Babazadeh *et al*., (2011), who found that, adding of synbiotic to broiler diets had gave higher ($p\leq0.05$) feed intake as compared to control diet. Also, the results were disagreed with the findings of Altaf *et al*., (2019), found addition of synbiotic significantly increased feed intake compared to control.

In the present study the results of final body weight (FBW) revealed that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p\leq0.05$) heaviest means values of FBW as compared to chicks fed on control. However, the chicks fed on 1.5g/kg SBYM had obtained significantly ($p\leq0.05$) heaviest mean value of FBW than those chicks fed on 0.5, 1.0g/kg SBYM and chicks fed on control diet, with an increasing estimated by about 12.04%, 7.53% and 23.58% respectively. The result agreed with the finding of Wang *et al*., (2016) and Babazadeh *et al*., (2011), who found that, inclusion of synbiotic had a beneficial effect on final body weight of broilers. In contrast the results were disagreed with the findings of Mora *et al*., (2019) and Sarangi *et al*., (2016), who found that, there were no significant difference in final body weight with or without synbiotic (probiotic + prebiotic) supplementation in broilers diets.

In this study application of graded levels of SBYM significantly ($p\leq0.05$) affected body weight gain (BWG), the results indicated that, chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p\leq0.05$) the heaviest means values of BWG as compared to chicks fed on control diet. However, chicks fed on 1.5g/kg SBYM had obtained significantly ($p\leq0.05$) the heaviest mean value of BWG as compared to those chicks fed on 0.5, 1.0g/kg

SBYM and control diet, with an increasing estimated by about 13.18%, 8.21% and 26.07% respectively.

The improvement in FBW and BWG by the addition of SBYM may be due to beneficial effects of synbiotic by their mechanism of action, and their ability to balance the gut environment and its microbiota (Dhama *et al*., 2011) by providing substrates for bacterial fermentation, generating antibacterial substances, competing for nutrients, modulating immune responses (Rooks and Garrett, 2016), competing with pathogens for adhesion receptors on the intestinal epithelium (Adil and Magray, 2012) and improves the growth of broilers (Mookiah *et al*., 2014). These results were consistent with the findings of Wang *et al*., (2018); Abdel-Hafeez *et al*., (2017) and Wang *et al*., (2016), who found that, inclusion of synbiotic had a beneficial effect on body weight gain of broilers. In contrast these results were disagreed with the findings of Mora *et al*., (2019); Sarangi *et al*., (2016) and Abudabos *et al*., (2015), who found that, there were no significant difference in body weight gain with or without synbiotic (probiotic + prebiotic) supplementation in broilers diets.

In this study the results deal with feed conversion ratio (FCR) recorded that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg) had obtained significantly ($p \leq 0.05$) better FCR as compared to chicks fed on control diet, however, chicks fed on 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) the best mean value of FCR as compared to chicks fed on 0.5g/kg SBYM. The improved FCR in synbiotic fed group might be attributed to the improvement of intestinal environment as it is reported that, feeding synbiotic in the diet reduces the intestinal pH and increases digestive enzyme activity in gastrointestinal tract (Samli *et al*., 2007). These results were consistent with the findings of (Dizaji *et al.*, 2012), who found that, the synbiotic fed birds had improved FCR as compared to control group. Similar to these findings of Oliva *et al*., (2016), indicated that, a

significant improvement in FCR in birds fed different growth promoters such as synbiotic than those fed with the control diet.In contrast these results were disagreed with the findings of Wang *et al*., (2018); Abdel-Hafeez *et al*., (2017) and Wang *et al*., (2016), who found that, the FCR did not significantly affects by the inclution of dietary synbiotic, compared with un-supplemented control in broiler chicken.

In this study the results of carcass dressing percentages showed that, the chicks fed on all levels of SBYM supplementations (0.5, 1.0 and 1.5g/kg), had obtained significantly ($p \leq 0.05$) the highest percentages values of dressing as compared to chicks fed on control diet, whereas, no significant differences ($p \geq 0.05$) were observed between all additives groups of SBYM. The results were consistent with the findings of Mokhtari *et al*., (2015), who found that, synbiotic additive increased carcass dressing significantly ($p \leq 0.05$) compared to control. In contrast these results were disagreed with the findings of Abdel-Hafeez *et al*., (2017); Wang *et al*., (2016); Sarangi *et al*., (2016) and Abudabos *et al*., (2015), who found that, there was no significant ($p > 0.05$) difference between all tested groups in the carcass traits with respect to dressing percentage of broiler chicks fed on synbiotic.

In this study the results of giblets percentages values (gizzard, liver and heart) were showed, no significant differences ($p \geq 0.05$) among the all treatment groups. However, the chicks fed on 1.5g/kg SBYM had obtained numerically the highest percentages values of gizzard, liver and heart while chicks fed on control diet noted numerically the lowest percentages values. These results were consistent with the findings of Sarangi *et al*., (2016), who found that, there was no significant ($p > 0.05$) difference between all tested groups in percentages of heart, liver and gizzard of broiler chicks fed on synbiotic. The results were partially consistent with the findings of Dizaji *et al.*, (2012), found no significant effect ($p \geq 0.05$) on gizzard and liver percentages of broilers fed synboitic diet. Also, the results were partially

in line with the findings of Abudabos *et al*., (2015), found that, addition of synbiotic did not affects liver percentage of broilers. On the other hand, the results were partially in contrast with the findings of Abdel-Hafeez *et al*., (2017) and Fatima, (2015), found that, application of synbiotic on broiler diets significantly ($p \leq 0.05$) affects liver and heart percentage.

In the present study the results of back, wings and neck percentages indicated, no significant differences ($p \geq 0.05$) among the all treatment groups of broiler chicks. However, chicks fed on 1.5g/kg SBYM had obtained numerically the highest percentages values of back, wings and neck as compared to other tested groups. These results were in the same line with the findings of Sarangi *et al*., (2016), found that, there was no significant ($p > 0.05$) difference between all tested groups in percentages of back, wings and neck of broiler chicks fed on synbiotic. Also, the results were partially consistent with the findings of Wang *et al*., (2016) and Fatima, (2015), who recorded that, there was no significant ($p > 0.05$) among all tested groups in wings percent of broilers fed on synbiotic.In contrast these results were partially disagreed with the findings of Fatima, (2015), found addition of synbiotic in broiler diets significantly affected back and neck percentages.

In this study the results of non- carcass components percents (head, legs, lung, kidney, intestine weights and abdominal fat) recorded, no significant differences ($p \geq 0.05$) were observed between all tested groups of broiler chicks. However, the chicks fed on 1.5g/kg SBYM had obtained numerically higher percentages values of non- carcass components which mentioned above, except abdominal fat recorded numerically the lowest percentage value as compared to other tested groups. This result was partially supported by the findings of Wang *et al*., (2016) and Abudabos *et al*., (2015), who found that, dietary synbiotic did not affect abdominal fat of broiler chicks. Also, the result was partially consisting with the findings of Fatima, (2015), found that, supplementing of synbiotic to diet did not

affected abdominal fat, head and legs percentages. The result was partially disagreed with the findings of Fatima, (2015), who reported that, supplementing of synbiotic significantly ($p \leq 0.05$) affected intestine weight percentage.

Meanwhile, the significant differences ($p \leq 0.05$) were found in the length of an intestine (cm), the results indicated that, chicks fed on 1.0 and 1.5g/kg SBYM had obtained significantly ($p \leq 0.05$) longest an intestine as compared to those chicks fed on 0.5g/kg SBYM and chicks fed on control diet. Improvement of an intestine length may be due to benefit adding of synbiotic (combination between probiotic and prebiotic) to the broiler diets which modulate and improve the growth of the intestinal mucosa layer and microbiota diversity (Pourabedin *et al*., 2014). Prebiotic improved villi height and crypt depth, and improve nutrient digestibility (Huang *et al*., 2015). These results were in the same line with the findings of Al-Baadani *et al*., (2016), who found that, length and surface area of intestine of broiler fed on synbiotic were higher, compared with the control. These results in contrast to the findings of Fatima, (2015), found addition of synbiotic in broiler diets did not affected length of an intestine.

In the present study the results illustrated that, application of graded levels of SBYM did not significantly affect commercial cuts and their meat (breast, thigh and drumstick), however, the chicks fed on 1.5g/kg SBYM had obtained numerically the heaviest percentages values of breast, thigh and drumstick as compared to other tested groups of broiler chicks. This result was supported by the findings of Sarangi *et al*., (2016) and Fatima, (2015), who found that, no significant ($p>0.05$) difference between all tested groups in percentages of breast, thigh and drumstick and their meat of broiler chicks fed on synbiotic. The results were partially consistent with the findings of Wang *et al*., (2016), who recorded no significant ($p>0.05$) among the all tested groups in thigh and drumstick percentages of broilers fed on synbiotic. Also, the results were partially in line with

the findings of Mokhtari *et al*., (2015), who found that, no significant ($p>0.05$) differences between all tested groups in breast percentages of broilers fed on synbiotic.

In the present study the results illustrated no significant differences ($p\geq0.05$) among the all dietary treatments of broiler chicks in the average subjective meat quality score value of colour, tenderness, juiciness and flavour using an eight-point scale, and score given for all attributes are above moderate acceptability level. However, chick fed on 1.0 and 1.5g/kg SBYM had obtained numerically the best desirable colour, tender, juicy and intense flavour as compared to the other tested groups. The results were in agreement with the findings of Fatima, (2015) and Marwa, (2015), who indicated that, there were no significant ($p>0.05$) difference between all tested groups in the average subjective meat quality score value of colour, tenderness, juiciness and flavour of broiler chicks fed on synbiotic. Also, the results were partially consistent with the findings of Wang *et al*., (2016), who recorded that, there were no significant ($p>0.05$) difference between all tested groups in the average subjective meat quality score value of tenderness of broilers fed on synbiotic.

In this study the results of meat chemical analysis of broiler chicks recorded no significant differences ($p\geq0.05$) were observed between all tested groups in percentages of meat chemical composition (dry matter, moisture, protein, ash and ether extract). However, chicks fed on 1.5g/kg SBYM recorded numerically the highest percentages values of moisture, protein, ash and ether extract and the lowest percentage value of dry matter as compared to other tested groups of broiler chicks. The result was disagreed with the findings of Fatima, (2015), who recorded that, application of synbiotic in broiler diets significantly affected meat chemical analysis percentages (moisture, protein, ash and ether extract).

In the present study the results revealed no significant ($p \geq 0.05$) differences were observed between all tested groups in serum total protein, albumin, creatinine, uric acid, urea, and glucose of broiler chicks. However, chicks fed on 1.5g/kg SBYM recorded numerically the highest values of serum total protein and glucose as compared to other tested groups. At the same time this group 1.5g/kg SBYM recorded numerically the lowest values of albumin, creatinine, uric acid and urea as compared to other tested groups of broiler chicks. These results were partialy in line with the findings of Abdel-Hafeez *et al*., (2017), who found that, there were no significant difference in serum total protein and glucose with synbiotic supplementations in broiler diets. The results were partially consistent with the findings of Fatima, (2015), who found addition of synbiotic in broiler diets did not affect serum protein and albumin.

In this study there were no significant ($p \geq 0.05$) treatments effect on cholesterol, HDL cholesterol, LDL cholesterol and triglycerides of broiler chicks. However, chicks fed on 1.5g/kg SBYM recorded numerically the lowest values of cholesterol, HDL cholesterol, LDL cholesterol and triglycerides as compared to the other tested groups. The results were in agreement with the findings of Beski and Al-Sardary, (2015); Fatima, (2015), found that, there were no significant differences ($p \geq 0.05$) between tested groups in triglycerides and HDL of broilers fed synbiotic. These results were in line with the findings of Abdel-Hafeez *et al*., (2017), who found that, cholesterol levels were not affected by dietary synbiotic. Also, the result was in agreement with the findings of Ashayerizadeh *et al*., (2011), who found HDL and LDL levels were not affected by dietary synbiotic. On the other hand, the results were in contrast with the findings of Beski and Al-Sardary, (2015); Fatima, (2015), who found that, addition of synbiotic significantly ($p \leq 0.05$) affected serum cholesterol and LDL of broiler chicks. Also, the results were in contrast with the findings of Ashayerizadeh *et al*., (2011), who observed

that addition of synbiotic to broilers diet significantly ($p \leq 0.05$) lower cholesterol and triglycerides compared to control.

The lower concentration of cholesterol in the groups fed synbiotic (probiotic + prebiotic) may be due to some microorganisms present in the probiotic had the ability of cholesterol utilization for their metabolism and depressed the cholesterol absorption from gastrointestinal tract (Mohan *et al*., 1995), in addition probiotic microorganism had the ability to inhibits the activity of hydroxyl-mthyl-glutarylcoenzymeA which involved in the cholesterol synthesis (Fukashima and Nakano, 1995). Prebiotic that present in the synbiotic mixture has hypo-cholesterolemic effects, there by reducing the absorption of lipids in the intestine through binding bile acids, increasing cholesterol elimination and hepatic synthesis of new bile acid (Zhang *et al*., 2003). Cholesterol is a precursor of bile acids; more molecules are spend for recovery of bile acids and thus reduced the cholesterol level in the serum (De Smet, 1994).

The lower serum LDL in the chicks fed on synbiotic may be due to that, the large portion of LDL is cholesteryl esters (CE) and free cholesterol with little triglycerides (McEneny *et al*., 2002). Synbiotic has the ability to decrease the concentration of CE in LDL (Min-Tze *et al*., 2007). Loss of CE from the core of LDL forms smaller and denser LDL particles. Although smaller LDL appeared more atherogenic than larger LDL particles (Haffner, 2002), smaller LDL formed could be removed from plasma easier than larger particles (Fernandez *et al*., 1993).

In this study the results indicated that, Aspartate amino-transferase AST and Alkaline phosphatase ALP enzymes were not significantly ($p \geq 0.05$) affected by application of graded levels of SBYM, however, the chicks fed on control diet obtained numerically the highest mean value of AST and ALP enzymes as compared to chicks fed on SBYM supplemented groups.The results were in agreement with the findings of Babazadeh *et al*., (2011), who found that,

application of synbiotic on broiler diets recorded no significant ($p \geq 0.05$) effects on AST and ALP enzymes between all tested groups. The results were partially consistent with the findings of Fatima, (2015), who found no significant effect on AST enzyme in broilers fed synbiotic. In contrast the results were partially disagreed with the findings of Fatima, (2015), who found adding of synbiotic in broiler diets significantly affect ALT enzyme.

In this study the results concerning serum minerals Ca and P recorded that, chicks fed on SBYM supplemented groups had obtained significantly ($p \leq 0.05$) higher means values of Ca and P as compared to control group, whereas, no significant differences ($p \geq 0.05$) were observed between all levels of SBYM supplementations in values of Ca and P. This result was partially consistent with the findings of Fatima, (2015), who found adding of synbiotic in broiler diet significantly affect serum Calcium (Ca).

The results of economical evaluation revealed that, chicks fed on dietary SBYM supplementations with all levels (0.5, 1.0 and 1.5g/kg) gained more net profit/bird as compared to chicks fed on control diet, also, recorded the highest values of profitability ratio/bird as compared to chicks fed on control diet, however, the group of chicks fed on 1.5g/kg SBTM was the highest of the tested groups (1.73). The improvement which occurred in values of net profit of treated groups may be attributed to improvement which occurred in body weight, body weight gain, feed conversion ratio, stimulation of birds immunity and reduction of mortality rate of broiler chicks (Mohamed, 2014). These results were in agreement with the findings of (Abdel-Hafeez *et al*., (2017); Marwa, (2015) and Fatima, (2015), who found that, supplementation of synbiotics in broiler diets had economic return and more profitable as compared to control group.

5. 4 Effect of dietary Probiotic, Prebiotic, Synbiotic and their Levels on performance and their interactive action of broilers

In the present studies the results of interaction between all treatments and their levels indicated no significant differences ($p \geq 0.05$) among the all dietary products with all inclusion levels in feed intake. The results also, showed chicks fed on all levels of probiotic, prebiotic and synbiotic had obtained significanty ($p \leq 0.05$) heaviest means values of body weight and body weight gain, and obtained the best feed conversion ratio as compared to control group. The best improvement of performance was obtained by synbiotic, followed by prebiotic and then probiotic, about the levels the improvement increased with increasing inclusion level, whereas, the best level 1.5g/kg followed by 1.0g/kg then 0.5g/kg.

CHAPTER SIX

CONCLUSION AND RECOMENDATIONS

Conclusion:

▪ Inclusion of bacterial probiotic Biogen.S (BPB), prebiotic Y-MOS (PYM) and synbiotic Biogen.S + Y-MOS 1:1 (SBYM) at all levels in broiler diets as natural feed additives significantly improved the performance without any effect on feed intake.

▪ The results of the present study illustrated no mortalities were recorded in all treatment groups throughout the experimental period.

Adding of dietary (BPB), (PYM) and (SBYM) in broiler diets significantly ($p \leq 0.05$) affect carcass dressing percentages.

▪ Inclusion of graded levels of all tested products made no changes in other meat quantity and quality.

▪ Application of all tested products did not affected serum metabolites, serum enzymes and serum minerals, except for the dietary (SBYM) at all levels which recorded higher values of minerals compared to control.

▪ The results of interaction between all treatments and their levels recorded significant improvement in body weight, wight gain and FCR at all inclusion levels compared to control.

▪ Using of dietary (BPB), (PYM) and (SBYM) at various inclusion levels in broiler diets economically is profitable compared to control.

Recommendations:

Practical implications:

▪ Based on the results of these studies, dietary (BPB), (PYM) and (SBYM) could be considered as potential natural growth promoters without any adverse effect.

▪ Based on the results of performance interaction between dietary (BPB), (PYM) and (SBYM), the dietary synbiotic could be the best natural growth promoter, and the level 1.5g/kg could be the best inclusion level.

▪ All levels of dietary (BPB), (PYM) and (SBYM) added to broiler diets in these studies were recommended economic – wise, but the level 1.5 g/kg of all tested products was more profitable.

Suggestion for future research:

▪ More trails are needed to clarify the effects of different products of probiotic, prebiotic and synbiotic as natural feed additives on performance, carcass characteristics, digestive system development, immune system, intestinal micro flora and blood constituent of poultry with regard to various management condition, including different stress factors, types of products, species and strain of bacteria, optimal dietary products application levels, dietary ingredients and nutrients contents.

▪ Further experiments are needed to test the synergistic effect of these products and their types to prove additive or otherwise.

▪ Finding of these studies point to the possibility of using those natural feed additives in laying hens as well as testing for egg production and quality.

▪ The future research also should focus on the use of other natural feed additive such as herbs and spices, essential oils extracted from aromatic plants, enzymes and organic acid in poultry production.

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APPENDIXES

Appendix -1

Weekly minimum and maximum experimental temperature during the 19th January to 23 February 2019. Temperature (ºC).

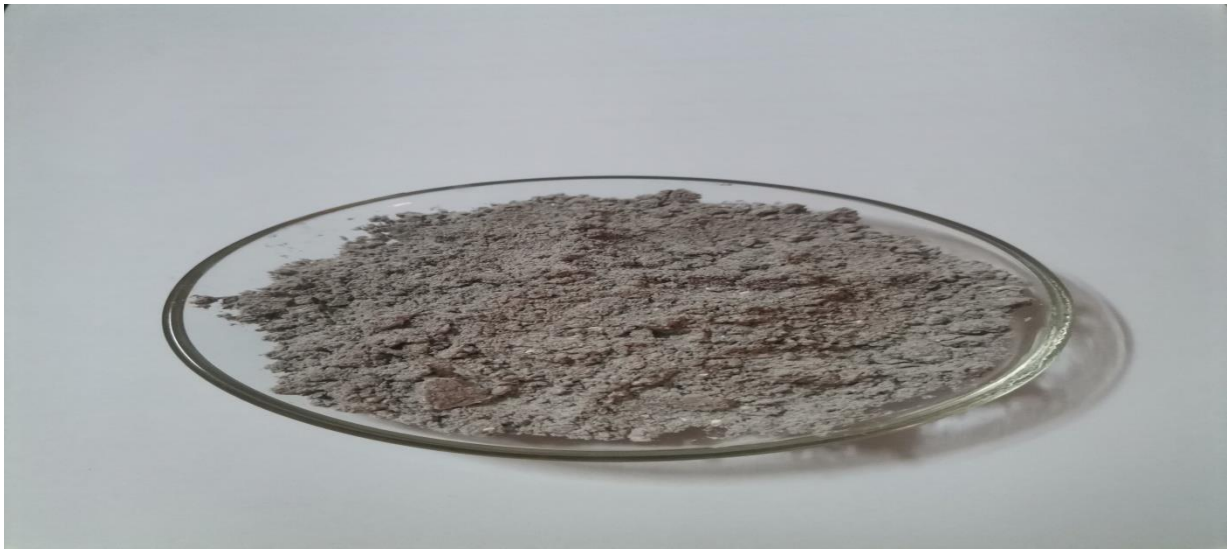
Weeks	Minimum	Maximum
1	24	34
2	22	30
3	20	26
4	18	22
5	16	18
Average temperature	20	26

Appendix -2

Commercial Probiotic used in experiment (1)

Appendix -3

Commercial Prebiotic used in experiment (2)



Appendix -4

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 tender	6-Moderately intense	6-Moderately desirable	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 -5

Cobb500 Broiler Performance & Nutrition Supplement

Performance objectives - metric

AS HATCHED

Age days	Weight for Age	Daily Gain (g)	Average Daily Gain (g)	Cumulative Feed Conversion	Daily Feed Consumption (g)	Cumulative Feed Consumption (g)
0	42					
1	52	10				
2	66	14				
3	81	15				
4	100	19				
5	122	22				
6	148	26				
7	**177**	**29**	**25.3**	**0.847**		**150**
8	208	31	26.0	0.865	30	180
9	242	34	26.9	0.888	35	215
10	279	37	27.9	0.914	40	255
11	320	41	29.1	0.938	45	300
12	364	44	30.3	0.962	50	350
13	410	46	31.5	0.988	55	405
14	**459**	**49**	**32.8**	**1.013**	**60**	**465**
15	511	52	34.1	1.039	66	531
16	567	56	35.4	1.063	72	603
17	626	59	36.8	1.088	78	681
18	688	62	38.2	1.112	84	765
19	753	65	39.6	1.135	90	855
20	821	68	41.1	1.158	96	951
21	**891**	**70**	**42.4**	**1.182**	**102**	**1053**
22	964	73	43.8	1.205	109	1162
23	1039	75	45.2	1.230	116	1278
24	1115	76	46.5	1.257	123	1401
25	1193	78	47.7	1.283	130	1531
26	1272	79	48.9	1.311	137	1668
27	1353	81	50.1	1.339	144	1812
28	**1436**	**83**	**51.3**	**1.367**	**151**	**1963**
29	1521	85	52.4	1.394	158	2121
30	1608	87	53.6	1.422	165	2286
31	1697	89	54.7	1.448	172	2458
32	1788	91	55.9	1.475	179	2637
33	1880	92	57.0	1.502	186	2823
34	1973	93	58.0	1.529	193	3016
35	**2067**	**94**	**59.1**	**1.556**	**200**	**3216**
36	2162	95	60.1	1.581	202	3418
37	2257	95	61.0	1.604	203	3621
38	2352	95	61.9	1.627	205	3826
39	2447	95	62.7	1.648	206	4032
40	2542	95	63.6	1.668	208	4240
41	2637	95	64.3	1.687	209	4449
42	**2732**	**95**	**65.0**	**1.705**	**210**	**4659**
43	2826	94	65.7	1.724	212	4871
44	2919	93	66.3	1.742	214	5085
45	3011	92	66.9	1.761	216	5301
46	3102	91	67.4	1.779	218	5519
47	3192	90	67.9	1.798	220	5739
48	3281	89	68.4	1.817	222	5961
49	**3369**	**88**	**68.8**	**1.836**	**224**	**6185**
50	3456	87	69.1	1.855	225	6410
51	3542	86	69.5	1.874	226	6636
52	3627	85	69.8	1.892	226	6862
53	3711	84	70.0	1.910	227	7089
54	3794	83	70.3	1.928	227	7316
55	3876	82	70.5	1.946	228	7544
56	**3958**	**82**	**70.7**	**1.964**	**228**	**7772**

Appendix -6

Cobb500 Broiler Performance & Nutrition Supplement

Yield Performance

Predicted carcass yields at given weights

AS HATCHED

Weight g	Weight lb	% Carcass	% Boneless Breast	% Whole Thigh	% Whole Drum Stick	% Wing
1600	3.528	71.9	20.70	13.78	8.77	7.78
1800	3.969	72.5	21.25	13.94	8.79	7.75
2000	4.410	73.1	22.12	14.08	8.81	7.72
2200	4.851	73.8	22.74	14.16	8.83	7.69
2400	5.292	74.4	23.31	14.28	8.85	7.66
2600	5.733	75.1	23.83	14.40	8.87	7.63
2800	6.174	75.9	24.26	14.50	8.89	7.60
3000	6.615	76.4	24.56	14.58	8.91	7.57
3200	7.056	77.0	25.11	14.66	8.93	7.54

FEMALES

Weight g	Weight lb	% Carcass	% Boneless Breast	% Whole Thigh	% Whole Drum Stick	% Wing
1600	3.528	72.0	21.53	14.02	8.52	7.84
1800	3.969	72.6	21.65	14.20	8.54	7.81
2000	4.410	73.2	22.40	14.36	8.56	7.78
2200	4.851	73.7	22.98	14.40	8.58	7.75
2400	5.292	74.5	23.46	14.52	8.60	7.72
2600	5.733	75.6	23.93	14.66	8.62	7.69
2800	6.174	75.8	24.31	14.76	8.64	7.66

MALES

Weight g	Weight lb	% Carcass	% Boneless Breast	% Whole Thigh	% Whole Drum Stick	% Wing
1600	3.528	71.8	20.35	13.54	9.02	7.71
1800	3.969	72.4	20.97	13.67	9.04	7.68
2000	4.410	73.0	21.84	13.79	9.06	7.65
2200	4.851	73.7	22.50	13.91	9.08	7.62
2400	5.292	74.3	23.15	14.04	9.10	7.58
2600	5.733	75.0	23.73	14.14	9.12	7.55
2800	6.174	75.6	24.21	14.24	9.14	7.52
3000	6.615	76.3	24.46	14.36	9.16	7.49
3200	7.056	76.9	24.95	14.48	9.18	7.46
3400	7.497	77.5	25.53	14.59	9.20	7.43
3600	7.938	78.2	26.10	14.71	9.22	7.40

- Eviscerated carcass is calculated with feet and shanks removed from the hock joint.
- % Boneless breast is as a percentage of live weight.

Appendix -7

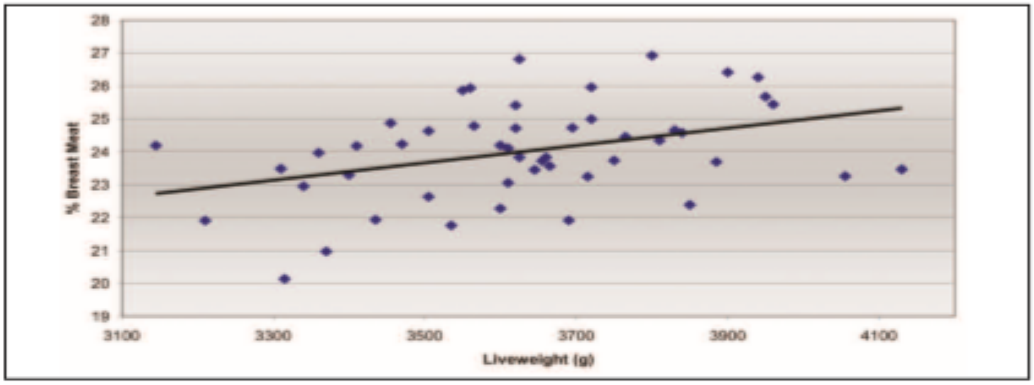
Cobb500 Broiler Performance & Nutrition Supplement

Yield Performance

Meat yield is dependent on many factors, but those that have the most influence are weight, age and nutrition.

Weight

- Carcass and breast meat yield increase as a function of liveweight at any given age.



Percent breast meat yield for birds from a single flock of males processed at 50 days.

Age

- Carcass and breast meat yield increase as a function of age.
- Older birds processed at the same weight will often yield more than their younger counterparts.

Sex	Age	Weight g (lb)	% Eviscerated Carcass
A/H	46	2826 (6.231)	74.6
A/H	43	2830 (6.240)	74.1
Difference	**3**	**-4**	**0.50**

Feed

- Carcass composition is affected by nutrition.
- Rations of varying nutrient density will affect yield in different ways.
- As protein is increased there is a corresponding increase in breast meat yield as a percent of live weight.

Appendix –8

CHICKS NORMAL PROFILE

METABOLITES:

Total Protein	(g/dl)	2.58 – 7.56
Albumin	(g/dl)	1.11 – 3.5
Globulin	(g/dl)	1.34 – 2.01
Total Bilirubin	(mg/dl)	0.015 – 0.61
Creatinine	(mg/dl)	2.0 – 3.56
Uric acid	(mg/dl)	4.58 – 8.3
Urea	(mg/dl)	4.67 – 12.95
Triglycerides	(mg/dl)	40 – 100.47
Cholesterol	(mg/dl)	102 – 203
Total Lipids	(mg/dl)	5.0 – 7.65
Glucose	(mg/dl)	219 – 247

ENZYMES:

Aspartate Amino Transferase (AST)	(iu/L)	16.72 – 54.0
Alkaline Phosphatase (ALP)	(iu/L)	36.9 – 244 86
Gamma-glutamyl Transferase (GGT)	(i.u.)	13.89 – 41.8
Lactate Dehydrogenase (LDH)	(i.u.)	63.2
Creatine Kinase (CK)	(i.u.)	129.6

MINERALS:

Calcium (Ca)	(mg/dl)	5.78 – 10.6
Phosphorus (P)	(mg/dl)	4.59 – 11.38

Iron (Fe)	(mg/dl)	111.09 – 119.05
Copper (Cu)	(mg/dl)	0.35 – 1.04
Manganese (Mn)	(mg/dl)	1.4 – 2.0
Zinc (Zn)	(mg/dl)	0.35 – 2.0

HEAMATOLOGY:

Red Blood Cell (RBC) ($x10^{12}$/l)	(cell/L)	2.0 – 2.35
White Blood Cell (WBC) ($x10^{9}$/l)	(cell/L)	1.14 – 1.17
Packed Cell Volume (PCV)	(%)	5 – 38
Hemoglobin (Hb)	(g/dl)	7.5 – 18.3
Mean Corpusular Volume (MCV)	(fl)	27.27 -138.2
Mean Corpusular Hemoglobin (MCH)	(pg)	32.42 -37.27
Mean Corpusular Hemoglobin Concentration (MCHC)	(g/dl)	24.79 – 137.5

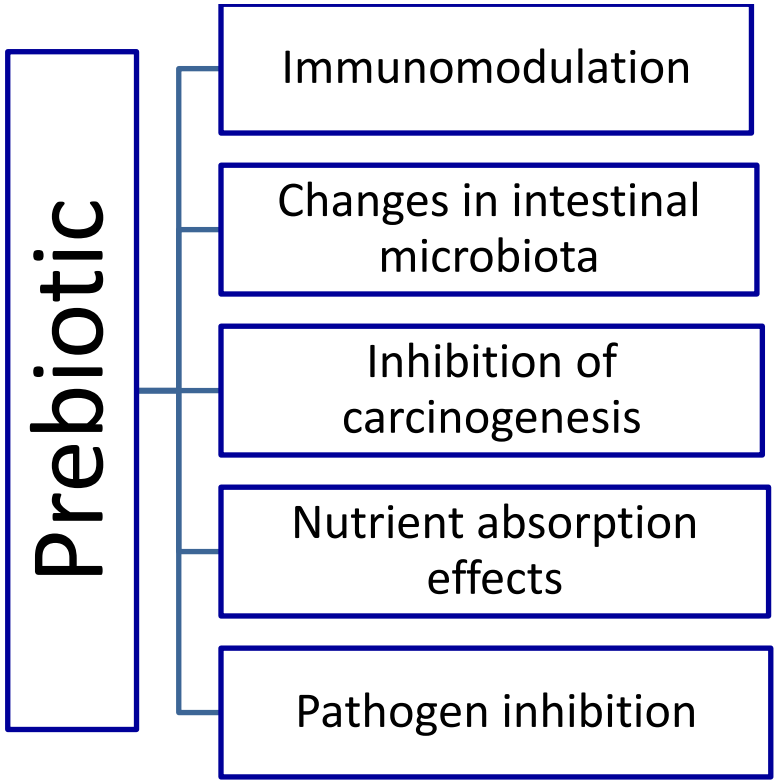
The Source:

AL- Amin. A. M. (2012), scientific issue.

Faculty of Veterinary Science, University of Khartoum.

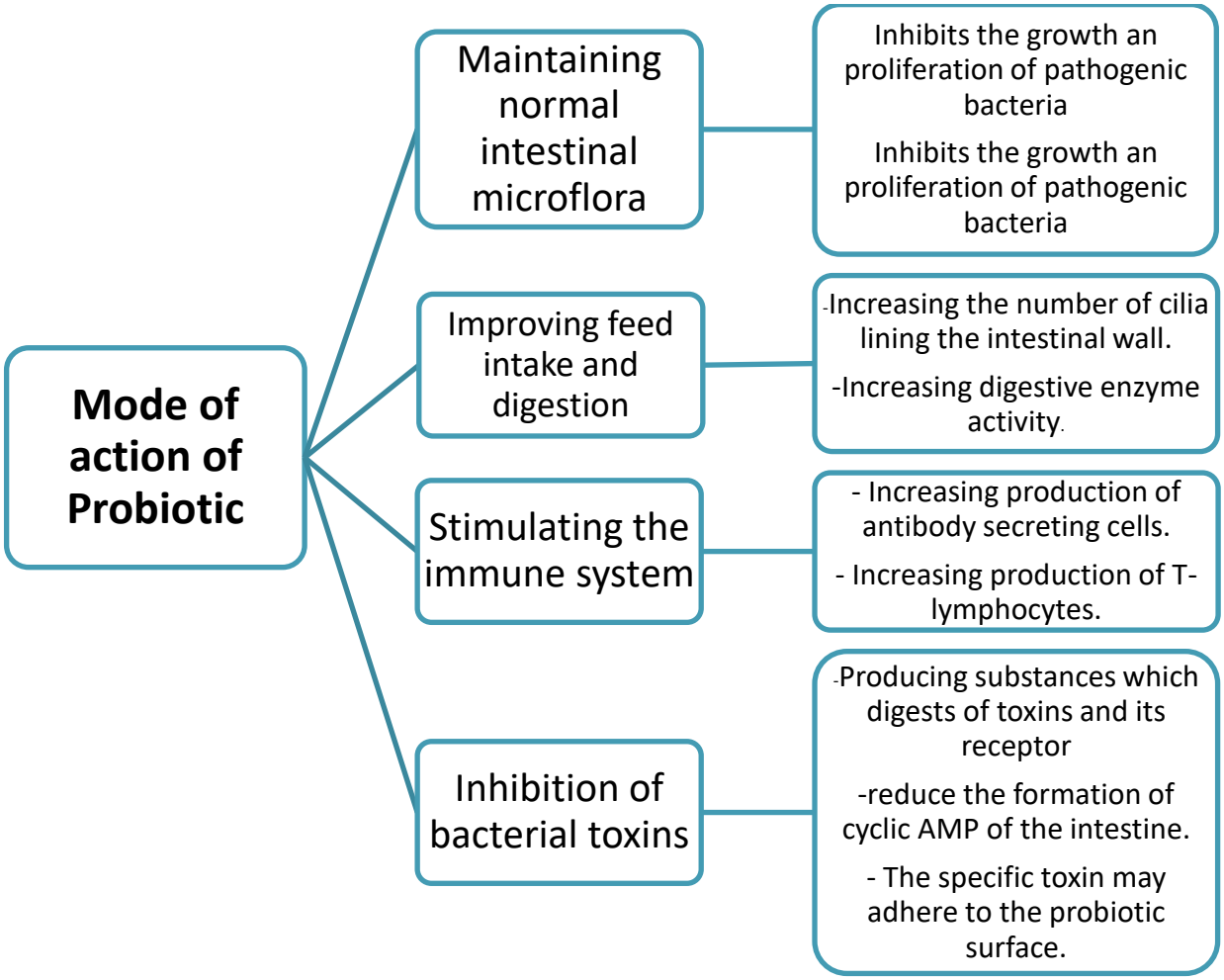
Appendix –9

Mode of Action of Prebiotic



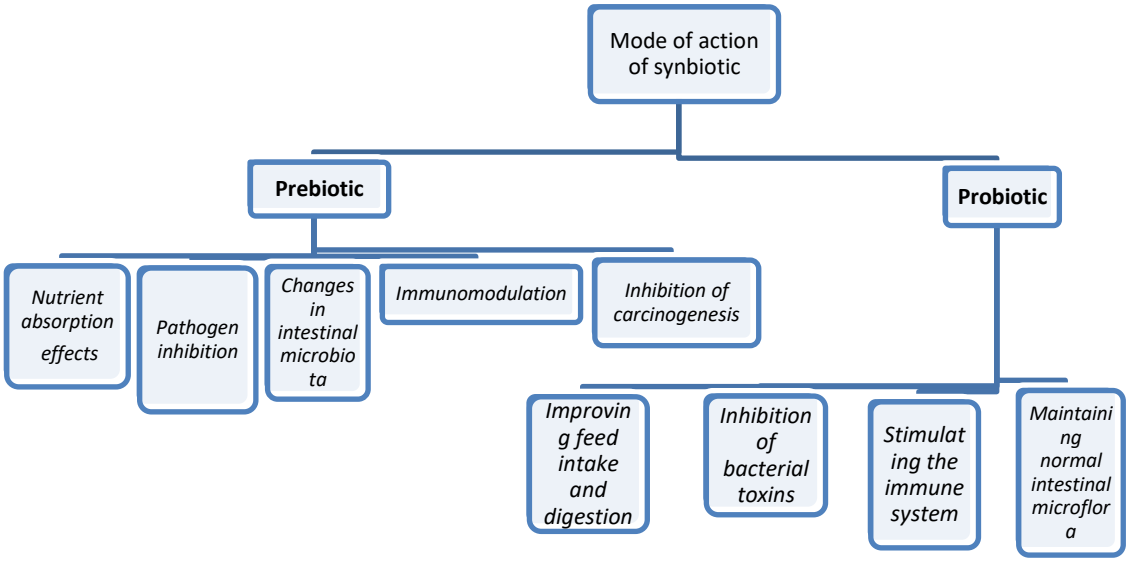
Appendix –10

Mode of action of Probiotic



Appendix –11

Mode of action of Synbiotic



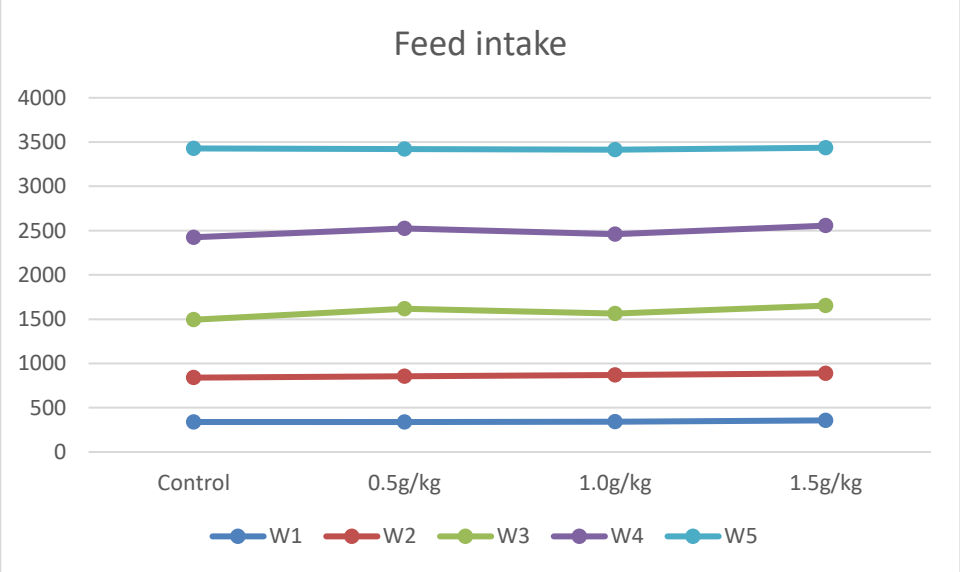
Appendix –12

Weekly tables and figures:

Table (1): Effect of graded levels of bacterial probiotic on feed intake (g) bird / week (b/w)

Items	W1	W2	W3	W4	W5
Control	339	840	1494	2425	3428
0.5g/kg	340	857	1619	2524	3421
1.0g/kg	344	871	1565	2460	3414
1.5g/kg	357	888	1653	2556	3436

Figure (1): Effect of graded levels of bacterial probiotic on feed intake (g) bird / week (b/w)

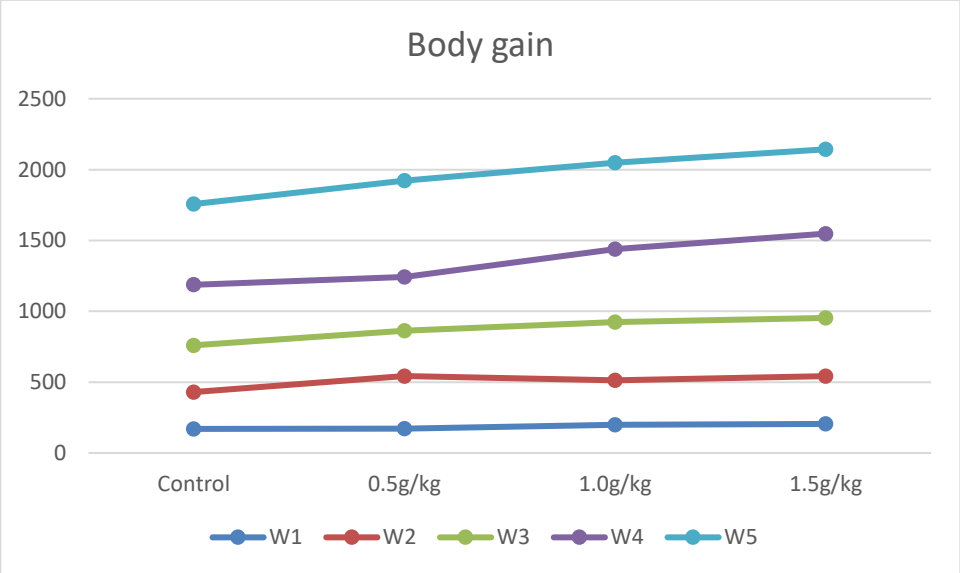


Appendix –13

Table (2): Effect of graded levels of bacterial probiotic on body weight gain (g) b/w

Items	W1	W2	W3	W4	W5
Control	170	430	760	1187^{c}	1757^{c}
0.5g/kg	172	543	864	1242^{b}	1922^{b}
1.0g/kg	200	512	924	1439^{ab}	2048^{ab}
1.5g/kg	205	542	953	1548^{a}	2143^{a}

Figure (2): Effect of graded levels of bacterial probiotic on body weight gain (g) b/w

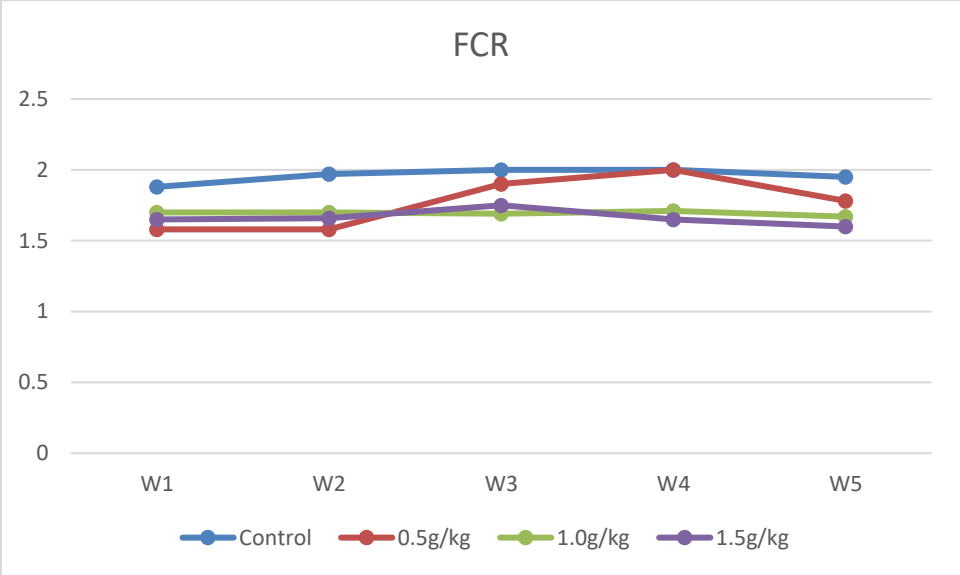


Appendix –14

Table (3): Effect of graded levels of bacterial probiotic on (FCR) b/w

Items	W1	W2	W3	W4	W5
Control	1.88^{b}	1.97^{b}	2.00^{b}	2.00^{b}	1.95^{c}
0.5g/kg	1.58^{a}	1.58^{a}	1.90^{b}	2.00^{b}	1.78^{b}
1.0g/kg	1.70^{a}	1.70^{ab}	1.69^{a}	1.71^{a}	1.67^{ab}
1.5g/kg	1.65^{a}	1.66^{a}	1.75^{a}	1.65^{a}	1.60^{a}

Figure (3): Effect of graded levels of bacterial probiotic on (FCR) b/w

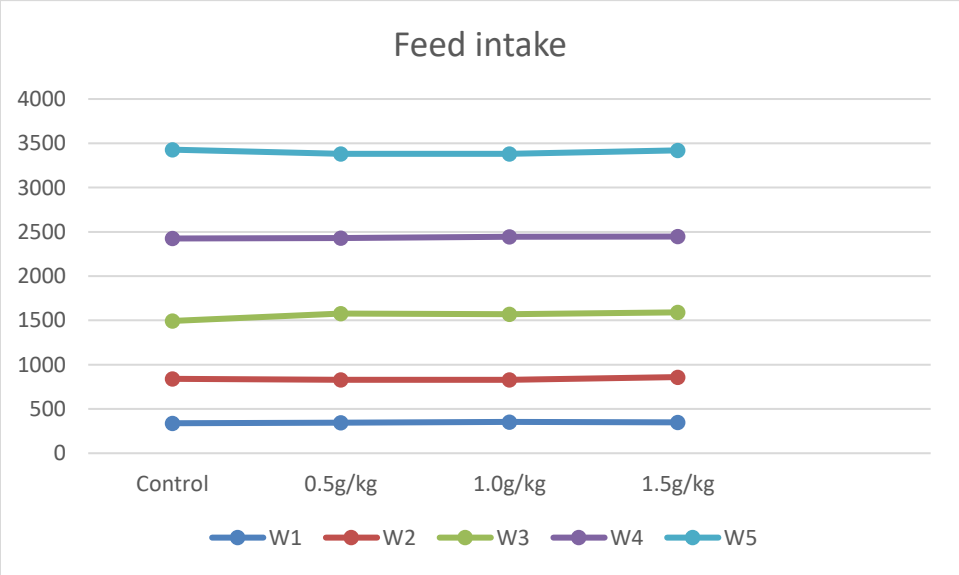


Appendix –15

Table (4): Effect of graded levels of prebiotic on feed intake (g) b/w

Items	W1	W2	W3	W4	W5
Control	339	840	1494	2425	3428
0.5g/kg	346	830	1576	2430	3381
1.0g/kg	354	829	1571	2445	3381
1.5g/kg	349	860	1591	2447	3420

Figure (4): Effect of graded levels of prebiotic on feed intake (g) b/w

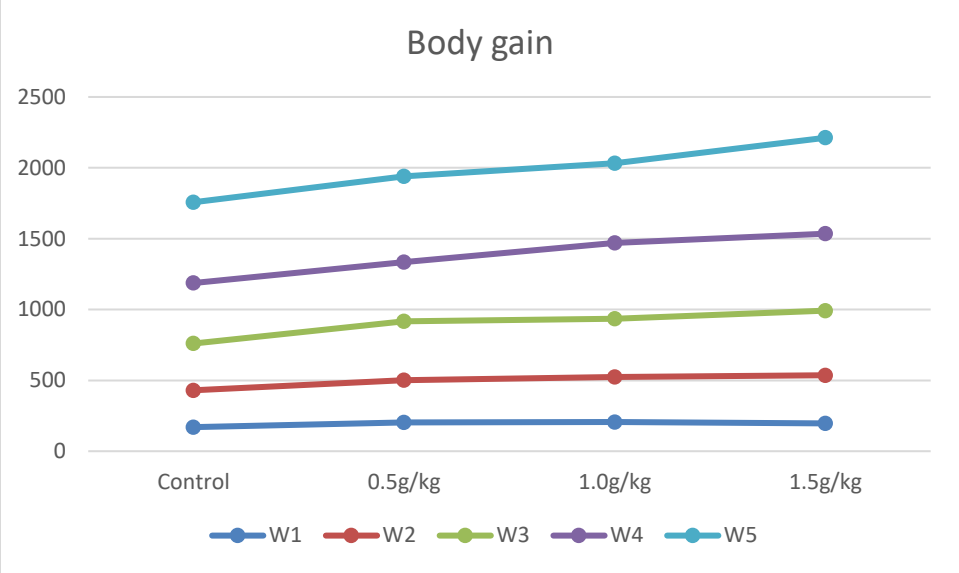


Appendix –16

Table (5): Effect of graded levels of prebiotic on body weight gain (g) b/w

Items	W1	W2	W3	W4	W5
Control	170	430	760	1187^{c}	1757^{c}
0.5g/kg	202	502	916	1334^{b}	1940^{b}
1.0g/kg	206	523	934	1469^{b}	2032^{b}
1.5g/kg	196	536	992	1535^{a}	2212^{a}

Figure (5): Effect of graded levels of prebiotic on body weight gain (g) b/w



Appendix –17

Table (6): Effect of graded levels of prebiotic on (FCR) b/w

Items	W1	W2	W3	W4	W5
Control	1.88[b]	1.97[b]	2.00[b]	2.00[c]	1.95[c]
0.5g/kg	1.71[a]	1.65[a]	1.72[a]	1.83[b]	1.74[b]
1.0g/kg	1.73[a]	1.58[a]	1.68[a]	1.67[a]	1.66[ab]
1.5g/kg	1.78[a]	1.60[a]	1.60[a]	1.60[a]	1.55[a]

Figure (6): Effect of graded levels of prebiotic on (FCR) b/w

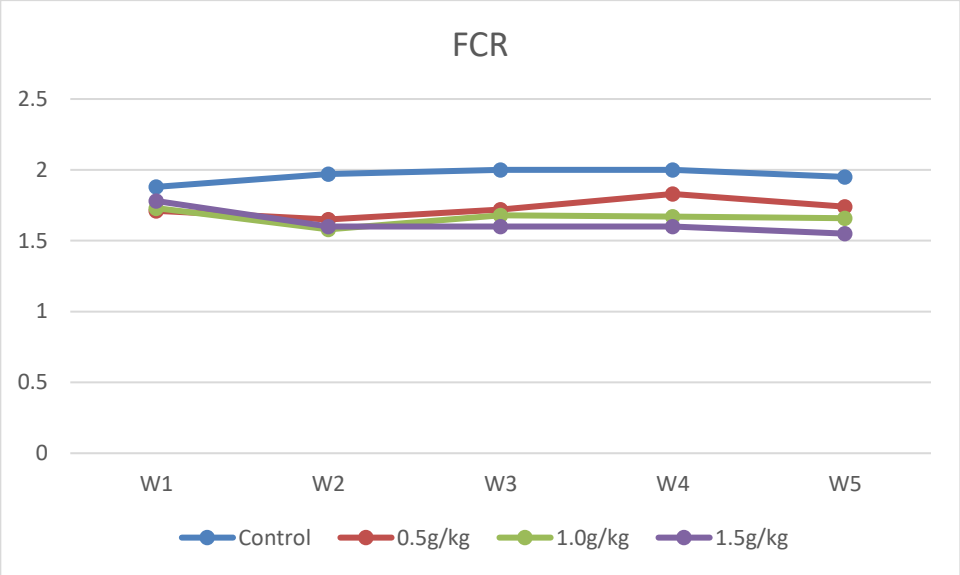


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Table (7): Effect of graded levels of synbiotic on feed intake (g) b/w

Items	W1	W2	W3	W4	W5
Control	339	840	1494	2425	3428
0.5g/kg	359	912	1678	2502	3383
1.0g/kg	364	849	1513	2409	3382
1.5g/kg	341	800	1559	2418	3390

Figure (7): Effect of graded levels of synbiotic on feed intake (g) b/w

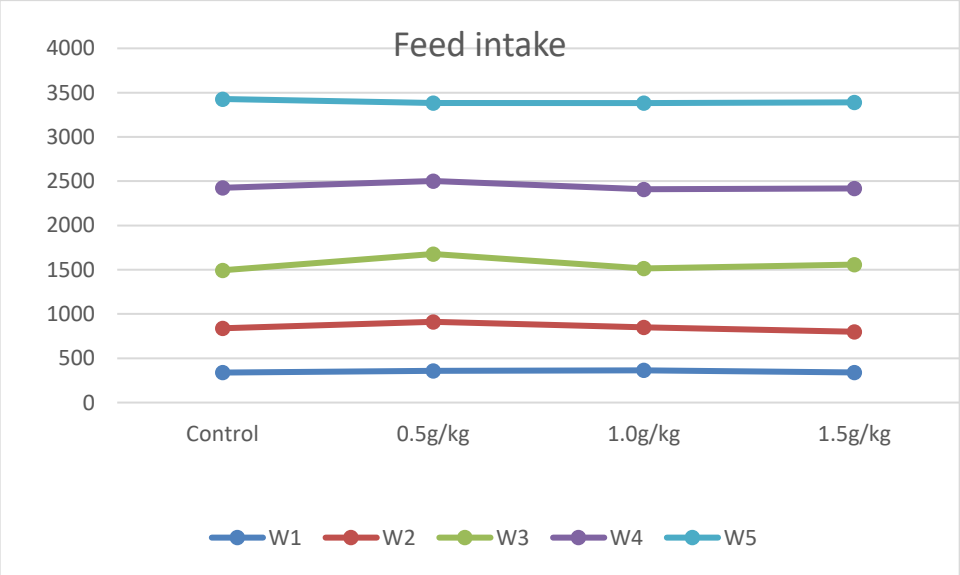

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Table (8): Effect of graded levels of synbiotic on body weight gain (g) b/w

Items	W1	W2	W3	W4	W5
Control	170	430	760	1187[b]	1757[c]
0.5g/kg	225	595	1009	1425[a]	1957[b]
1.0g/kg	198	543	977	1449[a]	2047[b]
1.5g/kg	212	567	1034	1569[a]	2215[a]

Figure (8): Effect of graded levels of synbiotic on body weight gain (g) b/w

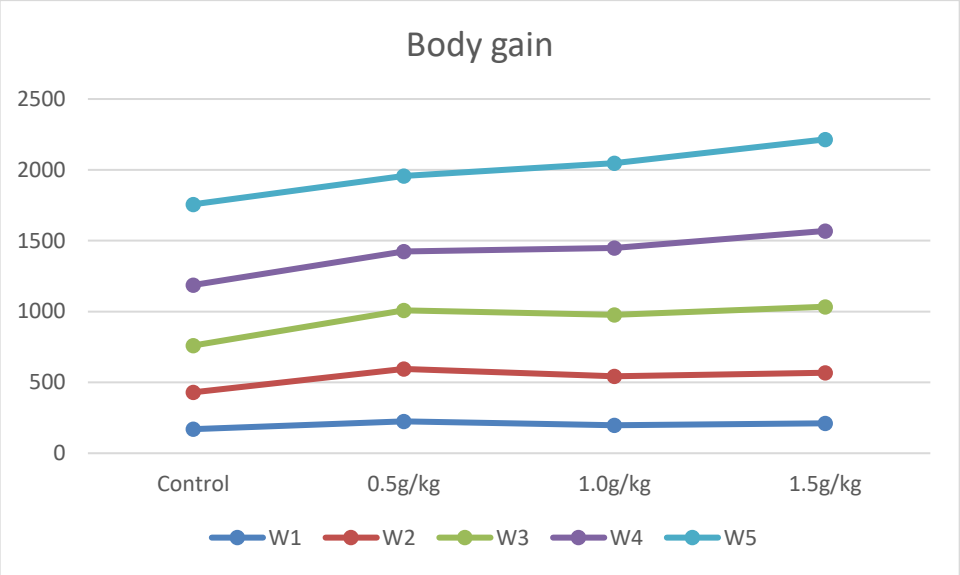

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Table (9): Effect of graded levels of synbiotic on (FCR) b/w

Items	W1	W2	W3	W4	W5
Control	1.88^{b}	1.97^{b}	2.00^{b}	2.00^{c}	1.95^{c}
0.5g/kg	1.59^{a}	1.53^{a}	1.66^{a}	1.78^{b}	1.73^{b}
1.0g/kg	1.89^{b}	1.56^{a}	1.55^{a}	1.62^{ab}	1.65^{ab}
1.5g/kg	1.62^{a}	1.42^{a}	1.51^{a}	1.55^{a}	1.53^{a}

Figure (9): Effect of graded levels of synbiotic on (FCR) b/w