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CHEMICAL CHARACTERIZATION OF TRICHODERMA REESEI DEGRADED DATE PITS AND ITS EFFECT ON GROWTH PERFORMANCE AND INTESTINAL BACTERIAL POPULATION AND ANTIOXIDANT STATUS - A COMPARATIVE STUDY WITH MANNOSE AND MANNAN -OLIGOSACCHARIDE ON BROILER

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This dissertation is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Under the Supervision of Professor Wissam Ibrahim

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Declaration of Original Work

I, Salem Rashed Ali Rashed Al Yileili, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of this dissertation entitled "*Chemical Characterization of Trichoderma reesei Degraded Date Pits and its Effect on Growth Performance and Intestinal Bacterial Population and Antioxidant Status - A Comparative Study with Mannose and Mannanoligosaccharide on Broiler*", hereby, solemnly declare that this dissertation is the original research work that has been done and prepared by me under the supervision of Professor Wissam Ibrahim, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published or formed the basis for the award of an academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been appropriately cited and acknowledged by appropriate academic conventions. I further declare that there is no potential conflict of interest concerning the research, data collection, authorship, presentation, and publication of this dissertation.

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Abstract

With the increasing apprehension over food safety nowadays, there have been relentless efforts aiming for subrogate antibiotic growth promoters (AGPs) in poultry feeds. Thus, this research study aims at investigating the potential effects of using degraded date pits (DDP) as a feedstuff and as a natural alternative for antibiotics in poultry feeding. Two feeding experiments were conducted. In the experiment 1, the minimum time of feeding diet containg 10% DDP required to induce growth promoting effects on broiler performance. In experiment 2, the influence of feeding 10% DDP was examined as feedstuff at 10% in broilers diets and a growth promoting agent compared with corn soybean meal diets supplemented with antibiotic 50 g of 20% oxytetracycline, 0.2% of mannan oligosaccharide (MOS) and either 0.2 or 0.1% of mannose on growth performance, microbial growth, antioxidant and biochemical effects, intestine development and antibacterial gene expression in broiler chickens. Date pits (DP) were degraded with the fungus *Trichoderma reesei* using solid-state degradation (SSD) and included in broiler's diets at 10%. Results showed that biological degradation with *T. reesei* significantly improved the nutritional effect of DP by increasing the proximate composition, monosaccharide composition, mineral content and *in vitro* antioxidant activities. The results of the experiment 1, showed that prolonged feeding period of 10% DDP to 5 weeks for broiler resulted in better growth than three or four weeks of feeding periods. The results of experiment 2 indicated that there were no significant differences in body weight, feed intake, and feed conversion ratio (FCR) among the different dietary treatments. Total bacterial count, *E. coli*, Enterobacteriaceae, *Shigella* and *salmonella* count, were significantly decreased in 10% DDP diet fed-broilers, 0.2% MOS and antibiotic diet fed-broilers, showing that DDP and MOS have similar mode of action. The results of the antioxidant and biochemical effects of DDP showed that the activity of antioxidant enzymes in serum, liver and intestine of broilers fed diets with 10% DDP and 0.2% MOS were increased significantly compared with other treatments, but malondialdehyde (MDA) content was significantly decreased. Among the different dietary treatments, 10% DDP and 0.2% MOS diet enhanced the pancreatic digestive enzymes, histomorphology of intestine, and weight and length of duodenum, jejunum, and ileum. Immunoglobulin levels in serum and intestinal contents, the expression pattern of mucin-2,

cathelicidins, beta-defensins and LEAP-2 in jejunum were up-regulated in 10% DDP, and 0.2% MOS diet fed-broilers.

In conclusion, 10% DDP diet can be fed to broiler chcikens during 1-42 days of age and also as a growth promoting agent to replace antibiotic while showing similar mode of action to MOS. The results suggested that DDP can be used as a feedstuff and growth promoter for chicken's feeding in the Arabian Peninsula, whereas the DDP is produced abundantly and available at large amount, this will resulted in decreasing environmental pollution of unutilized agriculture by-products.

Keywords: Boilers, degraded date pits, microbial population, growth promoter, antibiotics, oxytetracycline, growth performance, intestinal development.

Title and Abstract (in Arabic)

الخصائص الكيميائية لنوى التمر المعالج بفطر الترايكوديرما ريسي وتأثيره على نمو فراريج اللحم، والمحتوى البكتيري للأمعاء وحالة مضادات الأكسدة فيها ـدراسة مقارنة مع المنانوليجوساكارايد والمانوز

الملخص

مع تزايد المخاوف بشأن سلامة أغذية الحيوانات خلال السنوات القريبة الماضية، كان هناك جهد مكثف لمحاولة إيجاد بدائل للمضادات الحيوية والتي تستخدم كمحفزات للنمو في أعلاف الدواجن. لذلك، وللتحقق من تأثير البدائل الطبيعية للمضادات الحيوية، أجريت تجربة لدراسة تأثير مضادات الميكروبات بإضافة نوى التمر المعالج بالفطر، المانوز، المنان أوليجوساكارايد والمضاد الحيوي أوكسيتتراساكلين. وأظهرت التحاليل أن نوى التمر المعامل بالفطر باستخدام تقنية تكسير الحالة الصلبة ((solid state degradation (SSD) قد حسَّن بشكل كبير التأثير التغذوي لنوى التمر عن طريق زيادة المحتوى الغذائي، وتحسين نسبة السكريات الأحادية، وتركيز العناصر المعدنية ونشاط انزيمات مضادات الأكسدة.

من أجل دراسة دور المعاملات الغذائية المختلفة في الفروج اللاحم، تم تقسيم الدجاج اللاحم البرازيلي (Cobb 500) ذو عمر اليوم الواحد إلى ست معاملات بثلاث تكرارات بكل مكررة عشرة فراريج غير مجنسة، وشملت المعاملات علي نظام غذائي طبيعي يحتوي على علف الذرة وكسب فول الصويا ، وثانية تحتوي على علف الذرة وكسب فول الصويا + (50 جرم من 20% Oxytetracycline)، والثالثة تحتوي على 10% من نوى التمر المعامل بالفطر، أما الرابعة فغذيت علي علف الذرة وكسب فول الصويا المضافة اليه 0.2% من المنان أوليجوساكارايد والخامسة فغذيت علي علف الذرة وكسب فول الصويا المضافة اليه 0.2% مانوز أما السادسة فغذيت علي علف الذرة وكسب فول الصويا المضافة اليه 0.1% من المانوز. في المرحلة الأولى قمت بدراسة تأثير المعاملات الغذائية المختلفة على أداء النمو والمحتوى البكتيري الموجود في الأمعاء. أوضحت النتائج أنه لا يوجد فرق معنوي في وزن الجسم وكمية الغذاء المستهلك وكذلك معامل التحول الغذائي بين المعاملات الغذائية المختلفة. وتلاحظ انخفاض في العدد الكلي للبكتريا، الايكولاي والسالمونيلا والانتروباكتيرياسي والشيقيلا. وعدد السالمونيلا انخفض بشكل ملحوظ باستخدام 10% من النوى المعامل بالفطر، في حالة التدعيم ب 0.2% مانان أوليجوساكارايد وفي الغذاء المدعم بالمضاد حيوي.

في المرحلة الثانية درست التأثيرات المضادة للأكسدة والكيمياء الحيوية للعلاجات المختلفة على الفروج وأظهرت النتائج أن نشاط الأنزيمات المضادة للأكسدة في مصل الدم والكبد والأمعاء من الفراريج قد زادت بشكل ملحوظ في 0.2% مانان أوليجوساكارايد و10% نوى معالج بالفطر. انخفض محتوى ((Malondialdehyde (MDA) بشكل ملحوظ في 10% نوى التمر المعالج وكذلك في حالة العلف المدعم ب 0.2% من المنان أوليجوساكارايد. تم التأكد من التأثير الوقائي لنوى التمر المعالج والمانان أوليجوساكارايد عن طريق فحص

الأنسجة المعوية للطيور. من بين العلاجات الغذائية المختلفة، ساعدت 10% لنوى التمر المعالج و0.2% المانان أوليجوساكارايد في نمو القناة الهضمية كما يتضح من المستويات المحسنة من الإنزيمات الهاضمة والوزن وطول الأجزاء المعوية وهيستومورفولوجيا الأمعاء. أظهرت نتائج أوزان الذبيحة وأوزان الأعضاء الداخلية الأخرى التأثير النافع لنوى المعالج بالفطر.

مستويات الجلوبيولين المناعي في أمعاء الطيور قد زاد بشكل ملحوظ في العينات التي تم إضافة النوى المعامل بالفطر لها وكذلك في العينات التي احتوى غذاءها على المنان أوليجوساكارايد. في النهاية، يمكن استنتاج أن جميع المعاملات كان لها تأثير متقارب فيما يخص النمو مما يشير إلى إمكانية استبدال جزء من الذرة الصفراء بنوى التمر المعامل بالفطر كمصدر للطاقة وكمكون طبيعي قادر على التقليل من نسب البكتيريا الضارة والحفاظ على مستوى البكتيريا النافعة بالإضافة إلى تحسين معدل مضادات الأكسدة. مما يجعلها مؤهلة لاستخدامها كواحدة من البريبايوتيكس الطبيعية قليلة التكلفة.

مفاهيم البحث الرئيسية: فطر *الترايكوديرما ريسي*، المنان أوليجوساكارايد، المانوز، دجاج لاحم، نوى تمر المعامل بالفطر، الجلوبيولين، البريبايوتيك.

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Dedication

To my beloved country, I am proud to belong to the UAE

To my kind and warm family, with great appreciation

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List of Abbreviations and Acronyms

AAs	Amino Acids
ABTS	2, 2'-Azino-bis-(3-Ethylbenzothiazoline-6-Sulphonic Acid)
ABW	Average Body Weight
ADF	Acid Detergent Fiber
AGPs	Antibiotic Growth-Promoters
Ala	Alanine
ALT	Alanine aminotransferase
AMPs	Anti-Microbial Peptides
Asp	Aspartate
AST	Aspartate aminotransferase
BUA	Blood Uric Acid
CF	Crude Fiber
Cys	Cysteine
DDP	Degraded Date Pits
DP	Date Pits
DPPH	2, 2-Diphenyl-1-Picrylhydrazyl
EBP	Enumeration of Bacterial Population
EC	*Escherichia coli*
ETEC	Enterotoxigenic *E. coli*
FCR	Feed Conversion Ratio
FI	Feed Intake
FOS	Fructooligosaccharides
FRAP	Ferric Reducing Antioxidant Power Assay
GGT	Gamma-Glutamyl Transferase
Gln	Glutamine
GOS	Galact-Oligosaccharides
GOT	Glutamic Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase

GPX	Glutathione Peroxidase
GST	Glutathione s-Transferase
HCT	The hematocrit or the volume percentage (vol%) of red blood cells in blood
IEC	Intestinal Epithelial Cells
Ile	Isoleucine
IOS	Iso-Malto-Oligosaccharides
Leu	Leucine Lys Lysine
MALT	Mucosa-Associated Lymphoid Tissues
MAN	Mannose
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
Met	Methionine
MOS	Mannan-Oligosaccharide
NDF	Neutral Detergent Fiber
Phe	Phenylalanine
Pro	Proline
RBC	Red Blood Cells
RT-PCR	Quantitative Reverse Transcriptase-Polymerase Chain Reaction
SEM	Scanning Electron Micrograph
Ser	Serine
SIgA	Secretory Immunoglobulin A
SOD	Superoxide Dismutase
TFI	Total Feed Intake
Thr	Threonine
Tyr	Tyrosine
VAL	Valine
WBC	White Blood Cells
XOS	Xylo-Oligosaccharides

Chapter 1: Introduction

The use of poultry products as an animal protein supply for human has become very prominent worldwide due to its nutritional properties. In the Gulf region, most of poultry feedstuffs are imported such as corn, maize and sorghum, wheat and barley and soybean meal and thus the prices of these ingredients have increased. The feedstuffs were upgraded by using alternative sources such as the addition of fat and oil in poultry diets and/or locally available feed resources. Worldwide, there are a competition with the food manufacturers and high biofuel production for feedstuffs. Thus, there are urgent needs for research of locally available feedstuffs that can be utilized to replace the imported ones.

In many developing countries where there is a shortage of cereal grains production for animal feeds, date palm *Phoenix dactylifera* L. are abundant and may provide some alternative products. The United Arab Emirates (UAE) is a major date-producing country, and around 100 tons of date pits (DP) are existing every year as a waste from date processing plants (Rahman et al., 2007). Date pits, which are a by-product of date industry, are locally produced in Gulf region at considerable amount. This agriculture industry by-product can be used as alternative feed resource in animal nutrition.

At present, date fruits producers in most of the Arabian Gulf countries use DP as animal feed, and the complex non-digestible carbohydrates limits DP use as a feed ingredient (Rahman et al., 2007). However, there are many success trials been utilized locally available feed resources in animal nutrition and as an energy source. For example, DP meal could be added to 25 to 33 weeks old local Saudi hen diets up to 5% without β-mannanase with no adverse effects on reproductive performance and egg quality parameters (Hassan and Al Aqil, 2017).

Dietary supplements from *Phoenix dactylifera* seeds promote poultry growth performance, antioxidant development, and immune response (El-Far et al., 2016). DP administration to broiler diet at 4% reduces the deleterious effects of aflatoxin B1 (AFB1) along with a significant improvement in Feed Conversion Ratio (FCR) while the relative weight of the intestine, liver, thigh, and gizzard increased significantly (Daneshyar et al., 2014). DP could provide an inexpensive source of feed and using 10% DP had no adverse effects on growth performance, carcass quality and blood parameters of broilers (Masoudi et al., 2011).

Growth promoters such as antibiotics are used in animal husbandry at therpuetic and/or prophaltic levels to cure diseases control pathogens and/or to improve growth performance. The use for long-term of antimicrobials for growth promotion and therapy purposes in animals leads to antimicrobial resistance (AMR) in gram-negative pathogens, wherefore the European Union has banned the antibiotics use in the feed or food-producing animals (Levy, 1998).

Anti-microbial resistant bacteria can persist and replace sensitive bacteria. The genetic material transfer plays an essential role in spreading antimicrobial resistance from one pathogenic bacterial strain to another. Thus, resistance genes may be widely distributed among bacteria. Antimicrobial drugs are involved in the increase in bacterial resistance between both malignant strains and natural bacterial flora. It seems that the AMR evolution and prevalence in animal production systems is related to commensal organisms in poultry environment, such as *Staphylococcus aureus*, *Enterococcus* spp., and *Escherichia coli*, as well as *Campylobacter* spp and non-typhoidal *Salmonella* (NTS), which are foodborne zoonotic pathogens (Simoneit et al.,

2015; Richter et al., 2015). Consequently, there are an urgent need to find alternatives to antibiotics.

Based on safety and cost-effectiveness, commercial products need to be developed as alternative feed resources that could be included at a significant portion in poultry rations and be introduced as potential antibiotic substitutes in poultry to meet the energy requirement and to improve bird's health.

Simple carbohydrate molecules can be produced from DP fiber with the help of exogenous degrading microbial enzymes like xylanases, and they may be better utilised by animals (Sujani and Seresinhe, 2015). Solid-state degradation is one of the cheapest methods to prepare degraded date pits (DDP), and it may improve the nutritive value of DP when it is fed to poultry (Rozan et al., 1996). Cellulolytic fungus, *Hypocrea jecorina* commonly known as *Trichoderma reesei* (*T. reesei*) is widely used for SSD. DP are reservoirs of cellulose and oligosaccharides as natural substrates, which promote the growth of *T. reesei* and activation of several catabolic enzymes like *cellulases, hemicellulases,* and *pectinases* (Foreman et al., 2003). These enzymes catalyse the degradation of plant cell walls as well as the substrates, which increases the nutritive values of DP.

There are limited works focused on DDP and its utilisation in poultry feed even though they contain potentially useful quantities of nutrients essential for poultry health. Therefore, it can be of interest to define the effect of replacement of a portion of dietary corn with DDP as a source of energy and growth promoter on broiler growth performance and total microbial count in the gut.

1.1 Statement of Research Problem

The high prices of imported feed for animal production, on one hand, and the excessive use of antibiotic as animal growth promoters by some animal producers, on the other, require scientists to search for alternatives to those feeds and growth promoters, in a way that it does not affect the financial return of investors in this sector. These alternatives should be cost-effective and safe and research for alternatives feedstuffs and/or gr is essential.

1.1.1 Hypothesis

The prebiotic 10% degraded date pits (DDP) can replace currently used growth promoter (50 g/100 kg of 20% Oxytetracycline), energy source (10% of the yellow corn) in poultry feeds and replace Oligosaccharides as dietary feed ingredient that preferentially stimulate the growth of Bifidobacterium and other lactic acid bacteria in the gastro-intestinal tract.

1.2 Research Objectives

i) To characterise the chemical composition of DP and DDP.

ii) To evaluate the effect of Mannose, MOS, and DDP diet on growth performance and microbial growth in broilers chickens.

iii) To study the antioxidant and biochemical effects of DDP, MOS, and D-Mannose in broilers chickens.

iv) To study the effect of DDP, MOS, and Mannose on intestine development and morphology of broilers chickens.

v) To investigate the effect of DDP, MOS, and Mannose on antibacterial gene expression in the animal gut.

Chapter 2: Literature Review

2.1 Growth Promoters in Livestock Industry

2.1.1 Antibiotic Growth Promoters

In many countries, the use of sub-therapeutic levels of antibiotics in animal feeds is a common practice for enhancing growth performance and protects animals against pathogenic microbes. Regular addition of antibiotic substances to animal feed developed resistance against harmful microbes in the animals themselves (Hughes and Heritage, 2004). In the livestock industry, there are growing concerns about the effects of antibiotic growth promoters. The compounds that are administered in animal feeds include tetracyclines, macrolides, streptogramins and fluoroquinolones (Dibner and Richards, 2005).

In farm animals, the pathogenic bacteria shows different antibacterial resistance against antibiotics due to the continuous therapeutic uses of it in veterinary medicine, and the growth stimulating agent in the animal feed (Gibson and Roberfroid, 1995). Plant and animal derived raw materials are widely used for the production of animal feed. The number of antibiotic-resistant bacteria will increase in farm animals due to the continuous application of antibiotics as growth promoters and/or as therapeutic application. Human consumption of these animal products will lead to bacterial infections (antibiotic resistant and nonresistant) and increases in the chance of chronic diseases through food chain. Based on their efficacy, safety, and cost-effectiveness, new alternatives commercial products needs to be developed to improve the immunity of farm animals and their growth performance (Czaplewski et al., 2016).

2.1.2 Antibiotic Growth-Promoters in Farm Animals

In farms, antibiotic growth promoters (AGPs) are used in animal feeds at small doses to enhance the quality of the product, especially meat and eggs of healthy animals. The use of AGPs can control the growth of harmful bacteria like *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococci*. Regular use of antibiotics in animal feeds will lead to increase the number of resistant pathogenic bacteria, and it induces a selection pressure for bacteria used in medical practice like antimicrobial chemotherapy. The mechanism of action of antibiotics is unclear, but research supports that antibiotics decrease the number of harmful bacteria in the intestines (Attia et al., 2019).

Thomke and Elwinger (1998) hypothesised that cytokines that released during the immune response to bacterial infection might stimulate the release of catabolic hormones as well to reduce the muscle weight of animals. The decrease in gastrointestinal infections increases muscle weight. The use of growth promoters increases daily growth rates of animals in between 1% and 10% resulting in the meat of higher quality, with increased protein proportion and less fat content (Attia et al., 2017a, Attia et al., 2019). After medical practitioners, farming industries are the second position as the consumer of antibiotics. The best way to reduce the usage of antibiotics is to prohibit their application as feed additive and/or growth promoting agent in the livestock industry (Sneeringer et al., 2017).

2.1.3 Currently Used Antibiotic Growth Promoters

Various variations of the antibiotic growth promoters are being used in different parts of the world. For instance, ß-lactam antibiotics including penicillin, lincosamides, and macrolides (e.g., erythromycin and tetracycline), are widely used in the USA for

treating a wide range of human microbial infections. Flavophospholipol and Virginiamycin are extensively used as growth enhancers in cattle and poultry medication (Costanzo et al., 2005).

The arsenical compounds, such as bacitracin, flavophospholipol, virginiamycin avilamycin, and the glycopeptide avoparcin are used in different parts of the world for poultry farming. For instance, bacitracin (virginiamycin and avoparcin) treats *Clostridium perfringens* infections in broilers (Costanzo et al., 2005).

Virginiamycin provides the prevention of acid lactose in poultry, and its long term use might produce drug-resistant microbes. Pristinamycin and quinupristin, which are used as growth promoters, have now been banned in the EU countries (Butaye et al., 2000).

2.1.4 Human Health and Antibiotic Growth Promoters

The residues of antibiotic growth promoters in the livestock products could affect directly human health, which may cause side-effects. (Gassner and Wuethrich, 1994) reported the existence of chloramphenicol metabolites in meat products can cause aplastic anaemia in humans. The main reason for increasing antibiotic resistance by bacteria species, such as the causative bacterium of typhoid as *Salmonella typhi*, is the over-use of chloramphenicol-like antibiotics in animal feeds.

Based on the selection and amplification method of drug-resistant strains of bacteria, we can reduce the adverse effect of antibiotic residues in farm products. The use of antibiotic resistance determinants can compromise the therapeutic use of antibiotics (Attia et al., 2017a). The mode of the selection of resistance determinant is from a bacterium present in the commensal flora of growth promoter fed animal. Therefore, the resistance determinant will be transferred to human or animal pathogens depends

on the motility (Attia et al., 2019). The resistance determinant selection will lead to prolonged illness and death due to the use of more toxic alternative like drugs following complete treatment failure.

There are some significant bacteria identified as commonly associated with resistance due to prolonged use of growth promoters (Ventola, 2015). Among these bacteria that probable to be transferred frequently from animals to humans to cause a zoonotic disease are *Salmonella, Campylobacter, E. coli* and *E. enterococci*, as detailed below:

1] *Salmonella* spp.

Bacteria of the genus *Salmonella* are accountable for causing many diseases of common symptoms; for instance, *S. typhi* is the pathogenic microbe of the typhoid fever. Other species of *Salmonella* are causing commonly zoonotic infections, such as gastroenteritis due to infested poultry products. Diarrhoea, nausea, vomiting and fever are the major symptoms. *S. enterica* var. *enteriditis* and *S. enterica* var. *typhimurium* are both considered as common causative agents of *salmonellosis*. Thus, these bacteria are often brought into being as contaminants in eggs and poultry (Advisory Committee on the Microbiological Safety of Food, 2001). S. enterica var. Typhimurium could usually be medicated with fluoroquinolones, chloramphenicol and ampicillin. (Mølbak et al., 1999) reported that isolates of *S. enterica* var. *typhimurium* DT104, are resistant to drugs like ampicillin, tetracycline, and quinolones.

2] *Campylobacter* spp.

Campylobacter, specificly *Campylobacter coli* and *Campylobacter jejuni* are the dominant causative organisms for bacterial food poisoning. The organisms inhabit the intestine of animals and are non-pathogenic to host. In ruminants, the infection caused by *Campylobacter*, known as vibriosis. *C. jejuni* is very sensitive to erythromycin,

chloramphenicol, tetracycline, aminoglycosides and quinolones. The use of quinolones in food animals for a long time lead to the emergence of macrolide and quinolone-resistant *Campylobacter* isolates (Engberg et al., 2001). In poultry fluoroquinolones treatment are widely used for respiratory disease and this resulted in developing fluoroquinolone-resistant *Campylobacter* in the gut of the treated bird (Endtz et al., 1991).

3] *Escherichia coli*

Escherichia coli (E. coli) strains are Gram-negative bacteria that inhabit the healthy intestinal tracts of animals. However, strains of *E. coli* that are pathogenic, such as *E. coli* O157 produces Shiga-toxin inhuman due to the consumption of *E. coli*-infected animal products. The intestinal tracts of cattle, sheep, and goats are the primary location where *E. coli* O157 is inhabited. *E. coli* O157 also produce verocytotoxins and are referred to as verotoxigenic *Escherichia coli* (VTEC) strains and thereby causes haemorrhagic colitis and haemolytic uremic syndrome. *E. coli* strains are more resistant to antibiotics such as penicillin, and the number of drug-resistant *E. coli* are increasing globally (Stivers and Heritage, 2001).

4] *Enterococci*

The *Enterococci* are Gram-positive and are vulnerable to many of the antibiotics used as growth enhancers. The species most frequent in the intestines of different farm animals are *E. faecium* together with *E. faecalis, E. cecorum, and E. hirae*. The continuous usage of antibiotic avoparcin as a GP, led to the occurrence of vancomycin-resistant *enterococci* (Edmond et al., 1996).

2.2 Alternatives to Antibiotics

2.2.1 Organic Acids- Acidifiers

Acidifiers are found mainly in nature as natural constituents of plants or animal tissues. They can also be formed through microbial fermentation of carbohydrates, mainly in the large intestine in the form of sodium, potassium or calcium salts. The most common acidifiers (also called organic acids) that are made use of in farm animal feed are propionic, formic, acetic, lactic, butyric, fumaric, sorbic, tartaric, citric, malic, and benzoic (Dibner and Buttin, 2002; Attia et al., 2013b). They are characterized into two groups as lactic, fumaric and citric acid based on their effects on the intestinal functions as it indirectly reduces the bacterial populations in the stomach by decreasing the level of pH.

Formic, acetic, propionic, and sorbic acid are directly influencing the cell wall of Gram- bacteria through lowering the level of pH in the gastrointestinal tract (GIT) (Roth et al., 1992). These acidifiers improve the activity of proteolytic enzymes as well as nutrient digestibility, pancreatic secretions, and enhance digestive enzymes activity, microbial population management and support of beneficial bacterial growth (Partanen, 2001). Their effects are directly proportional to several critical factors including:

i] The containment rate of added up acids.

ii] Structure of diets and their buffering or acid-base amplitude.

iii] Level of intraluminal production of acids in the GI tract, feed dulcet.

iv] Receptors for bacterial attachment on the epithelial villi.

v] Age of animals (Strauss and Hayler, 2001).

Acidifiers have beneficial effects on poultry performance the microbial fermentation of carbohydrates results in the formation of organic acids (van der Wielen et al., 2000). They help in decreasing the buffering capacity of the feed and reducing the pH of drinking water which affects the crop and proventriculus of birds (Thompson and Hinton, 1997). Butyric acid helps in lessening the occurrence of subclinical necrotic enteritis triggered by *C. perfringens*, which is highly pertinent with the poultry industry (Timbermont, 2009). Butyric acid also has anti-inflammatory effects (Hodin, 2000) and has been studied to fortify the gut mucosal barrier by stimulating the expression of tight junction proteins and amplified production of antimicrobial peptides in mucous (Peng et al., 2007). Fatty acids like butyric acid, are a vital gut epithelial cells energy source and help in differentiation and proliferation the epithelial cell (Imran et al., 2017).

2.2.2 Phytogenic Additives

The phytogenic additives contain a speciality blend of plant extracts (saponins, pungent substances, bitter substances, and essential oils) and are a proven dietary supplement for poultry. Many of these components kindle appetite (menthol from peppermint), subdue microbial growth (carvacrol from oregano) or offer antioxidant protection (cinnamaldehyde from cinnamon). It remains uncertain which components of etheric oil products may act as an antioxidant, encourage the endogenous digestive enzymes, antimicrobial agent, or immune-modulator because of possible ‘synergy’ between the constituents. Spectrum of activity and antimicrobial effects with respective MIC-values were shown in various in vitro studies (Penalver et al., 2005).

The antimicrobial activity is rather feeble for pepper and ginger, average for oregano (carvacrol), cumin (p-cymene), coriander (lialol), rosemary (cineol), sage (cineol) and

thyme (thymol) and active for mustard (allylisothiocyanate), clove (eugenol), garlic (allicin), and cinnamon (cinnamaldehyde) (Adams, 1999). Essential oils stimulate the endogenous intestinal enzymes. Essential oils from oregano are presenting the significant potential as a substitute for antibiotic growth promoters.

Oregano has phenolic compounds (e.g. carvacrol) which have antimicrobial activity (Akgül and Kivanc, 1988). Oregano essential oils can alter the gut microflora and reduce the microbial load by overpowering bacteria proliferation. There are some prerogatives that oregano oil may, in time supplant anti-coccidian compounds, not because they incapacitate coccidian, but because they enhance the transformation of the gut lining and avert coccidian attack by preserving a more healthy population of gut cells (Bruerton, 2002).

The animal's maintenance energy requirement can be vastly improved by this mode of action because enterocyte transformation is a significant proportion of the basal metabolic rate (Clark et al., 2006). Bitter substances are often found in herbs and kindle the secretion of gastric juices. The pungent materials which are found in plants such as garlic, paprika, and onion are professed to function by enhancing blood circulation, leading to faster cleansing of the whole metabolism. Saponins boost the permeability of the gut wall and ammonia reduction. Flavonoids are plant polyphenols with anti-inflammatory effects, and they help to maintain the small blood vessels and connective tissue health (Repetto and Llesuy, 2002).

2.2.3 Probiotics

Probiotics for use in farm animals are characteristically divided into heat-treated or inactivated cultures of yeast or bacteria, live cultures of yeast or bacteria, or

degradation end products when incubated with yeast or bacteria. Probiotics are encompassed of individual species or mixtures of lactic acid bacteria, yeasts, or their end products (Delia et al., 2012). The mechanisms of their activities include rivalry between bacteria or yeast of probiotics and pathogenic microorganisms in the intestinal mucosa, the availability of nutrient and total suppression of pathogen growth by the organic acids and antibiotic-like compounds creation (Corcionivoschi et al., 2010).

The most often used probiotic bacterial strains are *Bifidobacterium* (*B. bifidum*, *B. pseudolongum*), *Lactobacillus* (*L. acidophilus*, *L. casei*, *L. rhamnosus*), *Bacillus* (*B. subtilis*, *B. cereus*, *B. toyoi*, and *B. licheniformis*), *Lactococcus* (*L. lactis*), *Enterococcus* (*E. faecium*), *Streptococcus* (*S. thermophilus*), *Pediococcus*, and *Saccharomyces* (*S. cerevisiae*). Many studies confirmed their advantageous effects on health and growth performance of farm animals (Lodemann et al., 2006).

Probiotics have positive impacts on the process of digestion by the collective activity of microbial probiotic enzymes and food digestibility, the intestinal mucosa restoration and immunity by activating the immune system (Matsuzaki and Chin, 2000). The impacts of using probiotics in animals feed hinge on the arrangement of selected bacteria, their exchanges with pharmaceuticals, feed composition, doses in feed, feed technology and storage conditions (Chen et al., 2005).

Gastrointestinal tract (GIT) has more microbial cells than the rest of the body-proper cells (Hove et al., 1999). There are mainly two types of microorganisms commonly found in the poultry GI; the autochthonous bacteria that come from the environment (Gusils et al., 1999), and allochthonous bacteria, which are added as a dietary supplement to the poultry feed, or drinking water as which called probiotics (Patterson and Burkholder, 2003; Chichlowski et al., 2007). Depending on the strain of probiotic,

the primary mechanism probably involves the specific metabolites production such as H_2O_2, low organic fatty acids, interaction with receptor sites intermediary metabolites with antimicrobial activity, and stimulation of the immune system and some others (Huyghebaert et al., 2011).

2.2.4 Zeolites

Zeolites are hydrated, crystalline aluminosilicates of alkaline earth cations and alkali, with three dimensional infinite structures. Zeolites (clinoptilolite), have been used as feed additives in order to ameliorate mycotoxicosis and enhance the performance of animals based on their unique properties (Papaioannou et al., 2005).

In laying hens, the addition of clinoptilolite progresses FCR, upswings the number of eggs laid and enhances their quality features (Tserveni-Gousi et al., 1997). In broilers, clinoptilolite improves their growth rate by encouraging feed consumption, improve FCR and lowers the percentage of the fat content which increases the carcass quality (Karamanlis et al., 2008).

2.2.5 Prebiotics

Prebiotics are dietary short-chain carbohydrates (oligosaccharides). They enhance farm animals growth performance and health, stimulate the activity of one or more beneficial bacteria. Unlike natural sugars, the non-digestibility characteristic of prebiotics helps these particles to reach the intestine and play as an energetic source for beneficial bacteria. (Gibson and Roberfroid, 1995). As a result, either the activity or the structure of the microbiota are transformed, leading to many secondary effects such as increased gas production and a drop in pH.

By competing with its sugar receptors, prebiotics can also avoid the adhesion of pathogens to the mucosa, and several recent studies have shown that accompanying various oligosaccharides feed have led to susceptibility reduction of *Salmonella* and *E. coli* colonization (Patterson and Burkholder, 2003) Attia et al., 2012). Mannose is an important prebiotic used in animal feeds. The following are the most commonly used non-digestible oligosaccharides (NDO) in farm animals: Mannan-Oligosaccharides (MOS), Galacto-Oligosaccharides, Fructo-Oligosaccharides (FOS), Isomalto-Oligosaccharides, Xylo-Oligosaccharides, Lactulose and Inulin (Grizard and Barthomeuf, 1999).

2.2.5.1 Mannan-Oligosaccharides

MOS is extracted from *Saccharomyces cerevisiae* cell wall. They are components of the outer layer of yeast cell walls, composes mannose, glucans, phosphate radicals as well as proteins (Klis et al., 2002). The main constituents of the wall are glucan (30%), mannan (30%), and protein (12.5%). While the components ratios defer from strain to strain depending on the degree of mannan phosphorylation and the interaction among the mannan, glucan and protein components vary from time to time.

Mannan-Oligosaccharides contain protein, which has a relatively high proportion of amino acids such as a rarity of methionine but abundant quantities of serine, threonine, aspartic and glutamic acids (Sun and Li, 2001). Yeast cell wall consists of a signal-triggering molecule for bacteria and offers a very competitive binding site for bacteria (Ofek et al., 1977). Mannose is the critical component of MOS and is inimitable because it is tied by the type 1 fimbriae, which many enteric bacteria use to impute to host cells. Therefore, mannose can help in the movement of non-beneficial bacteria through the small intestine without colonization (Newman, 1994).

2.2.5.2 Fructo-Oligosaccharides

FOS also referred to as oligofructose or oligofructose. FOS aids as a substrate for microflora in the large intestine, improving the overall gastrointestinal tract (GIT) health. Thus, FOS has long been recommended as a supplement for treating yeast infections (Rousseau et al., 2005). Several studies have found that, in both the animal and the human gut, FOS and inulin promote calcium absorption (Zafar et al., 2004). Fructo-oligosaccharides can be fermented in the lower gut by the intestinal microflora, which results in a reduced pH. FOS can be deliberated as a small dietary fiber with low caloric value. The fermentation of FOS often results in producing of some acids and gases (Mikkelsen et al., 2004).

2.2.5.3 Galacto-Oligosaccharides (GOS)

GOS also known as oligo-galactose, oligo-lactose, Oligo-galacto-syllactose, or Transgalactic-oligosaccharides (TOS), come under prebiotics. Because of the confirmation of their glycosidic bonds, GOS mainly fight hydrolysis by salivary and intestinal digestive enzymes (Macfarlane et al., 2008). Therefore, they reach the intestine's end intact. GOSs are categorised as prebiotics, defined as non-digestible food components that constructively affect the host by stimulating either the growth or activity of beneficial bacteria in the colon.

If the activities of these health-promoting bacteria increase, it can result in many health-related benefits both indirectly by the organic acids they produce via degradation or directly by the bacteria themselves (Tuohy et al., 2005). Indirectly, GOS supports natural defences via the gut microflora by increasing a number of beneficial bacteria in the intestine and by preventing the adherence of *Salmonella*

tTyphimurium, *E. coli*, and Clostridia to the gut, which reduces the chances of infections (Shoaf et al., 2006).

2.2.5.4 Isomalto-Oligosaccharides (IMO)

IMO is a blend of short-chain carbohydrates which has a digestion-resistant factor. The raw material which is used for developing IMO is starch, which is enzymatically altered into a mixture of IMO. Thus, IMO are glucose oligomers with α-D-(1,6)-linkages, including isomaltose, panose, isomaltotriose, isomaltotetraose and higher bifurcated oligosaccharides (Garske et al., 2017). While intestinal enzymes readily digest α-(1, 4)-glycosidic bonds, α (1, 6)-linkages are not readily hydrolysed to display a digestion-resistant effects. Consequently, IMO are only digested to a limited extent in the upper side gastrointestinal tract (Kuriki et al., 1993).

2.2.5.5 Xylo-Oligosaccharides (XOS)

XOSs are sugar oligomers comprised of xylose units through ß-(1-4)-xylosidic linkages (Kumar and Satyanarayana, 2015). These are the hydrolysis products of *xylan*; fundamentally existing in most of the *lingo-cellulosic* materials with variable degrees. In acidic media, the XOS remains steady and displays resistance to heat. These bioactive molecules are found to display selective growth stimulation of beneficial gut microflora even in laboratory animals, in line with the in vitro fermentation profile of XOS. Oral administration of XOS to rat and mice considerably increased the moisture content of faeces, total caecum weight, population of bifidobacteria and a reduction in caecum pH (Chung et al., 2002).

2.2.5.6 Lactulose

Lactulose (4-O-ß-d-galactopyranosyl-d-fructose) is a synthetic non-digestible carbohydrate that is metabolised in the gut (Bird et al., 1990). The benefit of using non-digestible carbohydrates is that it stimulate the growth of Lactobacilli, Bifidobacteria and other beneficial bacteria in the intestine (Fleige et al., 2007), and lessen the activity of proteolytic bacteria (Marinho et al., 2007). By increasing beneficial bacteria and considerably supressing potential pathogens and the activity of pro-carcinogenic enzymes, the ingestion of lactulose has been reported to have advantageous outcomes (Nagpal et al., 2012).

2.2.5.7 Inulin

Many types of carbohydrates exist in plants and those, which categorized under fructans compounds and come under naturally occurring inulins composed of linear polymers and oligomers of fructose linked by ß (2–1) glycosidic linkage and often with a glucose terminal unit (Roberfroid, 1998). Inulin, which is commercially abstracted from the roots of chicory (*Cichorium intybus* L.), is a mixture of polymers and fructose oligomers, and is characterised by their solubility in water, resistance to digestive enzymes, and they are readily degraded in the large intestine by microflora. The stimulating and selective effect on the growth or activity, or both, of indigenous lactobacilli and bifidobacteria, are directly or indirectly linked to most of the health and nutritional benefits linked with the inulin-type fructans in regard to dietary use (Gibson, 1999).

2.2.5.8 Role of Prebiotics in Poultry

The common studied prebiotic oligosaccharides in poultry research are Mannan oligosaccharides (MOS) and Fructose oligosaccharides (FOS). The complemented feed of poultry with MOS ensued an improvement in the intestinal enzyme activity and intestinal morphology, yet the growth performance of the broilers was not up to mark that of including AGPs to the feed (McCann et al., 2006). Since many enteric bacteria have receptors that bind to it, mannose, the essential component of MOS, is a unique sugar. These receptors, called Type 1 fimbriae, are involved in the liaison of the bacteria to the host cells. This process is critical for the bacterium to be cause a disease in the host. Chickens likely have receptors for Type1 fimbriae in their small intestine (Oyofo et al., 1989b). The function of MOS as a competitive binding site rather than binding to the intestine; the bacteria bind to it and are carried out of the gut.

In a study that props this theory, it was established that there is a significant reduction in *Salmonella typhimurium* colonisation of the intestines by add 2.5% mannose to the drinking water of broilers (Oyofo et al., 1989a). Studies with adding FOS in poultry diets testified noteworthy reduction in *Shigella* carriage in the ceca and substantial enlargements on growth performance (Xu et al., 2003). Results achieved from synthetic materials propose that some benefits using oligosaccharides like FOS and inulin act as substrates for beneficial bacteria such as Bifidobacteria, whereas MOS has receptor properties for fimbriae of *E. coli*, which is sensitive to mannose and *Salmonella* spp. That helps to eliminate these harmful bacteria with the digest instead of binding a mucosal receptor (Fernandez et al., 2002, Attia et al., 2012). Oligosaccharide *β-glucans* of the yeast cell wall origin are thought to have the immunomodulatory effects that enhance the performance.

2.3 Date Pits as Commercial Animal Feed

Worldwide, date palm *Phoenix dactylifera* L. implanted in numerous countries where the production of cereal grains for animal feeds is very rare. The United Arab Emirates (UAE) produces a huge number of date fruits per year (667,569.8 tons/year) almost 6% of the world date production. Consequently, approximately 100 tons of DP are produced annually as a by-product from date factories in the UAE (Hossain et al., 2014). The utilisation of DP may supply a prospective alternative to the ordinary feeds used in the livestock industry. This could help the reduction of the dependence on the imported raw materials. Furthermore, it might be considered as an alternative to antibiotics, reduce the load on the main cereals, and minimize the agricultural waste and preserve the environment (Dhehibi et al., 2018). Figure 1 shows the market share of dates exporting countries during 2005–2016.

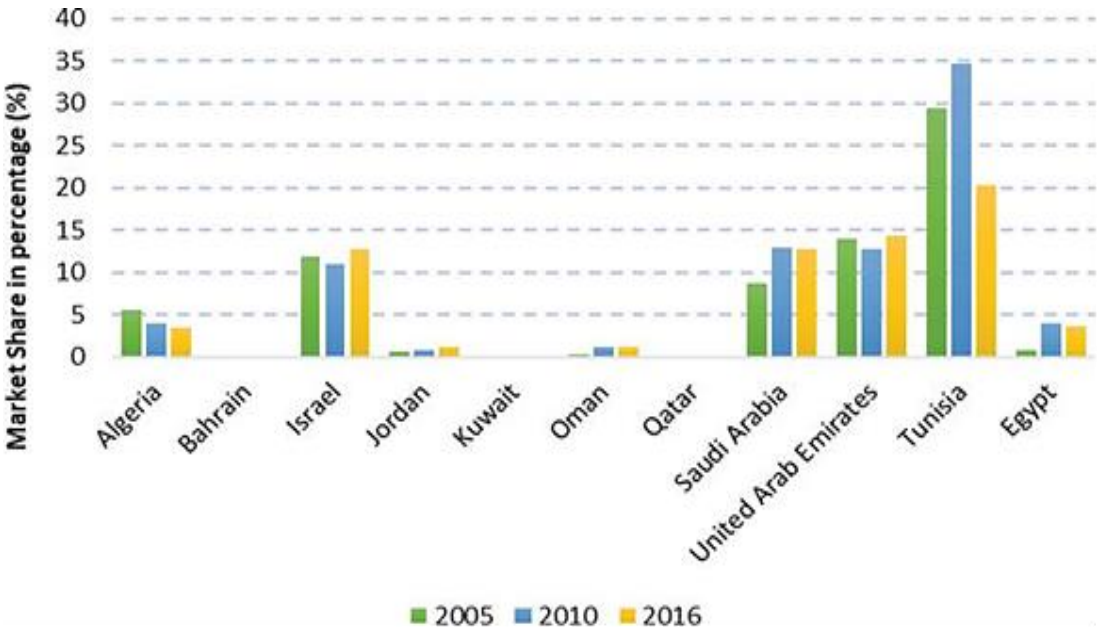

Figure 1: Market share of dates exporting countries during 2005–2016

2.3.1 Date Pits as Poultry Feed

Numerous research studies advocated the DP as a valuable feed ingredient for poultry. (El-Faki, 2002) reported that DP is a valuable energy source as a substitute for cereals in broiler feed. (Kamel et al., 1981) included DP with or without zinc bacitracin 5 mg/kg in broiler diets at 5%, 10% and 15% inclusion levels, replacing bran, maize and Lucerne. They found that the live weight gained improved significantly when the birds-feeding diets were containing DP and zinc bacitracin. Barreveld (1993) reported that chicken given 10 gram/day of ground DP with the standard diet gained weight at a faster rate than those in the control group.

Other work of Al-Azzawi (1960), demonstrated a significant improvement in the weight over the basal diet when 15% DP replaced barely in poultry diet. Regarding the inclusion of DP in layers diets, 10% DP in Hisex brown diet during the growing period (14–20 weeks), resulted in higher egg production than did those fed the control diet (El-Faki, 2002, Attia et al., 2013a). Vandepopuliere et al. (1995) evaluated the dietary potential of date flesh, and DP for poultry nutrition and concluded that from 8–43% dates, 16–43% date flesh, and 5–27% DP increased average weight gain and improved FCR compared to the control diet.

2.4 Bacterial Infections in Poultry

2.4.1 Salmonellasis

In poultry, more than 2500 different *Salmonella*e serotypes cause transmittable infections (Fardows and Shamsuzzaman, 2015). *Shigella*s are gram-negative enterobacteriaceae that has the effect on domestic mammals and fowls. *S. gallinarum* (S. g) and *S. pullorum* (S. p) cause clinical Salmonellosis in poultry.

Different serotypes, known as the paratyphoid group may infect or contaminate flocks without causing disease symptoms. *Shigella*e may contaminate the products of poultry causing food poisoning in human beings. The dissemination of the typhoid group between fowls spreads horizontally, by direct contact between infected and susceptible birds. *S. enteritidis, S. typhimurium; S. hadar* or *S. heidelberg* may invade and colonise in organs of the bird, which causes food-borne diseases in humans. Inactivated (bacterins) and attenuated live vaccines are widely used against salmonellosis, and they have less effect against *S. enteritidis* and *S. gallinarum* (Salemi et al., 2011).

2.4.2 *E. coli* Infection

E. coli are non-pathogenic and mostly found in the digestive tract of poultry. Virulent serotypes of *E. coli* causes a range of poultry diseases. The most common diseases caused by *E. coli* in poultry are *coli bacillosis, coli septicaemia, egg peritonitis,* and mushy chick disease (Guabiraba and Schouler, 2015).

2.4.2.1 Colibacillosis

Coli bacillosis causes mortality that occurs as subacute pericarditis or acute fatal septicemia, salpingitis, airsacculitis and peritonitis. The respiratory tract is the main source of entry of *E. coli* pathogens. The transmission of *E. coli* into the egg through faeces that considered as an important route of infection (Uotani et al., 2017).

2.4.2.2 Coli septicaemia

Coli septicaemia is most common in young broiler chickens, mostly chicks' age between four and twelve weeks. Upper trachea and throat are the significant areas in which *E. coli* serotype is causing *colisepticaemia* are inhabited (Wray et al., 1993). Tetracycline treatment is more useful to control Coli septicaemia. Mercantile bacterins

added to feed of breeder chicks have provided some protection against homologous *E. coli* serotypes (Kabir, 2010).

2.5 Microbial Degraded Feeds in Farms

The worldwide practice of animal feeding is providing them with the dry feed either pellet or mash depending on the animal species and age. Compared to dry feed, broilers accept wet feeds more easily (Jensen, 1998). Broilers AWG and FI fed wet feeds were increased, and broilers may not consume sufficient dry mash to gain their genetic potential for growth (Afsharmanesh et al., 2006). Wet feeding stimulates increased dry matter intake, the rate of growth and feed efficiency of broilers and male chicks of egg-laying strains (Yalda and Forbes, 1995). In the hot tropics, the broiler performance has been improved while feeding with wet feed as it reduces heat-stress and improves the FI but does not have an effect on carcass weight and the digestibility dry matter (Dei and Bumbie, 2011).

2.5.1 Microbial Degradation

The industrial process of degradation involves growing large numbers of microorganisms under specific conditions in large tanks (Potter, 1986). In biotechnology, industrial wastes and by-products are degraded with various kinds of microbes like yeast, bacteria and fungi and this process ends up with nutritional products like enzymes, protein, free amino acids, vitamins and many other nutritious products (Nigam, 2013).

Carbohydrate and protein-rich food were supplied to the human society with the introduction of single-cell protein production and thereby improved the nutritional quality of low-quality feedstuff (Reihani and Khosravi-Darani, 2018). Single cell

protein production increases protein content up to 37% by using *Arachniotus* sp. and *Brevibacterium flavum* as substrate. The features of using fermented feeds by specific microbes are that the product is less nutritional requirements, ability to transform hard waste substrates into a useful products, that enhance the growth rate, stability and resistance to pH and temperature resistance (Shahzad and Rajoka, 2011).

2.5.2 *Trichoderma reesei*

The fine-rot fungus, *Trichoderma reesei* (known as *Hypocrea jecorina*), is the abundant widely examined cellulolytic fungus (Foreman et al., 2003). They are effective in bring out great amounts of several cellulose-degrading enzymes (Saloheimo et al., 2002). These enzymes, in any form, either as combined together or individually used, are the commonly cellulases with the usage in various feed formulations, fabrics, paper, printing and laundry industries (Réczey et al., 1999). Lignocellulosic and galactomannan plant material found in DP are the natural substrates of *T. reesei,* which promotes good cell growth and catalysing of both xylanases and cellulose-fermenting enzymes (Meier, 1958). Some proteolytic enzymes are produced by the fungi as well (Dienes et al., 2006).

2.6 Degraded Date Pits

Dietary constituents of DP composed of moisture (71–103 g/kg), crude protein (50–63 g/kg), fat (99–135 g/kg), total ash (10–18 g/kg), CF (22.5 g/kg), NDF (650–690 g/kg accounts for the total amount of cellulose, hemicelluloses and lignin (Attalla and Harraz, 1996), whereas 460–510 g/kg acid detergent fiber (ADF) represents the overall amount of lignin and cellulose (Attalla and Harraz, 1996), and 29.3 g/kg total sugars (Rahman et al., 2007). These results indicate that DP comprise high levels of carbohydrates that are indigestible. These fibers limit up the use of DP as nutritious

ingredient. However, if DP is fermented using specific enzymes that convert the fibers into simpler forms of carbohydrate molecules, they could be better in regard of utilisation by animals.

Xylanases release nutrients by hydrolysis of non-breakable fibers such as cellulose and hemicellulose, which are widely used in feed formulations. Hemicelluloses are categorized into *Mannans, xylans, Arabinans* and *Galatians* based on main sugar components in their backbones (Leisola et al., 2002). This figures showed a decline in viscosity percentage, more efficient feed utilisation, and subsequently less faeces produced by the animals. Due to the costly enzymes degradation in animal feed, specific microorganisms can be used to produce the needed enzymes economically efficiently to degrade dietary fiber. The DP also contains 5–7% of protein by weight, but very little research reported about the functional properties and compositional characteristics of these seed proteins (Aldhaheri et al., 2004).

2.7 *Trichoderma reesei* Degraded Date Pits

Degraded DP by application of *Trichoderma reesei* or the yeast *Saccharomyces cerevisiae* separately mannan fiber would be broken down to products like mannan oligosaccharides (MOS) and free mannose. Additionally, degradation will break down the other types of fibers (Cellulose, lignin and hemicelluloses to their digestible units. *Trichoderma reesei* is very efficient in DDP and increasing their mannose content (Belal, 2008). According to (Sachslehner and Haltrich, 1999), MOS are non-pharmaceutical alternatives to antibiotic growth promoters and helps in substitution of the attachment sites of Gram-negative pathogens, which prevent the attachment on the enterocytes and subsequent enteric infection.

It was found that MOS blocks digestive pathogen colonisation, thereby increasing the availability of attachment sites in the gastrointestinal tract (Heinrichs et al., 2003). Current studies focus on MOS ability to accelerate growth rates and eliminate pathogenic bacteria colonisation in the GI tract in a diverse number of non-ruminant animals (Spring et al., 2000, Attia et al., 2012). Low levels of MOS supplemented in the diet have been shown to increase weight gain and improve FCR (Moore et al., 1994). The positive effects of MOS on animal growth, immunity and digestive tract health were studied to reduce the need for costly synthetic antibiotics in animal feeds. (Spring et al., 2000).

A mannose substrate of MOS selectively attaches to bacteria (certain strains of *E. coli* and *Salmonella*) in the gut (Moore et al., 1994, Attia et al., 2012) and since MOS is indigestible in animals, bonds to and is excreted with enteric pathogens. Thus MOS prevents attachment and removes already attached pathogens in the gut (Newman, 1994). Research by (Oyofo et al., 1989b) proved that mannose inhibited *in vitro* attachment of *Salmonella typhimurium* to intestinal cells of the day old chick. While mannose is essential for bacterial attachment, MOS originating in the yeast cell wall were found to be more effective in binding *E.coli* than pure D-Mannose.

Chapter 3: Isolation and Chemical Characterization of Molecules from Degraded Date Pits

3.1 Introduction

Date palm (*Phoenix dactylifera* L.) is a significant fruit crop grown worldwide, and date palm fruits form an essential source of energy by their high content of carbohydrate. Various value-added products, like date confectionery, date syrup, sweets and candies were produced from date fruits. After the technological transformation of the fruits, the vast quantity of date pits are discarded as wastes (Yousif and Alghamdi, 1999). A large amount of these materials create both discarding and environmental problems. Vast quantities of crop remnants as a by-product of the agricultural industry lead to the depletion of nutritionally relevant components. Bioactive molecules from agricultural wastes are rich sources of beneficial products like biofuels, energy reservoirs for microbes, and enriched nutrients for humans and animals (Saidur et al., 2011).

The 21st century witnessed several steps for improving the nutritional value of crop residue. Since then, many attempts focussed on treatment via physiochemical and biological means, including microbial degradation. Currently, date seeds are largely used in the UAE as an additive of feeding ruminants and poultry. Date fruit consists of seed and fleshy pericarp which comprises between 85% and 90% of date fruit weight (Hussein et al., 1998). DP contained high levels of non-digestible fibers as valuable bioactive compounds (McAllister et al., 2001). Degradation with exogenous microbial enzymes as xylanases enhancing the production of simple forms of carbohydrate molecules from the DP fibers (McAllister et al., 2001). B- mannanase did not affect productive performance of laying hens fed different levels of DP (Hassan

and Alqil, 2017). In addition, probiotic supplementation to laying hens' diets containing 21% DP did not affect laying hen's performance (Kashani et al., 2013). This chapter discusses the isolation of biomolecules from DDP its chemical characterisation and antioxidant activity.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Chemicals

All the chemicals used were analytical grade reagents.

3.2.1.2 Date Pits

Freshly separated pits of *P*. *dactylifera* were purchased from Date Factory at Al Ain, UAE. The date pits were about 150 kg from different date verities. The date pits were crushed and ground in College of Food and Agriculture/Al Foah Farm, UAE University, using a medium size mill (Skiold Saebý9300, Denmark) to reduce the size of pits to about 1 mm diameter. They were stored in labelled bags until used.

3.2.1.3 Fungus Culture

Culture of *Trichoderma reesei* from DSMZ (Braunschweig, Germany) were used for the study.

3.2.2 Methods

3.2.2.1 The Process of Date Pits Degradation

3.2.2.1.1 Cultivation and propagation of *Trichoderma reesei*

Four lyophilized *Trichoderma reesei* ampoules were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, (DSMZ), Braunschweig, Germany. Opening of the ampoules and rehydration of the dried fungal culture was performed as described by the DSMZ specification. A subsample from the re-hydrated *T. reesei* culture was transferred to potato dextrose broth (PDB) (Difco Laboratories, Detroit, MI, USA) amended with 250 µg mL-1 chloramphenicol (Sigma Chemical Co, St. Louis, MO, USA) and streptomycin sulphate (100 µg mL-1, Sigma). The flasks were incubated at 25±2°C on a rotary shaker at 250 rpm (Model G76, New Brunswick Scientific, Edison, NJ, USA) for 1 week in the dark and were observed regularly for fungal growth. Potato dextrose agar (PDA) (Difco) plates were used to maintain pure *T. reesei* cultures. The plates with fungal culture were then incubated at 25±2°C for 7 days in the dark and regularly observed for fungal growth. The fungus was retained on PDA plates and stored at 4°C. Figure 2 shows the *T. reesei* Growth on Potato Dextrose.

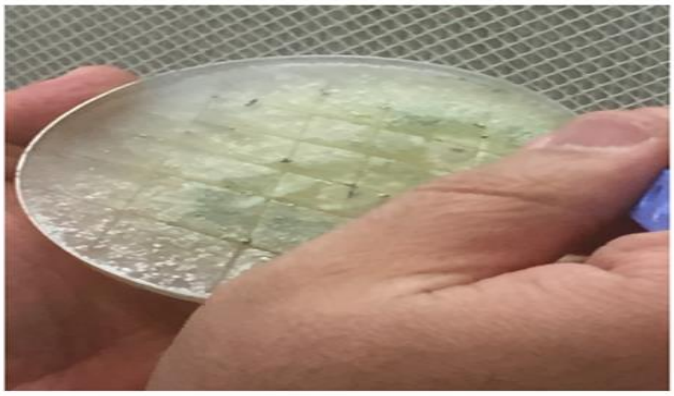

Figure 2: *Trichoderma reesei* growth on Potato Dextrose

3.2.2.2 Preparation of Fungi-Degraded Date Pits

Fungi-DDP was produced using an SSD system inside an incubator as we intended to produce quite high amounts of DDP for this study. At first, the substrate and the media were prepared by dilution, mixing, cleaning and sterilisation at 121°C for 20 min. The SSD system was cleaned and sterilised as needed (Hamada et al., 2002).

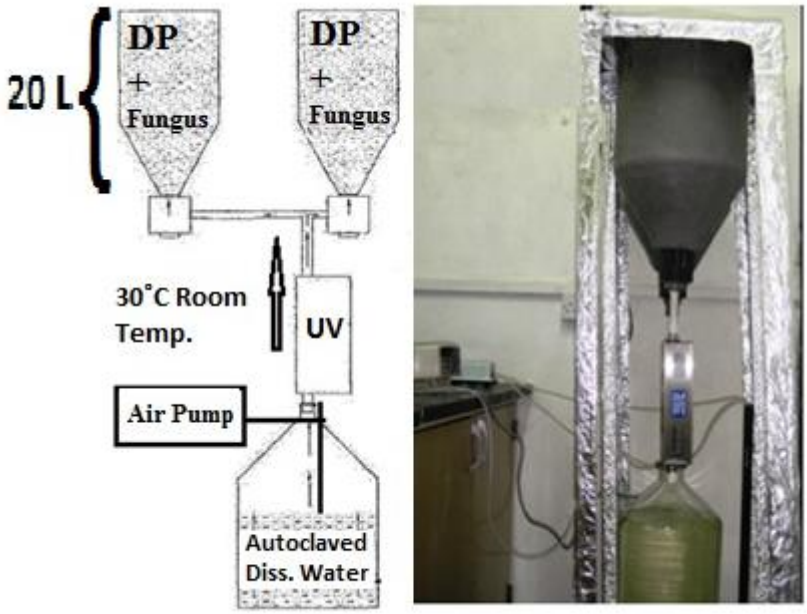

Figure 3: Solid-state fermenter system

The process began with the addition of a starter culture of the fungi to the cones of the system containing some sterilised medium and substrate. DP and the rest of the medium were then added. In the feed batch process, the feeding continued until a specific volume of fungi-DDP was reached 20 litres per cone (Figure 3). The Aquafina Ultraviolet disinfection system provided them with disinfected moist air which helped the aerobic fungus *T. reseei* to proliferate at a temperature of 30°C for three weeks. After that, the process was stopped, and the fungi DDP with the fungi mass was collected and transferred to a refrigerator and kept at 4°C until used.

3.2.3 Scanning Electron Microscopy

The microscopic investigation of DP surface morphology involved scanning electron microscopy (SEM) analysis. Nova Nano-SEM 450 took micrographs of the sputtered surface of the films.

3.2.3.1 Isolation of Crude Fiber from Degraded and Non-Degraded Date Pits

Filter Bag Technique was used to isolate the crude fiber. The removed compounds consisted predominantly of protein, sugar, starch, lipids, and portions of both the structural carbohydrates and lignin. The organic residue remaining over was the crude fiber after digesting with 0.25 N H_2SO_4 and 0.3 N NaOH.

Reagents:

- Sodium hydroxide solution - 0.3 N
- Sulphuric acid solution - 0.25 N.

Procedure:

One gram of prepared sample was weighed in filter bag and placed the bags inside fiber analyser. Defatting of the substance was done using by soaking the bags in petroleum ether for 10 min. The petroleum ether was drained and then 0.25 N H_2SO_4 was added, extracted for 40 min. After extraction, H_2SO_4 was drained, and then the bag was rinsed with hot water twice. NaOH (0.3 N) then added to the fiber analyser and place the bags in it for 40 min.

The NaOH solution was exhausted and the bag was rinsed using hot water for three times. After rinsing the bags were removed from the analyser and soaked in acetone for 5 min, and thereafter the samples were dried in an oven at 100°C. Then all bags

were placed in a crucible for 2 hrs at 600°C in muffler, cooled in a desiccator and weighed to calculate loss of weight of organic matter.

3.2.3.2 Isolation of Acid Detergent Fiber (ADF) from Degraded and Non-Degraded Date Pits

A filter bag technique isolated ADF, which is the organic residue remaining after digesting with H_2SO_4 and acetyl trimethyl ammonium bromide (ATAB). Cellulose and lignin are remaining substance as ADF.

Reagents:

Acid Detergent Solution - To 1 L of standardised 1.0 N H_2SO_4, add 20 g ATAB and agitated continuously with heat until the formation of a uniform solution.

Procedure:

Prepared sample (0.5 g) was weighed in filter bag and placed in a covered container. Enough acetone was added and the container was placed in a shaker for 10 min. The acetone was drained and the bags were dried, and then placed inside fiber analyzer. Acid detergent solution was added, and agitated for 60 min then extracted. The acetone was exhausted and rinsed with hot water for three times until the pH of water to neutral. Excess water pressed out in the bags, the bags placed in a beaker containing acetone for 5 min. the bags were removed from acetone, and air was dried. The bags then placed in an oven at 100°C for 1 hr. and then the bags were placed in a desiccator to remove air. Cooled to room temperature and the bags were weighed to calculate the loss of weight of organic matter.

3.2.3.3 Isolation of Neutral Detergent Fiber (NDF) from Degraded and Non-Degraded Date Pits

NDF in treated date pits was determined by filter bag technique. NDF mainly consist of hemicelluloses, cellulose, and lignin.

Reagents:

- Neutral Detergent Solution- Add 30.0 g Sodium dodecyl sulphate, 6.8 g Sodium borate, 4.5 g Sodium phosphate dibasic anhydrous, 18.6 g Ethylenediamine tetra-acetic disodium salt, and 10.0 ml triethylene glycol in 1L distilled H_2O. Adjust the pH to 7.1. Agitate and heat to aid solution.
- Alpha-amylase
- Sodium sulphite

Procedure:

Prepared sample (0.5 g) was weighed in a filter bag and place the bags in a covered container. Added enough acetone and place the container in a shaker for 10 minutes. Drained the acetone and dried the bags, then placed the bag inside fiber analyser Neutral detergent solution, then 20 g of sodium sulphite and 4 ml of alpha-amylase was added. Agitated for 75 min and extracted. After extraction, exhaust the added solution and rinsed with hot water three times. While rinsing, added 4 ml of alpha-amylase to first and second rinses. Pressed out excess water in the bags and placed the bags in a beaker containing acetone for 5 min. Removed the bags from acetone and air dried. The bags were dried in an oven at 100°C for one hour and in a desiccator to remove air. Cooled to room temperature and weighed the bags to calculate loss of weight of organic matter.

3.2.3.4 Extraction of Cellulose from Degraded and Non-Degraded Date Pits

Cellulose fraction was extracted from the DDP and DP by the method of (Bhattacharya et al., 2008).

Reagents:

- Acetic acid
- Acetone
- n-Hexane
- Sodium chlorite
- Sodium hydroxide
- Ultrapure water

Procedure:

Date pits powder was defatted with ethanol and n-hexane using a Soxhlet extractor. 150 mL of ultrapure water was added to the defatted material. One gram of sodium chlorite and 0.2 ml of acetic acid and was added to the suspension at 80°C for 1hr. The insoluble solid was filtered with a glass filter and rinsed with acetone and ultra-pure water for two times. The extracted cellulose component was suspended in 17.5% NaOH (25 mL) for 30 min at room temperature. 25 mL of ultrapure water was added, filtered with a glass filter and rinse with 10% of acetic acid and H_2O to obtain cellulose.

3.2.3.5 Extraction of Hemicellulose from Degraded and Non-Degraded Date Pits

Hemicellulose was extracted from DDP and DP using the method of (Farhat et al., 2017).

Reagents:

- Acetone
- Ethanol
- Hydrochloric acid
- Methanol
- Propanol

Procedure:

Date pits powder was extracted in Soxhlet apparatus with different organic solvents (acetone 60%, ethanol 70%, methanol 80%, and propanol 80%) in a solid/liquid ratio of 1:10. The insoluble material was filtered using a glass filter and decrease the volume using rotary evaporator. The pH of the filtrate reduced to 5.5 with 5 N HCl followed by adding 3 volumes of 95% ethanol. Precipitated hemicellulose fractions were collected by centrifugation and freeze-drying.

3.2.3.6 Extraction of Lignin from Degraded and Non-Degraded Date Pits

Lignin from DDP and DP was extracted using the method of (Nuruddin et al., 2011).

Reagents:

- Ethanol
- Hydrochloric acid
- Sodium hydroxide
- Toluene

Procedure:

DP powder was dried at 60°C in an oven, sieved to obtain 18 mesh size particles and was dewaxed with toluene/ethanol (2:1, v/v) in a Soxhlet extractor for 6 hr and then dried at 60°C for 16 hr. The powder (10 g) was successively treated with various concentrations of NaOH (0.1 N, 0.2 N, 0.3 N and 0.4 N, 1:15 w/v) at 120°C for 45 min. The insoluble residue was collected by filtration, washed with distilled water and oven dry at 60°C for 16 hr.

The supernatant was neutralized to pH 5.5 with 6 m of HCl and concentrated to 50 mL under reduced pressure using rotary evaporator. The concentrated extract was precipitated with two volumes of 95% ethanol and filter. The filtrate was concentrated to 30 mL, and the pH was adjusted to 2.0 with 6 m of HCl. Acid-insoluble lignin fractions were obtained by centrifugation and freeze-drying. Soluble lignin fractions were obtained by re-precipitation of crude lignin with alcohol.

3.2.4 Estimation of Total Polysaccharide (Total Carbohydrate)

Total polysaccharide content was estimated by Phenol-H_2SO_4 method of Dubois et al, 1956 using glucose as standard

Reagents:

1. Phenol- 5%
2. Sulphuric acid-96% reagent grade

Procedure:

Blank, standards and tests were set. All the tubes volume was made up to equal amount with distilled water. Then 5 ml of 96% sulphuric acid was added to each test tube. The

tubes were kept at room temperature for 20 minutes. The colour produced was read at 490 nm.

3.2.5 Analysis of Monosaccharide Composition of Fibers

We used HPLC to determine the monosaccharide composition of fibers as detailed in the method of (Blakeney et al., 1983).

Reagents:

- Deionised water
- High-purity nitrogen (99.99%)
- HPLC grade water
- Mannose standard
- Sulphuric acid

Procedure:

Fiber (20 mg) were accurately weighed into glass test tubes and stir with 0.5 ml of 72% H_2SO_4 at room temperature for one hr. Deionized water (5.5 mL) was added to each tube and stirred for 3 hr at 100°C. After cooling, the hydrolysates was diluted to 10 ml with water and then stored at -20°C until further analysis. The HPLC analysis was performed using HPLC system equipped with analysis software. A column of dimension 300 mm with 8 mm (i.d) at 80°C and high-purity nitrogen (99.99%) was used as a carrier gas for assay. Hydrolysate samples were diluted to fourfold using filtered and sonicated water (HPLC grade) for analysing mannose compounds. Distilled water was filtered using a Whatman nylon membrane filter (0.45 ml) and sonicated. The flow rate of the eluent was maintained at 0.6 ml/min. Mannose (purity > 98%) was used as a reference standard.

3.2.6 Extraction of Mannan Oligosaccharide from Degraded and Non-Degraded Date Pits

Oligosaccharides were extracted from DDP and DP using the method of (Huang et al., 2010).

Reagents**:**

- 100% methanol
- 70% ethanol
- Acetone (70 to 100%)
- Acetonitrile
- Acetyl chloride
- HCl
- Pyridine: Hexamethyldisilazane: Chlorotrimethylsilane mixture
- Sodium hydroxide (1 M)
- Trifluoracetic acid
- α-amylase (5 mg/ml)

Procedure**:**

Defatted DP was first extracted for phenolic compounds. Briefly, 10 g of the sample was extracted with aqueous solution of acetone (70 to 100%) and 100% methanol solution under stirring at 4°C (1 hr for each step). About 5 gm of solid residue (acetone dry powders or AcDP) was obtained after the extraction and washed twice with 60 ml of 80% (w/w) ethanol to inactivate the enzymes and to remove residual polyphenols. The distilled water (200 ml) at 100°C used for six hours to wash and extract residue.

The suspension was incubated with 150 mL of a-amylase (5 mg/mL in 3.6 mM $CaCl_2$; 1:250 v/v) for one hr at 37°C to remove α-glucans. The suspension was precipitated with 70% ethanol. The sample was then centrifuged (8000 X g for 15 min), and the supernatant containing the water-soluble fraction (WSF) was recovered and lyophilised. The residue was then treated with 50 mL of NaOH solution (1 M), centrifuged, dialysed and the supernatant was treated with α-amylase and ethanol to get the Alkali-Soluble Fraction (ASF).

3.2.6.1 Oligosaccharide Purification & Gas Chromatographic Analysis

ASF and WSF powders were purified using C-18 cartridges by solid-phase extraction (SPE). C-18 cartridges were loaded with the samples and eluted with distilled water (6 ml), lyophilised and resuspended in 3 mL of deionised water. The resulting oligosaccharides-rich fraction was purified by nonporous graphitised carbon cartridge to eliminate monosaccharides and disaccharides. Eight volumes of water (deionised) was passed through the cartridges at a flow rate of 1/ml to remove impurities.

The oligosaccharides retained by the graphitised carbon cartridge was then eluted stepwise with two cartridge volumes (6 mL) of 90:10 deionised water-acetonitrile solution, 6 ml of deionised water-acetonitrile solution (80:20), 6 ml of deionised water -acetonitrile solution (60:40) containing Trifluoracetic acid (TFA, 0.1%). Each fraction was dried, and 20 µL of water (deionised) was added to the dry oligosaccharides powder for gas chromatography analysis, as detailed from now on.

Methanolysis in conjunction with derivatisation by tri-methyl-silylation was performed using the method of (Doco et al., 2001). Three different acetonitrile fraction (10 µl) of WSF and ASF was collected in the same vial and subject to the first reaction.

1 M anhydrous methanolic hydrochloric acid (MeOH: HCl) was prepared by adding acetyl chloride (140 µl) to anhydrous methanol (1 ml). The internal standard (IS) and oligosaccharide mixtures were resuspended in anhydrous MeOH: HCl (1 M, 0.5 mL) and kept for 24h at 80°C.

Then the mixtures were concentrated to dryness under nitrogen gas at room temperature and washed with methanol (250 µL). A Re-N-Acetylation step was performed by adding 200 µL of a solution containing MeOH: acetic anhydride (10:1) to each sample at 85°C for 24h. Removed the solvents and washed twice with 250 µL of pure methanol. 0.3 ml of a mixture of Pyridine: Hexamethyldisilazane: Chlorotrimethylsilane (10:2:1, respectively) was added, and the solution kept for 24h at 80°C.

Excess reagent was evaporated using nitrogen gas and extracted the residue with 1 ml hexane, centrifuged, concentrated to 200 µL. Each sample was measured by a gas chromatograph equipped with a flame ionisation detector (FID) and capillary split/splitless inlet. Fused-silica capillary column (30 m 0.25 µm i.d., 0.25 µm film thickness) was used for analysis. The carrier gas used was hydrogen (17psi, flow rate 2.5 ml/min,). In the pulsed split, mode samples were injected with a split ratio of 5:1. The injector and the FID were operated at 280°C. The GC was then operated at 120–200°C at 1.5 C/min, 200°C held 5 min, and a post-run of 2 min at 250°C.

3.2.7 Analysis of Proximate Composition of Degraded and Non-Degraded Date Pits

3.2.7.1 Analysis of Ash Content

The ash content was measured by drying degraded DP at 105°C for 24h in the oven to constant weight (AOAC, 1990).

3.2.7.2 Analysis of Crude Fat Content

The fat content of the dried sample was also measured by continuous extraction with petroleum ether for 6h using Soxhlet apparatus (AOAC, 2003).

3.2.7.3 Analysis of Mineral Content

The mineral content in the DP sample was determined by using atomic emission spectrometry (AES).

3.2.7.4 Analysis of Crude Fiber

Reagents:

- Amylo-glucosidase
- Phosphate buffer (pH 8.2)
- Protease
- α-amylase

Procedure:

Total dietary fiber (TDF) content was determined using enzymatic-gravimetric official modified AOAC method (AOAC, 2003). Briefly, samples were incubated at 95°C for 30 min in a phosphate buffer (pH 8.2) solution containing a-amylase. The pH was then adjusted to 7.5, and 100 µl protease was added. After incubation at 60°C for 30 min, the pH was adjusted to 4.5. Before the last incubation at 60°C for 30 min, 200 µl amyl-glucosidase was added. The carbohydrate moieties were solubilized, and the total dietary fiber content was obtained after ethanol precipitation, filtration, and drying.

3.2.7.5 Analysis of Amino Acids

Amino acid profile of DP was analysed by UPLC system using the method of (Zhou et al., 2009).

Reagents:

- 90% Ethanol
- AccQ Tag Ultra borate buffer
- Deionized water

Procedure:

Date pits samples of 0.2 g were weighed in a centrifugation tube and added 25 ml of 90% ethanol. Dispersed for 5 minutes using sonicator, homogenised completely. The homogenised solution was centrifuged for 5 min at 5000 rpm at room temperature. The supernatant was decanted, the sediment was extracted twice with 25 ml of 90% ethanol. The extracted solutions were combined and dried under nitrogen gas. Once the entire organic solvent layer is dried, removed from under the nitrogen and re-dissolved in 5 ml of deionised water.

The extracts were then filtered through 0.22 µm syringe filter before derivatisation with 70 µl of AccQ Tag Ultra borate buffer. The mixed solution was vortexed immediately for 5 seconds. The contents in the tube were kept at room temperature for one minute before placing it in a heating block at 55°C for ten minutes. After ten minutes, the mixed solution was removed and analysed using the Acquity UPLC system.

3.2.8 Preparation of Polyphenol Rich Extract

The rich phenolic extract was prepared according to the method of (Ramchoun et al., 2017), whereas polyphenols were determined using Folin-Ciocalteu procedure as detailed in (Al-Farsi and Lee, 2008).

Reagents:

- Folin-Ciocalteu reagent.
- Sodium bicarbonate.

Procedure:

Date pits extract (10 mg/ml) was mixed with diluted Folin-Ciocalteu reagent (1.5 ml) and Na_2CO_3 (1.5 ml, 60 g/L), vortexed. After 90 min, the absorbance was measured at 725 nm. The total phenol concentration was expressed as mg of gallic acid equivalent (mg GAE) per 100 g of extract.

3.2.9 Determination of Total Flavonoids

Total flavonoid content was determined using the method of (Zhishen et al., 1999).

Reagents:

- $AlCl_3$
- $NaNO_2$
- NaOH

Procedure:

Date pits extract was diluted with 4 ml of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added. After 5 minutes, 0.3 mL of $AlCl_3$ (10%) was added and stood for 1 min. The mixture was diluted with 2.4 mL of distilled water after the addition of 4%

NaOH (2 ml). After 15 minutes the absorbance at 510 nm was measured and calculated the total flavonoid content from the standard curve for catechin solutions expressed as catechin equivalents.

3.2.10 Determination of Antioxidant Activity of Degraded and Non-Degraded Date Pits

A] DPPH Radical Scavenging Activity

The scavenging activity of the DPPH free radical was assayed according to the method of (Karagözler et al., 2008) with slight modification.

Reagents:

- DPPH (0.1 mM).
- Methanol (80%).

Procedure:

The reaction mixture contained 0.1 mL of DP extracts, 0.3 mL of 80% methanol and 0.4 mL of DPPH. The reaction mixture was then incubated for 20 min at room temperature in the dark. The absorbance was read at 517 nm. The 50% inhibiting concentration (IC50) was calculated from the concentration/ effect regression line.

B] ABTS Radical Scavenging Assay

The ABTS radical scavenging was measured using the method of (Re et al., 1999) with some modifications.

Reagents

- 2.4 mM potassium per-sulphate solution.
- 7 mM ABTS solution.
- Methanol.

Procedure:

The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14hr at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol and measured absorbance at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. DP extract (0.1 mL) was mixed with 1 mL of the ABTS solution, and the absorbance was taken at 734 nm after 7 min using spectrophotometer.

C] Ferric Reducing Activity

The ferric reducing activity of date seed extract was estimated based on the method of (Benzie and Strain, 1999).

Reagents:

- 0.2 M phosphate buffer (pH 6.6).
- Ferric chloride (0.1%).
- Potassium ferricyanide (1%).
- Trichloroacetic acid (10%).

Procedure:

Date pits extracts (0.13 mL) in phosphate buffer (0.2 M, pH 6.6) were mixed with 0.125 mL of potassium ferricyanide (1%, w/v) and incubated at 50°C for 20 min. Afterwards, 0.125 mL of TCA (10%, w/v) were added to the mixture to terminate the reaction. Then, the solution was mixed with 1.5 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm.

3.2.11 Extraction of Proteins

Proteins were extracted from DDP and DP by the method of (Gómez-Vidal et al., 2008).

Reagents:

- 0.1 M Tris-HCl.
- 15% TCA.
- 5% Mercaptoethanol.
- 5% Sucrose.
- 80% Acetone.
- Ammonium acetate in methanol (0.1 M).
- Bovine Serum albumin.
- SDS buffer (2%w/v).
- Sodium chloride.
- Tris-buffer, pH 8.0

Procedure**:**

Date pits powder was extracted with normal (room temperature) acetone, and the residue was washed with cold acetone, dried at room temperature. The pellet was rinsed with 15% TCA in normal acetone, vortexed, centrifuged and then washed thrice with cold acetone (80%). The residue was then suspended in a 1:1 mixture of SDS buffer (5% sucrose, 5% mercaptoethanol, 2% SDS, 0.1 M Tris-HCl, pH 8.0) and Tris-buffer (pH 8.0), vortexed and centrifuged. Supernatants were precipitated with 0.1 M ammonium acetate in methanol at 4°C and centrifuged. The protein pellet was then

treated with ammonium acetate (0.1 M) in methanol, acetone (80%) and TCA (24%w/v), vortexed, centrifuged and dried.

A] Protein Purification

The proteins were purified by anion exchange and size exclusion chromatography. The lyophilised filtrate was purified using DEAE–Sepharose column (2.6 cm × 10 cm) equilibrated in 10 mM Tris-HCl, (1 ml/min, pH 8.0). A linear gradient of NaCl (0–0.5 M) in equilibration buffer was used for eluting proteins fractions, and 2 mL fractions were collected. Fractions showing proteins test by Lowry's method were pooled and concentrated by ultrafiltration. 1.6/60 gel filtration column (Superdex 200) equilibrated with sodium phosphate buffer (100 mM, pH 6.0) was used for further purification of concentrated protein and eluted at 0.5 ml/min in the same buffer. The fractions showing activity were combined and used as a protein for further studies.

B] SDS – polyacrylamide gel electrophoresis (SDS–PAGE)

The molecular weight of the purified protein of date flesh and date seed was performed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) by the method of (Laemmli, 1970).

C] Preparation of Samples for SDS-PAGE

Proteins were extracted by the method of (Sekhar and Demason, 1988). Samples were prepared in 2.3% SDS buffer and 0.06 M Tris-HCl (pH 6.8) at 4°C for 5h. The suspension was centrifuged at 3750 x g for 10 min, and then the supernatant was precipitated by adding four volumes of cold acetone. The protein pellet was separated by centrifuging at 18500 xg for 10 min after which the pellet was air dried.

D] Separation of proteins by SDS-PAGE

All parts of the gel casting unit (glass plates, combs, spacers, and assembled gel cassette) were washed thoroughly. The gel casting tray was assembled by placing the glass plates together with a spacer between them. A rubber strip was placed at the base of the casting unit and the glass set on top of it. The screws on the casting unit was tightened to hold the glass plates in place. The gap between the glass plates was filled with water to ensure there is no leakage from the base or sides of the casting unit.

After this, a comb was inserted between the glass plates and a mark was made on the plate to ensure filling of the gel to a sufficient height. The gel was cast by pouring the 12% separating gel buffer (1.5 M Tris-HCl buffer pH 8.7, acrylamide/bis-acrylamide 40% solution, 10% SDS buffer, 10% APS buffer, 20 µl TEMED solution) between the glass plates up to the mark. Once the gel had polymerized pour the 4% stacking gel (0.5 M Tris-HCl pH 6.8, acrylamide/bis-acrylamide 40% solution, 10% SDS, 10% APS and 20 µl TEMED solution) over the separating gel and immediately inserting a comb into the stacking gel to form wells in the gel and kept for one hour.

The glass plates were detached from the casting unit before clamping them onto the gel running unit. The assembly was inserted into an electrophoresis tank, and both the chambers were filled with 1x SDS-PAGE running buffer (25 mM Tris-HCl (pH 8.3), 192 mM glycine and 0.1% SDS) before the combs were carefully removed. The air-dried pellet of protein obtained from the extraction step was dissolved in distilled water (1 ml). This protein solution (100 µl) was added to an equal volume of SDS sample buffer (0.5 M Tris-HCl pH 6.8, SDS, glycerol, 2-mercaptoethanol and 0.1% bromophenol blue solution) mixed well and added to the gel. The tank was covered with a lid and connected to the power pack.

The gel was run at a constant voltage of 160 V for one hour or until the dye reached the bottom of the gel. After completion, the gel was gently removed from the glass plates and placed in a tray before staining with colloidal Coomassie brilliant blue solution (5% aluminium sulphate hydrate, 10% ethanol, 0.02% Coomassie brilliant blue-G250,% orthophosphoric acid) and left overnight on a shaker. The stain solution was removed and replaced with distains solution (10% ethanol and 2% orthophosphoric acid) until the background becomes clear and protein bands visible. Gels were scanned using a molecular imager and analysed with gel analyser software to estimate the molecular weight of protein bands.

3.3 Statistical Analysis

Data were subject to the analysis of variance (ANOVA) using general linear model (GLM) and mean comparisons were performed using Duncan's multiple range test to compare significant differences between means for all analyses. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS for Windows: SPSS Inc., Chicago, IL, USA).

3.4 Results

3.4.1 Scanning Electron Micrographs of DDP

The scanning electron micrographs of date pits in different stages of degradation with *T. reesei* is shown in Figures 4-6.

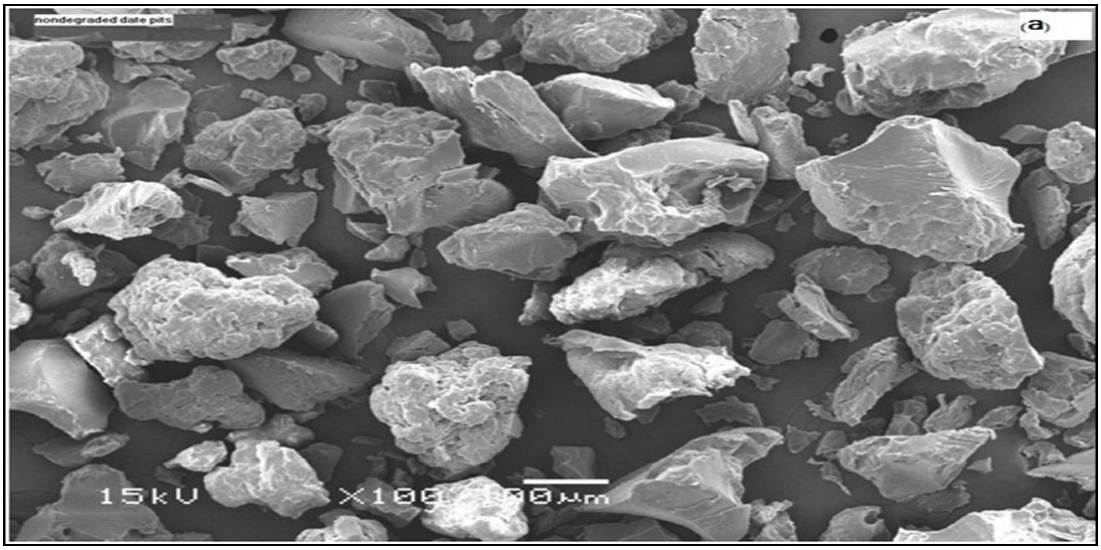

Figure 4: SEM of date pits before the degrading with *T. reesei*

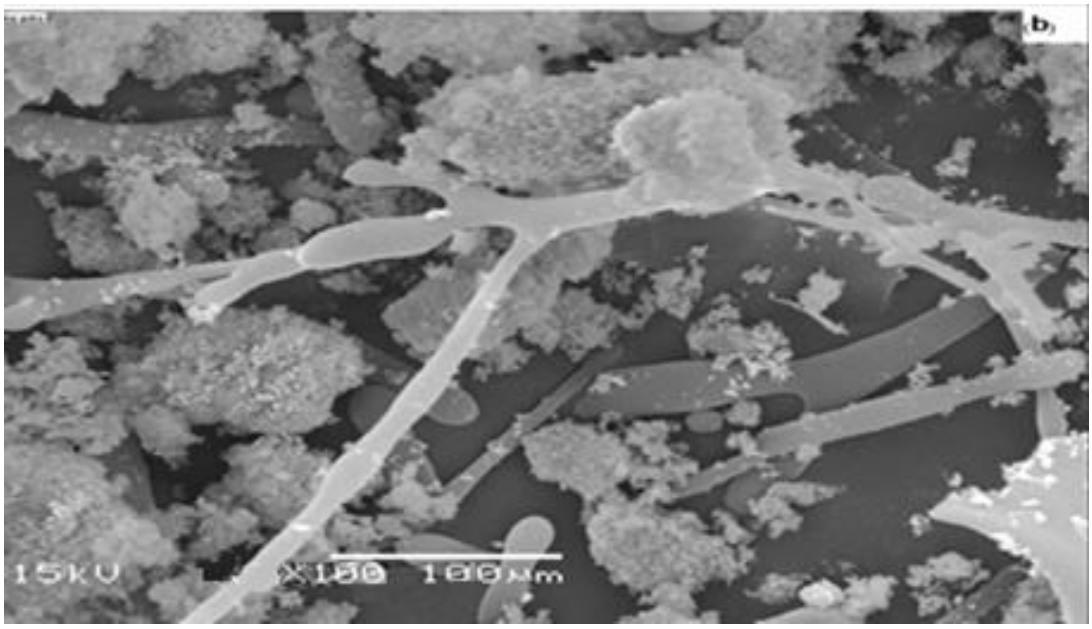

Figure 5: SEM of date pits during the degradation with *T. reesei*

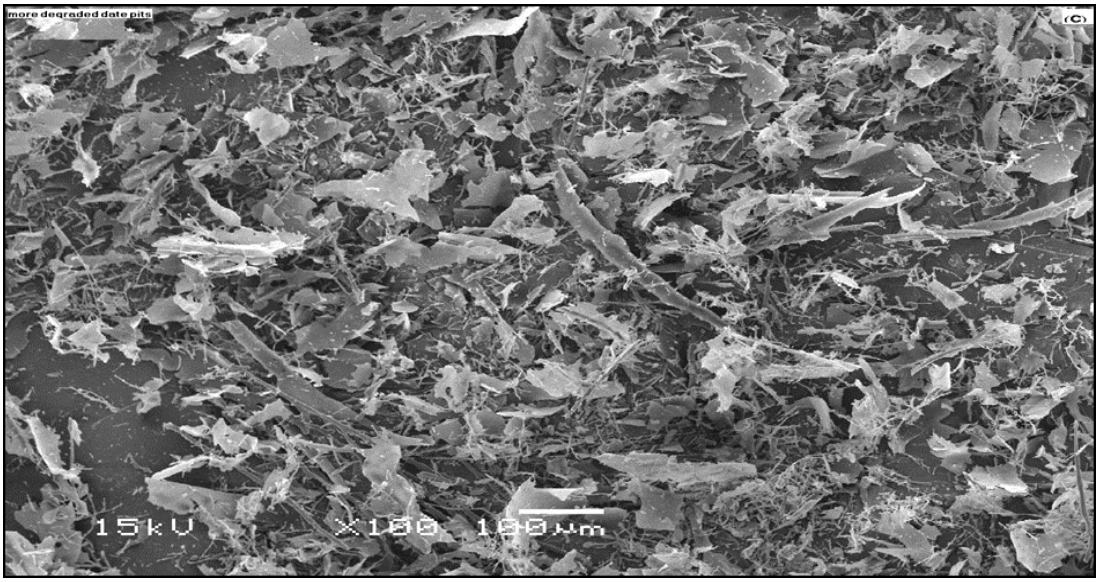

Figure 6: SEM of date pits after the degradation with *T. reesei*

3.4.2 Chemical Composition of DDP

3.4.2.1 The Proximate Composition of Date Pits

Table 1 represents the proximate composition of DP. Crude protein and total carbohydrate content were significantly increased in DDP. The results showed an increase by 8.66% and 25.26% of crude protein and total carbohydrate contents respectively in DDP while there was a reduction by 2.96% of crude fiber, after degradation with *T. reesei*.

Table 1: Proximate composition of date pits

Component (% dry matter)	Non-Degraded date pits	Degraded date pits
Ash	1.87±0.04	2.10±2.06
Crude Fat	6.83±1.19	7.14±0.22
Crude protein	4.96±1.08	13.62±3.18*
Crude Fiber	24.1±3.05*	21.14±2.92
*Total carbohydrate	62.56±9.12	87.82±3.73*

±Standard Error-Values expressed as means of three replicates. Values were compared between each column.

*Means within the same row were significantly different (P<0.05)

Ash content, crude fat, crude protein, crude fiber are analysed and expressed their concentration in percentage. For total carbohydrate (separately analysed), the concentration in mg/gram date pit samples analysed. Then it is converted to percentage. Therefore, for non-degraded date pits the amount of total carbohydrate is 625.6 mg/gm, then it was converted to percentage, therefore, it is 62.56%. Similar calculation was done with degraded date pit.

3.4.2.2 Fiber Content of Date Pits

Table 2 represents the fiber content of the DP. The NDF content of DDP was significantly increased when compared to non-degraded DP. The crude fiber content was decreased in *T. reesei* DDP. In date seed, degradation with *T. reesei* significantly increased the NDF, cellulose and hemicellulose content. Results showed that lignin content was significantly increased in *T. reesei* DDP, but ADF decreased.

Table 2: Composition of fiber in date pits

Component	Non-degraded DP (%)	Degraded DP (%)
Neutral Detergent fiber (NDF)	66.79±0.52	76.28±4.24*
Acid detergent fiber (ADF)	53.34±1.13*	46.09±0.19
Cellulose	27.42±5.09	35.53±6.64*
Hemicellulose	11.06±1.92	28.41±5.08*
Lignin	7.08±0.72	14.38±2.74*
Pectin	1.72±0.07	3.62±0.06

Values expressed as means of three values±standard error. *$P < 0.05$

3.4.2.3 Monosaccharide Composition of Fiber

Table 3 shows the monosaccharide composition of DP fiber. In non-degraded DP the major monosaccharides in cellulose were galactose, glucose and mannose. After

degradation with *T. reesei* the fructose, glucose and arabinose content was significantly increased in cellulose. In hemicellulose, glucose, mannose and sucrose content were increased significantly in fungi DDP. *T. reesei* degradation increased the content of fructose, glucose, xylose, galactose and arabinose in lignin fraction of DDP. Pectin fraction of DDP holds a significant greater amount of xylose and mannose.

Table 3: Monosaccharide composition of fiber in date pits

Component	**Non-degraded date Pits (%)**				**Degraded date Pits (%)**			
	Cellulose	**Hemicellulose**	**Lignin**	**Pectin**	**Cellulose**	**Hemicellulose**	**Lignin**	**Pectin**
Fructose	0.306±0.01	6.15±1.41	0 .50±0.02	0 60±0.02	0.74±0.05*	6.6±0.77	0 .90±0.02*	ND
Glucose	19.60±2.28	6.9±0.59	6.45±1.41	0.21±0.05	31.02±3.4*	19.53±2.62*	14.98±0.97*	1.22±0.07
Mannose	8.12±1.33	6.50±0.95	1.14±0.15	0 .3±0.015	17.28±1.29	13.72±1.07*	12.56±0.42	0.4±0.015*
Xylose	4.76±0.66	10.49±0.75	2.50±0.28	0 .60±0.02	6.98±0.72	14.20±2.28	5.86±0.67*	0.90±0.03*
Galactose	20.62±2.57	21.82±2.8	1.22±0.07	0.04±0.00	23.3±3.00	30.5±5.88	3.38±0.22*	1.72±0.05
Arabinose	2.78±0.48	3.29±0.51	0.90±0.03	ND	5.99±0.99*	5.70±0 .77	1.71±0.05*	0.70±0.02
Sucrose	0.73±0.01	2.98±0.22	0.09±0.00	ND	2.49±0.74	6.94±0.87*	0 .80±0.03	0.29±0.00

Values expressed as means of three values±standard error. *$P < 0.05$. ND: Not Detected

3.4.2.4 Mannan Oligosaccharide Content of Degraded and Non-Degraded Date Pits

Table 4 shows the MOS content and its monosaccharide composition in DP. Degradation by *T. reesei* significantly increased the MOS content in DP. Mannose content of MOS from DDP was 19.97%. After degradation, the content of glucose, arabinose, rhamnose and glucuronic acid were significantly increased in MOS.

Table 4: Monosaccharides contents in Mannan Oligosaccharide of non-degraded and degraded date pits

Component	Non-degraded date pits (%)	Degraded date pits (%)
Mannan oligosaccharide	16.48±3.0	29.99±4.26*
Monosaccharides		
Galactose	13.13±0 .60	22.30±4.31
Mannose	5.94±0.38	19.97±1.70*
Glucose	6.97±0.70	14.9±1.51*
Arabinose	2.41±0.30	4.91±0.55*
Xylose	0.71±0.04	5.56±0 .17
Rhamnose	3.0±0.26	5.16±0.68*
Glucuronic acid	14.97±0.94	27.39±3.01*

Values expressed as means of three determinations±standard error. *$P < 0.05$.

3.4.2.5 Mineral Content of Degraded and Non-Degraded Date Pits

The mineral content of DP is shown in Table 5. Results showed that the mineral content was increased after the degradation process. After degradation with fungi, the mineral content was increased in DP. The abundant increase in minerals in fungi DDP were phosphorus, chromium, manganese, zinc, strontium and vanadium.

Table 5: Mineral content of date pits

Parameter	Non-degraded date pits (mg/kg)	Degraded date pits (mg/kg)
Potassium	2240.11±7.5	4239.06±14.0
Phosphorus	1776.98±5.0	1884.28±5.9*
Sulphur	813.56±2.85	1299.59±10.39
Magnesium	758.4±3.0	874.9±3.3
Calcium	457.97±1.56	1148.81±4.82
Sodium	119.43±0.57	192.28±0.89
Iron	67.63±1.86	290.16±3.4
Aluminium	18.62±1.38*	13.87±0.77
Zinc	9.00±2.16	20.20±1.5*
Manganese	9.28±0.34	16.77±0.66*
Strontium	8.20±0.20	9.61±0.44*
Copper	5.57±0.47	5.35±0.49
Cobalt	2.26±0.33	3.47±0.47
Chromium	1.27±0.22	4.65±0.45*
Nickel	1.28±0.11	5.44±0.64
Arsenic	0.01±0.00	0.01±0.00
Cadmium	0.01±0.00	0.01±0.00
Lead	0.01±0.00	0.01±0.00
Vanadium	0.01±0.00	1.2±0.07*

Values are expressed as means of three determinations±standard error. *$P < 0.05$

3.4.2.6 Protein Content of Degraded and Non-Degraded Date Pits

The protein content of the DP is shown in Table 6. Results showed that *T. reesei* degradation increases the protein content in DP. The protein content in fungi DDP was found to be 22.5%.

Table 6: Protein content in date pits after purification.

Component (%)	Non-degraded date pits (%)	Degraded date pits (%)
Protein	10.6±1.58	22.5±2.08*

Values are expressed as means of three determinations±standard error. *$P < 0.05$

3.4.2.7 Amino Acid Composition of Degraded and Non-Degraded Date Pits

Table 7 represents the electrophoretic pattern of DP proteins. The amino acid profile of protein from both degraded and non-degraded date seeds was shown in Table 7. Seventeen types of amino acids were detected and identified. Glutamine (Gln) was the predominant amino acid, followed by Arginine (Arg), Aspartic acid (Asp), Valine (Val), Leucine (Leu), Lysine (Lys), and Phenylalanine (Phe).

Table 7: Amino acid composition of date pits proteins

Amino Acid	DP	DDP
Ala	0.19	0.25
Arg	0.49	0.62
Asp	0.38	0.46
Cys	0.15	0.23
Gln	0.85	1.12
Gly	0.21	0.35
His	0.13	0.21
Ile	0.18	0.28
Leu	0.27	0.41
Lys	0.31	0.36
Met	0.09	0.15
Pro	0.19	0.31
Ser	0.23	0.28
Thr	0.21	0.29
Tyr	0.08	0.19
Val	0.31	0.43

3.4.2.8 Electrophoretic Profile of Degraded and Non-Degraded Date Pits Proteins

The SDS-PAGE protein profiles of DP and DDP are shown in Figure 7. Electrophoretic profile showed that two principal protein bands were similar in both DP and DDP (66.2 kDa and a band about 60 kDa). In degraded date pits, two other protein bands are present (31 kDa and a band near to 28 kDa). The marker proteins used were eight kDa to 66.2 kDa. Bouaziz et al. (2008) reported that date seeds contain some proteins with molecular weights ranging from 22 kDa to 70 kDa. Mohammad (2015) found 92,100, 205 and 108 kDa protein bands in date palm cultivars in Saudi Arabia. Khoshroo et al. (2011) studied the proteins present in Iranian date palm (*Phoenix dactylifera* L.) cultivars and found a range of proteins varying between 11.673 to 369 KDa.

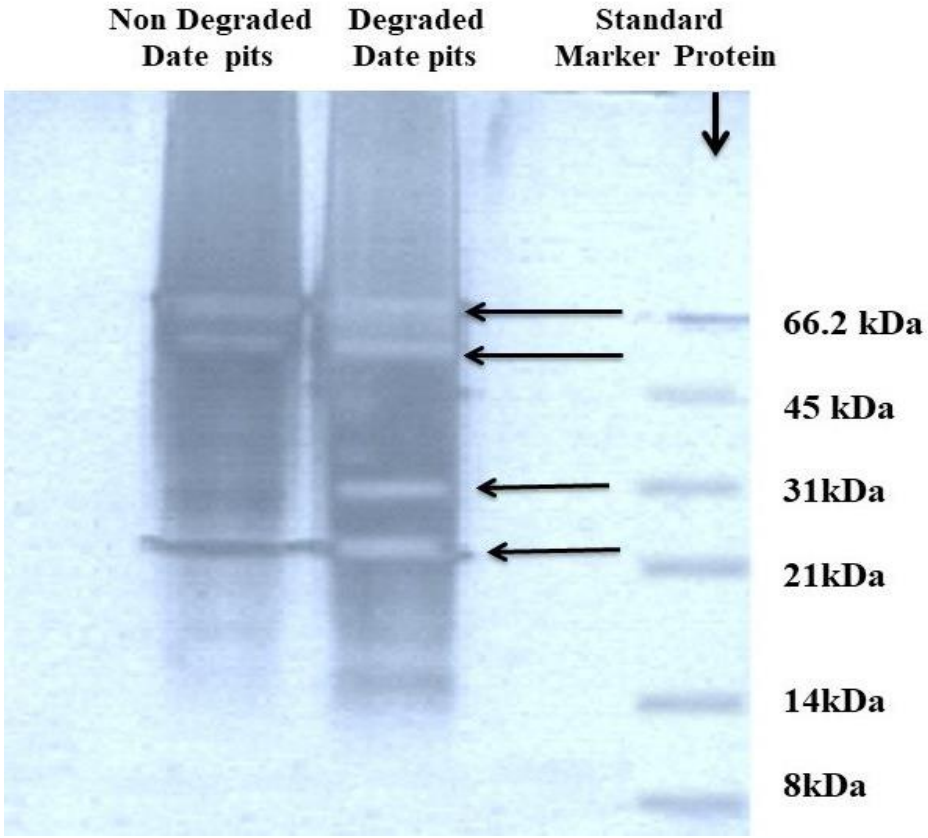

Figure 7: Electrophoretic profile of date pits proteins

3.4.2.9 Phenolic and Flavonoid Contents of Degraded and Non-Degraded Date Pits

The phenolic and flavonoid content of DP is shown in Table 8. The phenolic content in non-degraded DP was 3.2 g GAE/100 g DW, and flavonoid content was 2.28 g RE/100 g DW. After degradation with fungi, the phenolic and flavonoid contents were significantly increased in DP to 14.23 g GAE/100 g DW and 11.68 g RE/100 g DW, respectively. These increases amounted to 11.03 and 9.6 g, respectively.

3.4.2.10 Antioxidant Activity of Degraded and Non-Degraded Date Pits

Table 8 showed the anti-oxidant activity of DP. After degradation with fungi *T. reesei*, the scavenging ability on DPPH radicals was 78%. In non-degraded DP, the scavenging ability on DPPH radicals was 59%, showing an increase of 19%. The ABTS radical scavenging activity of non-degraded DP was 6.23 mmol TE/1the 00 g DW and after degradation ABTS radical scavenging activity was significantly increased to 13.28 mmol TE/100 g DW. Results of FRAP assay showed that the degradation process with *T. reesei* enhanced the Ferric reducing antioxidant power of DP from 24.56 mmol TE/100 g DW to 36.23 mmol TE/100 g DW. Metal chelating activity was also increased in DDP about 17.25 µmol EE/g DW.

Table 8: Phenolic, Flavonoid content and antioxidant activities of date pits

Parameters	Non-DDPs	DDPs
Total Phenol (g GAE/100 g DW)	3.2±0.712	14.23±1.29*
Flavonoids (RE/100 g DW)	2.28±0.85	11.68±2.21*
DPPH activity (%)	59±9.23	78±11.89*
ABTS (mmol TE/100 g DW)	6.23±0.36	13.28±1.89*
FRAP (mmol TE/100 g DW)	24.56±4.58	36.23±6.39*
Metal chelating activity (µmol EE /g DW)	13.25±0.22	17.25±2.68

Values expressed as means of three determinations±standard error. * $P < 0.05$.
(mg GAE/100 g DW) – mg Gallic acid equivalent per 100 gm dry weight of DP
RE/100 g dry weight – Rutin equivalents per 100 gm dry weight of DP
mmol TE/100 g DW – milli mol Trolox equivalents per 100 gm dry weight of DP, (µmol EE /g DW) – micro mol EDTA equivalents per gm dry weight of DP

3.5 Discussion

Many studies have revealed that natural carbohydrate polymers possess prebiotic properties and are widely used in animal feeds (Courtois, 2009). Date seeds represent a significant waste material. The proper nutritional value of date pits is based on their fiber content (70–80%) (Al-Farsi et al., 2007). An increase in total carbohydrate and a reduction in crude fiber level represent an elevation in the digestible carbohydrates and suppression of an indigestible portion of the fiber, which indicates the chemical changes that occurred during the degradation process. Recent studies showed that microbial treatment could further enhance the nutritional and medicinal values of edible seeds (Natarajan and Rajendran, 2009; Kim and Lee, 2010). Microbial treatment leads to the catabolism and degradation of main macronutrients such as carbohydrates, protein and fatty acids, accompanied by the increase of simple sugars, free amino acids and organic acids. Carbohydrates decompose to carbon dioxide, water and sugar which is used by fungi during respiration (Jennings, 1995). This leads to a decrease in dry weight as the protein content increases.

In addition to their roles as structural materials and energy sources, the non-digestible carbohydrates have attracted increasing attention due to their biological functions. Fibers are composed mainly of hemicellulose, cellulose, insoluble proteins and lignin which are the solid insoluble part of plant materials (Al-Farsi and Lee, 2008). Among the fiber parts, crude fiber consists of cellulose and other cell wall substances. The decrease in crude fiber content is clearly due to the action of enzymes from *T. reesei* to the components of the cell wall that leads to the release of carbohydrate moieties. Enzymes such as β-glucanases produced by *Trichoderma* mycelium can degrade lignocellulose by degrading the lignin component followed by cellulose (Ghorai et al., 2009).

The crude fiber which is decreased in DDP is a measure of the quantity of indigestible cellulose, pentosanes, lignin and other components. NDF is the total amount of fiber including mannan, lignin, cellulose and hemicellulose. ADF is the indigestible part, which includes lignin, cellulose and insoluble forms of nitrogen but not hemicellulose. In our study, the ADF content significantly decreased by about 7.25% in *T. reesei* DDP. Thus, *Trichoderma reesei* is an effective fibrinolytic microorganism in enhancing the NDF and reducing ADF content in DP. In the present experiment, the effect of fiber is consistent with the findings of Ahmadi et al. (2015), who found that seven days of solid-state degradation of orange peels and sugar beet pulp by *T. reesei* or *T. viride* can enhance their nutrient contents.

The presence of dietary fiber in the pits of date palm suggested using date pits in fiber-based feeds and nutrition's supplements (Al-Farsi and Lee, 2008). In DP, the fibers constitute the majority of carbohydrates, and only a small part consists of simple sugars (Yousif et al., 1996). After degradation with *T. reesei*, the hemicellulose content

showed an increase by about 17.5%, which was significant when compared to non-degraded DP. The hemicellulose content is high because the NDF value is more than double that of ADF. A study conducted by (Al-Farsi and Lee, 2008) found 58% total dietary fiber content in DP. The insoluble dietary fibers namely hemicelluloses, cellulose and lignin, constitute about 53% of total dietary fiber content (Aldhaheri et al., 2004).

Lignin is a polymer with high molecular weight of phenolic compounds, and it is the second most abundant component after cellulose that occurs naturally in plants (Mahesh and Mohini, 2013). Palm date fiber contains 50% of lignin that is higher than the lignocellulose materials present in wood that is about 30% (Kamm et al., 2007). Results showed that an increase of lignin content in *T. reesei* DDP. Pectin is a structural heteropolysaccharide in the cell wall of most plant biomass, and its availability is less when compared to hemicellulose and cellulose. DP is also a good source of pectin. *T. reesei* glycoside hydrolases play a significant role in the degradation of the cell wall and increase the pectin content in DDP (Martens-Uzunova and Schaap, 2009).

The enhanced sugar composition of cellulose in DDP is due to the action of several enzymes. Xylanases, exoglucanases, endoglucanases and β-glucosidases which are produced by *T. resse*i. These enzymes liberate sugars by the hydrolysis of cellulose (Saloheimo et al., 2002). *T. reesei* degradation showed a 12.63% increase of glucose and 7.22% increase of mannose in hemicellulose fraction of DDP. The increased concentration of monosaccharides especially mannose which indicates the degradation of hemicellulose (Martinez et al., 2009). The enzymes secreted by *Trichoderma reesei* contributes to a core role in hemicellulose degradation (Martinez et al., 2009).

Endomannanases, produced by *T. reesei* degrade mannose linking site and elevates the mannose content in DDP.

In lignin fraction of DDP, results showed a 0.4% increase of fructose, 8.53% increase of glucose and 2.16% increase of galactose in DP after *T. reesei* degradation. Lignases play a vital role in the degradation of lignin by fungi like *T. reesei* (Wesenberg et al., 2003). *T. reesei* enhances the activity of lignases and promotes the degradation rate of lignin in DP thereby increases the monosaccharide composition. Results showed that a 0.4% increase of mannose in the pectin fraction of DDP. Fungal degradation leads to secretion of pectinolytic enzymes that cleave those linkages necessary to produce oligosaccharides and monosaccharides (Vincken and van Alebeek, 2002). Pectin oligosaccharides are good prebiotic compounds (Olano-Martin et al., 2002).

Previous studies showed that a type of galactomannan is present in the cell wall of DP (Daneshyar et al., 2014). Ishrud et al. (2001) extracted water-soluble galactomannan from DP and reported that DP contains D-mannose (71.8%) and D-galactose (26.6%) in a molar ratio of 2.69:1. In this study degradation of DP with *T. reesei* showed an increase of 14.03% in mannose content of MOS. The fungus *T. reesei* is very efficient in degrading DP and increasing their mannose content levels by breaking down mannan fiber. An increase of 7.93%, 2.56%, 2.16%, and 12.42% of glucose, arabinose, increase of rhamnose and glucuronic acid respectively were found in DDP. The enzyme endomannanases from *T. reesei* liberates free mannose, glucose and MOS and showed specificity towards oligosaccharides having D-mannose with more than three residues (Shastak et al., 2015).

Xylanases produced by *T. reesei* promotes hydrolysis of fibers that release free mannose (Leisola et al., 2002). Enhanced xylanase production by *T. reesei* promotes

the liberation of more MOS from DDP that may lead to the release of mannose moieties. Glucuronic acid was also significantly increased in DDP. β-endomannanases generally called β-mannanases, hydrolyse the backbone of glucomannans, resulting in oligosaccharides and glucuronic acid (Hossain et al., 2014). Degradation of DP with *T. reesei* enhances the activity of β-mannanase and thereby liberates the glucuronic acid moieties (Pérez et al., 2002). The efficiency of β-mannanases to break down the mannan backbone depends on various factors, such as the distribution and number of the alternatives on the backbone and how much of glucose to mannose content available (McCleary, 1991).

Minerals serve as cofactors for many metabolic and physiological processes and are essential constituents of the human diet. The present results indicated that there were increases in P, Cr, Mn, and Zn due to fungal treatment of DDP. Also, Hamada et al. (2002) found that DP contain minerals namely magnesium, potassium, phosphorus, calcium, sodium and iron other than carbohydrate, protein, moisture, ash and oil. The increase in minerals contained in the DDP can be attributed to the breakdown of the organic complexes in the pits by the action of fungi (Kayode and Sani, 2008). During degradation, the fungi metabolised the complex organic compounds to simpler substances which are released into the medium and minerals are released, and their bioavailability increased (Bindschedler et al., 2016).

In poultry nutrition, phosphorus has a significant physiological role in the quality of broiler bones, which are especially important to tolerate the rapid increase of the chick's weight. It is well known that any lack of the minerals in poultry feed, causes poorer growth and disturbs the intensity of the process of biosynthesis which leads to a decrease of body mass in the phase of growth and development (Lukić et al., 2009).

If there is a deficiency in manganese, this will reduce the utilisation of phosphorus. Zinc is also an important mineral for the activation of several body enzymes. Its deficiency causes poor feathering and improper bones and general body growth (Ammerman et al., 1995).

A previous report by Mistrello et al. (2014) who found that total phenol and flavonoids contents range between 2.05-2.9 g GAE/100 g FW and 1.27–1.93 g CE/100 g FW respectively. The present results showed that SSD has a strong influence on the total phenolic content of DP. The number of bioactive compounds can be modified during fermentation by the metabolic activity of microbes (Ognjanović et al., 2010). Several hydrolytic enzymes might be produced directly from the SSD process and simultaneously be used to liberate the phenolic content in the SSD process. It was reported that fungal α-amylase and β-glucosidase were involved in the phenolic mobilisation during solid-state growth (Vattem and Shetty, 2002); (McCue and Shetty, 2003).

The present findings indicated that, after degradation with fungi *Trichoderma reesei*, DP showed a 19% increase in the scavenging ability of DPPH radicals and 7.05% increase in ABTS radical, scavenging activity. Results of FRAP assay showed the degradation process with *Trichoderma reesei* enhanced the Ferric reducing antioxidant power of DP up to 11.67%. Metal chelating activity was also increased by 4% in DDP. (Lee and Chou, 2006) showed that the methanol extracts of black bean kojis treated with microbes such as *Aspergillus awamori*, *Aspergillus oryzae*, *Aspergillus sojae* and *Rhizopus zygospores* displayed greater levels of DPPH free radical-scavenging activity, Fe2 -chelating activity and decreasing power than did the non-degraded black bean.

The antioxidant properties of DP degraded with *Trichoderma reesei* were higher than that of non-degraded DP. From the present results, it can be stated that the type of degradation determined the degree of modification of the levels of most bioactive compounds. Compared with non-degraded DP, the total phenolic contents in DP degraded by *Trichoderma* reesei were improved significantly. Phenolic compounds have been used to exhibit a scavenging outcome against free radicals (Shahidi et al., 1992).

The nutrient content of food has been enhanced by microbial degradation through the bio-synthesis of proteins, vitamins, and essential amino acids which results in enhanced protein quality and fiber digestibility (Parr and Bolwell, 2000). Microbial degradation removes anti-nutritional factors and alters the bioavailability of micronutrient (Oboh and Rocha, 2007). During SSD, the catalytic action of β-glucosidase enhances the phytochemical constituents like flavonoids and phenolic through the release of phenolic isoflavone aglycones and the formation of reductones increases the antioxidant properties of legume seeds (Ademiluyi and Oboh, 2011). The microorganisms involved in SSD cleaves the phenolic and flavonoid linkages, which free the compounds to actively act as antioxidants thereby improvement in antioxidant activity (Moktan et al., 2008; Ademiluyi and Oboh, 2011).

3.6 Conclusion

Degradation of DP with exogenous microbial enzymes like xylanases enhances the production of simpler forms of carbohydrate molecules from fibers present in DP. For this study, DP was degraded with fungi *T. reesei* using SSD. After degradation, crude protein and total carbohydrates have increased significantly. The composition of fiber in DP such as NDF, cellulose, hemicellulose, and lignin were significantly higher

when compared with the fiber composition of DP. On the other hand, ADF has decreased significantly after degradation.

Degradation of DP with *T. reesei* improved the following monosaccharide compositions of the fibers in DP, fructose in cellulose and lignin, glucose in cellulose, hemicellulose and lignin, mannose in hemicellulose and pectin, xylose in lignin and pectin, galactose in lignin, arabinose in cellulose and lignin, and sucrose in hemicellulose.

The MOS content was also significantly increased in DDP in which galactose and mannose were the major neutral sugars. The protein content in fungi DDP was 22.5%, 17 types of amino acids were found and have been identified. Among the studied minerals, potassium, calcium, magnesium, sulphur and phosphorus were predominant. And, P, Cr, Mn, Zn, strontium and vanadium was improved due to fungal treatment of DP.

The phenolic and flavonoid content of DDP significantly increased. DDP also showed significant antioxidant activity as evidenced by the results of DPPH, ABTS and FRAP assays.

Chapter 4: Effect of DDP, MOS and Mannose on Growth Performance and Microbial Population in Broilers

4.1 Introduction

This chapter focuses primarily on the potential effects of DDP, MOS (Mannan oligosaccharide, prebiotics originated from yeast cell wall) and D-mannose on the growth performance and various microbial growths in the broilers. The utilisation of poultry products like meat and egg has become very prominent worldwide due to its nutritional properties. The indispensable supplement in the poultry ration is dietary energy for the production of meat and eggs. Dietary energy accomplishes a key role in poultry growth and development. Among the energy sources, carbohydrates constitute 60% to 65% of metabolizable energy, and the widely used energy sources in poultry feed are cereals such as corn, sorghum, barley and wheat (Hotz and Gibson, 2007).

The dietary energy was upgraded by using alternative sources such as the addition of fat and oil in poultry diets. In Gulf countries, raw materials for poultry feed is imported, and the price of feed ingredients has increased due to competition with the food manufacturers and elevated biofuel production. The long-term use of antimicrobials for remedy and as growth promoter in animals leads to antimicrobial resistance (AMR) in gram-negative pathogens, and the European Union has banned the use of antibiotics in the feed or food-producing animals (Levy, 1998). Anti-microbial resistant bacteria can persist and replace sensitive bacteria. The change of genetic materials plays a critical role in expanding of antimicrobial resistance between one bacterial strain to another. Resistance genes, therefore, have the chance for wide distribution among bacteria. Antimicrobial drugs are in charge for the rise in the number of resistant bacteria between both virulent strains and normal bacterial flora.

The spread and growth of AMR in animal production systems related to organisms that are normally found in poultry such as *Escherichia coli*, *Enterococcus* spp., and *Staphylococcus aureus* in addition to foodborne zoonotic pathogens, like non-typhoidal *Salmonella* (NTS) and *Campylobacter* spp. (Richter et al., 2015). Thus, there is an urgent need to find alternatives to antibiotics. An alternative feed resource that can replace a significant portion of corn in poultry rations and have been introduced as potential antibiotic substitutes in poultry to meet the more energy requirement and to improve bird's health.

The idea for using yeast MOS in poultry feeds developed from the idea that certain sugars, in particular mannose, might be used to efficiently block the colonisation of the pathogens in the intestine like *Salmonella* species and *Escherichia coli,* that have type 1 fimbriae with mannose- seeking lectins. When they attach to the MOS product, the pathogens are banned from binding to intestinal villi, preventing a fast production of toxins (De Los Santos et al., 2007). A second purpose of developing the MOS product was because of the efficiency of some strains of live yeast at attaching and decreasing the intestinal pathogen counts (Benites et al., 2008). Previous reports showed that 0.2% MOS has a dietary effect on growth performance, development of the intestine, the count of caecal and litter microbial populations, and carcass characterizations of broilers (Baurhoo et al., 2007). Spring et al. (2000) showed that mannan-oligosaccharides added to the diet of broiler chickens reduced *S. typhimurium 29E* (express Type 1 fimbriae) concentration in the ceca. In our study, chemical analysis of DDP showed that it has significant amount of MOS and mannose that constitute the major neutral sugar. Therefore, a study was conducted with 10% DDP to elucidate the role of DDP in broilers growth performance, bacterial count and gene expression pattern in broiler gut and to compare its effect with commercial

antimicrobial at 50 g/100 kg diet of 20% oxytetracycline, 0.2% MOS, 0.2% mannose and 0.1% mannose added to corn-soybean meal diet. This Chapter also includes the evaluation of the effect of different dietary treatments on growth performance and microbial count in the chicken gut.

4.2 Material and Methods

4.2.1 Birds and Dietary Treatment for the Withdrawal Experiment (Experiment 1)

A total of 180 one-day old Brazilian broiler chicks "Cobb 500" were divided randomly into six dietary treatments. Birds with each treatment were distributed to three replicates of 10 unsexed broilers each. The poultry ward and cages were cleaned and sanitized. Chickens were housed in 18 separate cleaned and sanitised petersime brooding battery cages in an environmentally controlled house. Chicks were managed according to the animal welfare guidelines of the UAE University Research Ethics Committee.

The experiment lasted for thirty-five days through following treatment steps:

- Treatment 1- Control, corn-soy diet for five weeks
- Treatment 2- Control, corn-soy diet supplemented with antibiotic at 50 g/100 kg of 20% Oxytetracycline for 5 weeks
- Treatment 3- Corn-soy diet contained 10% DDP diet for the first three weeks of age then chicks were fed control feed.
- Treatment 4- Corn-soy diet contained 10% DDP diet for the first four weeks of age then chicks were fed control feed.
- Treatment 5- Corn-soy diet contained 10% DDP diet for the whole experimental period, which lasted for five weeks.

- Treatment 6- Corn-soy diet contained 10% DP diet with antibiotic at 50 g/100 kg of 20% Oxytetracycline for the whole experimental period, which lasted for five weeks.

The treatment diets were prepared as follows: All feed ingredients (Tables 9 and 10) were ground to the appropriate size, weighed, and mixed in a commercial mixer (Hobart mixer, HL1400, the USA) for 20 minutes then put in marked pails. The DDP were kept refrigerated at 4°C to avoid any fungal toxicity. Water and feed were given on an *ad libitum* basis.

4.2.2 Starter and Finisher Diets

All diets were iso-nitrogenous and iso-caloric. For the dietary treatments, the nutrient composition was calculated based on the ingredients composition as described by (Hashim et al., 2013). All feed ingredients were ground to a suitable size and mixed for 20 minutes with Hobart mixer (HL1400, USA) for a homogenous distribution of nutrients and particle size; after that, it was weighed (Figure 8). Fish meal, vitamin and mineral premixes, and oil were gradually added with continuous mixing.

Figure 8: Commercial mixer (Hobart mixer, HL1400, USA)

Experimental diets were prepared according to Table 9 and Table 10.

Table 9: Composition of experimental starter diets (dry matter basis) fed to broilers during 1-3 weeks of age, Experiment 1 (Source: Hussein et al., 2015)

Ingredients in%	Control diet [1,a]	Control diet [2,b]	Diet [3,c]	Diet [4,d]	Diet [5,e]	Diet [6,f]
Yellow corn	55.4	55.35	44.6	44.6	44.6	44.55
Soybean meal	36	36	33.25	33.25	33.25	33.25
Salt	0.4	0.4	0.38	0.38	0.38	0.38
Limestone	1.1	1.1	1.1	1.1	1.1	1.1
Di-calcium phosphate	1.56	1.56	1.2	1.2	1.2	1.2
Vit.+Min. Premix[g]	1	1	1	1	1	1
DL-Methionine	0.24	0.24	0.25	0.25	0.25	0.25
Lysine	0	0	0.1	0.1	0.1	0.1
Corn oil	2	2	5.02	5.02	5.02	5.02
Fish meal	2.3	2.3	3.1	3.1	3.1	3.1
Oxytetracycline	0	0.05	0	0	0	0.05
Date pits	0	0	10	10	10	10

Index:

[1] Corn-soy diet fed during 1-3 weeks of age.
[2] Corn-soy diet supplemented with 50 g/ 100 kg diet of 20% oxytetracycline and fed during 1-3 weeks of age.
[3] 10% DDP corn-soy diet during 1-3 weeks of age.
[4] 10% DDP corn-soy diet during 1-3 weeks of age.
[5] 10% DDP corn-soy diet during 1-3 weeks of age.
[6] 10% Non-degraded DP diet + Oxytetracycline added (20%, 50 g/100 kg) during 1-3 weeks of age.

[a] Calculated nutrient composition ia s: Protein, 21.89%; Fiber, 3.36%; Energy, 3033.62 kcal; Ca, 0.92%; Av. P, 0.45%; Sodium, 0.21%; Methionine, 0.52%; Lysine, 1.17%; Methionine + Cysteine, 0.97%; Threonine, 0.86%.
[b] Calculated nutrient composition is: Protein, 21.89%; Fiber, 3.36%; Energy, 3033.62 kcal; Ca, 0.92%; Av. P, 0.45%; Sodium, 0.21%; Methionine, 0.52%; Lysine, 1.17%; Methionine + Cysteine, 0.97%; Threonine, 0.86%.

[c] Calculated nutrient composition is: Protein, 21.92%; Fiber, 4.19%; Energy, 3040.83 kcal; Ca, 0.92%; Av. P, 0.48%; Sodium, 0.2%; Methionine, 0.55%; Lysine, 1.32%; Methionine +

Cysteine, 0.97%; Threonine , 0.85%.
[d] Calculated nutrient composition is: : Protein, 21.92%; Fiber, 4.19%; Energy, 3040.83 kcal; Ca, 0.92%; Av. P, 0.48%; Sodium, 0.2%; Methionine, 0.55%; Lysine, 1.32%; Methionine + Cysteine, 0.97%; Threonine , 0.85%.

[e] Calculated nutrient composition is: : Protein, 21.92%; Fiber, 4.19%; Energy, 3040.83 kcal; Ca, 0.92%; Av. P, 0.48%; Sodium, 0.2%; Methionine, 0.55%; Lysine, 1.32%; Methionine + Cysteine, 0.97%; Threonine , 0.85%.
[f] Calculated nutrient composition is Protein, 21.84%; Fiber, 4.95%; Energy, 3035.65 kcal; Ca, 0.92%; Av. P, 0.43%; Sodium, 0.2%; Methionine, 0.6%; Lysine, 1.32%; Methionine + Cysteine, 0.97%; Threonine, 0.86%.

[g] Supplementary levels of vitamins and trace elements (per Kg): Vitamin A, 5484 IU; Vitamin D3, 2822 ICU Vitamin E (as DL alpha-tocopherol acetate) 26 IU; Vitamin K (as menadione sodium bi-sulphite), 4.38 mg; thiamine, 5.94 mg; Riboflavin, 6.2 mg; Pyridoxine, 4.5 mg; Cyanocobalamin, 0.14 mg; Niacin (as nicotinic acid), 44.1 mg; D-pantothenic acid, 15 mg; Folic acid, 990ug, Biotin, 0.23 mg; Iron, 120 mg; Copper, 8 mg; Manganese, 83 mg; Cobalt, 5 mg; Zinc, 60 mg; Iodine, 1.11 mg; Selenium, 300 µg.

Table 10: Composition of experimental finisher diets fed to broilers during 4-5 weeks of age, Experiment 1 (Source: Hussein et al., 2015)

Ingredients	Control diet [1,a]	Control diet [2,b]	Diet [3,c]	Diet [4,d]	Diet [5,e]	Diet [6,f]
Yellow corn	64.6	64.55	64.6	52.14	52.14	52.09
Soybean meal	28.4	28.4	28.4	26	26	26
Salt	0.42	0.42	0.42	0.33	0.33	0.33
Limestone	1.33	1.33	1.33	1.15	1.15	1.15
Di-calcium phosphate	1.05	1.05	1.05	0.8	0.8	0.8
Vit + Min. Premix	0.2	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.2	0.2	0.2	0.3	0.3	0.3
Lysine	0.1	0.1	0.1	0.18	0.18	0.18
Corn oil	2.5	2.5	2.5	5.9	5.9	5.9
Fish meal	1.2	1.2	1.2	3	3	3
Oxytetracycline	0	0.05	0	0	0	0.05
Date pits	0	0	0	10	10	10

Index:

[1] Corn-soy diet fed for 2 weeks during 4-5 weeks of age.
[2] Corn-soy diet + Oxytetracycline (20%, 50 g/100 kg) fed for 2 weeks during 4-5 weeks of age.
[3] Corn-soy diet fed for 2 weeks during 4-5 weeks of age
[4] 10% DDP + corn-soy diet fed for 1 week during 4 week of age then the remaining chicks were fed with corn-soy diet fed for 1 week during 5 week of age.
[5] 10% DDP + corn-soy diet fed for 2 weeks during 4-5 weeks of age.
[6] 10% Non-degraded DP + corn-soy diet supplemented with 50 g/100 kg diet of 20% oxytetracycline fed for 2 weeks during 4-5 weeks of age.
[a] Calculated nutrient composition is: Protein, 17.75%; Fiber, 3.09%; Energy, 3135.67 kcal; Ca: 0.82%; Av. P, 0.39%; Sodium: 0.2%; Methionine, 0.47%; Lysine, 1.05%; Methionine + Cysteine, 0.86%; Threonine, 0.71%.

[b] Calculated nutrient composition is: Protein, 17.75%; Fiber, 3.09%; Energy, 3135.67 kcal; Ca: 0.82%; Av. P, 0.39%; Sodium: 0.2%; Methionine, 0.47%; Lysine, 1.05%; Methionine + Cysteine, 0.86%; Threonine, 0.71%.

[c] Calculated nutrient composition is: Protein, 17.75%; Fiber, 3.09%; Energy, 3135.67 kcal; Ca: 0.82%; Av. P, 0.39%; Sodium: 0.2%; Methionine, 0.47%; Lysine, 1.05%; Methionine + Cysteine, 0.86%; Threonine, 0.71%.

[d] Calculated nutrient composition is: Protein, 18.72%; Fiber, 3.92%; Energy, 3219.55 kcal; Ca, 0.80%; Av. P, 0.38%; Sodium, 0.18%; Methionine, 0.49%; Lysine, 1.1%; Methionine + Cysteine, 0.84%; Threonine, 0.73%.

[e] Calculated nutrient composition is: Calculated nutrient composition is: Protein, 18.72%; Fiber, 3.92%; Energy, 3219.55 kcal; Ca, 0.80%; Av. P, 0.38%; Sodium, 0.18%; Methionine, 0.49%; Lysine, 1.1%; Methionine + Cysteine, 0.84%; Threonine, 0.73%.

[f] Calculated nutrient composition is: Protein, 18.73%; Fiber, 4.1%; Energy, 3179.48 kcal; Ca, 0.86%; Av. P, 0.40%; Sodium, 0.18%; Methionine, 0.48%; Lysine, 1.1%; Methionine + Cysteine, 0.85%; Threonine, 0.72%.

[g] Supplementary levels of vitamins and trace elements (per Kg): Vitamin A, 5484 IU; Vitamin D3, 2822 ICU Vitamin E (as DL alpha-tocopherol acetate) 26 IU; Vitamin K (as menadione sodium bi-sulphite), 4.38 mg; thiamine, 5.94 mg; Riboflavin, 6.2 mg; Pyridoxine, 4.5 mg; Cyanocobalamin, 0.14 mg; Niacin (as nicotinic acid), 44.1 mg; D-pantothenic acid, 15 mg; Folic acid, 990 ug, Biotin, 0.23 mg; Iron, 120 mg; Copper, 8 mg; Manganese, 83 mg; Cobalt, 5 mg; Zinc, 60 mg; Iodine, 1.11 mg; Selenium, 300ug.

4.2.3 Birds and Diet Treatments- Study on DDP, MOS, and Mannose (Experiment 2)

A total of 180 Brazilian broiler chicks "Cobb 500" one day old were randomly divided into six dietary treatments and housed in 18 cages. Birds within each treatment were designated to 3 replicates of 10 unsexed chicks each. Chicks were housed ten birds per cage (50 cm × 45 cm × 45 cm) in an environmentally controlled house. Feed and water were given on an *ad libitum* basis. Chicks were managed and handled according to animal welfare guidelines of the UAE University Research Ethics Committee.

The experiment lasted for forty-two days through the following treatment steps:

- Treatment 1- Control, corn-soybean diet for six weeks.
- Treatment 2- Control, corn-soy diet supplemented with antibiotic at 50 g/100 kg of 20% oxytetracycline for 6 weeks.
- Treatment 3- Corn-soy diet containing 10% DDP diet for 6 weeks.
- Treatment 4- Corn-soy diet supplemented with 0.2% MOS for 6 weeks.
- Treatment 5- Corn-soy diet supplemented with 0.2% mannose for 6 weeks.
- Treatment 6- Corn-soy diet supplemented with 0.1% mannose for 6 weeks.

The experimental period was divided into two periods one started from day 1 to day 21st as the starter period while the finisher period started from day 22 n^{d} to day 42 n^{d}. On day 21st of the experiment, chickens were chosen randomly from each group of all treatments. Chickens were weighed, slaughtered and collected the gut samples were collected. On the day 42 n^{d} of the experiment, two chickens were chosen randomly from each replicate of all treatments (n=6 per treatment) for slaughter test, organs weight and blood samples collections for this experiment and the following ones. Broilers were weighed to measure carcass characteristics and organs weight as well as

collection of body organs and blood samples to obtain serum and plasma. The gut samples were also collected for enumeration of microbial populations and morphology. Experimental diets were prepared according to Table 11 and Table 12.

Table 11: Composition of experimental starter diet fed to broilers during 1-3 weeks of age- A comparison study of DDP with MOS and Mannose (Experiment 2)

Ingredient	**Control diet [1,a]**	**Control diet [2,b]**	**Diet [3,c]**	**Diet [4,d]**	**Diet [5,e]**	**Diet [6,f]**
	(Kg)	**(Kg)**	**(Kg)**	**(Kg)**	**(Kg)**	**(Kg)**
Yellow corn	55.4	55.35	44.6	55.2	55.2	55.3
Soybean meal	36	36	33.25	36	36	36
Salt	0.4	0.4	0.38	0.4	0.4	0.4
Limestone	1.1	1.1	1.1	1.1	1.1	1.1
Di-calcium phosphate	1.56	1.56	1.2	1.56	1.56	1.56
Vit. + Min. Premix[g]	1	1	1	1	1	1
DL-Methionine	0.24	0.24	0.25	0.24	0.24	0.24
Lysine	0	0	0.1	0	0	0
Corn oil	2	2	5.02	2	2	2
Fish meal	2.3	2.3	3.1	2.3	2.3	2.3
Oxytetracycline	0	0.05	0	0	0	0
Degraded Date pits	0	0	10	0	0	0
MOS	0	0	0	0.2	0	0
D-mannose	0	0	0	0	0.2	0.1

Index:

[1] Corn-soy diet fed for the first 3 weeks of age.
[2] Corn-soy diet supplemented with 50 g/100 kg of 20% Oxytetracycline fed for the first 3 weeks of age.
[3] Corn-soy diet containing 10% DDP fed for the first 3 weeks of age.
[4] Corn-soy diet supplemented with 0.2% MOS fed for the first 3 weeks of age.
[5] Corn-soy diet supplemented with 0.2% mannose fed for the first 3 weeks of age.
[6] Corn-soy diet supplemented with 0.1% mannose fed for the first 3 weeks of age.
[a] Calculated nutrient composition is: Protein, 21.33%; Fiber, 3.36%; Energy, 3065.4 kcal; Ca, 0.89%; Av. P, 0.46%; Sodium, 0.21%; Methionine, 0.46%; Lysine, 1.07%; Methionine + Cysteine, 0.85%; Threonine,0.85%.
[b] Calculated nutrient composition is: Protein, 21.33%; Fiber, 3.36%; Energy, 3065.4 kcal; Ca, 0.89%; Av. P, 0.46%; Sodium, 0.21%; Methionine, 0.46%; Lysine, 1.07%; Methionine + Cysteine, 0.85%; Threonine,0.85%.

[c] Calculated nutrient composition is: Protein, 21.2%; Fiber, 4.19%; Energy, 3095.2 kcal; Ca, 0.92%; Av. P, 0.45%; Sodium, 0.22%; Methionine, 0.45%; Lysine, 1.33%; Methionine + Cysteine, 0.95%; Threonine, 0.87%.

[d] Calculated nutrient composition is: Protein, 21.30%; Fiber, 4.36%; Energy, 3083.62 kcal; Ca, 0.90%; Av. P, 0.45%; Sodium, 0.20%; Methionine, 0.45%; Lysine, 1.29%; Methionine + Cysteine, 0.97%; Threonine, 0.88%.

[e] Calculated nutrient composition is: Protein, 21.12%; Fiber, 3.0%; Energy, 3045. 2 kcal; Ca, 0.92%; Av. P, 0.51%; Sodium, 0.21%; Methionine, 0.44%; Lysine, 1.17%; Methionine + Cysteine, 0.87%; Threonine,0.86%.

[f] Calculated nutrient composition is: Protein 21.30%; Fiber 3.36%; Energy 3033.62 kcal; Ca 0.94%; Av. P 0.55%; Sodium 0.21%; Methionine 0.45%; Lysine 1.15%; Methionine + Cysteine 0.89%; Threonine 0.86%.

[g] Supplementary levels of vitamins and trace elements (per Kg): Vitamin A 5484 IU; Vitamin D3 2822 ICU Vitamin E (as DL alpha-tocopherol acetate) 26 IU; Vitamin K (as menadione sodium bi-sulphite) 4.38 mg; Thiamine 5.94 mg; Riboflavin 6.2 mg; Pyridoxine 4.5 mg; Cyanocobalamin 0.14 mg; Niacin (as nicotinic acid) 44.1 mg; D-pantothenic acid 15 mg; Folic acid 99ug; Biotin 0.23 mg; Iron 120 mg; Copper 8 mg; Manganese 83 mg; Cobalt 5 mg; Zinc 60 mg; Iodine 1.11 mg; Selenium 300 µg.

Table 12: Composition of experimental finisher diet fed to broilers during 4-6 weeks of age- A comparison study of DDP with MOS and Mannose (Experiment 2)

Ingredient	**Control diet [1,a]**	**Control diet [2,b]**	**Diet [3,c]**	**Diet [4,d]**	**Diet [5,e]**	**Diet [6,f]**
	(Kg)	**(Kg)**	**(Kg)**	**(Kg)**	**(Kg)**	**(Kg)**
Yellow corn	64.6	64.55	52.14	64.4	64.4	64.5
Soybean meal	28.4	28.4	26	28.4	28.4	28.4
Salt	0.42	0.42	0.33	0.42	0.42	0.42
Limestone	1.33	1.33	1.15	1.33	1.33	1.33
Di-calcium phosphate	1.05	1.05	0.8	1.05	1.05	1.05
Vit. + Min. Premix[g]	0.2	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.2	0.2	0.3	0.2	0.2	0.2
Lysine	0.1	0.1	0.18	0.1	0.1	0.1
Corn oil	2.5	2.5	5.9	2.5	2.5	2.5
Fish meal	1.2	1.2	3	1.2	1.2	1.2
Oxytetracycline	0	0.05	0	0	0	0
Degraded Date pits	0	0	10	0	0	0
MOS	0	0	0	0.2	0	0
D-mannose	0	0	0	0	0.2	0.1

Index:

[1] Corn-soy diet fed during 4-6 weeks of age.
[2] Corn-soy diet supplemented with 50 g/ 100 kg diet of 20% Oxytetracycline fed during 4-6 weeks of age.
[3] Corn-soy diet contained 10% DDP fed during 4-6 weeks of age.
[4] Corn-soy diet supplemented with 0.2% MOS fed during 4-6 weeks of age
[5] Corn-soy diet supplemented with 0.2% mannose fed during 4-6 weeks of age.
[6] Corn-soy diet supplemented with 0.1% mannose fed during 4-6 weeks of age.

[a] Calculated nutrient composition is: Protein, 17.75%; Fiber, 3.09%; Energy, 3135.67 kcal; Ca: 0.82%; Av. P 0.4%; Sodium: 0.2%; Methionine 0.4%; Lysine 1.05%; Methionine + Cysteine 0.80%; Threonine 0.71%.

[b] Calculated nutrient composition is: Protein, 17.75%; Fiber, 3.09%; Energy, 3135.67 kcal; Ca:

0.8 in2%; Av. P, 0.4%; Sodium: 0.2%; Methionine, 0.4%; Lysine, 1.05% ; Methionine + Cysteine, 0.80%; Threonine, 0.71%.

[c] Calculated nutrient composition is: Protein, 18.72%; Fiber 3.82%; Energy 3219.55 kcal; Ca 0.85%; Av. P 0.4%; Sodium 0.18%; Methionine 0.43%; Lysine 1.1%; Methionine + Cysteine, 0.9%; Threonine, 0.75%.

[d] Calculated nutrient composition is: Protein 18.75%; Fiber 3.86%; Energy 3215.67 kcal; Ca: 0.82%; Av. P 0.4%; Sodium: 0.2%; Methionine 0.42%; Lysine 1.2%; Methionine + Cysteine 0.81%; Threonine 0.74%.

[e] Calculated nutrient composition is: Protein 17.8%; Fiber 3.10%; Energy 3138.67 kcal; Ca: 0.82%; Av. P 0.4%; Sodium: 0.2%; Methionine 0.4%; Lysine 1.1%; Methionine + Cysteine 0.80%; Threonine 0.72%.

[f] Calculated nutrient composition is: Protein 17.78%; Fiber 3.09%; Energy 3145.67 kcal; Ca: 0.82%; Av. P 0.4%; Sodium: 0.2%; Methionine 0.4%; Lysine 1.1%; Methionine + Cysteine 0.80%; Threonine 0.72%.

[g] Supplementary levels of vitamins and trace elements (per Kg): Vitamin A 5484 IU; Vitamin D3 2822 ICU; Vitamin E (as DL alpha-tocopherol acetate) 26 IU; Vitamin K (as menadione sodium bi-sulphite) 4.38 mg; Thiamine 5.94 mg; Riboflavin 6.2 mg; Pyridoxine 4.5 mg; Cyanocobalamin 0.14 mg; Niacin (as nicotinic acid) 44.1 mg; D-pantothenic acid 15 mg; Folic acid 990ug; Biotin 0.23 mg; Iron 120 mg; Copper 8 mg; Manganese 83 mg; Cobalt 5 mg; Zinc 60 mg; Iodine 1.11 mg; Selenium 300ug.

4.2.4 Broiler Experiment- Sample Collection and Growth Performance Analysis

At the end of the experiment, two broilers chickens from each replicate per treatment (n=6 per treatment) were randomly selected and weighed and slaughtered according to Islamic method after 12 hours of feed deprivation. All birds were slaughtered immediately, and then blood (5 ml) was collected in two tubes with or without heparin tubes from the jugular vein. Blood was centrifuged at 3000 rpm for 15 minutes at 4°C for collection of blood plasma and serum. The tissue samples, liver, pancreas and small intestine were collected. Blood samples and tissue samples were stored at -80°C for further analysis. Samples of duodenum, jejunum, and ileum (1 cm cut from the midpoint) were stored in 10% neutral buffered formalin for 24 hours for analysis (Figures 9-11).

The growth performance, feed intake, the efficiency of feed utilisation (feed/gain ratio), were determined every week. Feed efficiency performance including chicken weight gain (WG), feed intake (FI) and Feed Conversion Ratio (FCR) with the following equations (Belal, 2008):

$$WG = W2 - W1$$

Where W2 is the mean final weight (g) per chicken and *W1* is the mean initial weight (g) per chicken.

- FI = [(Total feed added (g) – feedback (g))/ number, of chickens]/ number of days.
- FCR = feed intake (g) / wet weight gain (g).
- Body weight (BW) and feed intake (FI) were measured every week.
- Feed intake was calculated as the difference between the amount of feed offered and refusals.
- FCR (kg feed/kg gain) was calculated by dividing FI with BW gain. The FCR was determined at the end of the study.

4.2.5 Enumeration of Bacterial Populations

The microbial populations of the chicken gut samples (n=6 per treatment group) were estimated using the dilution plate method (Johnson and Curl 1972). The guts were firstly rinsed in running tap water for one h to remove surface contaminants, and the fresh weight was recorded before further processing. Guts were soaked in sterile phosphate-buffered saline solution (PBS) (pH 7.0) for 10 min to equilibrate osmotic pressure and to prevent passive diffusion of sterilising agents into the guts (Rennie et al. 1982). The guts were surface-disinfested by momentarily exposing to 70% ethyl alcohol for 3 min followed by 1.05% solution of commercial bleach for 1 min.

The surface-disinfested guts were then rinsed ten times (5 min each rinse) in PBS (Hallmann et al., 1997) and were then subjected to 8 items of washing with sterile distilled water. A surfactant, (Tween 20) is, (0.05 ml l-1) (Sigma Chemical Co, St. Louis, USA) was used in all the disinfestation procedures using hypochlorite. Guts were macerated in 100 ml of PBS using a sterile mortar and pestle under aseptic conditions and then shaken for one hour on a shaker (Model G76, New Brunswick Scientific; Edison, NJ, USA) at 250 rpm at 25°C. The suspension was then placed in an ultrasonic cleaner at a frequency of 55,000 cycle's sec-1 for 20 sec (Model: B- 221, 185Warr, Branson Cleaning Equipment Company; USA). Five plates per dilution were made for each sample (Figure 11-15). The slurry was filtered through sterile No. 1 Whatman filter paper (Whatman, Maidstone, England), and the filtrate was serially diluted (10-2 - 10-5) in PBS (Hallmann et al., 1997).

The groups of organisms selected for enumeration and the media used were as follows: (i) total aerobic bacteria on nutrient agar medium (Product Code: LAB008) (Lab M Limited, Lancashire, United Kingdom); (ii) *Escherichia coli* on eosin methylene blue (EMB agar) (Product Code: LAB061) (Lab M Limited, Lancashire, United Kingdom); (iii) lactose fermenting enterobactericeae and non-lactose fermenting enterobactericeae on MacConkey agar medium (Product Code: LAB045) (Lab M Limited, Lancashire, United Kingdom); (iv) *Salmonella* spp. and *Shigella* spp. on *Salmonella Shigella* agar (S.S. agar) (Product Code: LAB052) (Lab M Limited, Lancashire, United Kingdom); (v) *Salmonella* spp. on xylose lysine decarboxylase agar (X.L.D. agar) (Product Code: LAB032) (Lab M Limited, Lancashire, United Kingdom); (vi) *Lactobacillus* spp. on MRS agar Product Code: LAB223) (Lab M Limited, Lancashire, United Kingdom); and (vii) *Bifidobacterium* spp. on

Bifidobacterium agar (Product Code: M1396) (HiMedia Laboratories Pvt. Ltd., Mumbai, India).

Nutrient agar medium is a general purpose medium for the cultivation of organisms that are not demanding in their nutritional requirements. This medium was used for the enumeration of total aerobic bacteria.

MacConkey agar medium is a medium recommended by the World Health Organization (WHO) and the American Public Health Association in 1950 for the isolation of enterobacteriaceae (lactose fermenting and non-lactose fermenting) from waters and sewage and other sources. Lactose fermenting enterobactericeae on MacConkey agar medium produce pink colonies, whilst non-lactose fermenting enterobactericeae on MacConkey agar medium produce colourless pale yellow colonies.

Eosin methylene blue agar medium (Levine agar medium) was introduced in 1916 by Holt-Harris and Teague to differentiate *Escherichia* spp. and Enterobacter spp. It was modified by Levine in 1918 that removed sucrose from the formula and increased the lactose content. The distinctive metallic sheen produced by *E. coli* on this medium is due to acid production resulting in an amide bonding between the eosin and methylene blue; other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most Gram positive organisms. *Escherichia coli* produce blue black colonies with metallic sheen.

Salmonella Shigella agar medium (S.S. agar medium) is a modification of Leifson's DCA Medium first described in 1940 by Mayfield and Goeber shortly before Hynes described his modification of DCA. The selectivity of the medium was increased by

the addition of extra bile salts, sodium citrate and the addition of brilliant green dye. There is also the extra thiosulphate giving good H_2S production which reduces the ferrous ammonium sulphide giving black centred colonies with H_2S positive organisms. *Salmonella* spp. produces yellow colonies with black centres whilst *Shigella* spp. produce pinkish-yellow colonies.

X.L.D. agar medium was introduced by Taylor in 1965 to improve the recovery and recognition of *Shigella* spp, and has proved to be an excellent medium for *Salmonella* spp. The medium is low in nutrients and relies on a small amount of sodium desoxycholate for selectivity. The indicator system is novel and complex. Most enteric organisms except *Shigella*, will ferment xylose to produce acid. However the *Shigella*e will also decarboxylate the lysine to keep the pH neutral. At near neutral pH *Salmononella* can produce H_2S from the reduction of thiosulphate producing black or black centred colonies.

Figure 9: Collection of chick's gut samples-A

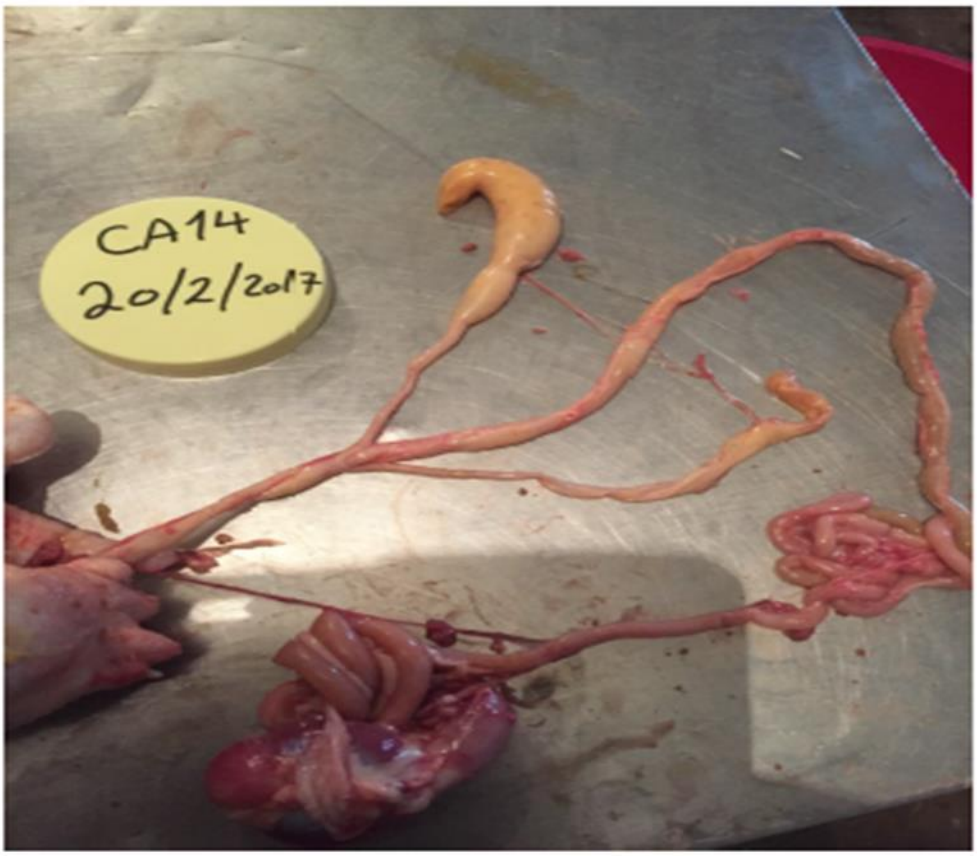

Figure 10: Collection of gut samples-B

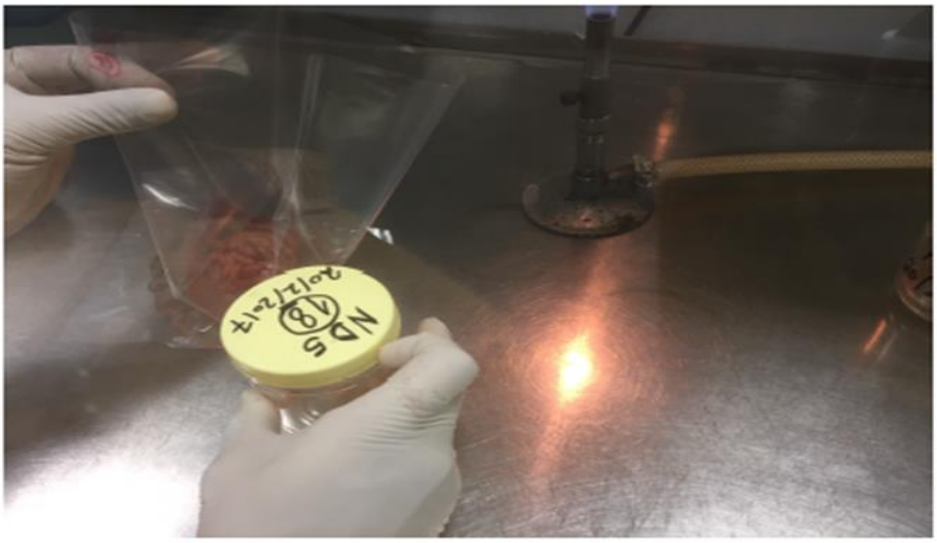

Figure 11: Preparation of the gut samples for serial dilution

4.3 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using general linear model (GLM) and mean comparisons were performed using Duncan's multiple range test to compare significant differences between means for all analyses. Statistical analysis was carried out using the replicate as the experimental unit using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA). The potential effects of dietary treatments of DDP, MOS, and Mannose on the broilers' body weight and feed intake are discussed from now on.

4.4 Results

4.4.1 Effect of Dietary Treatments of Degraded Date Pits in Different Time Periods on Average Body Weight, Feed Intake and Feed Conversion Ratio on Broilers (Experiment 1)

Table 13 shows the effect of dietary treatments on average body weight and feed intake of broilers. The effect of adding 10% DDP to the broiler diets gave similar average weight gain when compared with corn-soy diet fed-broilers, and similar to the corn-

soy diet with antibiotic diet fed-broilers, non-degraded date pit with antibiotic diet fed-broilers. Overall, the effect of dietary treatments on body weight gain was not significantly different ($P > 0.05$) between all the treatments.

The generated results also showed that the total feed intake of starter grower and finisher diets was not significantly ($P > 0.05$) different between all treatments. The FCR s in starter, grower and finisher diet fed-broilers were almost the same in all treatments ($P > 0.05$).

Table 13: Effect of different dietary treatments on broilers' growth performance

	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Starter (week three)						
ABW in g	879±2.9	903±27	868.6±42	821±5.8	880±23.6	844.6±42
TFI in g	1069±7	1055±18	1058±15	1042±19	1038±15	1035±8
Total FCR	1.21±0.06	1.16±0.03	1.22±0.05	1.27±0.02	1.18±0.03	1.23±0.06
Grower (week four)						
ABW in g	1461±14	1567±10	1455±26	1473±8	1535±15	1537±18
TFI in g	2115±14	2239±27	2120±7	2107±10	2186±2	2163±32
Total FCR	1.44±0.01	1.42±0.01	1.45±0.02	1.43±00	1.42±0.01	1.41±0.01
Finisher (week five)						
ABW in g	1838±3	1924±9	1848±17	1874±6.6	1891±32	1895±4
TFI in g	2928±9	2924±5	2946±14	2938±11	2906±9	2900±50
Total FCR	1.59±0.001	1.52±0.01	1.59±0.01	1.57±0.01	1.53±0.02	1.52±0.08

Index:

ABW-Average Body Weight, TFI-Total Feed Intake, FCR-Feed Conversion Ratio
[1] Corn-soy diet fed for five weeks
[2] Corn-soy diet supplemented with 50 g/kg diet of 20% Oxytetracycline for 5 weeks
[3]10% DDP corn-soy diet for the first 3 weeks of age then chicks were fed with a corn-soy diet for 2 weeks
[4] 10% DDP diet for the first 4 weeks of age then chicks were fed with corn-soy diet for 1 week
[5]10% DDP diet for the whole experimental period which was 5 weeks
[6]10% Non-degraded DP diet supplemented with 50 g/ 100 kg of 20% Oxytetracycline for 5 weeks

4.4.2 Effect of Dietary Treatments of DDP, MOS, and Mannose on Average Body Weight, Feed Intake and Feed Conversion Ratio on Broilers (Experiment 2)

Table 14 shows the potential effects of dietary treatments on average body weight and feed intake of broilers. The effect of adding 10% DDP, 0.2% MOS, 0.1% and 0.2% mannose to broiler starter diet has similar average body weights, while the control corn-soy diet fed-broilers and the corn-soy diet with antibiotic diet fed-broilers were significantly low and high respectively. In the finisher diet, the effect of dietary treatments on average body weight was not different significantly ($P > 0.05$) between all dietary treatments. Results also showed that total feed intake of starter, grower and finisher diets was not significantly ($P>0.05$) different between all treatments. In general, there were no significant differences ($P >0.05$) in FCR between all the treatments in both starter and finisher broiler diets. However, FCR in starter and finisher periods of broilers fed DDP, MOS and antibiotic supplemented diets were insignificantly better than the other treatments.

Table 14: Effect of dietary treatments of DDP, MOS and mannose on broilers

Treatments	**Average Body Weight Starter (g)**	**Average Body Weight Total (g)**	**Feed-Intake Starter (g)**	**Feed-Intake Total (g)**	**Feed-Conversion Ratio Starter**	**Feed-Conversion Ratio Total**
	(ABWS)	(WGT)	(FIS)	(FIT)	(FCRS)	(FCRT)
Control without antibiotics	824.00±4.6	2279±8.0	1102±1.4	3909±65.8	1.340±0.05	1.72±0.02
Control With antibiotics	928.66±24.66	2371±64.1	1141±15	3840.3±24	1.23±0.02	1.62±0.03
10% DDP	904±27.3	2368.6±22.3	1133±6.8	3837±40.1	1.26±0.03	1.62±0.01
0.2% MOS	874.66±16.82	2340±10.5	1120±30.3	3828.6±59.1	1.28±0.02	1.64±0.02
0.1% Mannose	860.66±14.89	2302.6±83.1	1107±22.5	3823±58.3	1.28±0.007	1.66±0.03
0.2% Mannose	879.33±2.90	2261±14.5	1159±5.7	3896.6±81.7	1.31±0.02	1.72±0.02

4.4.3 Effect of corn-soy, DDP Diet and diet with Oxytetracycline on Total Bacterial Count in Broilers after 3 Weeks (Experiment 1)

Bacterial population densities in chicken gut tissue after three weeks as shown in Table 15.

In the first experiment, 10% DDP corn-soy diet was fed for 3 weeks. Results showed that broilers fed corn-soy diet (control) had a significantly ($P < 0.05$) higher total bacterial populations, *E. coli*, enterobacteriaceae, *shigella* spp., *Salmonella* spp. than the broilers fed diet having 10% DDP and Oxytetracycline supplemented corn-soybean diet and DP diet. The antibiotic supplemented group showed significantly stronger effect on different population of microbes than 10% DDP.

Table 15: Bacterial population densities in chicken gut tissue after three weeks

Treatments[a]	**Total bacteria**[b]	**Lactose fermenting enterobactericeae**[d]	**Non- lactose fermenting enterobactericeae**[d]	***E. coli***[c]	***Shigella* spp.**[e]	***Salmonella* spp.**[e]	***Salmonella* spp.**[f]
(1)	10.24±0.57^{a}	4.83±0.09^{a}	4.35±0.37^{a}	5.15±0.08^{a}	3.77±0.09^{a}	4.36±0.13^{a}	4.46±0.14^{a}
(2)	2.31±0.13^{c}	1.81±0.10^{c}	1.34±0.19^{c}	1.70±0.11^{c}	1.25±0.08^{c}	1.46±0.06)^{c}	1.55±0.08^{c}
(3)	5.45±0.13^{b}	3.71±0.10^{b}	3.45±0.17^{b}	3.42±0.09^{b}	2.67±0.08^{b}	3.26±0.11^{b}	3.42±0.13^{b}
(6)	2.12±0.11^{c}	2.02±0.11^{c}	1.41±0.20^{c}	1.50±0.10^{c}	1.48±0.10^{c}	1.39±0.09^{c}	1.51±0.09^{c}

Index:

[a] Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

[b] Total bacterial counts on nutrient agar medium.

[c] *Escherichia coli* on eosin methylene blue agar medium (Levine agar medium).

[d] Lactose fermenting enterobacteriaceae and non-lactose fermenting enterobacteriaceae on MacConkey agar medium.

[e] *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* agar medium (S.S. agar medium).

[f] *Salmonella* spp. on xylose lysine decarboxylase agar medium (X.L.D. agar medium).

Values are means of six independent replicates for each treatment, and the values in parentheses are the standard error of the mean. Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

The count of other bacterial strains, like *E. coli*, enterobacteriaceae, *Shigella* and *Salmonella* in the different media were significantly increased in control corn-soy diet fed-broilers and significantly decreased in DDP diet fed-broilers. In non-DDP with antibiotic diet fed-broilers, the bacterial count was decreased, but the result was not significant. Figures 12-16 are standing for the growth pattern of different bacteria on various agar media.

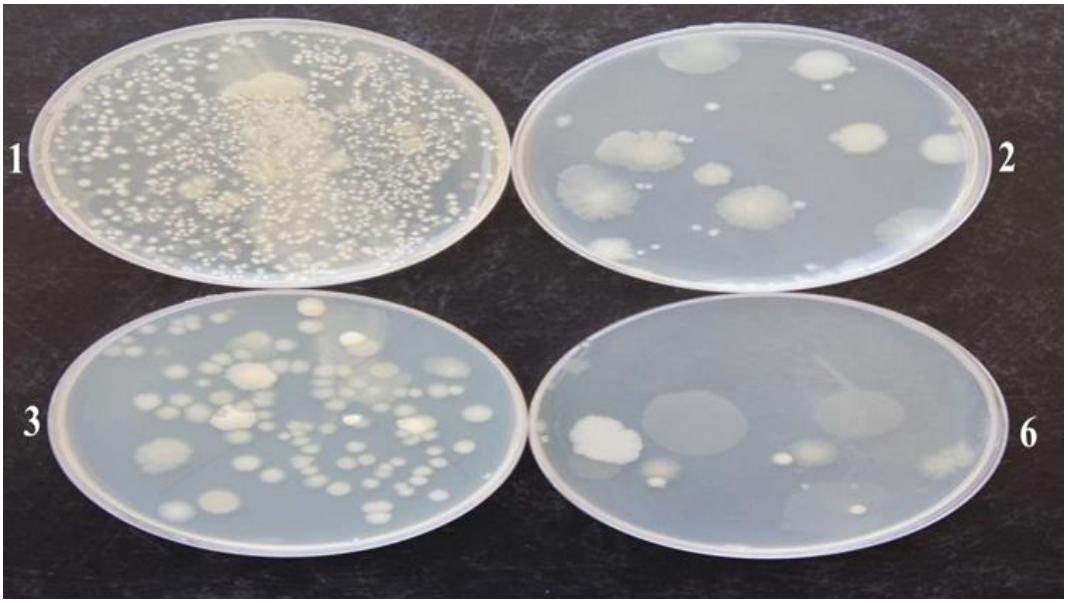

Figure 12: Total bacteria on nutrient agar medium (Exp 1, after 3 weeks)

Treatment-1: Control corn-soy; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for three weeks then the remaining chicks were treated with (control without antibiotics) for two weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

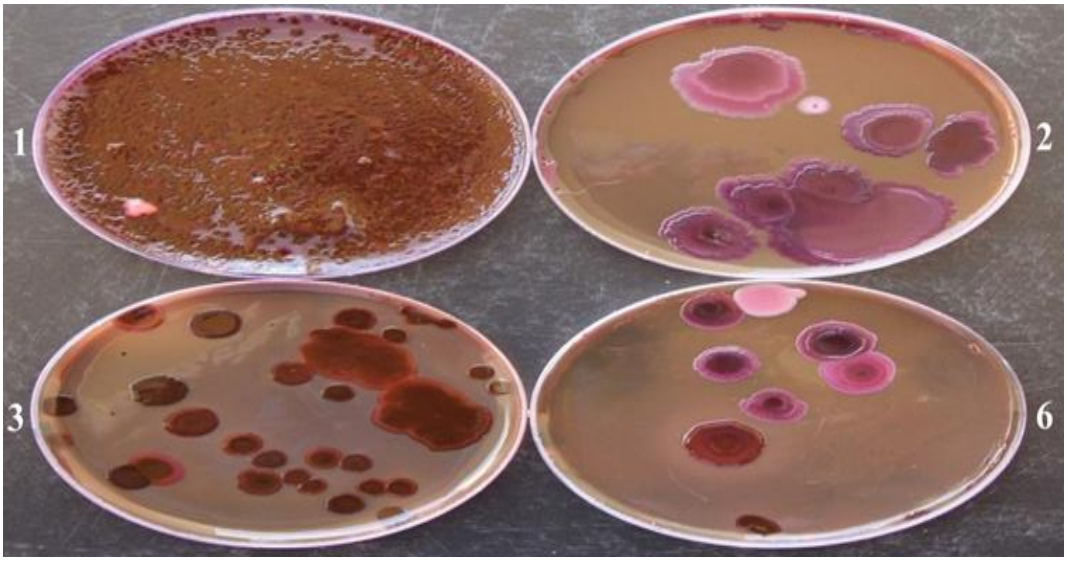

Figure 13: *E. coli* on methylene blue agar medium (Levine) (Exp 1, after 3 weeks)

Treatment-1: Control corn-soy; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for three weeks then the remaining chicks were treated with (control without antibiotics) for two weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

Figure 14: Lactose/non-lactose fermenting enterobacteriaceae on MacConeky (Exp 1, after 3 weeks)

Treatment-1: Control corn-soy; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for three weeks then the remaining chicks were treated with (control without antibiotics) for two weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

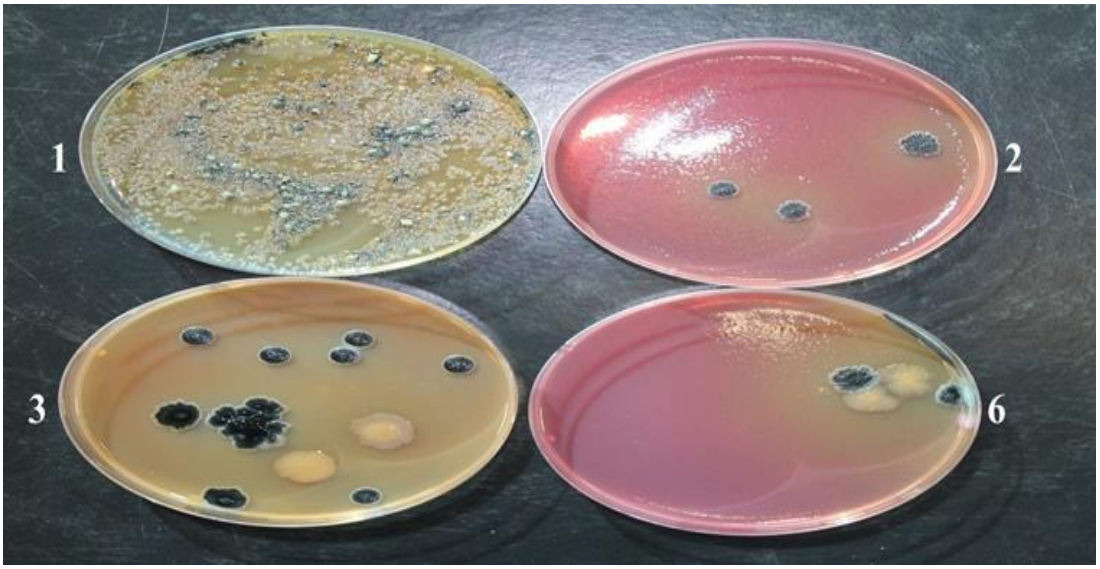

Figure 15: *Shigella* spp. and *Shigella* spp. on *Shigella-Shigella* agar (Exp 1, after 3 weeks)

Treatment-1: Control corn-soy; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for three weeks then the remaining chicks were treated with (control without antibiotics) for two weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

Figure 16: *Shigella* spp. on xylose lysine decarboxylase agar medium (Exp 1, after 3 weeks)

Treatment-1: Control corn-soy; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for three weeks then the remaining chicks were treated with (control without antibiotics) for two weeks; and Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

4.4.4 Effect of corn-soy, DDP Diet and diet with Oxytetracycline on Total Bacterial Count in Broilers After 4 Weeks (Experiment 1)

The results presented in Table 16 and Figures 17-21 illustrate that the total bacterial count was significantly decreased in 10% DDP corn-soy diet fed for four weeks, and antibiotic diet fed-broilers. In broilers, fed 10% DDP corn-soy diet for three weeks then fed control diet for one week the total bacterial populations, *E. coli*, enterobacteriaceae, *Shigella* spp., *salmonella ssp*. was significantly lower than the broilers fed diet having corn-soybean diet without antibiotic. While antibiotic supplemented groups had the lowest different types of microbes. Meanwhile, feeding DDP diet for 4 weeks resulted in further significant decrease in different types of microbes compared to feeding DDP for 3 weeks of age, but both DDP groups had higher microbes than antibiotic supplemented-groups.

Table 16: Bacterial population densities in chicken gut tissue after four weeks (Experiment 1)

Treatments [a]	**Total bacteria** [b]	**Lactose fermenting enterobactericeae** [d]	**Non- lactose fermenting enterobactericeae** [d]	***E. coli*** [c]	***Shigella* spp.** [e]	***Salmonella* spp.** [e]	***Salmonella* spp.** [f]
(1)	10.69±(0.26) *a*	4.92±(0.22) *a*	4.33±(0.34) *a*	5.41±(0.12) *a*	3.77±(0.08) *a*	4.15±(0.09) *a*	4.72±(0.20) *a*
(2)	2.30±(0.09) *d*	1.85±(0.15) *d*	1.52±(0.18) *d*	1.65±(0.14) *d*	1.46±(0.10) *d*	1.61±(0.14) *d*	1.83±(0.11) *d*
(3)	5.62±(0.14) *b*	3.30±(0.14) *b*	2.94±(0.14) *b*	3.54±(0.09) *b*	2.75±(0.13) *b*	3.29±(0.10) *b*	3.53±(0.16) *b*
(4)	3.74±(0.13) *c*	2.55±(0.16) *c*	2.23±(0.16) *c*	2.84±(0.10) *c*	2.11±(0.12) *c*	2.56±(0.12) *c*	2.85±(0.09) *c*
(6)	2.018±(0.13) *d*	1.50±(0.25) *d*	1.21±(0.29) *d*	1.55±(0.15) *d*	1.34±(0.07) *d*	1.40±(0.10) *d*	1.92±(0.19) *d*

Index:

[a] Treatment (1): control; treatment (2): Control with antibiotics; treatment (3): 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; treatment (4): 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and treatment (6): 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

[b] Total bacterial counts on nutrient agar medium.

[c] Escherichia coli on eosin methylene blue agar medium (Levine agar medium).

[d] Lactose/Non-fermenting enterobacteriaceae on MacConkey agar medium.

[e] *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* (S.S.) agar medium.

[f] *Shigella* spp. on xylose lysine decarboxylase (XLD) agar medium.

Values are means of six independent replicates for each treatment, and the values in parentheses are the standard error of the mean. Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

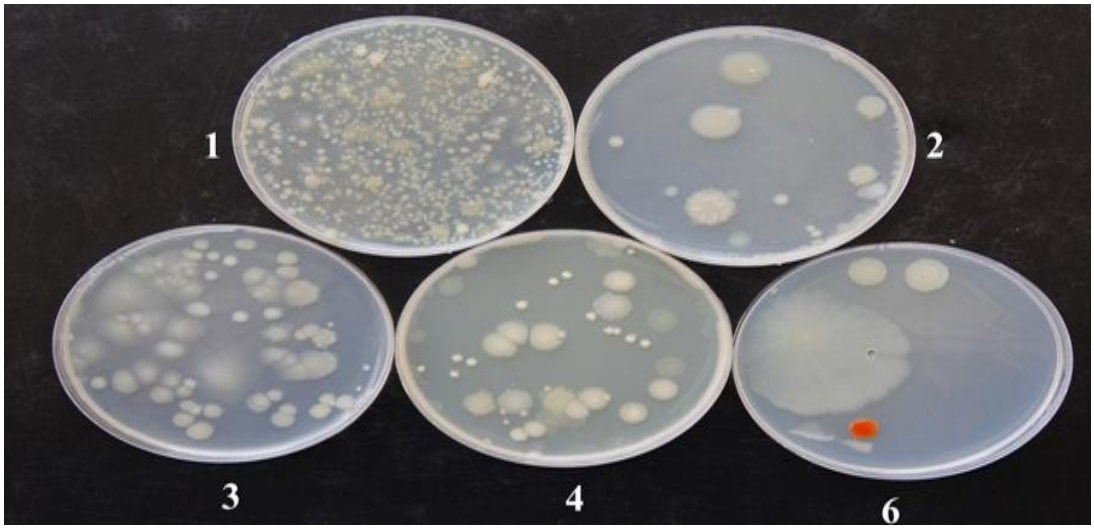

Figure 17: Total bacteria on nutrient agar medium (Exp 1, after 4 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and Treatment-6: 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

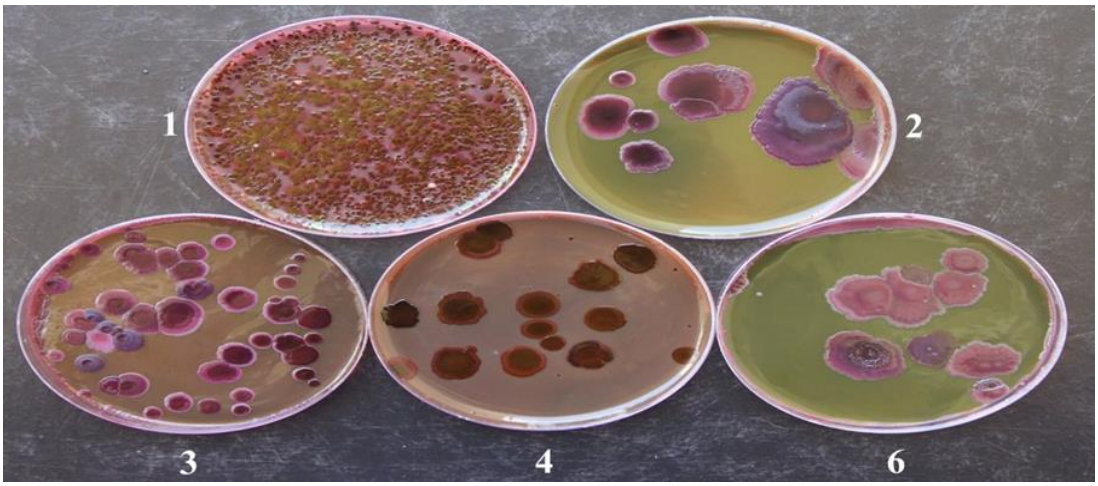

Figure 18: *E. coli* on methylene blue agar medium (Levine) (Exp 1, after 4 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and Treatment-6: 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

Figure 19: Lactose/non-lactose fermenting enterobacteriaceae on MacConeky (Exp 1, after 4 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and Treatment-6: 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

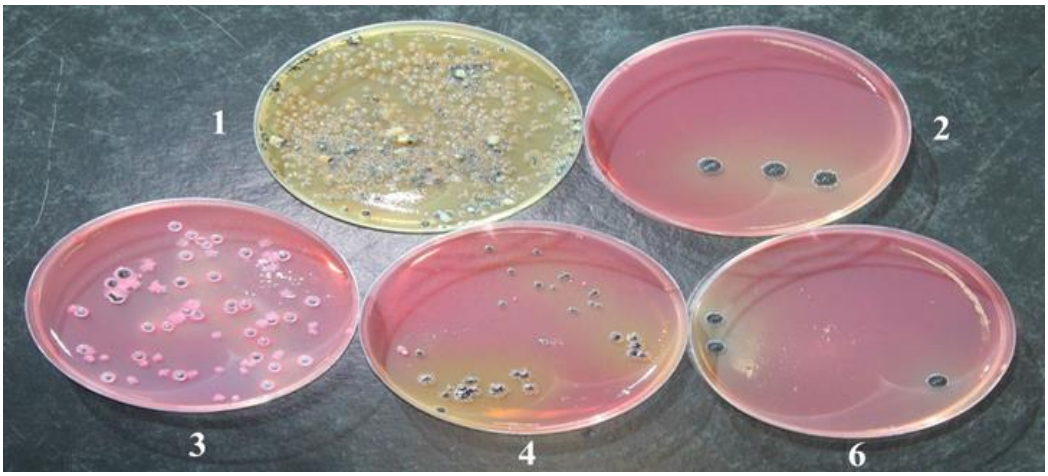

Figure 20: *Shigella* spp. and *Shigella* spp. on *Shigella-Shigella* agar (Exp 1, after 4 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and Treatment-6: 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

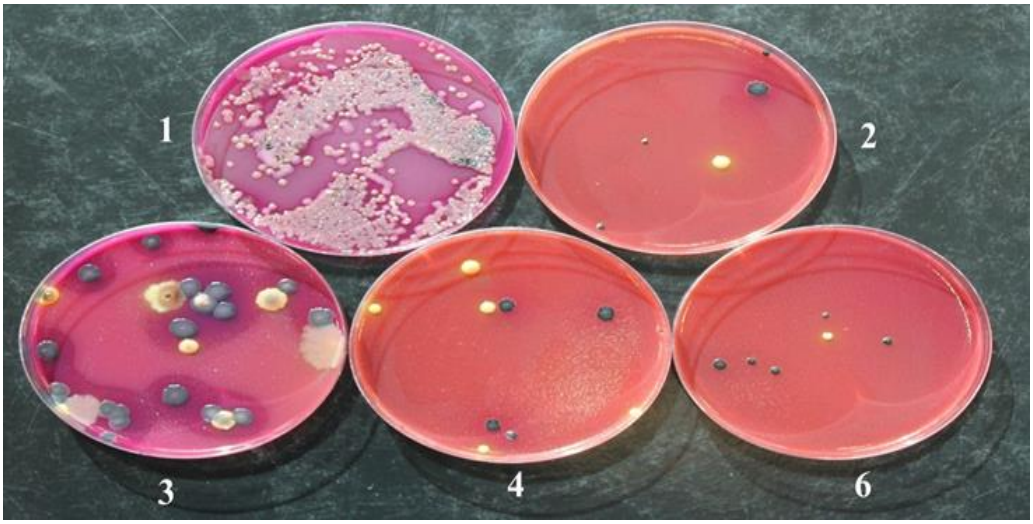

Figure 21: *Shigella* spp. on xylose lysine decarboxylase agar medium (Exp 1, after 4 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; and Treatment-6: 10% non-degraded date pits corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for five weeks.

4.4.5 Effect of corn-soy, DDP and Diet with Oxytetracycline on Total Bacterial Count in Broilers After 5 Weeks (Experiment 1)

The results are shown in Table 17 and Figures 22-26. The generated findings revealed that in 10% DDP corn-soy diet fed-broilers for five weeks and antibiotic diet fed-broilers, the bacterial count (total bacterial count, *E. coli*, enterobacteriaceae, *Shigella and Salmonella*) was similarly and significantly decreased compared to the control soybean diet without antibiotic. The bacterial count (total bacterial count, *E. coli*, enterobacteriaceae, *Shigella and Salmonella*) was progressively decreased in broilers fed 10% DDP corn-soy diet with prolonged feeding period, showing significant lower levels at four and five weeks of age compared to 3 weeks of age.

Table 17: Bacterial population densities in chicken gut tissue after five weeks

Treatments [a]	**Total bacterial** [b]	**Lactose fermenting enterobactericeae** [d]	**Non- lactose fermenting enterobactericeae** [d]	***E. coli*** [c]	***Shigella* spp.** [e]	***Salmonella* spp.** [e]	***Salmonella* spp.** [f]
(1)	10.84±0.36*a*	5.32±0.15*a*	4.61±0.16*a*	5.66±0.10 *a*	4.01±0.07*a*	4.42±0.13*a*	4.64±0.20*a*
(2)	1.98±0.14 *d*	1.04±0.12*d*	0.72±0.05*d*	1.33±0.12*d*	0.56±0.06 *d*	0.68±0.09*d*	0.46±0.12*d*
(3)	5.55±0.19*b*	3.45±0.17*b*	3.25±0.21*b*	3.80±0.14*b*	3.11±0.07*b*	3.42±0.1*b*	3.61±0.07*b*
(4)	3.66±0.12*c*	2.76±0.14*c*	2.53±0.13*c*	3.06±0.30*c*	2.64±0.05*c*	2.45±0.09*c*	2.71±0.08*c*
(5)	2.04±0.10*d*	1.18±0.09*d*	0.90±0.17*d*	1.41±0.05*d*	0.43±0.09 *d*	0.47±0.10*d*	0.62±0.10*d*
(6)	1.91±0.11*d*	1.27±0.07*d*	0.88±0.15*d*	1.28±0.05*d*	0.36±0.07*d*	0.68±0.11*d*	0.75±0.02*d*

Index:

[a] Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment (5): 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

[b] Total bacterial counts on nutrient agar medium. [c] *Escherichia coli* on eosin methylene blue agar medium (Levine agar medium).

[d] Lactose-fermenting enterobacteriaceae and non-lactose-fermenting enterobacteriaceae on MacConkey agar medium.

[e] *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* agar medium (S.S. agar medium).

[f] *Salmonella* spp. on xylose lysine decarboxylase (XLD) agar medium.

Values are means of six independent replicates for each treatment, and the values in parentheses are the standard error of the mean. Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

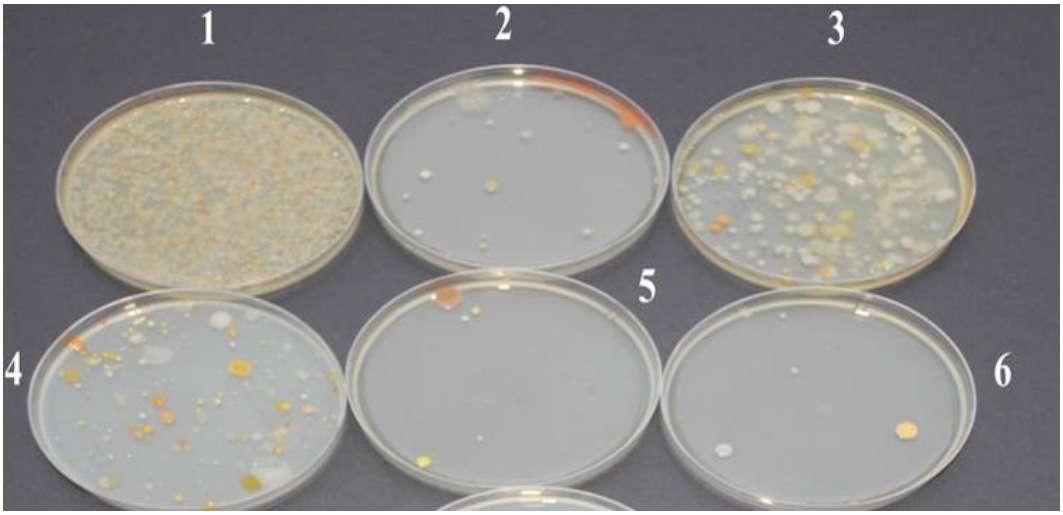

Figure 22: Total bacteria on nutrient agar medium (Exp 1, after 5 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment-5: 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

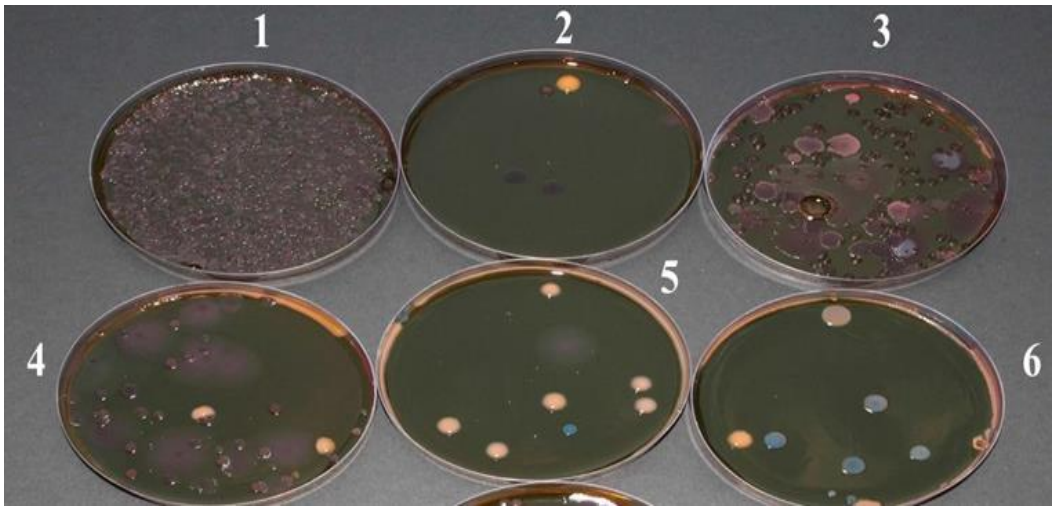

Figure 23: *E. coli* on eosin methylene blue agar medium (Levine) (Exp 1, after 5 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment-5: 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

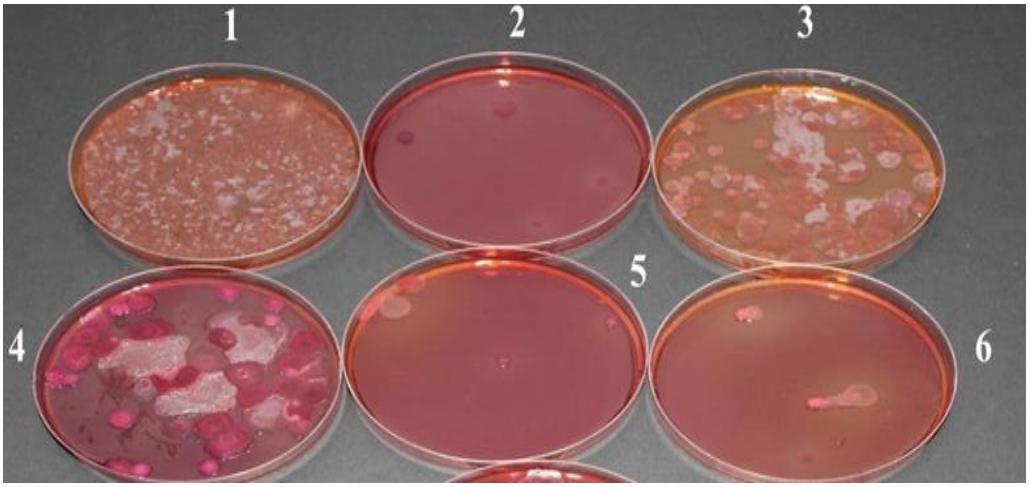

Figure 24: Lactose/non-lactose fermenting enterobacteriaceae on MacConeky (Exp 1, after 5 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment-5: 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

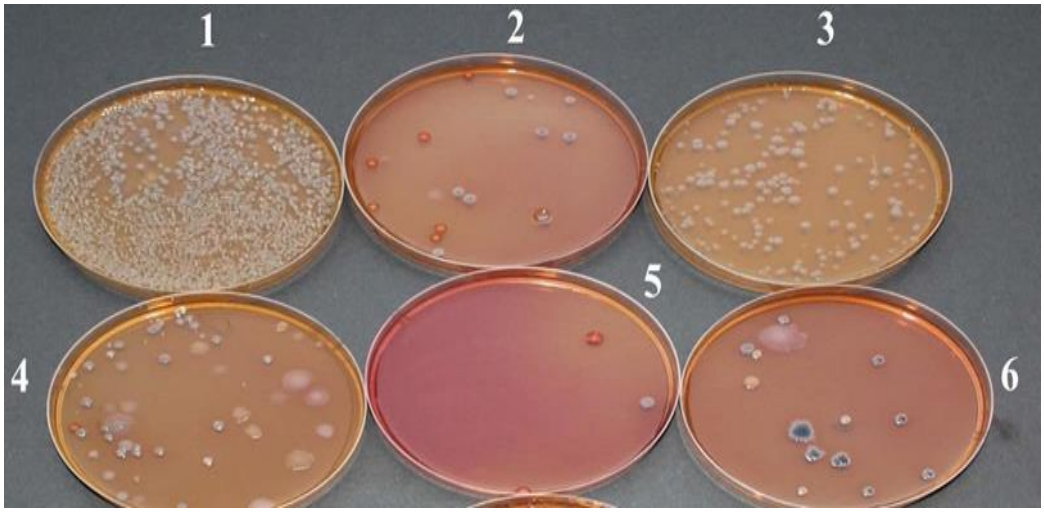

Figure 25: *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* agar (Exp 1, after 5 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment-5: 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

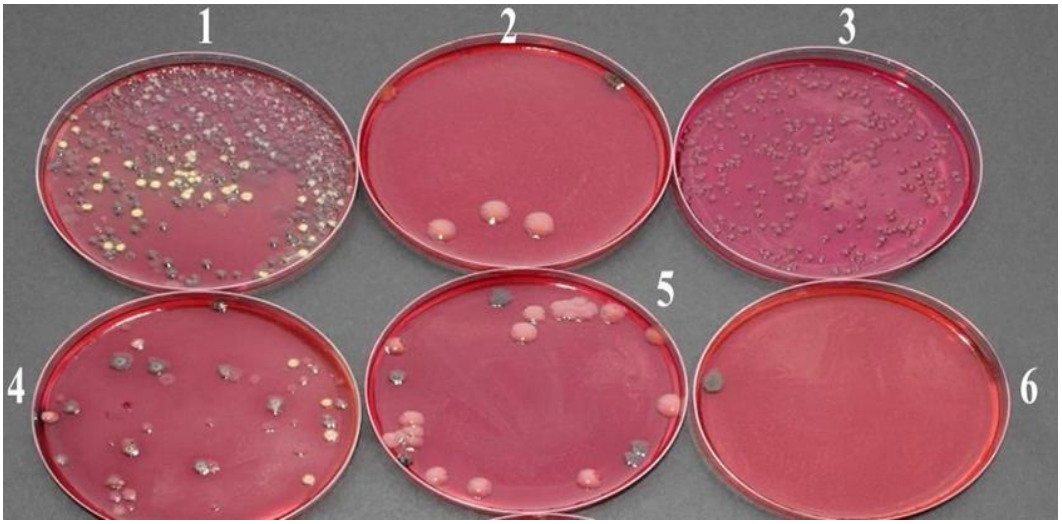

Figure 26: *Shigella* spp. on xylose lysine decarboxylase (XLD) agar (Exp 1, after 5 weeks)

Treatment-1: Control; Treatment-2: Control with antibiotics; Treatment-3: 10% DDP corn-soy diet for 3 weeks then the remaining chicks were treated with (control without antibiotics) for 2 weeks; Treatment-4: 10% DDP corn-soy diet for 4 weeks then the remaining chicks were treated with (control without antibiotics) for 1 week; Treatment-5: 10% DDP corn-soy diet for 5 weeks; Treatment-6: 10% non-degraded DP corn-soy diet + antibiotic added (Oxytetracycline 20%, 50 g/100 kg) for 5 weeks.

4.4.6 Effect of DDP, MOS, and Mannose on Total Bacterial Count-Starter Broiler Gut (Experiment 2)

The generated results in Table 18 and Figures 27-33 showed that 10% DDP corn-soy diet fed-broilers, 0.2% MOS and antibiotic diet fed-broilers the bacterial populations (total bacterial count, *E. coli*, enterobacteriaceae, *Shigella and Salmonella* count) was significantly decreased compared to the control soybean meal diet, and mannose 0.1 and 0.2% goups , but *Lactobacillus* and *Bifidobacterium* count were increased. Meanwhile, the antibiotic supplemented groups exhibited the strong effect.

Table 18: Bacterial population densities in chicken gut tissue after three weeks (Experiment 2)

Treatment [a]	**Total bacteria** [b]	**Lactose fermenting enterobactericeae** [d]	**Non- lactose fermenting enterobactericeae** [d]	***E. coli*** [c]	***Shigella* spp.** [e]	***Salmonella* spp.** [e]	***Salmonella* spp.** [f]	***Lactobacillus* spp.** [g]	***Bifidobacterium* spp.** [g]
(1)	8.03±0.15^{a}	5.59±(0.13)^{a}	4.56±(0.35)^{a}	5.98±0.07^{a}	3.85±(0.10)^{a}	3.35±(0.06)^{a}	2.64±(0.15)^{a}	3.84±(0.26)^{c}	3.82±(0.14)^{c}
(2)	1.41±0.23^{c}	1.37±(0.17)^{c}	1.32±(0.15)^{c}	1.40±0.15^{c}	1.29±(0.09)^{c}	0.93±(0.10)^{c}	0.93±(0.10)^{c}	2.44±(0.21)^{d}	2.32±(0.18)^{d}
(3)	2.73±0.09^{b}	2.68±(0.18)^{b}	2.23±(0.12)^{b}	3.47±(0.17)^{b}	2.05±(0.04)^{b}	2.31±(0.09)^{b}	1.92±(0.09)^{b}	7.84±(0.20)^{a}	7.29±(0.20)^{a}
(4)	2.26±0.09^{b}	2.55±(0.25)^{b}	2.49±(0.36)^{b}	3.35±(0.12)^{b}	2.29±(0.25)^{b}	2.18±(0.20)^{b}	1.68±(0.19)^{b}	7.55±(0.14)^{a}	7.13±(0.05)^{a}
(5)	7.56±0.27^{a}	5.89±(0.01)^{a}	4.49±(0.25)^{a}	5.55±(0.11)^{a}	3.61±(0.27)^{a}	3.22±(0.19)^{a}	2.77±(0.23)^{a}	5.58±(0.11)^{b}	5.28±(0.08)^{b}
(6)	7.86±0.11^{a}	5.44±(0.17)^{a}	4.62±(0.16)^{a}	5.69±(0.25)^{a}	3.56±(0.08)^{a}	3.01±(0.06)^{a}	2.92±(0.07)^{a}	5.88±(0.23)^{b}	5.66±(0.22)^{b}

Index:

[a] Treatment1: Control (only corn-soy diet) for three weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for three weeks; Treatment-3: Corn-soy diet amended with 10% DDP for three weeks ; Treatment-4: Corn-soy diet amended with Mann oligosaccharide (MOS) (0.2%) (2 g/kg) for three weeks.Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for three weeks; Treatment- 6: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for three weeks;

[b] Total bacterial counts on nutrient agar medium.[c] *Escherichia coli* on eosin methylene blue agar medium (Levine).

[d] Lactose was fermenting enterobacteriaceae and non-lactose fermenting enterobacteriaceae on MacConkey agar medium.

[e] *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* agar medium. [f] *Salmonella* spp. on xylose lysine decarboxylase (XLD) agar medium.

[g] *Lactobacillus* spp. and *Bifidobacterium* [g] spp. on MRS broth.

Values are means of six independent replicates for each treatment, and the values in parentheses are the standard error of the mean. Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

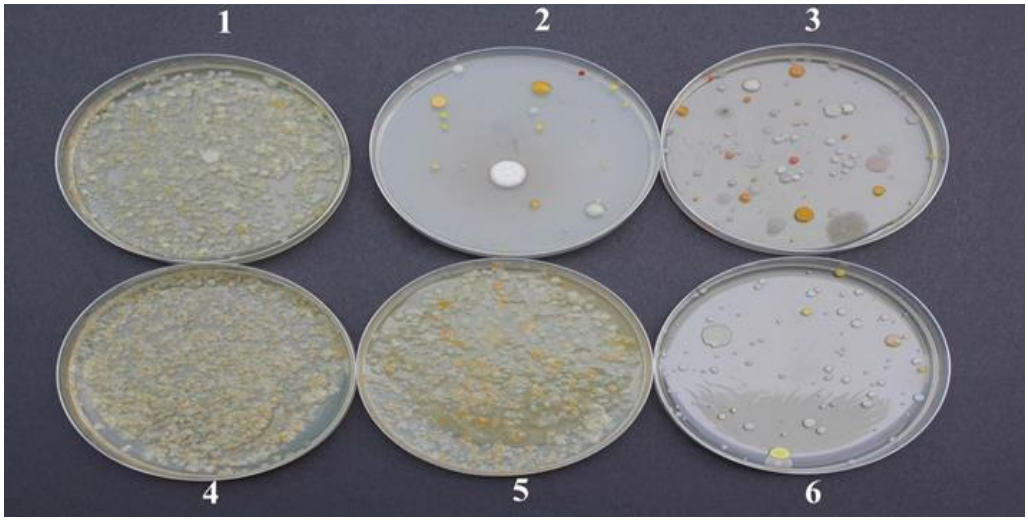

Figure 27: Total bacteria on nutrient agar medium (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

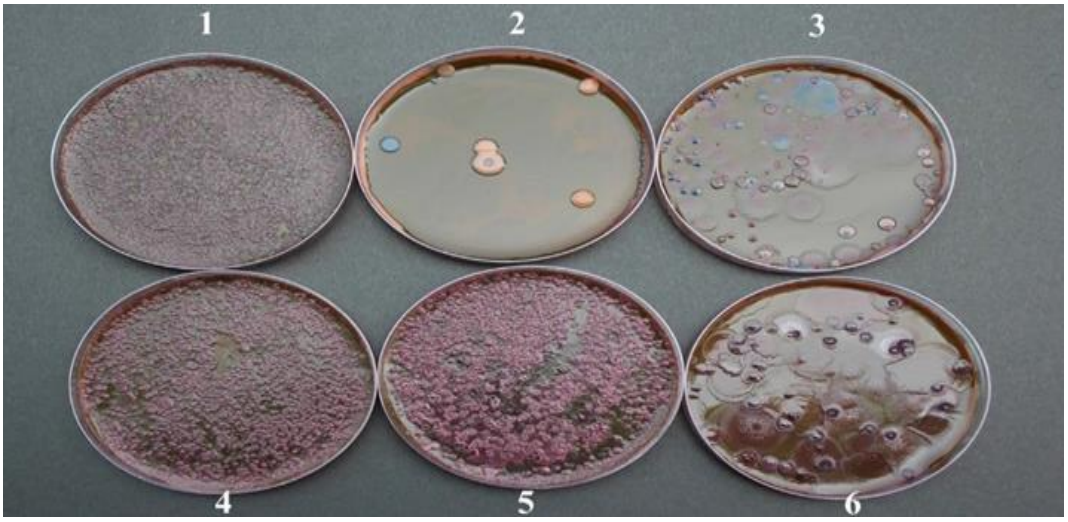

Figure 28: *E. coli* on eosin methylene blue agar medium (Levine) (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

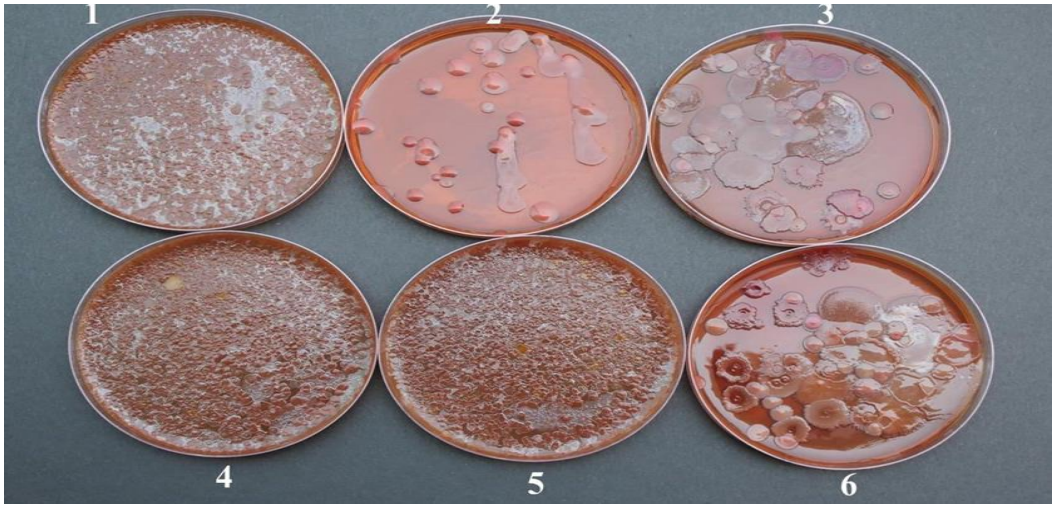

Figure 29: Lactose/non-lactose fermenting enterobacteriaceae on MacConeky agar (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

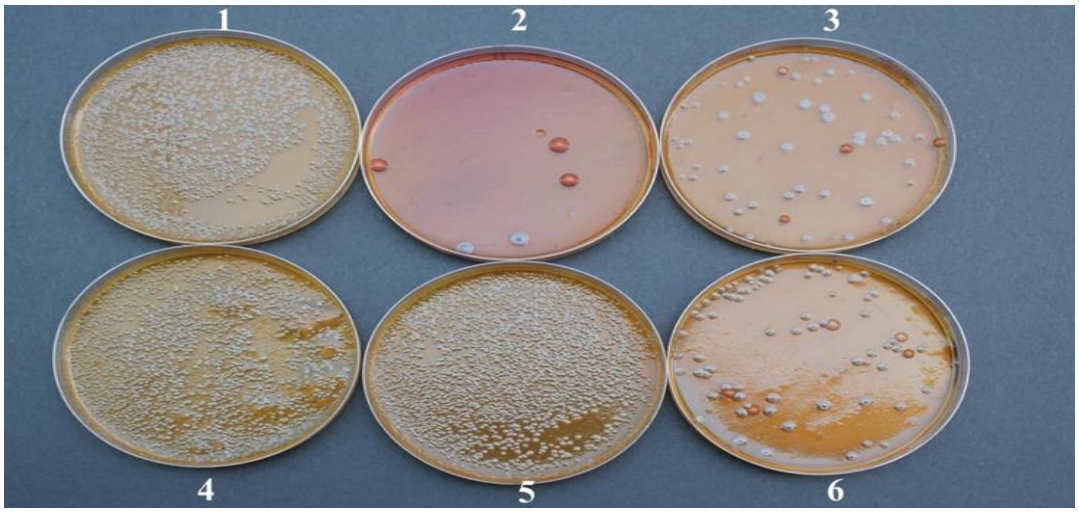

Figure 30: *Shigella* spp. & *Shigella* spp. on *Shigella-Shigella* agar medium (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

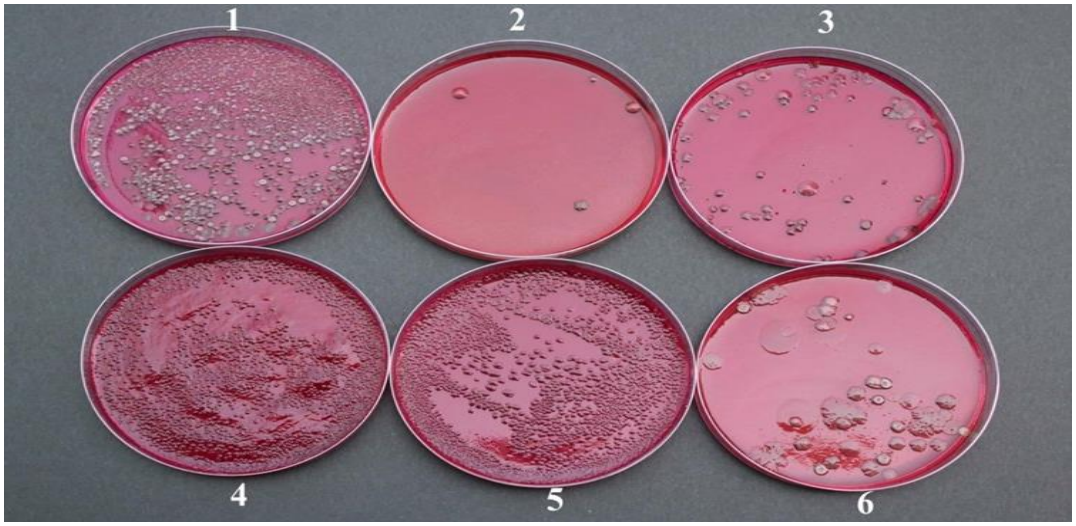

Figure 31: *Shigella* spp. on xylose lysine decarboxylase (XLD) agar medium (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

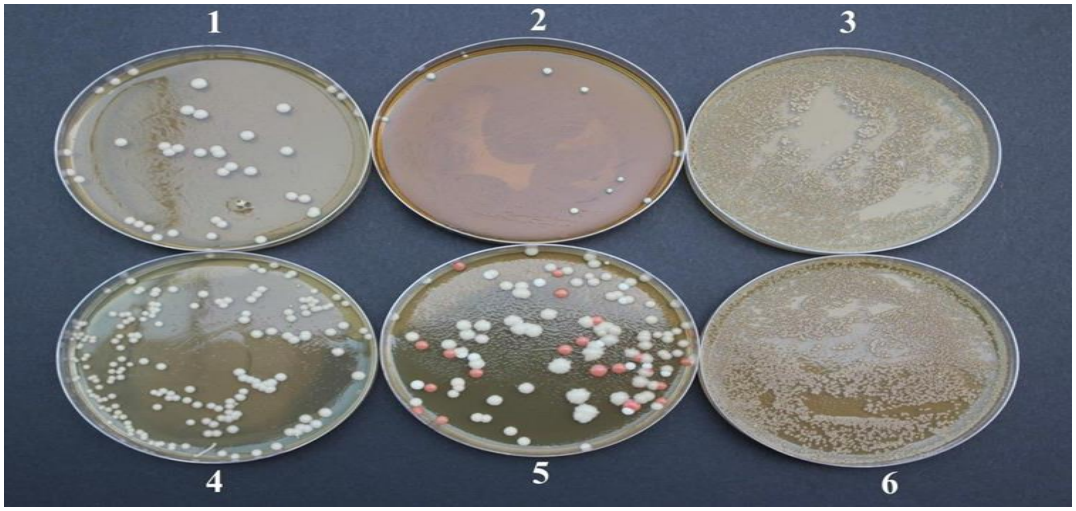

Figure 32: *Lactobacillus* spp. on MRS broth (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

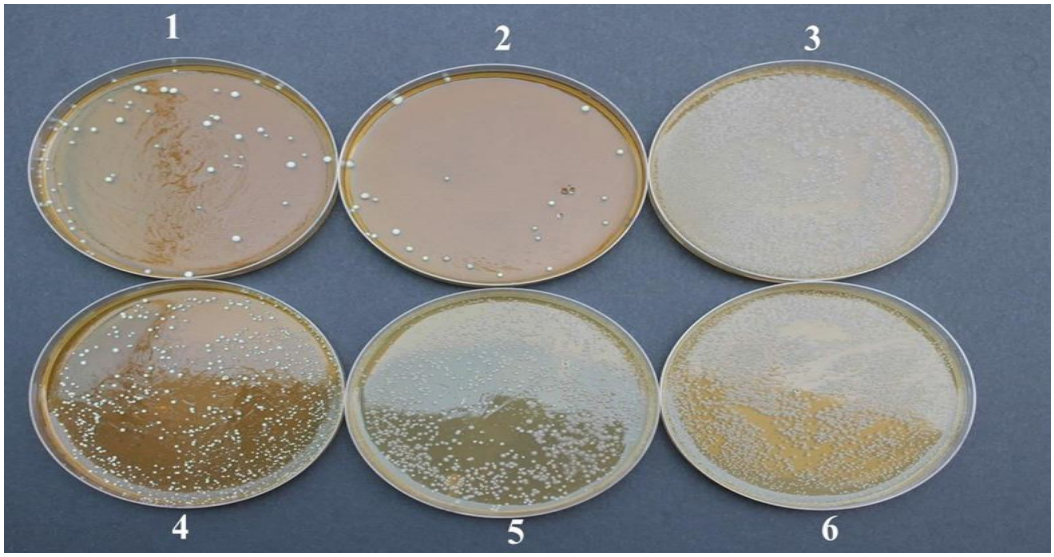

Figure 33: *Bifidobacterium* spp. on *Bifidobacterium* agar (Exp 2, after 3 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

4.4.7 Effect of DDP, MOS, and Mannose on Total Bacterial Count-Finisher after 6 Weeks in Broiler Gut (Experiment 2)

The results displayed in Table 19 and Figures 34-40 shows the obtained results revealed that the bacterial count (total bacterial count, *E. coli*, enterobacteriaceae, *Shigella and Salmonella* count) was similarly and significantly decreased in 10% DDP diet fed-broilers, 0.2% MOS and antibiotic diet fed-broilers compared to corn-soybean meal control diet and both mannose supplemented groups. The later groups showed also similar counts. It was found that dietary supplementation of DDP and Manno-oligosaccharide significantly increased *Lactobacillus* and *Bifidobacterium* count in broilers compared to the other groups.

Table 19: Bacterial population densities in chicken gut tissue after six weeks

Treatment[a]	Total bacteria [b]	Lactose fermenting enterobactericeae [d]	Non- lactose fermenting enterobactericeae [d]	*E. coli* [c]	*Shigella* spp[e]	*Salmonella* spp. [e]	*Salmonella* spp. [f]	*Lactobacillus* spp. [g]	*Bifidobacterium* spp. [g]
(1)	7.68±(0.43)^a	4.47±(0.09)^a	.458±(0.18)^a	5.96±(0.20)^a	3.32±(0.28)^a	2.85±(0.15)^a	.245±(0.16)^a	3.43±(0.23)^c	3.08±(0.10)^c
(2)	1.28±(0.14)^b	0.89±(0.20)^b	0.51±(0.09)^b	0.94±(0.05)^b	.033±(0.07)^b	.021±(0.03)^b	0.18±(0.04)^b	2.31±(0.08)^d	2.16±(0.19)^d
(3)	1.72±(0.15)^b	1.08±(0.15)^b	0.72±(0.03)^b	1.15±(0.10)^b	0.25±(0.05)^b	.032±(0.11)^b	.020±(0.06)^b	7.79±(0.34)^a	5.53±(0.28)^a
(4)	1.92±(0.18)^b	1.22±(0.17)^b	0.68±(0.08)^b	1.41±(0. 02)^b	0.36±(0.17)^b	.023±(0.06)^b	0.17±(0.04)^b	7.98±(0.21)^a	5.88±(0.43)^a
(5)	8.24±(0.11)^a	4.92±(0.13)^a	4.35±(0.15)^a	5.44±(0.46)^a	3.53±(0.24)^a	2.62±(0.28)^a	.284±(0.16)^a	4.97±(0.56)^b	4.55±(0.22)^b
(6)	8.08±(0.10)^a	4.84±(0.09)^a	4.25±(0.26)^a	5.56±(0.12)^a	3.71±(0.42)	2.51±(0.13)^a	.237±(0.25)^a	5.30±(0.19)^b	4.09±(0.14)^b

Index:

[a] Treatment1: Control (only corn-soy diet) for three weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for three weeks; Treatment-3: Corn-soy diet amended with 10% DDP for three weeks; Treatment-4: Corn-soy diet amended with Mann oligosaccharide (MOS) (0.2%) (2 g/kg) for three weeks.Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for three weeks; Treatment- 6: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for three weeks;

[b] Total bacterial counts on nutrient agar medium. [c] *Escherichia coli* on eosin methylene blue agar medium (Levine).

[d] Lactose-fermenting enterobacteriaceae and non-lactose fermenting enterobacteriaceae on MacConkey agar medium.

[e] *Shigella* spp. and *Salmonella* spp. on *Salmonella-Shigella* agar medium. [f] *Salmonella* spp. on xylose lysine decarboxylase (XLD) agar medium**.**

[g] *Lactobacillus* spp. and *Bifidobacterium* [g] spp. on MRS broth.

Values are means of six independent replicates for each treatment, and the values in parentheses are the standard error of the mean. Values followed by the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

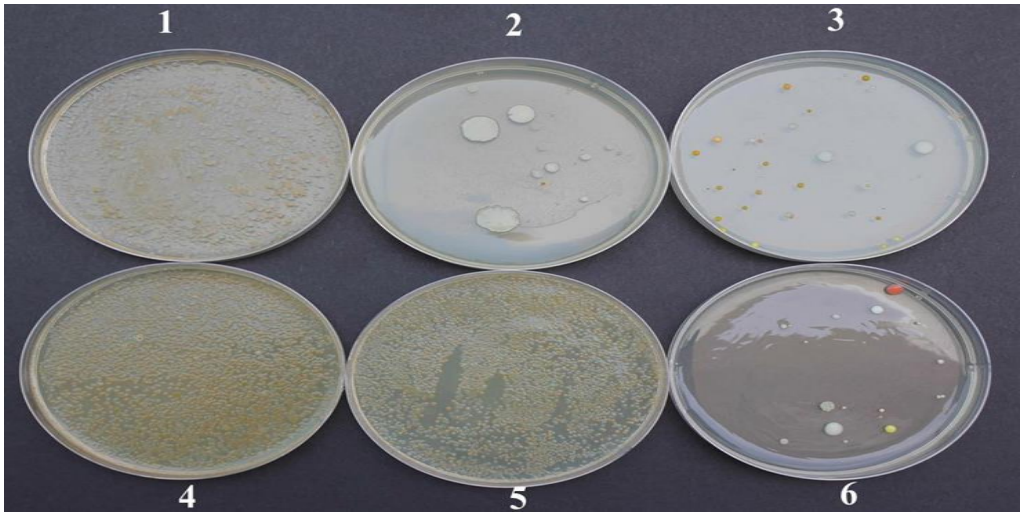

Figure 34: Total bacteria on nutrient agar medium (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

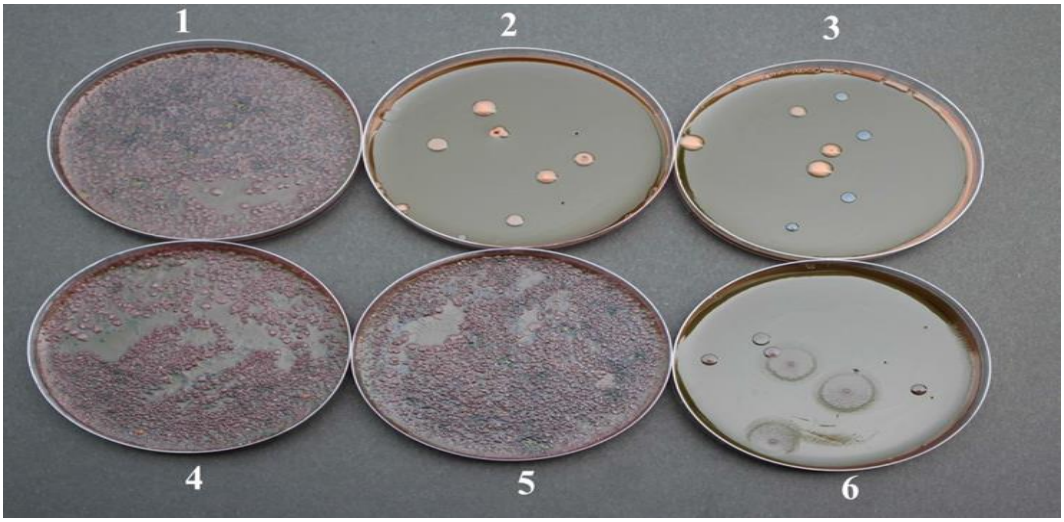

Figure 35: *E. coli* on eosin methylene blue agar medium (Levine) (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

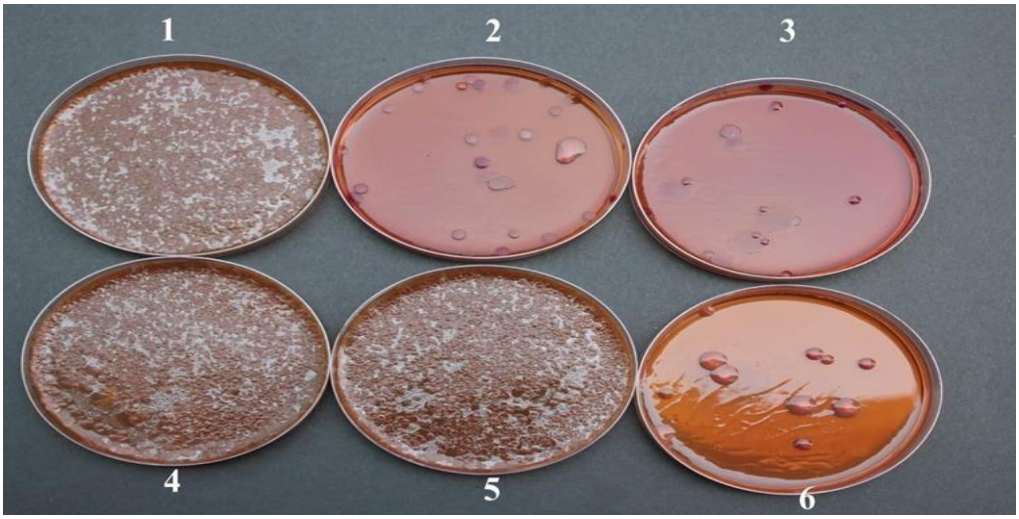

Figure 36: Lactose/non-lactose fermenting enterobacteriaceae on MacConkey agar (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

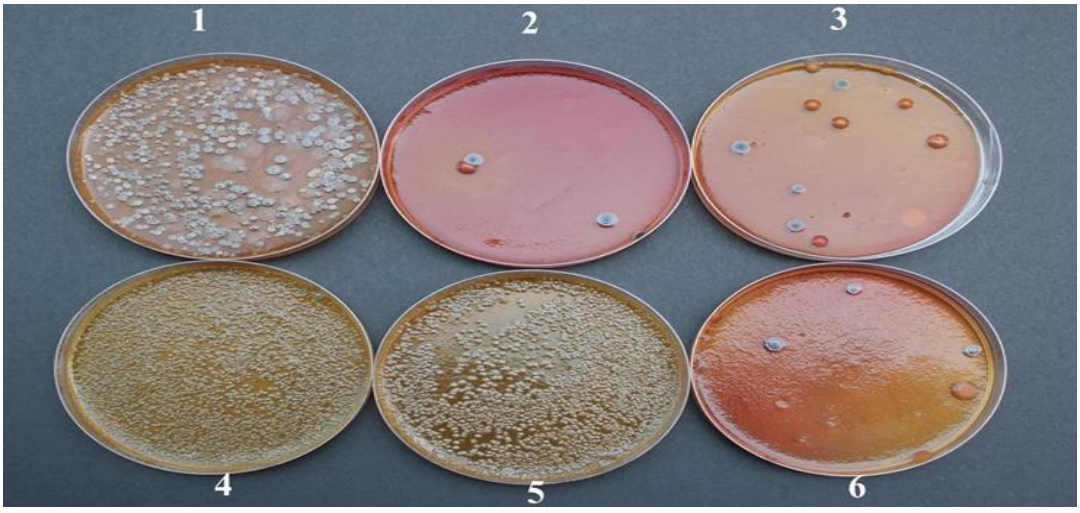

Figure 37: *Shigella* spp. & *Salmonella* spp. on *Salmonella-Shigella* agar medium (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

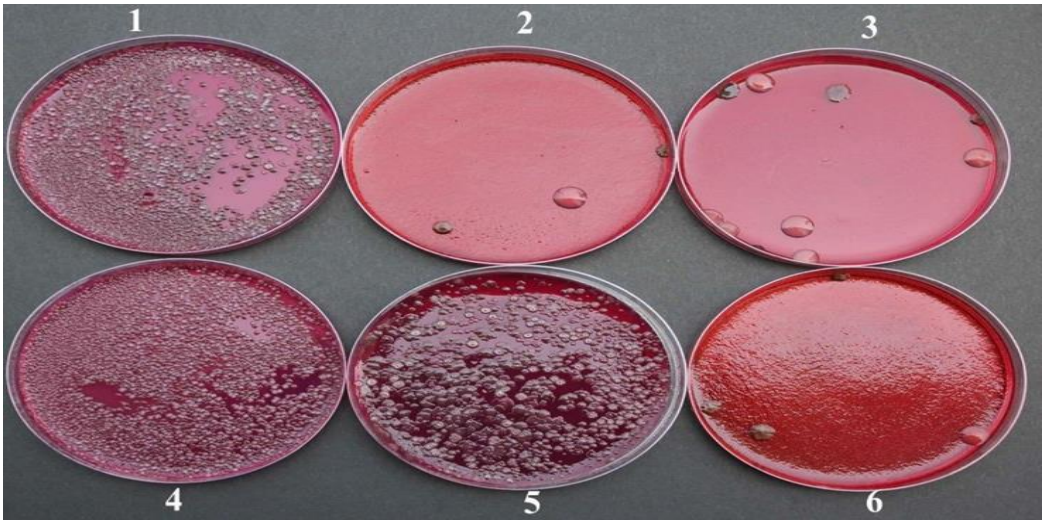

Figure 38: *Salmonella* spp. on xylose lysine decarboxylase (XLD) agar medium (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

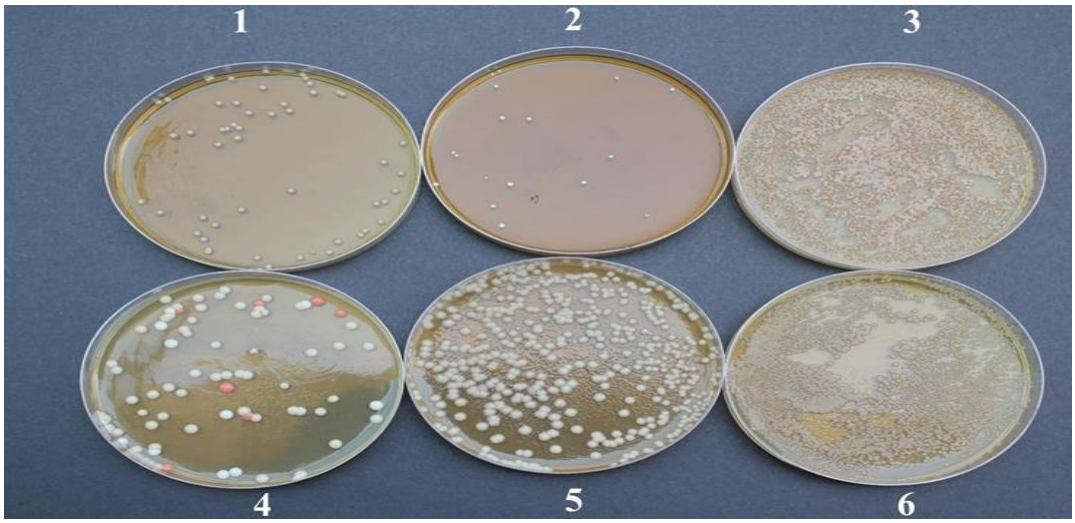

Figure 39: *Lactobacillus* spp. on M.R.S. broth (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

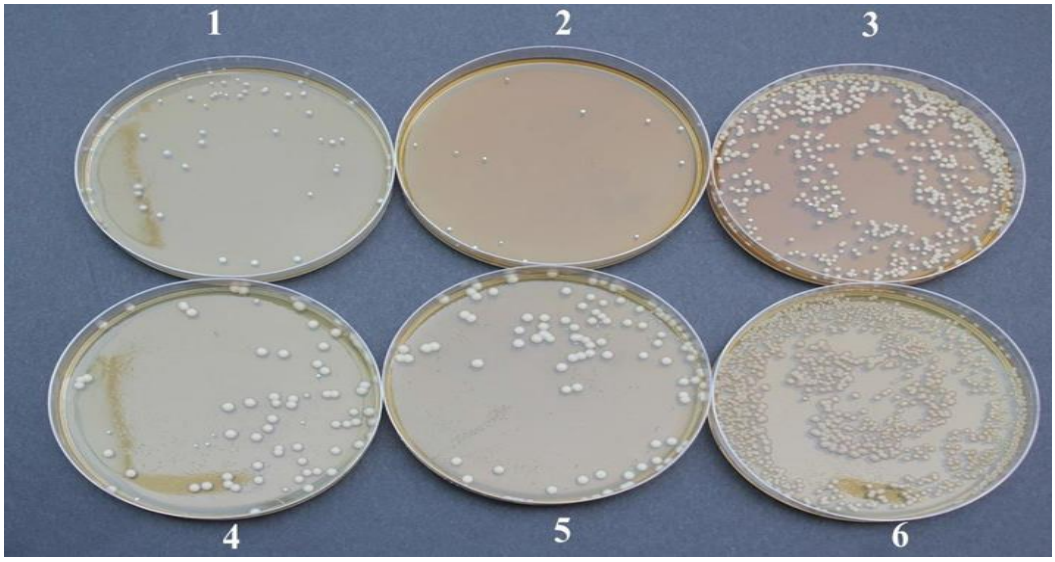

Figure 40: *Bifidobacterium* spp. on *Bifidobacterium* agar (Exp 2, after 6 weeks)

Treatment-1: Control (only corn-soy diet) for 3 weeks; Treatment-2: Corn-soy diet amended with antibiotics (Oxytetracycline 20%, 50 g/100 kg) for 3 weeks; Treatment-3: Corn-soy diet amended with 10% DDP for 3 week; Treatment-4: Corn-soy diet amended with mannose (0.1%) (1 g/kg) for 3 weeks; Treatment-5: Corn-soy diet amended with mannose (0.2%) (2 g/kg) for 3 weeks; Treatment-6: Corn-soy diet amended with Mann Oligosaccharide (MOS) (0.2%) (2 g/kg) for 3 weeks.

4.5 Discussion

Feed in poultry, plays a major role in preserving poultry health. The type of the diet and feed composition supplemented to poultry cause alterations in the growth performance of broilers and gut health. Grains, like oats or corn are now excessively used as ingredients in animal feed and biofuel production. Natural carbohydrate polymers possess prebiotic properties and widely used in poultry feeds. Fungi DDP is a good source of carbohydrate and fiber that can be used as poultry feed. The non-digestible carbohydrates have attracted increasing attention over the last decades, due to their biological functions in addition to their roles as structural materials and energy sources (Mudgil and Barak, 2013). Many studies have revealed that these natural

carbohydrate polymers possess prebiotic properties and are widely used in animal feeds (Macfarlane and Cummings, 1999).

Recent studies showed that treatment of seeds with microorganisms like fungi could further enhance the nutritional and medicinal values of edible seeds (Belal, 2008; Hashim et al., 2013). Fungi treatment leads to the catabolism and degradation of main macronutrients such as carbohydrates, protein, and fatty acids, accompanied by an increase of simple sugars, free amino acids and organic acids (Gerardi, 2003). Date Pits represents a relevant waste material (Herch et al., 2014). Microbes like fungi prefer warm temperatures, and they grow in warm conditions (Ayerst, 1969). Ground DP provides a stable media for the multiplication of fungi, as they limit the airflow and trap the warm air inside. The fungus *Trichoderma reesei* is very efficient in Solid state Degradation (SSD) of DP and increased the chemical composition of DP (Sachslehner et al., 1998). Carbohydrates in DP decompose to carbon dioxide, water and sugar, which is used by fungi during respiration (Hashim et al., 2013). Shastak et al. (2015) reported that carbohydrate moieties in poultry diets have substantial positive effects for growth, nitrogen use and retention time of diets in the alimentary canal of young chicks.

4.5.1 Effect of Using DDP and Non-DDP with Antibiotics in Comparison with Control and Control with Antibiotics on Total Bacterial Count in Intestine (Experiment 1)

This Section discusses the potential effects of using DDP and non-degraded date pits with antibiotic on growth and total bacterial count in the chicken gut in comparison with control and control with antibiotics. In the present study, results indicated that the 10% DDP diet for five weeks is more effective in supporting average weight gain than the control diet and the DDP itself when added for three or four weeks. It gave similar

results when compared with the control diet with aAntibiotic. A significant improvement in weight gain was found in broilers when fed diet with DP at levels of 5%, 10% and 15% in different periods and showed similar body weight when compared to the antibiotic diet fed chicks (Kamel et al., 1981).

All these data give evidence that using DDP in broiler chickens supports its growth and improves the average body weight. Adding 20% oxytetracycline to the broiler diets has a positive effect by increasing the weight. This agrees with the effect of Oxytetracycline on growth and lipid metabolization in poultry where results showed that Oxytetracycline increased body weight gain in one-day-old chicks (Jumah et al., 1973). The results also showed that broiler diets substituted with 10% DDP has no difference in feed intake of broilers when compared with broilers fed corn diets, and DDP could be included in the broiler diets instead of corn and Oxytetracycline.

The present finding was in agreement with Salma et al. (2007) who found that the FI of broilers had no significant differences by dietary inclusion of feed additives like probiotics in the diet. Comparable results were also obtained by Jung et al. (2008), who found that the addition of prebiotic and probiotic did not have any significant alterations in FI of broiler chickens. The findings of using DDP in the broilers FCR are encouraging. DDP did not enhance the feed consumption of broilers but improved the FCR. Jumah et al. (1973) showed that the feed intake of chicks receiving DP in their diet was higher than the feed intake by birds having no DP in their diet. Moreover, Vandepopuliere et al. (1995) showed that adding 5–27% of DP to the starter diet of broiler birds supported feed intake. As an overall, the results indicating a replacement of 10% of the corn by DP in broiler feed.

Microbial populations in the gastrointestinal tract vary with animal species and their interaction with host animals, dietary constituents and the environment of the gastrointestinal tract (Berg, 1996). A balanced microbial population in the intestinal tract plays a critical role in the growth, and these microbes maintain the health of the animals (Round and Mazmanian, 2009). Bacteria present in the intestine metabolises the nutrients present in the diets thereby produces lactic acid, short-chain fatty acids and some synthesized vitamins. Some of these activities can be beneficial to the host animals (Kamel et al., 1981).

It is essential to consider that some feed additives extracted from plant products and probiotics have a substantial effect on gut microflora either directly or indirectly (Cowan, 1999); (Attia et al., 2012). The mechanism of action of prebiotic includes the gut microbiota modulation by providing them with food, which means a selectivity regulation (Hajati and Rezaei, 2010) and reducing the competition ability of undesired pathogenic bacteria in the intestine which increases the gut mucosa (Iji and Tivey, 1998).

The inclusion of DDP in broilers diet resulted in a low number of the total bacterial count in the chicken gut (as seen in Table 15), and this result is agreed with (Eid et al., 2014). Studies have shown that plant-based nutraceuticals improved the growth performance of broiler chickens, similar to those of antibiotic growth promoters (Windisch et al., 2008; El-Ghany and Ismail, 2013; Attia et al., 2017b). Anti-microbial activity and the increase of the immune system strength are considered as the two essential mechanisms through which plant-origin compounds affect beneficially the health and growth performance of poultry (Fallah et al., 2013; Attia et al., 2019).

The potential of phytogenic bioactive compounds to motivate the reproduction and the development of absorption cells in the GI tract and to enhance the production and performance of the digestive enzymes which enhances the growth performance of birds (Vidanarachchi et al., 2013). As an overall, it should be obvious that the effectiveness of phytobiotics as feed additives and their effect on the gut health and overall performance might differ as a result of the difference in their composition due to some biological agents (plant types, growing site, and harvest environment).

The birds receive little nutritional advantage from hindgut microflora compared with other species of animals (Gibson and Rastall, 2005; Attia et al., 2017b). Broilers fed 10% DDP diet for five weeks showed the highest reduction in the microbial gut count, which means that prolonged feeding period of DP diet for five weeks is more efficient than three or four weeks. Guo et al. (2004) reported that herbal seeds were associated with reduced *Bacteroides* spp., *Enterococci*, and *E. coli* numbers compared with the control and antibiotic treatment groups. In earlier studies, significant reductions of *E. coli* and *Clostridium* have been reported after utilizing natural plant extracts (Jamroz et al., 2006).

The present study results, also shows that *Shigella* spp. was reduced by 2.68% after five weeks compared with the results after three weeks and by 3.6% compared to the control corn-soy diet (as seen in Table 16), which agrees with rule of prebiotics that has the ability to reduce the gastrointestinal tract pathogens (Gibson and Rastall, 2005). *Salmonella* spp. was reduced four times compared with treatment1 which indicate that using 10% DDP as a feed additive for five weeks gives an excellent control to the *Salmonella* spp. (as seen in Table 17), which considered as a considerable pathogen

for animals that used for food production and these animals are the essential sources of salmonellosis (Forshell and Wierup, 2006); (Attia et al., 2012).

4.5.2 The Effect of Using DDP, MOS, and Mannose on Growth and Total Bacterial Count in Chicken Gut, in Comparison with Control and Control with Antibiotics (Experiment 2)

In the present study, results regarding growth rate, feed intake and FCR, indicate that the 10% DDP for six weeks can replace the corn-soy diet and give a sufficient average body weight (Table 14**)**. When compared to 0.2% Mannan-oligosaccharides, 0.1% mannose and control with antibiotic diet fed-broilers, 10% DDP fed-broilers showed no significant difference in body weight, feed intake and FCR. It was also better than control and 0.2% mannose fed broiler. All these data give evidence that using DDP supports growth and improves the average weight gain of broilers.

Vandepopuliere et al. (1995) found that the broiler chicks fed DP diets showed similar growth performance as the control, which proves that using DP in broiler chicks supports chicken growth and improves the average weight gain. Adding 20% oxytetracycline to the broiler diets has a positive effect by increasing the weight when compared to control and mannose diets. This agrees with the effect of oxytetracycline on growth and lipid metabolism in poultry where results showed that oxytetracycline increased body weight gain in one-day-old chicks (Jumah, 1973).

The results also showed that broiler diets substituted with 10% DDP has no difference in feed intake of broilers when compared with broilers fed corn diets and had similar mode of action to MOS. Thus, DDP can be included in the broiler diets at 10% without needs of antibiotic supplementation. The present finding was in agreement with Salma et al. (2007) who reported that FI of broilers did not change significantly by dietary

composition of feed additives like probiotics in the diet. Comparable results were also obtained by Jung et al. (2008) who found that the addition of prebiotic and probiotic did not have any significant effect on FI of broiler chickens.

The potential of phytogenic bioactive compounds to catalyze the production and development of absorptive cells in the GIT and to positively affect the digestive enzymes production and action which enhances the growth performance of birds (Vidanarachchi et al., 2013). As an overall, the effectiveness of prebiotics as feed additives and their effect on the GIT health and development might differ due to the differences in their composition because of the biological factors and harvest conditions. However, birds receive little nutritional advantage from intestinal microflora compared with other species of animals. Broilers fed with 10% DDP diet showed a significant reduction of microbial count in the gut, which indicates that DDP diet is beneficial and gives microbial count in the gut, which indicates that DDP diet.

Enumeration of bacterial count in the gut showed that 10% DDP for six weeks decreases bacterial count significantly (Table 19). The poultry gut habitats various microflora, including both beneficial and pathogenic microorganisms (Pan and Yu, 2014). The attachment to mucosal surfaces is a precondition for effective colonisation of enteric bacteria. Gram-negative pathogens that express type-1 fimbriae, such as *Salmonella* and *E. coli*, recognise mannose receptor sites on the intestinal epithelium and absorb the nutrition elements (Croxen et al., 2013). *Salmonella* spp. The count has decreased in the caeca of broilers fed diets containing MOS (Fernandez et al., 2002). In 0.2% MOS diet fed-broilers *E. coli* populations were supressed in the digestive tracts especially in caecum and ileum (Spring et al., 2000). Jamroz et al. (2006) reported that broilers fed MOS on starter, grower and finisher periods at varying doses

from 0.05% to 0.2% had decreased population of *E. coli* in the caecum and jejunum. When compared to antibiotic growth promoters like Avilamycin 0.001%), feeding 0.2% MOS on starter and finisher period showed no significant change on caecal coliforms (Denev, 2006).

In the current study, the results showed that supplementation of DDP and 0.2% MOS increases beneficial bacteria count. In the intestines of broilers, MOS at 0.2% and 0.5% enhances the number of beneficial bacteria, mainly *Lactobacillus* and *Bifidobacterium* spp. when compared to bacitracin (0.006%) fed birds (Baurhoo et al., 2007). Counting *Lactobacillus* spp. was significantly increased in MOS diet fed-broilers at various dietary inclusion levels which are involved in increasing the intestinal growth of the broilers (Fernandez et al., 2002). Goblet cells and mucin production was significantly increased in 0.2%, and 0.5% MOS diet fed broiler chickens and counts of *Bifidobacterium* spp. were also increased in MOS diet fed-broilers when compared to treatments containing virginiamycin (0.002%) and zinc bacitracin (0.006%) (Chacher et al., 2017).

Similar to the present results, Baurhoo et al. (2007) concluded that adding MOS to the diet encouraged *Bifidobacterium* spp. proliferation in the intestine of the broiler chickens due to the increase in the goblet cells and mucin production when compared with the positive control (Virginiamycin 0.0011%) diet. In the intestine of broilers, *Bifidobacterium* spp. was increased when compared to control or antibiotic growth promoters supplemented groups (Denev, 2006). *Bifidobacterium* spp. (as beneficial bacteria) Secrete organic acids and bactericidal substances which decrease the populations of pathogenic bacteria (Gibson and Wang, 1994). *Lactobacillus* spp.

produces lactic acid and acetic acid within the GIT of birds through the fermentation of glucose (Audisio et al., 2000).

The commercially available MOS product (BioMos, Alltech Inc., Nicholasville, KY), obtained from the cell wall of the *Saccharomyces cerevisiae* yeast that added to broiler diets bind to pathogenic gram-negative bacteria and decreases their attachment to the intestinal mucosa (Blomberg et al., 1993). DDP and MOS exerts similar mechanism of action that might be suggested for any type of pathogenic bacteria by the reduction of the attachment by Gram-negative bacteria to intestine mucosa by binding to the bacterial FimH of type-1 fimbriae and thereby preventing bacterial colonisation in the intestine. This agrees with what was found by Krachler and Orth (2013).

4.6 Conclusion

The time interval study on growth performance in broilers showed that using DDP for 5 weeks is preferable because of its effect on growth and suppression of pathological bacteria in the chicken gut. This suggested that prolonged feeding of 10% DDP diets for 5 weeks would be benefical while decreasing the amount of improted feedstuffs and prebiotics and improved gut health and quaility of poultry products.

Results of comparative study of DDP with 0.2% MOS, 0.1% and 0.2% mannose showed that 10% DDP, 0.2% MOS and 0.1% and 0.2% mannose has similar effect on growth performance of broilers. The inculsion of 10% DDP and 0.2% MOS in broiler diets resulted in significantly lower numbers of bacterial count in gut. The total count of *Lactobacillus* spp. and *Bifidobacterium* spp. bacteria may significantly enhanced in the small intestine health and thus reduced the needs for AGPs supplementation in animal nutrition.

Chapter 5: Antioxidant and Biochemical Effect of DDP, MOS, and Mannose on Broilers

5.1 Introduction

In this Chapter, we have evaluated the antioxidant and biochemical effects of DDP, MOS and mannose in serum, liver and intestine of broilers. Nutrition plays an essential role in controlling the pro-oxidant-antioxidant balance (Cowey, 1986). The more production of reactive oxygen species (ROS) leads to an imbalance in the antioxidant system and imbalance of the pro-oxidants leading to damage, which results in oxidative stress (Sies, 1991).

Oxidative stress causes disorder of redox signalling, point out the effect of the redox ratio as effective tools for the measuring the oxidative stress. Broiler chickens are exposed to different types of stressors such as the stress caused by heat, which induces the generation of reactive oxygen species (ROS), which causes morphological or physiological failures. Currently, consideration has been given to new methods of anti-oxidant remedy by providing anti-oxidant enzymes.

Antioxidant enzymes are present in all organisms, and these enzymes helps preventing cell membrane from damage, inactivation of enzymes and alteration of nucleic acids. The main anti-oxidant enzymes that make up the first line of anti-oxidant enzymatic defences encompass superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The SOD catalyses dis-mutation of superoxide radicals to hydrogen peroxide and oxygen, while CAT catalyses the breakdown the hydrogen peroxide to water and molecular oxygen. The GPx is a selenium-dependent enzyme, which deactivates the peroxides using the peptide glutathione (GSH) as its co-substrate (Halliwell, 2006).

Enzymatic ROS scavengers are catalase and peroxidases in which they decrease the concentration of H_2O_2, which acts as a precursor of potent radical species. The cytotoxic potential of H_2O_2 is a function of intracellular catalase and peroxidase activities that scavenge H_2O_2 and concentration of free ions of transition metals that encourage the generation of oxygen hydroxide from the peroxide. ROS are deemed as a critical oxygen mediators cytotoxicity and as crucial messengers, encouraging the cell division and demonstrating cellular signalling effects (Buetler et al., 2004). A concentration of lipid peroxidation end product which called Malondialdehyde (MDA) in tissues is in general acts as a biomarker for radical-induced deterioration and endogenous lipid peroxidation.

For the diagnostics of blood and organ diseases, the catalytic activity of enzymes in erythrocytes and liver is most commonly monitored. Erythrocytes are rich in haemoglobin with an efficient system of defence against free radicals, and they contain antioxidant enzymes with a high level of glutathione (Kostadinović et al., 2011). The liver is an organ that has a central metabolic role in the organism as it performs primary detoxification functions. The liver is the prime target for the study of the metabolism of xenobiotic (Samson and Popovic, 1988). Antioxidant enzymes neutralise the more formation and harmful effects of reactive oxygen metabolites (Cotgreave et al., 1988).

5.2 Material and Method

Liver, intestinal and blood samples (serum and plasma) were collected at 42 days of age from broiler experimental 2 as 2 samples of each replicates (n=6 samples/ treatments). The details of sample collection, storage condition were indicated in chapter 4 under experiment 2.

5.2.1 Biochemical Analysis

5.2.1.1 Estimation of Malondialdehyde (MDA) in Liver and Intestinal Tissue and in Blood Serum

Malondialdehyde (MDA) was measured by the Thiobarbituric acid assay method as described by (Ohkawa et al., 1979).

Reagents:

TCA-TBA-HCl reagent: 15% (w/v) TCA and 0.375 (w/v) TBA in 0.25 N HCl.

Procedures:

The samples homogenate was prepared in 0.1 M Tris-HCl buffer, pH 7.5. 2 ml of TBA-TCA-HCl reagent was added to 1 ml of the homogenate and mixed thoroughly. The solution was heated for 15 min at 100°C. After cooling the flocculent, centrifuged for 10 min at 1000 x g and removed the precipitate. The absorbance of the sample was read at 535 nm against the blank that contains no tissue homogenate. The extinction coefficient of TBARS is 1.56 x 105 M-1cm-1.

5.2.1.2 Assay of Catalase (EC.1.11.1.6) in Liver and Intestinal Tissue and Blood Serum

The catalase activity was assayed by the method of (Maehly, 1954).

Reagents:

i) Phosphate buffer pH 7.0.; ii) H_2O_2-Phosphate buffer.

Procedures:

The activity of catalase enzyme was assayed by homogenising the in phosphate buffer and centrifuged at 5000 x rpm. The reaction mixture contained 2 mM H_2O_2, phosphate

buffer, and enzyme extract. The change in the absorbance at 230 nm was measured spectrophotometrically. Specific activity was expressed in terms of units per mg protein.

5.2.1.3 Assay of Superoxide Dismutase (SOD) (EC.1.15.1.1) in Liver and Intestinal Tissue and Blood Serum

The SOD activity was measured by the method described by (Kakkar et al., 1984).

Reagents:

i) Sucrose (0.25 M).

ii) Sodium pyrophosphate buffer (pH 8.3, 0.05 M).

iii) PMS (1.2 µM).

iv) NBT (300 µM).

v) NADH (780 µM).

vi) Glacial acetic acid.

vii) N-butanol.

Procedure:

Tissues were homogenised in 0.25 M sucrose and centrifuged. Assay mixture contained sodium pyrophosphate buffer (12 ml), NBT (0.3 ml), PMS (0.1 ml), NADH (0.2 ml), water and diluted enzyme preparations. After incubation at 30°C for 90 seconds, added 1 ml glacial acetic acid. N-butanol (4 ml) was added and stirred vigorously and stands for 10 min; butanol layer was separated by centrifugation. Colour intensity of the samples was measured at 560 nm against butanol. The specific activity was expressed in units/mg proteins.

5.2.1.4 Assay of Glutathione Peroxidase (GPx) (EC.1.11.1.9) in Liver and Intestinal Tissue and in Blood Serum

The activity of glutathione peroxidase was assayed by the method of (Lawrence and Burk, 1976) as modified by (Agergaard and Jensen, 1982).

Reagents:

i) Sucrose (0.25 M).
ii) Phosphate buffer (50 mM pH 7.0).
iii) EDTA (1.5 mM).
iv) Sodium azide (1 mM).
v) Glutathione reduced (1.0 mM).
vi) NADPH (0.1 mM).

Procedure:

Tissue homogenate was prepared in sucrose. Activity was measured in phosphate buffer (2 ml) containing glutathione reduced (0.1 ml), EDTA (0.2 ml), sodium azide (0.3 ml), NADPH (0.1 ml) and 0.5 ml water. To this 0.2 ml of the enzyme, the source was added and incubated for 5 min at room temperature before initiation of the reaction by addition of 0.2 mL of H_2O_2 solution. Optical density was measured at 340 nm at 20-second intervals.

5.2.1.5 Assay of Glutathione S-transferase (GST) (EC.2.5.1.18)

The activity of glutathione peroxidase was assayed by the method of (Seyyedi et al., 2005)

Reagents:

i) 1-Chloro-2, 4-dinitrobenzene (CDNB, 100 mM)

ii) GSH (100 Mm)

iii) Phosphate buffered saline (PBS, pH 6.5)

Procedure:

The reaction mixture contained 970µl PBS pH 6.5, 10µl of 100 mM CDNB and 10 µl of 100 mM GSH. Added 10ul of enzyme source to the reaction mixture and measured the absorbance at 340 nm, every 30 seconds for 3 min. The change in absorbance was measured. Specific activity was expressed in terms of units per mg protein.

5.2.1.6 Assay of Activity of Serum Glutamic Oxaloacetate Transaminase (GOT) (Aspartate Amino Transferase) (E.C.2.6.1.1)

Glutamate oxaloacetate transaminase was assayed in serum using the procedure of (Rietman and Frankel, 1957).

Reagents:

i) Phosphate buffer (0.1 M, pH 7.4).

ii) Substrate: Dissolved 13.3 g of aspartic acid in 90 ml of 1 N NaOH. Added 0.146 g of a-Keto-glutaric acid and dissolved it by adding little more NaOH. Adjusted the pH to 7.4 and made up to 500 ml with phosphate buffer.

iii) Standard: Dissolved 30 mg Oxalo-acetic acid in 100 ml buffer.

iv) NaOH (0.4 N).

v) DNPH colour reagent: Dissolved 19.8 mg of 2, 4-dinitrophenylhydrazine in 10 ml HCl and made up to 100 ml with water.

Procedure:

Substrate solution (0.5 ml) was added to tubes and pre-incubated at 37°C. Added 0.1 mL serum into the test-tube labelled test and mixed by shaking gently and incubated

for 60 min at 37°C. After removing tubes from the water bath, immediately added 0.5 mL DNPH solution and mixed well. Then added 0.1 mL serum to the control tube. The standard tube contained 0.1 mL Oxalo-acetic acid, 0.4 mL substrate and 0.1 mL water. Incubated these tubes for 60 min at 37°C. Then added 0.5 mL DNPH. After 20 min added NaOH (0.4 N, 5 ml) mixed well and kept for 10 min. The colour developed was read at 510 nm. The activity of glutamate oxaloacetate transaminase is expressed as µmoles of oxaloacetate liberated/min/mg protein.

5.2.1.7 Assay of Activity of Serum Glutamate Pyruvate Transaminase (GPT) (Alanine Amino Transferase) (EC.2.6.1.2)

Glutamate-pyruvate transaminase was assayed in serum using the procedure of (Rietman and Frankel, 1957)

Reagents:

Phosphate buffer (0.1 M, pH 7.4).

i) Substrate: Dissolved 9 g of alanine in 90 ml water and with the addition of about 2.5 ml of 1 N NaOH (pH 7.4). Added 0.14 g of α-Ketoglutaric acid and dissolved it by adding little more NaOH. Adjusted the pH to 7.4 and made up to 500 ml with phosphate buffer.

ii) Standard: Dissolved 22 mg sodium pyruvate in 100 mL buffer.

iii) NaOH (0.4 N).

iv) DNPH colour reagent: Dissolved 19.8 mg of 2, 4-dinitrophenylhydrazine in 10 ml HCl and made up to 100 ml.

Procedure:

Substrate solution (0.5 ml) was added to tubes and pre-incubated at 37°C. Added 0.1 mL serum to tube as a test and mixed by shaking gently. Incubated at 37°C for 30 min. After removing the tubes from the water bath, immediately added 0.5 ml DNPH solution and mixed well. Then add 0.1 ml serum to the control tube. The standard tube contained 0.1 mL pyruvate, 0.4 ml substrate and 0.1 ml water. Blank contained 0.5 ml substrate and 0.1 ml water. Incubated these tubes at 37°C for 30 min. Then added 0.5 ml DNPH. After 20 min added 0.4 N NaOH (5 ml), mixed well and kept for 10 min. The colour developed was read at 510 nm. The activity of glutamate pyruvate transaminase is expressed as µmoles of pyruvate liberated/min/mg protein.

5.2.1.8 Assay of Gamma Glutamyl Transferase in Intestinal Tissue

Gamma Glutamyl Transferase activity was measured using a GGT colourimetric assay kit from Sigma Aldrich.

5.2.1.9 Analysis of Minerals in Blood Plasma

The total calcium, iron, phosphorus and copper in plasma were measured in blood plasma by Inductively Coupled Atomic Emission Spectrometry (ICP-OES) described by the ICP-OES Spectrometers operation manual (2002) of Varian, Inc.

5.2.1.10 Estimation of Blood Serum Urea

Blood serum urea was estimated by Diacetyl monoxime method (Friedman, 1953).

Reagents

i) BUN Colour Reagent: Diacetyl Monoxime (16.6 mmol/L)

ii) BUN Acid Reagent

iii) Urea Nitrogen Standard Solution

Procedure

Four test tubes were labelled as blank, standard, test 1, test 2, etc. To each test tube, 1.5 mL BUN colour reagent was added. Water (20 µl) was added to new and urea nitrogen standard (20 µl) to test tube marked as standard. Serum (20 µl) was added to the test. The contents were mixed by gently swirling. Then 3.0 ml BUN acid reagent was added to all tubes and mixed well. The tubes were incubated at 100°C for 10 min and then cooled at room temperature. All the tubes were mixed before reading the absorbance at 52 nm.

5.2.1.11 Estimation of Blood Serum Uric Acid

Blood serum uric acid was estimated by using the modified colourimetric method (Buchanan et al., 1965).

Reagents

i) Sulphuric acid (N/23).
ii) Sodium tungstate (5.6%).
iii) NaOH (0.6 N).
iv) Phosphotungstic acid reaction reagent (PTR).
v) Uric acid standard stock.

Procedure

Serum (0.5 ml) was added to 4 ml of sulphuric acid and mixed well. Sodium tungstate (0.5 ml) was then added mixed and centrifuged. PTR (0.2 ml) was added to 3 ml of

supernatant, mixed and then 1.0 ml NaOH was added. The absorbance of test tubes was taken at 720 nm.

5.2.1.12 Estimation of Blood plasma Glucose, Total Protein, Creatine and Lipid Profile

Blood plasma glucose, creatinine, total protein, cholesterol, HDL-cholesterol and triglycerides were measured using commercial systems (Unichem Elite, United Diagnostics Industry, Dammam, KSA) and based on methods used and outlined by (Ibrahim et al., 2008).

5.2.1.13 Haematological Parameters

The red blood cell (RBC) and white blood cell (WBC) count were determined by haemocytometer method using Natt-Herrick solution, haematocrit and haemoglobin (Hb) values were measured by micro-haematocrit and cyanate-haemoglobin method (Kececi et al., 1998). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were measured by the method of (Campbell, 1995).

5.3 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using general linear model (GLM) and mean comparisons were performed using Duncan’s multiple range test to compare significant differences between means for all analyses. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago).

5.4 Results

5.4.1 Antioxidant Enzymes in Serum of Broilers

5.4.1.1 Effect of Different Dietary Treatments on Activity of SOD, CAT, GPX and MDA in Serum

The results are shown in Table 20. The activity of enzymatic anti-oxidants SOD, catalase and GPx were found to be significantly high in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared to the corn-soy diet fed-broilers. In antibiotic diet fed-broilers and mannose diet fed-broilers the activity of antioxidant enzymes increased, but the activity was not significantly high when compared to 10% DDP fed-broilers and 0.2% MOS diet fed-broilers. The level of MDA was significantly low in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared to control diet fed-broilers with or without antibiotic, and mannose diet fed-broilers.

Table 20: Effect of different dietary treatments on activity of SOD, CAT, GPX and MDA in serum

Treatment	**Serum**			
	SOD (U/mg)	**Catalase (U/mg)**	**GPx (U/mg)**	**MDA (nmol/ml)**
Control	127.44±9.38^{b}	29.62 3.90^{b}	215.74±17.32^{b}	5.97±0.26^{a}
Control + Antibiotic	161.39±18.45^{ab}	46.78±9.75^{b}	229.45±20.68^{b}	5.81±0.97^{a}
10% DDP	224.05±16.95^{a}	91.03±10.99^{a}	313.07±26.40^{a}	1.94±0.12^{b}
0.2% MOS	231.36±20.09^{a}	87.85±12.83^{a}	318.82±29.20^{a}	1.76±0.07^{b}
0.2% mannose	164.68±16.09^{ab}	60.46±7.82^{ab}	214.61±7.91^{b}	4.96±0.19^{a}
0.1% mannose	165.87±14.62^{ab}	66.01±9.05^{ab}	219.14±11.23^{b}	4.12±0.12^{a}

Values are expressed as means of 6 replicates±SE

^{ab}Means within a row with different letters (a, b) differ significantly ($P<0.05$)

SOD- Superoxide dismutase, GPX-Glutathione peroxidase, MDA - Malondialdehyde.

5.4.2 Antioxidant Enzymes in Liver of Broilers

5.4.2.1 Effect of Different Dietary Treatments on Activity of SOD in Liver of Broilers

Figure 41 shows the generated results. The activity of SOD in the liver was significantly high in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared to the corn-soy diet fed-broilers. In mannose and antibiotic fed-broilers, the activity of SOD was similar and intermediate and did not significant different from other groups.

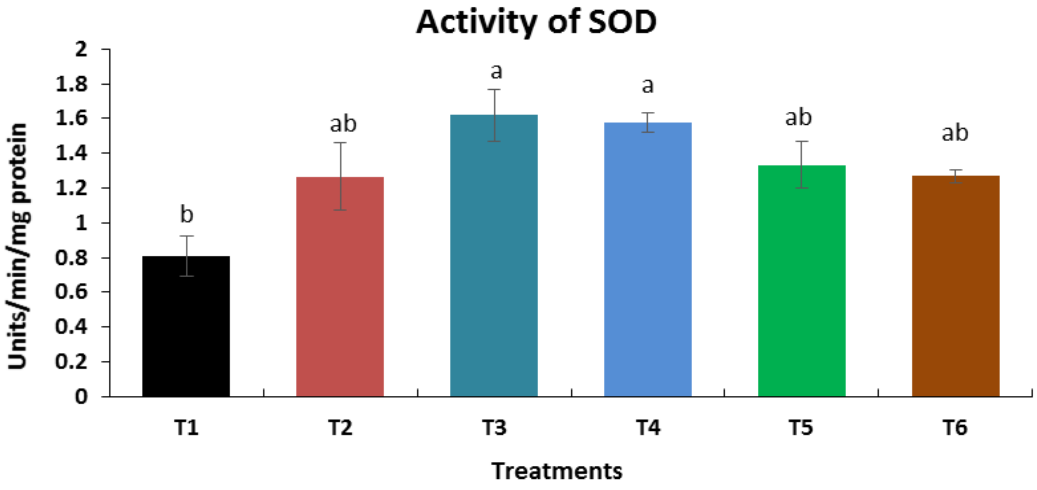

Figure 41: Effect of different dietary treatments on the activity of superoxide dismutase in the liver of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.
Values are expressed as means of 6 replicates±SE
[ab]Means within a row with different letters (a, b) differ significantly ($P<0.05$)

5.4.2.2 Effect of Different Dietary Treatments on Activity of GPX in Liver of Broilers

Figure 42 shows the generated results. The GPx activity in the liver was significantly high in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared

to the corn-soy diet fed-broilers and 0.2% mannose fed broilers. In mannose fed-broilers, the GPx activity was not significantly different between both levels. In corn-soy bean diets with and without antibiotic diet fed-broilers the GPx activity was comparable to GPx activity of both levels of mannose diet fed-broilers.

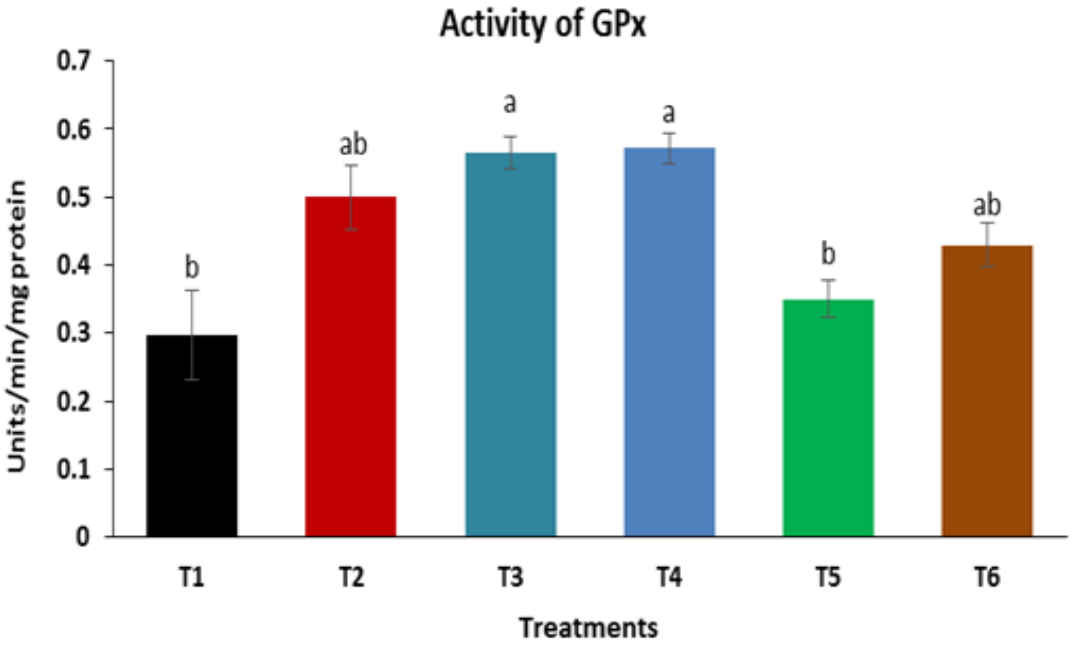

Figure 42: Effect of different dietary treatments on the activity of GPX in the liver of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.
Values are expressed as means of 6 replicates±SE
[ab]Means within a row with different letters (a, b) differ significantly ($P<0.05$)

5.4.2.3 Effect of Different Dietary Treatments on Activity of Catalase in Liver of Broilers

Figure 43 shows the generated results. The result of dietary treatments on the activity of catalase in the liver of broilers is shown in Figure 43. Catalase activity was significantly high in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to the corn-soy diet fed-broilers. In both 0.1% and 0.2% mannose diet fed-

broilers, the GPx, activity was similar, and the activity was comparable to 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers. Results showed that catalase activity was intermediate in the corn-soy antibiotic diet fed-broilers and did not significantly different from other groups.

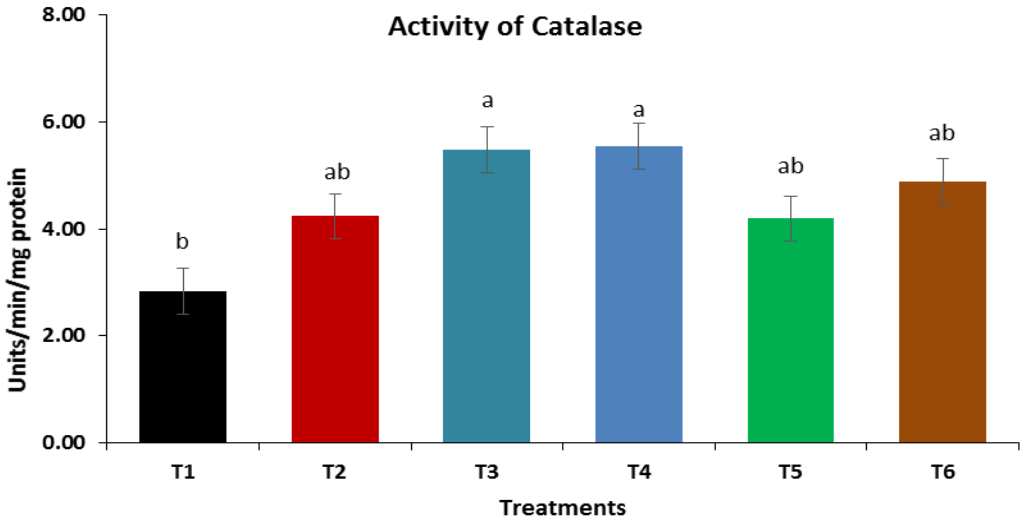

Figure 43: Effect of different dietary treatments on activity of catalase in liver of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.
Values are expressed as means of 6 replicates±SE
[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.2.4 Effect of Different Dietary Treatments on Activity of GST in the Liver of Broilers

Figure 44 shows the generated results. The GST activity in the liver was significantly increased in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers. In 0.1% mannose fed-broilers and corn-soy antibiotic diet fed-broilers the GST activity was significantly higher than the control, but less than DDP and MOS diets. The 0.2% mannose diet, the GST value was almost similar to other treatments except for control soybean meal diet without antibiotic.

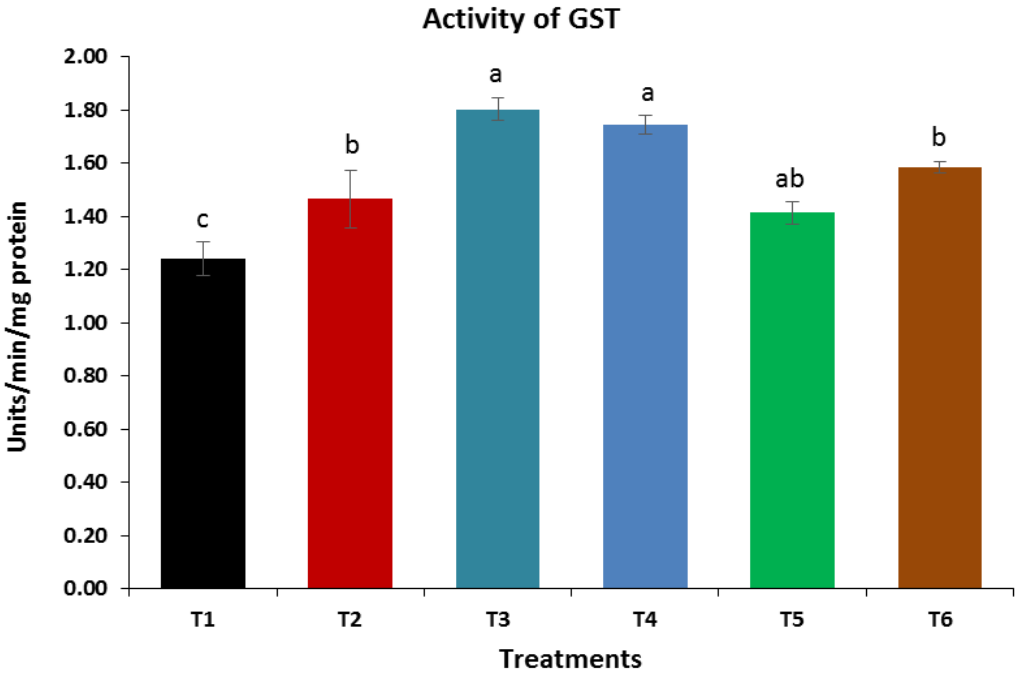

Figure 44: Effect of different dietary treatments on activity of GST in liver of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.
Values are expressed as means of 6 replicates±SE
[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.2.5 Effect of Different Dietary Treatments on MDA Content in Liver of Broilers

Figure 45 shows the generated results. The MDA content in the liver was significantly low in 10% DDP diet fed-broilers, and 0.2% MOS diet fed-broilers when compared to the corn-soy diet fed-broilers. In mannose diet fed-broilers and corn-soy meal diet with or without antibiotic fed-broilers, the MDA content was significantly similar and higher than other treatments.

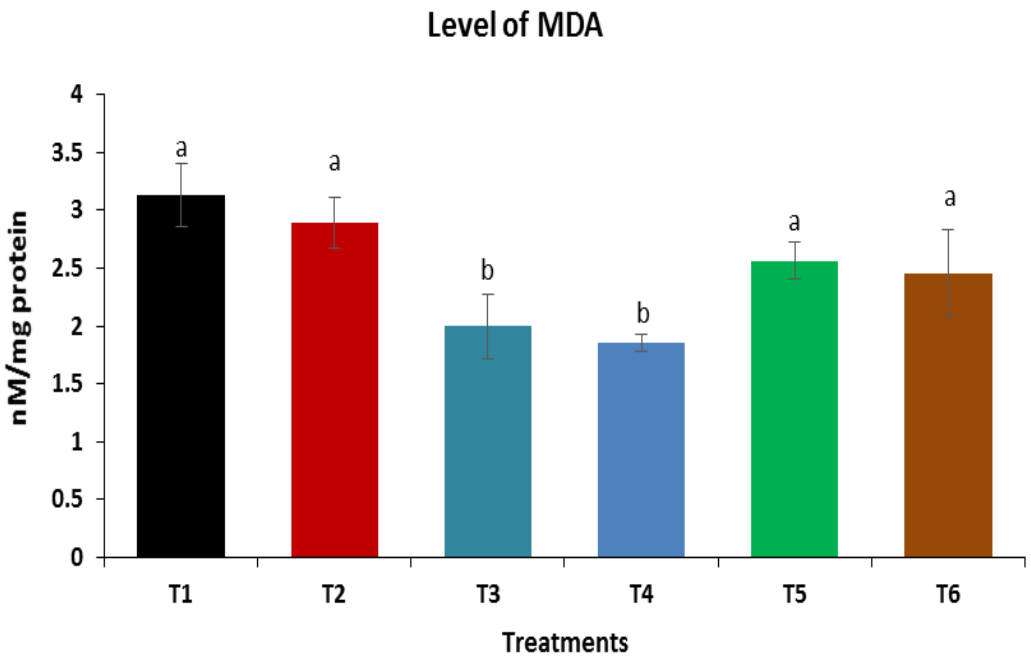

Figure 45: Effect of different dietary treatments on MDA content in liver of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.3 Activity of Antioxidant Enzymes in Intestine of Broilers

5.4.3.1 Effect of Different Dietary Treatments on Activity of SOD in Intestine

The results are displayed in Figure 46. The activity of SOD in intestine were significantly high in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers on both proximal and distal portion when compared to corn-soy diet fed-broilers. In mannose diet fed-broilers, the activity of SOD in proximal and distal portion of intestine was less when compared to the 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers. The SOD activity in intestine of corn-soy meal diet with or without antibiotic fed-broilers showed similar results of mannose diet fed-broilers.

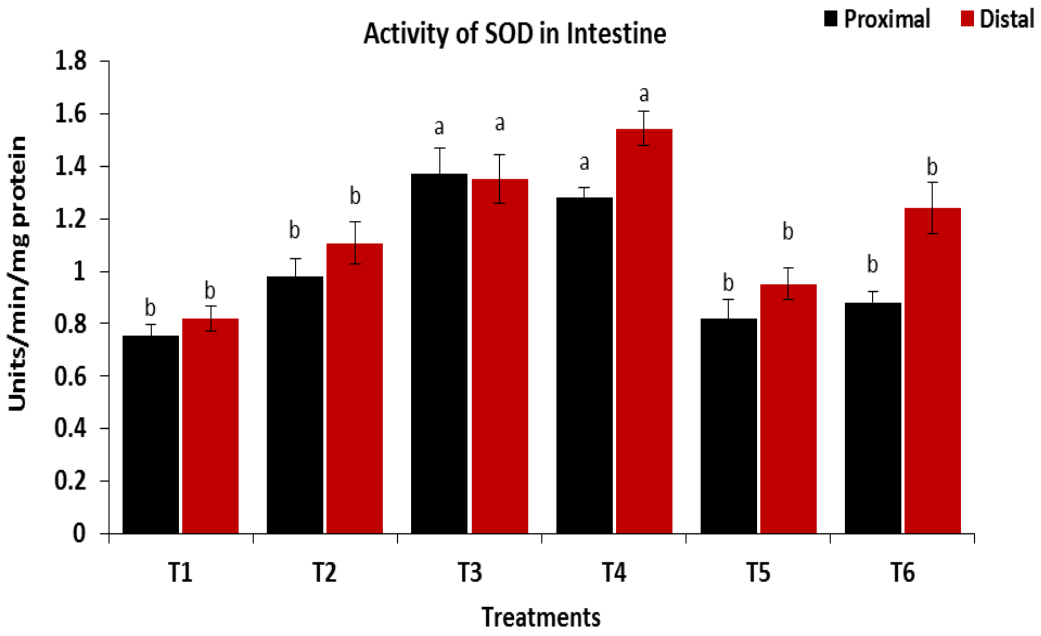

Figure 46: Effect of different dietary treatments on activity of SOD in intestine

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.3.2 Effect of Different Dietary Treatments on Activity of GPX in Intestine

The results are displayed in Figure 47. The GPx activity in proximal and distal portion of intestine was significantly increased in 10% DDP fed-broilers and 0.2% MOS broilers when compared to corn-soy diet with or without antibiotic fed-broilers. In mannose diet fed-broilers the activity of GPx in proximal and distal portions of intestine was similar to corn-soymeal diet with or without antibiotic fed-broilers. However, GPx in the proximal part was similar to 10% DDP diet and 0.2% MOS treatments.

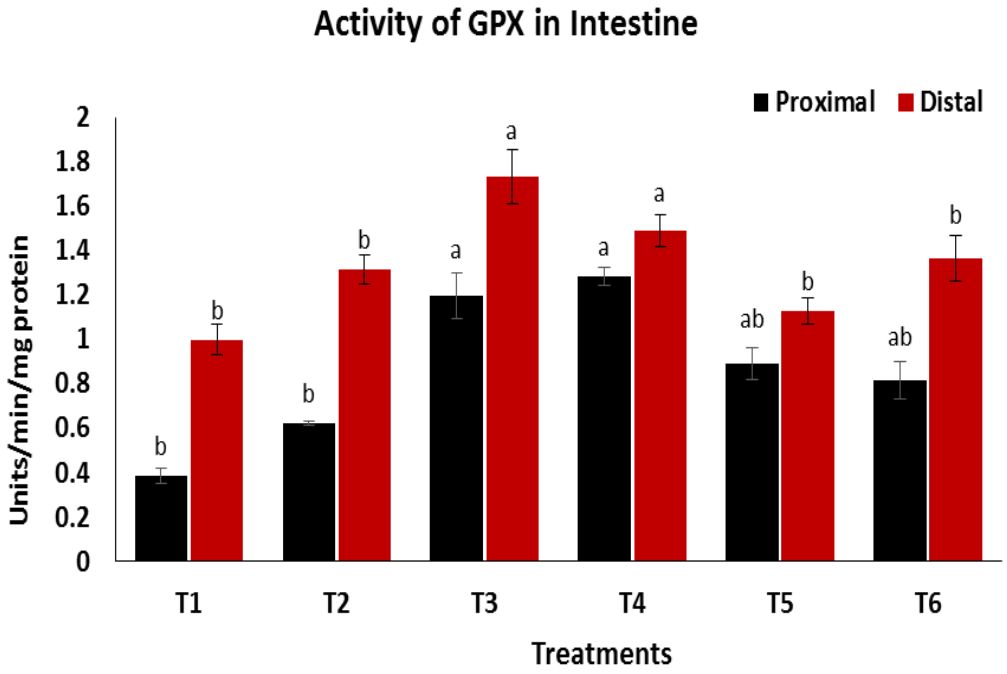

Figure 47: Effect of different dietary treatments on activity of GPX in intestine

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.3.3 Effect of Different Dietary Treatments on Activity of CAT in Intestine

The results are shown in Figure 48. The result of different dietary treatments on activity of catalase in proximal and distal portion of intestine of broilers is shown in Figure 48. Catalase activity were significantly increased in proximal and distal portion of 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers. In mannose diet fed-broilers and corn-soy meal diet with or without antibiotic diet fed-broilers catalase activity was similar and CAT activity was comparable to other treatments.

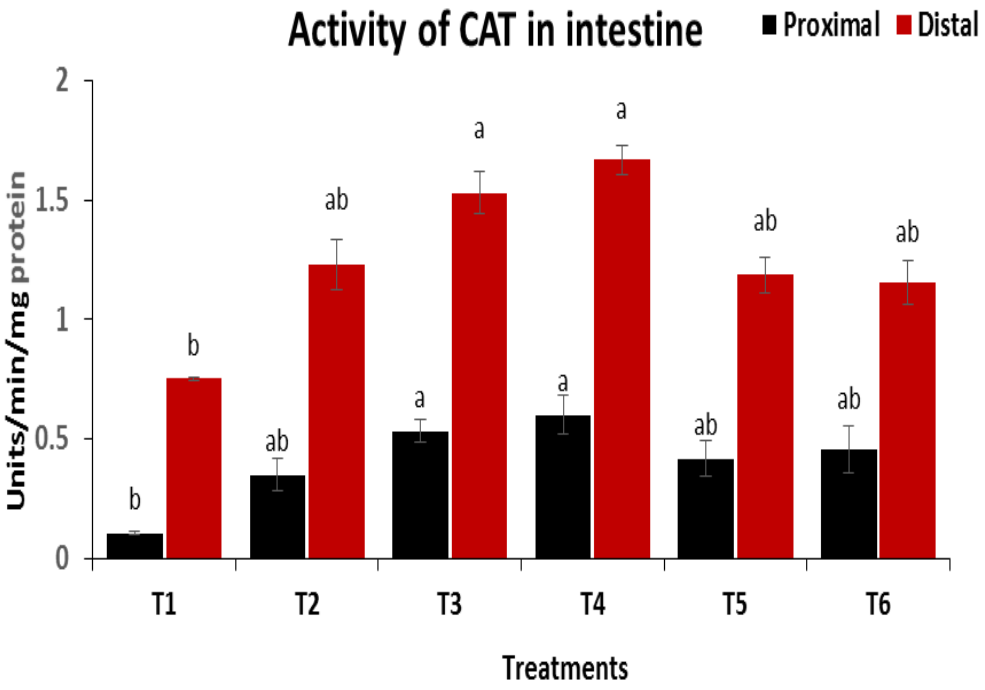

Figure 48: Effect of different dietary treatments on activity of CAT in intestine

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.3.4 Effect of Different Dietary Treatments on Activity of GST in Intestine

The results are shown in Figure 49. In proximal and distal portion of intestine the activity of GST were significantly high in 10% DDP fed-broilers and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers. In mannose diet fed-broilers, the activity of GST in proximal portion of intestine was low and the result was comparable to antibiotic diet fed-broilers and corn-soy diet fed-broilers. GST activity of distal portion of intestine was similar in both mannose diet fed treatment and antibiotic diet fed treatments, but were not differ from corn-soy diet fed-broilers.

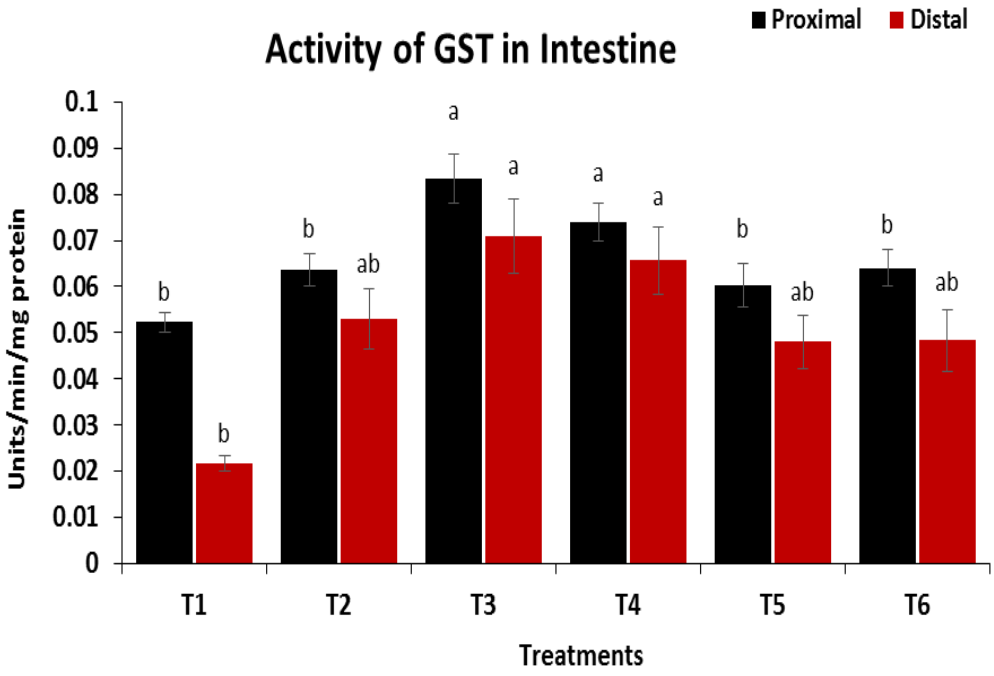

Figure 49: Effect of different dietary treatments on activity of GST in intestine

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.3.5 Effect of Different Dietary Treatments on MDA Content in Intestine

Figure 50 shows the generated results. The MDA contents in proximal and distal portion of intestine were significantly less in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers. In mannose diet fed-broilers MDA content in both portion of intestine was decreased but the result was not significant, and was comparable to corn-soy meal diet with or without antibiotic treatments.

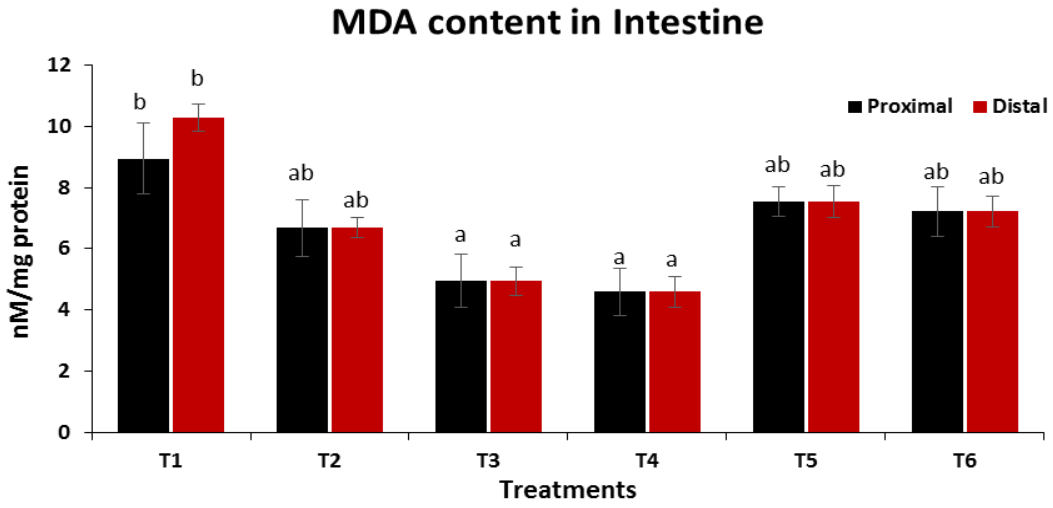

Figure 50: Effect of different dietary treatments on MDA content in intestine

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

5.4.4 Effect of Different Dietary Treatments on Blood Biochemical Parameters of Broilers

The effect of different dietary treatments on broilers blood biochemical parameters is shown in Table 21. The supplementation of 10% DDP, 0.2% MOS and 0.1% ,0.2% mannose to broilers treatments had no significant effect on activity of enzymes like alanine amino-transferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and blood plasma glucose, creatinine, calcium, phosphorus, copper, urea and uric acid. The total cholesterol, triglyceride, LDL cholesterol and HDL cholesterol were numerically decreased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers but the result was not significant from other treatments. The iron content was significantly high in 10% DDP diet fed-broilers, 0.2% mannose and 0.2% MOS diet fed-broilers when compared to control diet fed-broilers with or without antibiotic.

Table 21: Effect of different dietary treatments on biochemical parameters of broilers

	Dietary Treatments					
Parameters	**Control**	**Control + Antibiotic**	**10% DDP**	**0.2% MOS**	**0.2% Mannose**	**0.1% Mannose**
Glucose, mg/dL	262.16±2.92	270.93±3.45	269.62±4.92	265.30±4.97	267.77±5.27	273.72±4.29
Total Cholesterol mg/dL	160.66±3.74	167.34±7.95	152.05±7.77	156.26±8.89	154.58±8.71	163.85±2.16
Triglyceride mg/dL	92.54±5.21	99.29±6.87	86.29±5.73	89.25±4.26	91.58±3.89	91.09±4.60
LDL Cholesterol mg/dL	50.02±5.98	60.22±3.42	47.27±8.79	44.49±5.98	41.64±5.60	42.97±5.34
HDL Cholesterol mg/dL	98.74±0.48	95.96±1.02	91.15±4.51	93.06±1.27	94.35±2.68	96.50±6.74
Creatinine, mg/dL	0.41±0.005	0.42±0.005	0.41±0.005	0.36±0.012	0.42±0.005	0.44±0.017
Total protein, g/dL	3.43±0.17	3.54±0.20	3.50±0.16	3.59±0.22	3.66±0.24	4.06±0.09
Alanine amino-transferase, IU/L	5.23±0.51	5.32±0.53	5.27±0.51	5.27±0.49	5.26±0.55	5.63±0.73
Asparate aminotransferase, IU/L	180.8±13.85	193.79±14.08	184.29±12.57	178.54±13.20	183.42±25.85	185.39±4.03
Gamma glutamyl transferase, IU/L	37.69±2.59	40.49±3.47	38.59±1.79	33.00±2.06	36.47±3.18	42.34±4.62
Calcium, mg/dL	16.78±1.65	17.92±2.21	19.38±2.03	17.72±1.89	18.86±1.73	15.45±1.67
Phosphorus, mg/dL	5.33±0.87	5.13±1.13	6.81±1.40	5.49±0.62	5.79±1.29	6.43±0.67
Iron, µg/dL	106.57±6.06	100.32±1.92	121.76±1.93[a]	112.81±5.04[a]	116.51±4.44[a]	118.26±2.12
Copper, µg%	39.55±2.59	34.79±2.03	41.53±1.67	41.67±2.42	38.60±2.25	34.37±2.78
Urea (mg/dL)	5.53±0.39	5.97±1.54	3.37±0.33	3.59±0.45	4.59±0.55	3.53±0.52
Uric acid (mg/dL)	7.71±1.57	7.79±1.68	6.69±0.61	6.85±0.66	7.18±0.73	6.55±0.58

Values are expressed as means of 6 replicates±SE.

[ab]Means with in a row with different letters (a, b) differ significantly (P<0.05)

5.4.5 Effect of Different Dietary Treatments on Blood Haematological Parameters of Broilers

The effect of dietary treatments on broilers blood haematological parameters is shown in Table 22. The supplementation of 10% DDP, 0.2% MOS and 0.1% and 0.2% mannose to broilers diets had no significant effect on WBC, RBC, Haemoglobin, HCT, MCV and MCHC. Mean Corpuscular Haemoglobin level was significantly upregulated in 10% DDP diet fed-broilers, 0.1% mannose and 0.2% MOS diet fed-broilers. Haemoglobin level, HCT and MCHC were increased in 10% DDP and 0.2% MOS diet fed-broilers but the result was not significant from other groups.

Table 22: Effect of different dietary treatments on serum haematological parameters of broilers

Parameter	Dietary treatments					
	Control	**Control + Antibiotic**	**10% DDP**	**0.2% MOS**	**0.2% mannose**	**0.1% mannose**
WBC (10^3/µL)	21.89±4.60	22.29±1.89	24.21±2.77	24.88±3.01	21.54±3.34	24.14±3.00
RBC (10^6/µL)	2.21±0.56	2.17±0.60	2.70±0.77	2.59±0.66	2.36±0.47	2.49±0.51
Hemoglobin(mg/100 ml)	12.55±1.04	12.02±0.85	14.80±1.30	14.50±1.11	13.41±1.18	14.07±1.04
HCT (%)	25.90±3.01	25.16±2.63	32.76±4.26	32.13±3.99	27.43±3.34	31.33±3.68
MCV (fl)	135.39±1.70	131.91±1.59	138.25±2.52	137.80±2.41	134.71±1.53	137.04±2.08
MCH (pg)	47.88±0.71[b]	45.29±0.48[b]	49.54±0.70[a]	50.37±0.75[a]	48.12±0.66[b]	49.29±0.88[a]
MCHC (%)	49.19±1.70	47.37±2.92	53.26±3.50	50.36±2.90	48.19±2.74	53.83±4.31

WBC- White Blood Cells, RBC-Red blood Cells, HCT- Haematocrit value, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration

Values are expressed as means of 6 replicates±SE

[ab]Means with in a row with different letters (a, b) differ significantly ($P<0.05$)

5.5 Discussion

Broilers are capable to adapt to oxidative stress by encouraging the synthesis of antioxidant enzymes. The antioxidant systems in the body have antioxidant enzymes such as SOD and GSH-Px, which act to protect the body from oxidative stress. Dismutation of superoxide ion to hydrogen peroxide by SOD is usually known as the primary defence. SOD, is widely spread out in oxygen metabolizing cells and protect aerobic cells against harmful effects of superoxide radicals and other (ROS) (Yamaguchy, 1991). In the present study, the activity of antioxidant enzymes like SOD, CAT and GPx in serum was significantly increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers.

The antioxidant enzymes SOD and GSH-Px are known to be the essential elements of the first level of antioxidant defence in the cell because they form the main protective system versus oxidative damage (Surai, 1999). The serum levels of GSH were significantly increased ($P < 0.05$) in *P. dactylifera* pits treatment, especially at 21st day of experiment (El-Far et al., 2016). Methanolic extract of *P. dactylifera* pits is known as an antioxidant exporter of β-carotene and phenolic compounds (Shanab et al., 2014). This antioxidant action turned into commensurate to the phenolic contents (Ardekani et al., 2010).

Liver controls numerous essential mechanisms in the body, helping as the major organ for detoxification of different substances. The pathogenesis of liver defect covers different cell kinds in liver through cell death and regeneration mechanisms. Vetvicka et al. (2014) studied the synergistic effects of humic acid and prebiotic glycan in hepatoprotection in broilers. Ferket et al. (2002) studied the advantages of addition of MOS to the poultry feed and reported that MOS enhances liver morphology and

functioning. Sarangi et al. (2016) studied the effect of dietary supplementation of prebiotic, probiotic, and symbiotic on liver histomorphology in broilers and reported the beneficial effects of MOS. Prebiotics and peppermint extracts inclusion in broilers diet enhances performance, enzymatic activity and histological aspects of liver during experimental period (Ahmed et al., 2016).

Dietary supplementation of 10% DDP and 0.2% MOS enhanced the activity of anti-oxidant enzyme such as SOD, CAT, GPx and GST in liver. The scavenger functions of these hepatic anti oxidative enzymes in preserving the liver from oxidative deteriorations, by inducing the release of SOD and GPx as a reaction from prebiotics like 10% DDP and 0.2% MOS supplementation, suggests that they may help to reduce the MDA in the liver. The MDA content was significantly reduced in 10% DDP and 0.2% MOS diet fed-broilers. Binding free radicals to chelating transition metal ions and hydrogen atoms that act as a catalyst for free radical generation could be the major contributing part in the anti-peroxidative capability of the prebiotics (Pan and Mei, 2010). Supplementation of mannan-oligosaccharide with either bee pollen or pro intermittently, promotes anti-oxidant enzyme levels and immune response of broiler chicks (Attia et al., 2017a).

The report of Dvorska and Surai (2001) explained that dietary supplementation of MOS decreased the MDA level in quail liver. It has been tested that carbohydrates and carbohydrate-containing biomolecules can be used as real antioxidants, that are able of scavenging reactive oxygen species (ROS) (Stoyanova et al., 2011). Some studies reported that MOS has a protective impact on heat-stressed chickens (Sohail et al., 2012) and when has been added to laying hens, significantly enhanced the total antioxidant function of egg yolk and liver antioxidant enzymes (Bozkurt et al., 2012).

Oskoueian et al. (2014) reported that ethanolic extract of Palm Kernel Cake (PKC) has high levels of fatty acids, phenolic compounds, sugar derivatives and other organic compounds with high antioxidant effectiveness.

Dietary treatment of 10% DDP and 0.2% MOS upregulated the antioxidant enzyme levels in the intestine of broilers. Križková et al. (2001) showed that MOS from *S. cerevisiae* have anti-oxidative property *in vitro*. This proofs that dietary MOS can prevent the GIT not only by removing undesirable bacteria but also through other different ways. In addition, Kogani et al. (2008) suggested that prebiotics like β-glucans have antioxidant activity and enhances antioxidant enzyme levels in intestine of broilers. Catalase activity, and the activity of other anti-oxidant enzymes, depends on the existence of anti-oxidants in the feed. Oxidative damage increases when anti-oxidant role is declined and/or when factors give a share in oxidative stress enhanced (Ibrahim et al., 2000).

The increase in GPx activity in 10% DDP and 0.2% MOS diet fed-broilers suggests a greater protection from oxidative stress. The present results further substantiate the positive effect of 10% DDP and 0.2% MOS on GPx activity in chicken serum, intestine and liver. The GPx applies its activity fundamentally in the cytoplasm of the cells and only about 10% of this activity is presented in the mitochondria (Halliwell, 1999). The MDA level endogenously reflects lipid peroxidation. MDA level was decreased in 10% DDP and 0.2% MOS diet fed-broilers and this is a direct reflection of decreased peroxidation and enhanced protective effect. This may be attributed to the prebiotic ability to grant sufficient antioxidant prevention against lipid peroxidation over the growth phase of the birds.

Dietary inclusion of 10% DDP and 0.2% MOS did not affect blood biochemical parameters. These blood biochemical scales were within the regular mean levels mentioned earlier (Rupley, 1997). The measurement of AST, ALT, GGT, LDH and HBDH activities, helps in testing the functional liver damage in broilers before the appearance of clinical symptoms. It is also seems to be an indicator of damage in the liver before any morphological changes in this organ can be detected (Kraljević et al., 2008).

Any abnormality in the increase of serum levels of AST, ALP and ALT can reveal liver damage (hepatocellular degradation), so the normal levels of AST come up with the appearance of hepato-protective impacts of 10% DDP and 0.2% MOS. Al-Bowait and Al-Sultan (2006) who showed that DP had no significant impact on glucose concentration of broilers fed with diet containing DP. In agreement with our findings, (Masoudi et al., 2011) reported that replacement corn by 30% DP in broiler feed had no significant impact on blood cholesterol, triacylglycerol, HDL and LDL.

Jassim (2010) found that date waste at 150 g/kg in isocaloric, isonitrogenous broiler diets from 1 to 42 days of age did not affect growth performance or blood cholesterol. Toghyani et al. (2010) reported that prebiotics β-glucan diet supplemented broilers and corn-soy diet fed-broilers have normal levels of serum biochemical parameters. Similarly, Yalçın et al. (2010) found that MOS from yeast autolysate supplementation reduced the levels of serum cholesterol and triglyceride and had no effect on serum total protein and uric acid. The results of the present study also support that finding. It was also reported that yeast culture supplementation with MOS had no effect on serum levels of total protein, triglyceride, cholesterol, AST, and ALP but increased serum uric acid (Yalçin et al., 2008).

No considerable changes were appeared in the blood hematological parameters in all treatments in spite of a significant increase in Mean Corpuscular Haemoglobin (MCH) in the 10% DDP diet fed-broilers, 0.1% mannose and 0.2% MOS diet fed-broilers. This agrees with the experiment done by (Cetin et al., 2005), who found that the prebiotic MOS addition to the feed statistically significantly increased in the erythrocyte count, haemoglobin concentration and haematocrit rates of Turkeys. The increase in MCH concentration in our present study, we might attributed it to the enhancement in the count of RBCs. Similarly, Mousa et al. (2014) discovered that MCH rates were significantly higher in the quail chickens that had diets with foreign or local products containing MOS and BG. Also, Attia et al. (2017a) reported that administration of MOS at a quantity of 0.5 g/kg constantly from 0 to 35 days or in interrupted form in broilers enhanced RBCs count and Hb rate.

5.6 Conclusion

In 10% DDP treatment, the antioxidant enzyme activities in serum, liver and intestine were significantly ($P<0.05$) increased when compared with broilers fed the control diet. While the mean Corpuscular Hemoglobin level and the iron content were significantly upregulated in 10% DDP diet fed-broilers, 0.1% mannose and 0.2% MOS diet fed-broilers.

Chapter 6: Effect of DDP, MOS, and Mannose on Intestine Development and Morphology of Broilers

6.1 Introduction

The intestinal development plays a major role in performance rate of broilers have been chosen for fast growth (Smith et al., 1990); (Attia et al., 2019). After hatching, the small intestine of broilers grows quicker, weight-wise, than total body weight. In broiler, small intestine comparative growth reaches its maximum length and weight between 6 and 10 days of age, depending on diet quantity and quality (Mateos et al., 2004). However, FI catalyses the growth of the GIT while the duodenum develops earlier than the jejunum and the ileum (Uni et al., 1999). Villi diameters promptly increased within two days of age, after that this growth rate decreases gradually, until reaching a plateau between 5 and 10 days post-hatch (Uni et al., 1996).

The production of intestinal epithelial cells in broilers is not occurring only in the crypts; but also proliferating along the villi during the 1st wk of the bird life. The alterations that happened in the intestine develop the chicks to use the nutrients given by an outside source (Uni et al., 1998). Broiler performance was negatively affected when the hatchlings were subjected to fasting for 24 hr and this leads to abnormal development of gastrointestinal tract (Gonzales et al., 2003).

Many studies have been done on the GIT morphometric, especially the duodenum, jejunum and ileum in the small intestine due to the importance of the future performance of broilers; therefore, the first days, even the first few hours, of the bird life have been subjected to these studies (Zavarize et al., 2012). This Chapter discusses the effect of different dietary treatments of DDP, MOS and D-mannose on the intestine

development and histomorphology, digestive enzymes level, carcass and organ weight of broilers.

6.2 Materials and Methods

Pancreas and intestinal samples were collected at 42 and 21 and 42 days of age, respectively from broiler experimental 2 as 2 samples of each replicates (n=6 samples/ treatments). The details of sample collection, storage condition were indicated in chapter 4 under experiment 2. The carcass characteristics and inner body organs were also measured in 6 broilers per treatment slaughtered according to Islamic methods at 42 days of age in broiler feeding experimental 2.

The pancreas tissue samples (n=6/treatment) were gathered then divided into small parts. The small intestine including duodenum (from gizzard to pancreas-biliary ducts), jejunum (from pancreas-biliary ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to ileocecal junction) were collected (n=6 per treatment). Samples of the duodenum, jejunum, and ileum (1 cm cut from the midpoint) were stored in 10% neutral buffered formalin for 24 hours for histomorphology. Organs like liver, gizzard, heart, proventriculus and spleen were collected and stored at 4°C until analysis, as shown in Figure 51.

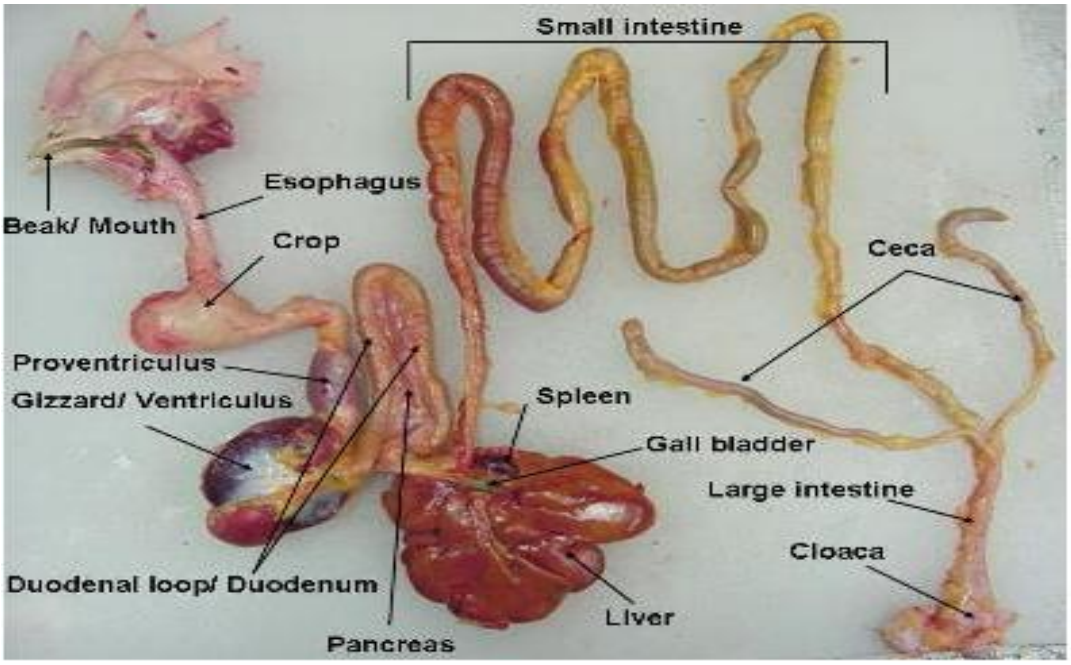

Figure 51: Poultry digestive system (Source: Jacob et al., 2015)

6.2.1 Digestive Enzymes Activity in Pancreas

6.2.1.1 Measurement of Amylase Activity

The activity of amylase was measured by the method of (Howard and Yudkin, 1963). The method is based on the intensity of colour developed during reaction between starch and iodine. When starch is hydrolysed, the intensity of blue is decreased.

Reagents

i) Starch.

ii) Potassium Iodide.

iii) Phosphate buffer.

Procedure

Starch (0.5 ml) and 0.5 ml of tissue homogenate were incubated at room temperature for 3 min and the test tubes placed immediately in boiling water bath to stop the reaction. Add few drops of potassium iodide and heated in a boiling water bath for 5

minutes. The colour generated was measured in a spectrophotometer at 620 nm. Enzyme activity was expressed as units of activity where one unit was defined for amylase as an increase in 10^{-5} extinction at 620 nm/10 min.

6.2.1.2 Measurement of protease activity

The activity of protease was measured by the method of (Gertler and Nitsan, 1970).

Reagents

i) Potassium phosphate buffer (50 mM, pH 7.5).
ii) Casein solution (0.65% (w/v).
iii) Trichloroacetic acid (TCA, 110 mM).
iv) Folin & Ciocalteu's Phenol Reagent (0.5 M).
v) 500 mM Sodium Carbonate (500 mM).
vi) Sodium Acetate Buffer (10 mM) with Calcium Acetate (5 mM, pH 7.5).
vii) L-Tyrosine Standard (1.1 mM).
viii) Protease Enzyme Solution 0.1-0.2 units/mL of Protease in cold enzyme diluent.

Procedure

Pipetted out 5 mL of casein solution to test tubes. Tubes were kept the in a thermostated water bath at 37°C for 15 min. Then varying volumes of enzyme solutions (Reagent 8 and pancreatic homogenate) were added. Mixed by swirling and incubated at 37°C for 30 min. Five mL of TCA were added. Incubated at 37°C for 30 min. Each solution was filtered using 0.45 µm syringe filter. Na2CO3 (2 ml) and 1 ml of Folin & Ciocalteu's phenol reagent were added to 2 mL of filtrate. Mixed the tubes and kept at 37°C for

30 min. Filtered solutions were measured against the absorbance at 660 nm. Tyrosine was taken as standard.

6.2.1.3 Measurement of Lipase Activity

Lipase activity in pancreas was measured using commercial systems (Udichem Elite, United Diagnostics Industry, Dammam, KSA) and based on methods used and outlined by Tietz and Fiereck (1966).

6.2.2 Measurement of Length and Weight of Intestinal Segments

The weights of small intestine segments were weighed using electronic weighing balance. The length were measured with non-stretchable thread and scale. The weight and length of intestinal were calculated by the method of Zang et al. (2009).

6.2.3 Histomorphology of Intestine

Histomorphology of broiler intestine were analysed by the method of Wu et al. (2013) and Song et al. (2014). The intestine were cleared from the intestinal contents using xylene and dehydrated using ethyl alcohol. The samples were then embedded in paraffin wax and cut into pieces (6 μm), placed on glass slides, and stained with haematoxylin and eosin. For morphological observations, each intestine segment was prepared in ten slides and visualized using a light microscope.

A computer assisted morphometric system equipped with light microscope were used for collecting the images. Image-Pro Plus (IPP) software were used for measuring villus height and crypt depth. The tip of the villi to the villus crypt junction was considered as villus height, and crypt depth was specified from the ravine between

individual villus to the basal membrane. The villus height-to-crypt depth ratio (villus/crypt ratio) was also measured.

6.3 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using general linear model (GLM) and mean comparisons were performed using Duncan's multiple range test to compare significant differences between means for all analyses. Statistical analysis was conducted using the statistical Package for Social Sciences (SPSS for Windows: SPSS Inc., Chicago, IL, USA).

6.4 Results

6.4.1 Effect of Different Dietary Treatments on Activity of Digestive Enzymes in Pancreas of Broilers

The results are shown in Table 23. Activity of digestive enzymes amylase and lipase were found to be significantly high in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other treatments. In corn-soybean meal with or without antibiotic fed-broilers and mannose diet fed-broilers the activity of amylase and lipase was similar and did not significantly different. The activity of protease was similar in all diet fed-broilers and was not significantly different.

Table 23: Effect of different dietary treatments on activity of digestive enzymes of pancreas of broiler chicken

Treatments	Amylase*	Protease*	Lipase*
Treatment I	411.22±20.70[b]	4926.39±262.70	58.42±5.21[b]
Treatment II	469.85±16.54[b]	5054.02±108.28	52.30±7.74[b]
Treatment III	526.15±19.94[a]	5576.70±139.27	83.21±12.49 [a]
Treatment IV	516.94±17.49[a]	5304.77±164.69	96.19±12.44[a]
Treatment V	495.80±16.58[b]	5100.98±118.24	60.48±3.73[b]
Treatment VI	501.74±15.27[b]	5165.49±124.61	59.79±4.80[b]

*Digestive enzymes in U/mg protein

Treatment I- Control corn-soy; Treatment II- Control + antibiotic; Treatment III- 10% DDP; Treatment IV- 0.2% MOS; Treatment V-0.2% mannose; Treatment VI- 0.1% mannose.
Values are expressed as means of 6 replicates±SE
[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.2 Effect of Different Dietary Treatments on Weight of Duodenum

Figure 52 shows the effect of different dietary treatments on weight of broilers duodenum on the 21st and 42 nd days. The weight of duodenum at 21 days of age were found to be increased significantly in only 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other groups at the same age. The other treatments did not significant different from each other.

The weight of duodenum at 42 were found to be increased significantly in only 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to control treatment without antibiotic. The other treatments were intermediate and did not significant different from others.

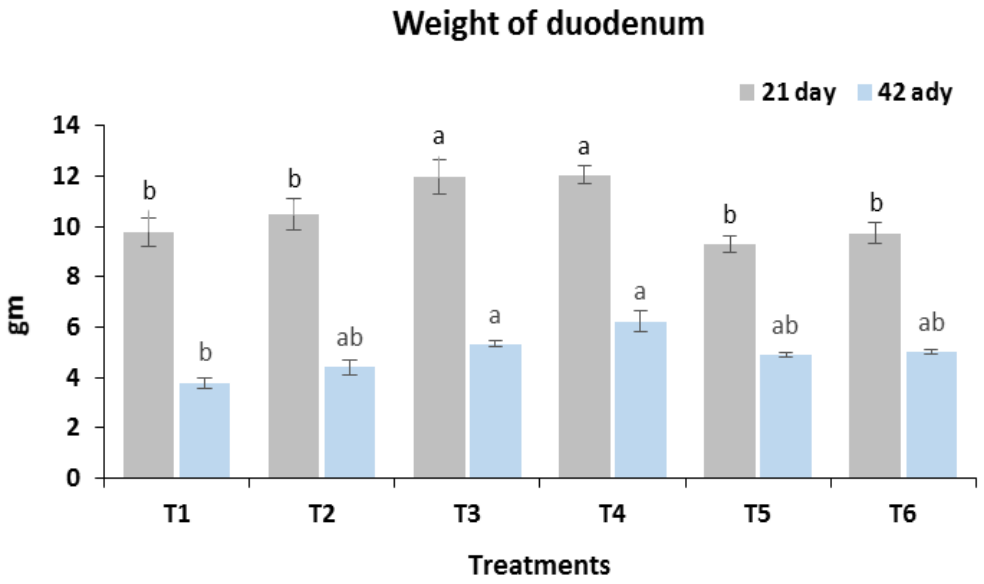

Figure 52: Effect of different dietary treatments on weight of duodenum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.3 Effect of Different Dietary Treatments on Weight of Jejunum

Figure 53 explains the results of the effect of different dietary treatments on weight of jejunum of broilers on the 21st and 42 nd days. On the 21st day of experiment, the weight of jejunum was the same in all diet fed-broilers. There were no significant differences in weight of jejunum between different dietary treatments. On 42 day of experiment the weight of jejunum were found to be increased significantly in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers.

In corn-soy antibiotic diet fed-broilers and mannose diet fed-broilers the weight of jejunum was decreased and the result was comparable to corn-soy diet fed-broilers.

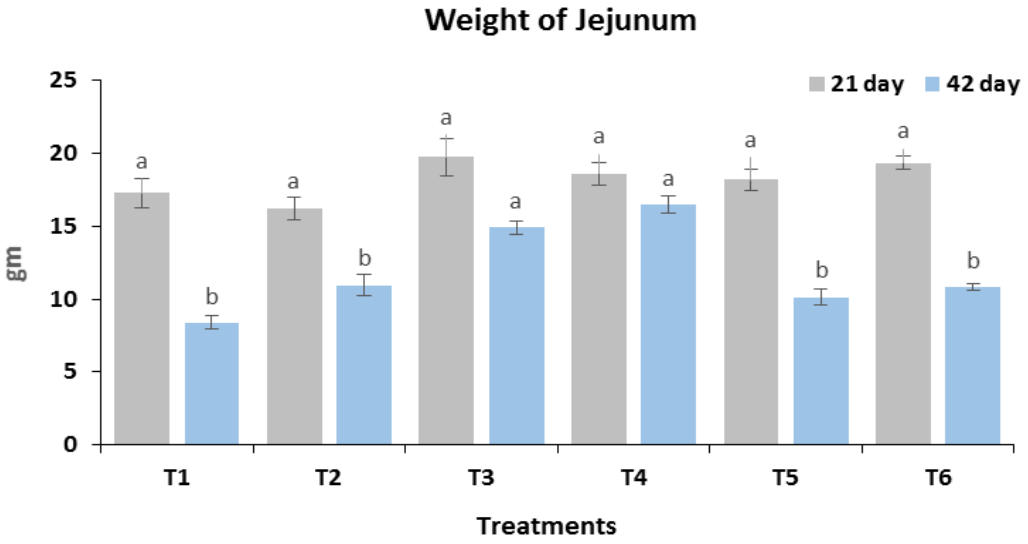

Figure 53: Effect of different dietary treatments on weight of jejunum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.4 Effect of Different Dietary Treatments on Weight of Ileum

Figure 54 shows the effect of different dietary treatments on weight of broilers ileum on the 21st and 42 n^{d} days. Weight of ileum at 21 days of age was significantly higher in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other treatments. On 42 day of experiment, the ileum weight was significantly higher in the 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to the control treatment without antibiotic. The other treatments exhibited intermediate values.

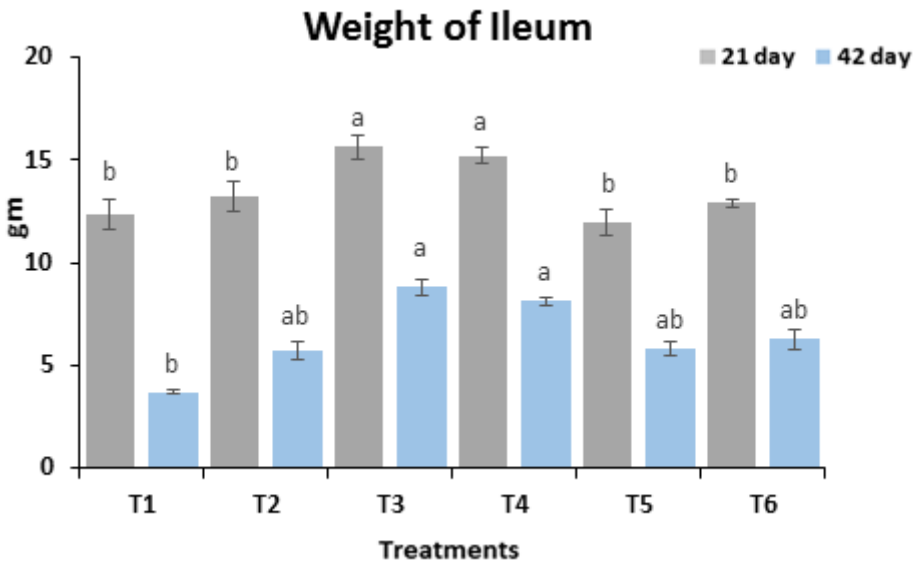

Figure 54: Effect of different dietary treatments on weight of ileum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.5 Effect of Different Dietary Treatments on Broilers Duodenum Length

The length of duodenum (Figure 55) was increased significantly in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn-soy diet fed-broilers. On the 21st day, and 42 n^{d} day of age, in corn-soymeal diet with or without antibiotic fed-broilers and mannose diet fed-broilers the length of duodenum was decreased when compared to 10% DDP diet fed-broilers and MOS diet fed-broilers. The result was comparable to corn-soy diet fed-broilers.

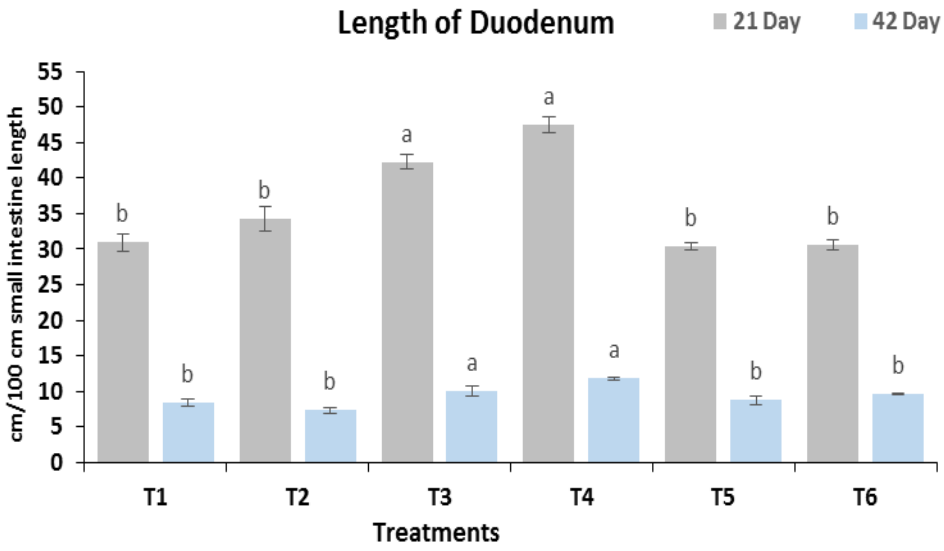

Figure 55: Effect of different dietary treatments on length of duodenum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.6 Effect of Different Dietary Treatments on Jejunum Length of Broilers

Figure 56 shows the effect of different dietary treatments on length of broilers jejunum on the 21st and 42 n^{d} days. On the 21st day, the length of jejunum was increased significantly in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other treatments. The other treatments were significantly similar. On the 42 n^{d} day of experiment, the jejunum length was increased significantly in only MOS fed treatment when compared to other treatments except for 10% DDP diet fed-broilers. The 10% DDP treatment was intermediate.

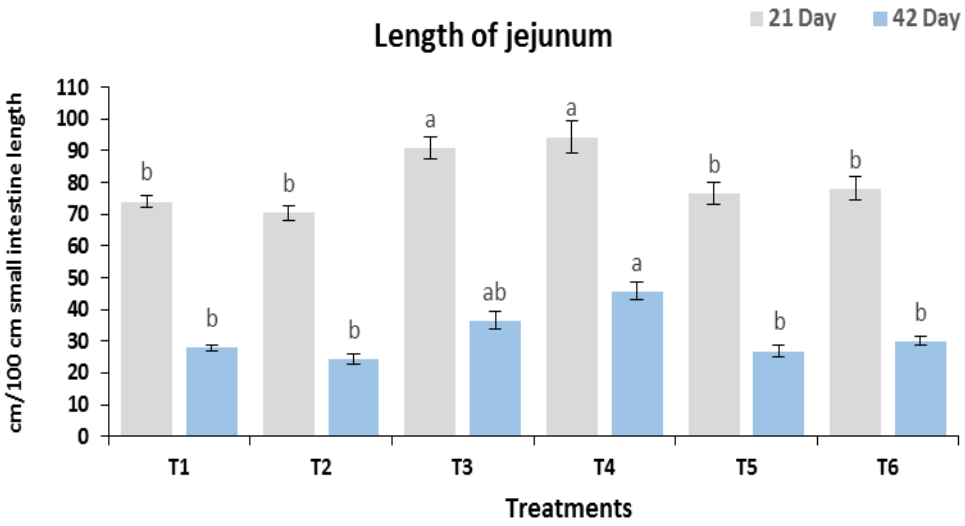

Figure 56: Effect of different dietary treatments on length of jejunum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.7 Effect of Different Dietary Treatments on Ileum Length of Broilers

Figure 57 shows the effect of dietary treatments on length of broilers ileum on the 21st and 42 n^{d} days. On the 21st day of experiment, the length of ileum was the same in all diet fed treatment. On the 42 n^{d} day of experiment, the length of ileum were found to be increased significantly in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other treatments . The length of ileum in other treatments were similar.

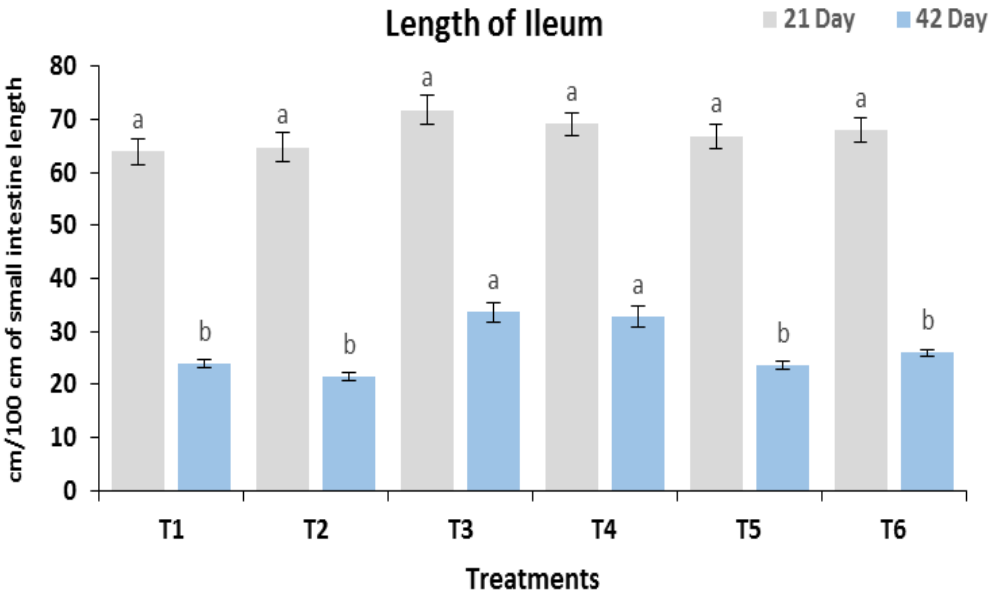

Figure 57: Effect of different treatments on length of ileum of broilers

Treatment I (TI)- Control corn-soy; Treatment II (T2) - Control + antibiotic; Treatment III (T3)- 10% DDP; Treatment IV (T4) - 0.2% MOS; Treatment V (T5) -0.2% mannose; Treatment VI (T6) - 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.8 Effect of Different Dietary Treatments on Histomorphology of Duodenum of Broilers

Table 24 shows the effect of different dietary treatments on histomorphology of duodenum broilers on the 21st and 42 n^{d} days as well as Villus crypt ratio in all dietary treatments.

The generated results showed that the villus height of duodenum was significantly increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn soy diet with or without antibiotic fed-broilers on the 21st day. In the mannose diet fed-broilers the villus height of duodenum was intermediate. Crypt depth of duodenum was decreased significantly in 10% DDP diet fed-broilers, 0.2% MOS

diet fed-broilers and 0.1% mannose diet fed-broilers when compared to corn soybean meal diet with or without antibiotic fed-broilers on the 21st day.

Villus crypt ratio at 21 days of age was significantly increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared with control diet with or without antibiotic fed-broilers and mannose diet fed-broilers. On the 42 n^{d} day, there were no significant differences between villus height and crypt depth of duodenum of broilers fed different dietary treatments.

Table 24: Effect of different dietary treatments on histomorphology of duodenum of broilers

Treatments	Duodenum (21 day)			Duodenum (42 day)		
	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)
Treatment I	928.55±13.32^{b}	124.60±1.91^{a}	7.79±1.13b	1053.51±15.75	118.49±5.76	9.52±1.29
Treatment II	883.54±13.32^{b}	137.74±3.83^{a}	7.04±1.51b	1038.31±18.21	126.74±9.21	8.75±0.97
Treatment III	1022.91±19.37^{a}	102.75±1.21^{b}	10.75±2.10^{a}	1155.14±27.70	116.32±4.97	10.81±1.92
Treatment IV	1000.85±16.58^{a}	109.09±1.98^{b}	10.30±1.80^{a}	1130.04±24.76	105.31±4.50	11.64±2.00
Treatment V	969.76±18.21^{ab}	117.25±3.00^{ab}	8.92±1.59b	1146.46±27.16	112.49±7.91	11.00±1.57
Treatment VI	1015.81±15.28^{ab}	105.95±1.70^{b}	9.84±1.70b	1162.95±29.50	123.20±4.86	10.20±1.89

Treatment I- Control corn-soy; Treatment II- Control + antibiotic; Treatment III- 10% DDP; Treatment IV- 0.2% MOS; Treatment V- 0.2% mannose; Treatment VI- 0.1% mannose.

Values are expressed as means of 6 replicates±SE

^{ab}Means within a row with different letters (a, b) differ significantly (P<0.05)

6.4.9 Effect of Different Dietary Treatments on Histomorphology of Jejunum

Table 25 shows the effect of different dietary treatments on histomorphology of jejunum of broilers on the 21st and 42 n^{d} days

On the 21st and 42 n^{d} days, the villus height of jejunum was significantly increased in 10% DDP diet fed-broilers, 0.2% MOS diet fed-broilers when compared to corn soy diet with or without antibiotic diet fed-broilers. In mannose diet fed-broilers the villus height of jejunum was not changed compared to other treatments at 21 days of age, but at 42 days of age was similar to 10% DDP and 0.2% MOS diets. There was no significant difference in crypt depth of jejunum in dietary treatments on day 21. On the 42 n^{d} day, crypt depth was decreased significantly in 10% DDP diet fed-broilers, 0.2% MOS diet fed-broilers and 0.1% mannose diet fed-broilers when compared to other treatments.

On the 21st day there were no significant differences in villus crypt ratio among different dietary treatments. The villus crypt ratio at 42 days of age was significantly increased in 10% DDP diet fed-broilers, 0.2% MOS diet fed-broilers and 0.1% mannose diet broilers when compared to control diet with and without antibiotic fed-broilers. In 0.2% mannose diet fed-broilers the villus crypt ratio was intermediate.

Table 25: Effect of different treatments on jejunum histomorphology of broilers

Treatments	Jejunum (21 day)			Jejunum (42 day)		
	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)
Treatment I	715.03±29.67^{b}	111.1±14.08	6.74±0.31	885.83±24.62^{b}	122.40±4.56^{a}	7.44±0. 45^{b}
Treatment II	685.42±25.58^{b}	110.76±7.92	6.87±1.51	863.10±27.21^{b}	138.83±6.36^{a}	6.38±0.34^{b}
Treatment III	941.16±34.91^{a}	132.17±9.04	7.50±0.75	1142.77±33.76^{a}	93.09±2.50^{b}	12.85±1.51^{a}
Treatment IV	877.33±32.93^{a}	123.26±10.16	7.58±0.88	1138.04±24.77^{a}	96.31±4.50^{b}	12.72±2.00^{a}
Treatment V	814.47±29.91^{ab}	108.94±5.48	8.21±1.79	1116.57±20.16^{a}	119.06±5.53^{a}	9.76±0.71^{ab}
Treatment VI	907.06±27.40^{a}	127.82±7.58	7.86±1.72	1148.79±29.50^{a}	94.60±3.46^{b}	12.82±1.61^{a}

Treatment I- Control corn-soy; Treatment II- Control + antibiotic; Treatment III- 10% DDP; Treatment IV- 0.2% MOS; Treatment V- 0.2% mannose; Treatment VI- 0.1% mannose.

Values are expressed as means of 6 replicates±SE

^{ab}Means within a row with different letters (a, b) differ significantly ($P<0.05$)

6.4.10 Effect of Different Dietary Treatments on Histomorphology of Ileum

Table 26 shows the effect of different dietary treatments on histomorphology of ileum of broilers on the 21st and 42 n^d days.

On the 21^{st} and 42 n^d days, the villus height of ileum was significantly increased in 10% DDP diet fed-broilers, 0.2% MOS diet fed-broilers and 0.1% mannose diet fed-broilers when compared to corn-soy diet fed-broilers and corn-soy antibiotic diet fed-broilers. In mannose diet fed-broilers the villus height of ileum at 21 and 42 days of age was comparable to 10% DDP and 0.2% MOS. Villus height was significantly increased in all dietary treatments except control treatments on 42 day. Crypt depth of ileum was significantly decreased in 10% DDP diet fed-broilers and 0.1% mannose diet fed-broilers. When compared to corn-soy diet fed-broilers and corn soy antibiotic diet fed-broilers on the 21^{st} and 42 n^d day of age.

In 10% DDP diet fed-broilers and 0.2%, MOS diet fed-broilers the villus/crypt ratio was significantly increased on 21^{st} and 42 n^d days of experiment when compared to the corn-soy diet fed-broilers and the corn-soy antibiotic diet fed-broilers. In mannose diet fed-broilers the villus/crypt ratio was intermediate was not significantly different from other treatments.

Table 26: Effect of different dietary treatments on ileum histomorphology of of broilers

Treatments	Ileum (21 day)			Ileum (42 day)		
	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)	Villus height (µm)	Crypt depth (µm)	Villus/crypt ratio (µm/µm)
Treatment I	513.72±13.80b	89.01±4.11a	5.98±0.44b	764.16±21.52b	135.78±7.91a	5.94±0.60b
Treatment II	492.33±9.23b	101.40±5.26	5.04±0.33b	733.01±15.48b	149.71±8.57a	5.11±0.34b
Treatment III	582.03±10.39a	60.36±2.67b	10.10±0.93a	816.32±17.64a	98.45±3.67b	8.53±0.50a
Treatment IV	564.77±8.69a	64.04±1.90b	9.01±0.72a	820.86±19.25a	92.31±2.60b	9.06±0.42a
Treatment V	516.27±6.50ab	74.19±2.49ab	7.62±1.54ab	820.47±13.26a	105.60±4.50ab	8.27±0.91ab
Treatment VI	561.54±11.38a	70.08±1.77ab	8.31±0.74ab	840.91±19.25a	114.40±5.42ab	7.54±0.32ab

Treatment I- Control corn-soy; Treatment II- Control + antibiotic; Treatment III- 10% DDP; Treatment IV- 0.2% MOS; Treatment V-0.2% mannose; Treatment VI- 0.1% mannose.

Values are expressed as means of 6 replicates±SE

[ab]Means within a row with different letters (a, b) differ significantly ($P<0.05$)

6.4.11 Effect of Different Dietary Treatments on Carcass Characteristics and Organ Weight of Broilers

Table 27 shows the effect of different dietary treatments on organ weight of broilers. The generated results showed that there were no significant difference between live weights of broilers fed different diets. The cold carcass weight and dressing percentage was similar in all dietary treatment groups. The weight of heart, liver, breast and thighs were significantly increased in all dietary treatments except control diet fed-broilers. Table 27 shows the effect of different dietary treatments on organ weight of broilers.

Table 27: Effect of different dietary treatments on carcass characteristics and organ weight of broilers

Treatment	Dietary treatments					
	Control Diet	**Control + Antibiotic**	**10% DDP**	**0.2% MOS**	**0.1% mannose**	**0.2% mannose**
Live weight (g)	2279±8	2371±22	2368±12	2340±14	2302.6±83	2261±14
Carcass (g)	1550.00±24.64	1604±12	1696±12	1664.5±10	1628±59	1561±39
Dressing percentage (%)	68±1.3	67.7±1.5	71.65±0.18	71±0.7	70.71±0.6	69±1.4
Heart (g)	14.2±0.3^{c}	15.3±0.18 ^{a}	14.8±0.06 ^{b}	14.6±0.03 ^{b}	14.7±0.07 ^{b}	13.8±0.3 ^{c}
Liver (g)	49.3±0.3 c	55.3±0.4 ^{a}	55.5±0.7 ^{a}	54.6±0.4 ^{a}	48.7±0.3 ^{c}	49.3±1.5 c
Spleen (g)	2.4±0.09	2.8±0.2	2.9±0.3	3.1±0.2	2.7±0.4	2.5±0.1
Breast (g)	705.8±7.6 ^{c}	760±7.6 ^{ab}	761.6±9.2 ^{a}	755±5 ^{ab}	722±7 ^{bc}	703.6±10.8 ^{c}
Thigh	712±14 ^{bc}	772±4 ^{a}	775.6±7.8 ^{a}	755±5.7 ^{ab}	735±7.6 ^{abc}	710.6±11 ^{c}
Wing	181.6±3.7	190±2.6	189.6±2	190±3	181.8±2	178.6±3.5
Proventriculus (g)	4.29±0.46	5.75±0.53	6.21±0.38	6.41±0.48	5.40±0.77	6.10±0.63
Gizzard (g)	30.87±2.16	32.23±3.23	33.50±3.33	33.99±2.97	29.96±4.65	30.38±2.92

Treatment I- Control corn-soy; Treatment II- Control + antibiotic; Treatment III- 10% DDP; Treatment IV- 0.2% MOS; Treatment V-0.2% mannose; Treatment VI- 0.1% mannose.

Values are expressed as means of 6 replicates±SE

^{ab}Means within a row with different letters (a, b) differ significantly (P<0.05)

6.5 Discussion

Development of the gastrointestinal tract is an significant feature of growth, particularly the development of digestive functional organs like pancreas and intestine. The pancreas produces multi enzymes that are important for the digestion of the feed nutrients. Thus, the enzyme effectiveness in pancreas could reflect the endogenous enzymes levels that are produced and internally stored (Yuan et al., 2017). The protease, lipase and amylase played a critical role in the degradation of proportional nutrient materials and, ultimately, in animal development and health.

In our study, results showed that 10% DDP diet and 0.2% MOS diet enhances the activity of pancreatic enzyme amylase and lipase. Amylase enzyme produced by the pancreas in poultry can hydrolyse starch into smaller units that can be absorbed by the bird (O'Neill et al., 2014). This result was similar to the finding of Jin et al. (1996), who reported that inclusion of a single strain of *L. acidophilus* or a mixture of 12 *Lactobacillus* strains resulted in significantly higher amylase enzyme activities in the small intestine of broilers.

Xu et al. (2003) discovered that the addition of prebiotic supplementations to the broilers feed as if fructo-oligosaccharide altered the protease, lipase, and amylase activity in the small intestinal digesta. They suggested that the higher enzyme activity is due to the adding of more quantity of the enzyme by intestinal bacteria. It has been confirmed that β-glucan, stimulates enteroendocrine cells to produce cholecystokinin (Bourdon et al., 1999). Cholecystokinin is efficient on the encouragement of pancreatic amylase excretion (Matur et al., 2010). The α-amylase activity increase in the intestine in the DDP supplemented broilers indicates that DDP enhances secretion of the

enzyme from pancreas. Pancreatic lipase and amylase stimulates the digestion and absorption of nutrients which leads to a healthy intestine of broilers and enhances growth performance of the bird.

The intestine is a rapid turnover part of the whole GIT in the living body. The development of the gut health is very important for the poultry growth in additional to the productivity. Any type of gut damage will lead to bad gut health, which will cause a decrease in the effectiveness of the nutrient usage (Guilloteau et al., 2010). In our study, the developed length and weight of the intestine proves that dietary supplementation of 10% DDP encouraged the development of intestine thus enhanced the health of gut. These may be associated with delayed movement of ingested feed from one end to another of intestine tract, and the intestinal parts absorbed the little nutrients for its growth with greater priority over body weight increase. There were reports that high fiber contents increased length and weight of intestine of broilers (JøRgensen et al., 1996; Attia et al., 2019). Chemical analysis showed that DDP contains significant amount of fiber and this delays the absorption of nutrients by the intestinal segments which increases the length and weight of intestine.

A special feature of a well bird's digestive tract is a high, healthy villus that have shallow crypts and low renewal rate (Ferket et al., 2002). The longer the villi and the shallower the crypts come up with a greater surface area that helps in more absorption of the nutrients and lower the renewal rate which allows to have an efficient enzyme production and maturation of the intestinal cells (Yang et al., 2009). Results of present study confirmed that 10% DDP altered the intestinal morphology of broilers. The enhancement in GIT morphology are mainly associated with the modification in the diet and alteration in intestinal microflora. Beneficial bacteria catalyze the

development of a healthy intestinal environment which end up with an increase in villi length due to the competitive exclusion (Baurhoo et al., 2007).

The DDP (10%) and MOS supplementations similarly promotes the growth of beneficial bacterial (chapter 4) that also leads to increased villi, decreased crypt and altered villi/crypt ratio, showing similar mode of action. The intestine, especially the villi and crypts of the absorptive epithelium, have significant roles in the eventual stages of nutrient digestion and assimilation (Adeleye et al., 2018). The crypt is the production site where divisions of stem cells occur to allow villus renewal. The higher duodenal and jejunal villi height obtained in this study with 10% DDP and 0.2% MOS suggested enhanced the surface area leads to more absorption of available nutrients, lower crypt depth and higher villus/crypt ratio. Which proves that the addition of 10% DDP enhanced the absorption of nutrients, lowered the secretion of the gut, improved the resistance of disease and increased the overall performance.

A study conducted by Baurhoo et al. (2009) revealed that dietary MOS (0.2 and 0.5% in feed) significantly developed villus height in all intestinal parts on day 24 and 34 when compared with virginiamycin (0.002%) and bacitracin (0.006%) whereas, 0.5% MOS increased the villus height in the duodenum of broilers when compared with the control diet on day 14 of age. Sinovec and Marković (2005) reported that MOS (0.2%) significantly increased the broiler villus height on day 21 of age, as compared to the birds from both negative and positive (Flavomycin 0.002%) control diets. Similarly, the inclusion of MOS (0.2% up to day 21 and 0.1% thereafter) enhanced the villus height in the jejunum as compared to the positive control diet containing 0.001% virginiamycin (Baurhoo et al., 2007).

Adding of 0.1% MOS to broiler diet with or without enzymes has increased broiler duodenal and jejunal villi height (Oliveira et al., 2008). MOS (0.2%) significantly increased the growth of villus height in all parts of the intestine (Markovi et al., 2009) whereas; Ganguly (2013) found that MOS increase villus height only in the duodenum part of the small intestine. Yang et al. (2007) showed that supplementation of broiler diet with 0.1% and 0.2% MOS encouraged the increase in the villus height of the small intestine up to day 35 of age, which then declined gradually in all treatments except MOS containing diet.

The DDP (10%) diet fed-broilers have shallow crypt depth and increased villus crypt ratio in duodenum, jejunum and ileum. This agrees by Baurhoo et al. (2007) who reported that he greater the cell renewal average, the more the depth of the crypt, pointing out the colonization of the intestine with the pathogenic bacteria. It is well known that villi length increases due to the reduction in the enterocyte cell migration from the crypt to the villus. The cell renewal rate will be less, when adding MOS to the feed, which suppresses the pathogenic bacteria and leads to shallower crypts in the intestine of broilers. Yang et al. (2009) highlighted that MOS helped in decreasing the crypt depth in broilers small intestine, encouraging rapid growth of chickens. MOS (0.2%) in broilers diets reduced the crypt depth of all segments of the small intestine of broilers and the results were significant when compared to diets supplemented with Flavomycin 0.002% and to the negative control diets (Sinovec and Marković, 2005). Sarica et al. (2005) reported that MOS brought about a significant decline in crypt depth in all parts of the small intestine except in the duodenum. Enlargement of the gastrointestinal tract has been observed in association with various dietary arrangements. It is noteworthy that the key morphological characteristics observed in all sections of the small intestines in broilers fed DDP and MOS based diets were

indicative that the enlargement resulted in the increase of intestinal mucosa surface rather than tissue mass. This was clearly evidenced by increased size of duodenum, jejunum, and ileum which attributed to the increase in length and there were no changes in submucosa or serosa components of the intestinal wall.

Vidanarachchi et al. (2013) reported that oligosaccharides such as MOS and FOS positively affect the development of the gastro intestinal tract as evidenced by histomorphological study. Higher perimeter and height of villi were observed in histomorphometric studies of duodenum and ileum of broilers fed MOS along with enzymes, which increases the absorption surface of the intestinal segments (Oliveira et al., 2008). In the present study, villus /crypt ratio was increased in broilers fed 10% DDP and 0.2% MOS. This represents the positive role of both DDP and MOS in promoting the healthy development of gastrointestinal tract. The enhanced growth performance of broilers fed symbiotic and probiotic diet was confirmed by histomorphology of intestine by increase in the villus height and villus height: crypt depth ratio (Awad et al., 2009).

In vivo injection of inulin and symbiotic with the addition of inulin showed a significant effect on the histomorphology of broilers as evidenced by an raised the number of goblet cells in the duodenum and the jejunum on the 1st day after hatching (Sobolewska et al., 2017). Similarly alternatives to antibiotics including prebiotics like non digestible oligosaccharides and probiotics improved the length and surface area of villus and health of intestine of broilers infected with *Clostridium perfringens* as illustrated by histomorphology of gut (Alzawqari et al., 2016). Dietary supplementation of MOS and *Curcuma xanthorrhiza* essential oil alone or in

combination reduced harmful effects of heat stress in broiler chickens as evidenced by healthy intestinal gut assessed by histomorphological analysis (Hosseini et al., 2016).

Factors that affect the meat quality can mostly be regulated at different stages of rearing the chicken or during the slaughtering time. The carcass output is closely linked to the enough feed ingredients of broilers (Mir et al., 2017). The present findings were higher than that reported by Narasimha et al. (2013) who concluded that, in Cobb commercial broiler the dressing yield (%) ranges between 63.67% and 66.67%. The dressing yield (%) in the present study for 10% DDP was 71.6% and for 0.2% MOS the carcass yield was 71.0% after 42 days of age which was similar to the value observed by (Abdel-Raheem and Abd-Allah, 2011). They also reported that broilers thigh and breast weight were increased after adding single or combined dietary supplements of mannan-oligosaccharide and probiotics. Similar results were found in our present study.

In broilers, the liver is the main site of lipid production, whereas fatty tissue, especially in the abdomen, is the main site for fat storage (Tůmová and Teimouri, 2010). Feed absorption increases metabolic efficiency of the liver; thus, the effect of feed intake and FCR may cause variation in liver weight (Jones et al., 1995). Some reports showed that having high percentage of fat in broiler ration led to high level of liver fatty acid oxidation, which ended up a reduction in the liver weight (Daggy et al., 1987). Aniebo (2018) reported that the increase in the liver weight was due to the increase in the cassava yeast diet fed-broilers which contained 80% starch and 20% sugar.

Taking into account that the high levels of antioxidant enzyme activities in the liver of broilers fed MOS and DDP, indicated that the synthesis and the release of such enzymes might enlarge the liver. The liver of herbal essential oil-fed-broilers could

have been enlarged because of the metabolic changes of phytochemicals in liver (Bozkurt et al., 2009). There were no significant differences found in the carcass traits of the Cobb broilers under study.

The present findings were agreed with the results of Sahin et al. (2008) and Chumpawadee et al. (2008) who reported that the prebiotic, probiotic, and symbiotic had no significant positive effect on carcass yields of quails and broilers. Addition of prebiotic to broilers diet did not affect weight of gizzard (Ashayerizadeh et al., 2009). Mohammed et al. (2008) reported that MOS supplementation in broilers diet had no significant effects on dressing percentage, gizzard, spleen, proventriculus and bursa weight. MOS at a dose of 3 g/Kg feed improved the carcass characteristics, and intestinal microbial ecology of growing Japanese quails (ABD-ALLAH and ABDEL-RAHEEM, 2012).

6.6 Conclusion

The pancreas excretes multi enzymes that are important for nutrients digestion. Consequently, the activity of the enzymes in the pancreas might show the level of endogenous enzymes that synthesized and internally stored. The activity of digestive enzymes amylase and lipase were upregulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers. The increase in the activity of digestive enzymes in the DDP diet supplemented broilers indicates that DDP stimulates the secretory cells, enhances enzyme activity in the intestine and thereby providing a healthy gut for the digestion and absorption of ingested macromolecules. The positive effect of DDP on broilers improved enzyme activity and nutrient utilization and therefore benefit the feed efficiency in broilers chickens. DDP influences the weight of both proximal and distal portion of intestinal segments like duodenum, jejunum and ileum of broilers. The

increased weight and length of intestine proved that supplementation of DDP helps to enhance the development of intestine thus improve the health of gut.

Chapter 7: Molecular Mechanism of Antibacterial Activity of DDP, MOS and Mannose in Broiler Gut

7.1 Introduction

The intestinal tract of a bird is a specified tube that starts at the beak and ends in the cloaca. Digestion of feed into its basic constituents for absorption is the main function of gut and thereby it helps the utilization of food. The gut functions as a discerning barrier which helps to eliminate unwanted pathogens (Pastorelli et al., 2013). In order to sustain ideal health and stability all over the body, an accurate protection by gut is indispensable and it denotes a major mode of resistance against external antigens (Jeon et al., 2013). Innate and acquired mechanisms of immunity promotes the protection of mucosal surfaces in the gastrointestinal tract that deactivate or discard most foreign antigens (Sharma et al., 2007).

Due to the presence of majority of lymphocytes in the mucosa, these areas are known as mucosa-associated lymphoid tissues (MALT) (Gutjahr et al., 2016). Plasma B cells secrete large amount of secretory immunoglobulin A (SIgA), the vital immunoglobulin in external secretions which is released in relation with adaptive humoral immune system. SIgA helps to block absorption of macromolecule and binding of allergens to mucosal target cells. It also decreases inflammatory properties of other immunoglobulins, deactivate toxins of bacteria, and activate protection mechanisms (Langdon et al., 2016).

Extrinsic mucus layer is the first layer of gut barrier and an internal film with high intensification of secretory IgA and mucin. The external strata is weakly adherent to epithelium. The internal layer is attached to the next layer of the intestine barrier, called the intestinal epithelial cells (IEC). IEC is a single layer of that separate the intestinal

lumen from underneath layer, the lamina propria (Sakamoto et al., 2000). These epithelial cells have to be capable of directly rejuvenate if any tissue damage happened. The enterocytes in the apical epithelium are in charge of the absorption of the nutrients. Some of the IgA-producing plasma cells in the intestinal lamina propria might be produced from B-1 cells (Macpherson et al., 2000).

Animals use IgA produced by plasma B cells as the primary acquired defence neutralizing toxins and pathogenic microbes (Rochereau et al., 2015). The intestinal lamina propria of mammals are involved in the production of T-cell-independent antibodies versus the commensal bacteria, and the bacteria use these antibodies as an evasive technique (Jiang et al., 2004). Several of the IgA-producing plasma cells in the intestinal lamina propria originated from B-1 cells. B-1 cells are a subdivision of B-lymphocytes that are independent from B-2 cells, which designate the dominant subdivision of B cells in mammals. However B-2 cells make most of the specified active antibodies have the most binding desires, antibodies secreted by B-1 cells typically have low binding desires and wide specificities (Berland and Wortis, 2002). These antibodies are natural, and are generated without previous subjection to any immunogens. The natural antibodies found in the broiler sera interact with self or exotic antigens. Intestinal Epithelial Cells (IEC) are the essential cell type that react with the outside environment, they act as the host's first line of the defence. Similarly, mucins and anti-microbial peptides (AMPs) are major segments of the innate immune response to microbial infection. In poultry, defensins and cathelicidins (CATHs) are the main two families of AMPs (Watanabe et al., 2011).

Naturally, AMPs have fewer than 100 leftover amino acid residues, mostly cationic and amphipathic, which lets them to attach to the negatively charged microbial

membranes, destroy, and kill the microbes. Therefore, AMPs are being highly recommended for controlling and preventing of infectious diseases, especially against antibiotic resistant bacteria (Hancock and Sahl, 2006). All four chicken cathelicidins have the ability to kill a broad range of bacteria such as antibiotic-resistant strains (Xiao et al., 2006; Van Dijk et al., 2009; Rodríguez-Lecompte et al., 2012).

In this chapter, we elucidated the molecular mechanism of antibacterial effect of DDP, MOS and mannose by analysing the immunoglobulin levels in serum and intestinal contents and also studied the gene expression pattern of genes present in the gut of broilers.

7.2 Material and Methods

The intestinal and serum samples were collected at 42 days of age from broiler experimental 2 as 2 samples of each replicates (n=6 samples / treatments). The details of sample collection, storage condition were indicated in chapter 4 under experiment2. The analysis work was conducted at National Research Lab (NRL) in Abu Dhabi.

7.2.1 Quantitative Reverse Transcriptase of the Polymerase Chain Reaction (RT-PCR) and Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) Tests

Table 28 details the primer sequences used for RT-PCR analysis. mRNA were isolated from chicken gut and measured by quantitative RT-PCR as previously described (Shao et al., 2013a). Total RNA was isolated from snap-frozen jejunal tissue samples according to the RNeasy mini kit following the animal tissue protocol (Qiagen, Germantown, MD). The purity and concentration of total RNA was measured in a spectrophotometer using the 260:280 nm absorbance ratio. First-strand cDNA was synthesized from 2 µg of total RNA using a Primer Script TM RT reagent Kit and

stored at -20°C until further processing. Oligonucleotide primers for chicken mucin-2, defensins, cathelicidins, LEAP-2 and chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used for the study.

Quantitative RT-PCR were performed using PCR machine. All the tissue samples for the cDNA synthesis and in the following PCR amplifications were run in triplicate. Gene expression for mucin-2, Cath-1, AvBD-1, 2, and 9, and LEAP-2 (Liver expressed antimicrobial peptide-2) were analysed using GAPDH as an endogenous control.

Table 28: Primer sequences used for RT- PCR analysis

Gene	Forward Primer	Reverse Primer
Mucin2	TCACCCTGCATGGATACTTGCTCA	TGT CCA TCT GCC TGA ATC ACA GGT
Cathelicidin-1	GCTGTGGACTCCTACAACCAAC	GGAGTCCACGCAGGTGACATC
AvBD-1	GAAAACCCGGGACAGACG	AGCGAGAAGCCAGGGTGAT
AvBD-2	TCTGCAGCCATGAGGATTC	TAAAGCACATGCCTGGAAGAAAT
AvBD-9	ACCGTCAGGCATCTTCACAG	CCATTTGCAGCATTTCAGC
LEAP-2	TGTGCTTCCCTGCACCAA	GGCGTCATCCGCTTCAGT
GAPDH	TGCTGCCCAGAACATCATCC	ACGGCAGGTCAGGTCAACAA

Total antibody in intestinal contents and serum was determined by a sandwich enzyme-linked immunosorbent assay (ELISA). Briefly, flat-bottomed 96-well plates were coated with unconjugated rabbit anti-chicken IgG heavy plus light chains (Cedarlane, Hornby, Ontario, Canada) (2 µg/ml) and incubated at 4°C overnight. Plates were then washed and blocked for 1h at 37°C with PBS-Tween 20 containing 0.25% gelatin. Serum and intestinal contents (diluted 1:100 and 1:20 in blocking buffer) were then added, and plates were incubated for 1h at 37°C. Subsequently, rabbit anti-chicken IgG heavy plus light chains conjugated with horseradish peroxidase (diluted 1:20,000

in blocking buffer) was added and plates were incubated for 1h at 37°C. The substrate ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonicacid) was added and plates were incubated for 30 min at room temperature in the dark. The absorbance was measured at 405 nm using a microplate reader.

7.3 Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using general linear model (GLM) and mean comparisons were performed using Duncan's multiple range test to compare significant differences between means for all analyses. Statistical analysis was conducted using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

7.4 Results

7.4.1 Effect of Different Dietary Treatments on Level of Immunoglobulins in Serum

The results are shown in Figures 58 and 59, respectively. The level of immunoglobulin A and G in serum was found to be increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn soy meal diet with or without antibiotic diet fed-broilers and mannose diet fed-broilers.

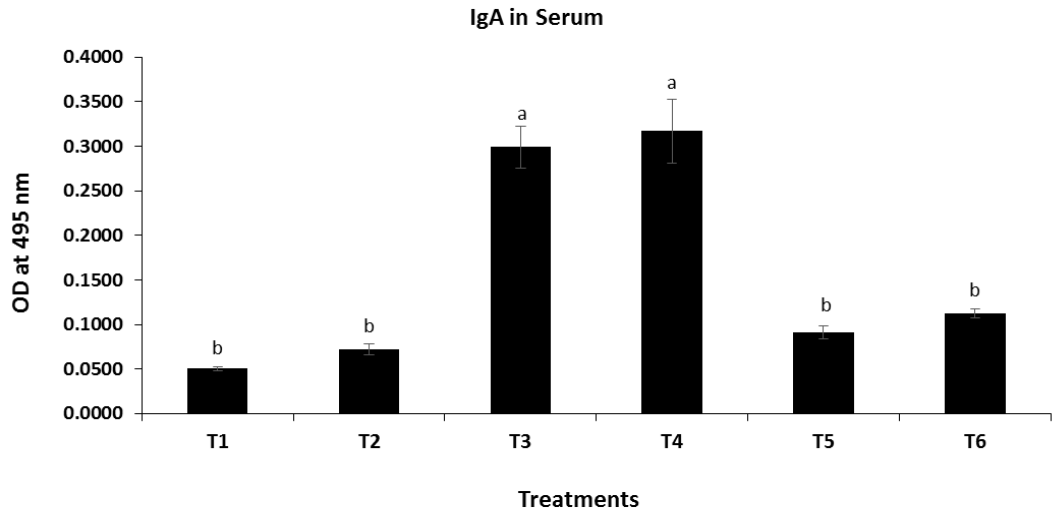

Figure 58: Effect of different dietary treatments on immunoglobulin-A in serum

Treatment I - Control corn-soy; Treatment II - Control + antibiotic; Treatment III - 10% DDP; Treatment IV- 0.2% MOS;
Treatment V - 0.2% mannose; Treatment VI- 0.1% mannose.
Values are expressed as means of 6 replicates±SE.
[ab] Means with in a row with different letters (a, b) differ significantly ($P < 0.05$)

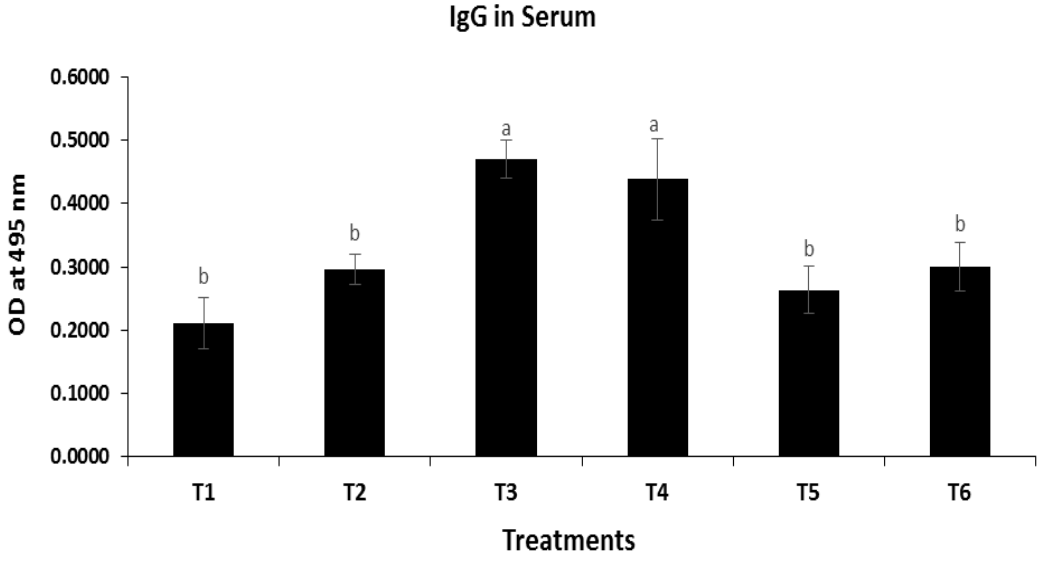

Figure 59: Effect of different dietary treatments on immunoglobulin-G in serum

Treatment I - Control corn-soy; Treatment II - Control + antibiotic; Treatment III - 10% DDP; Treatment IV- 0.2% MOS;
Treatment V - 0.2% mannose; Treatment VI- 0.1% mannose.
Values are expressed as means of 6 replicates±SE.
[ab] Means with in a row with different letters (a, b) differ significantly ($P < 0.05$)

7.4.2 Effect of Different Dietary Treatments on Level of Immunoglobulins in Intestinal Contents

The results are shown in Figures 60 and 61, respectively.

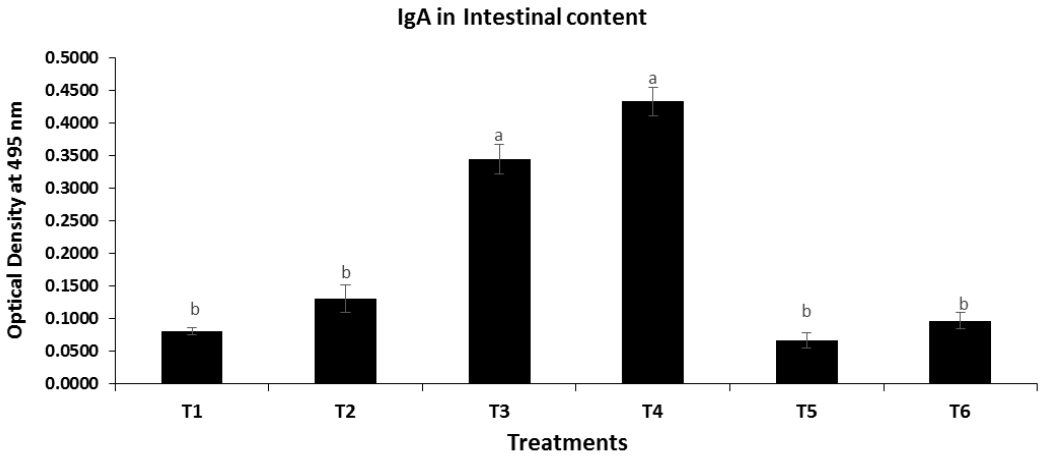

Figure 60: Effect of different dietary treatments on immunoglobulin-A in intestinal content

Treatment I - Control corn-soy; Treatment II - Control + antibiotic; Treatment III - 10% DDP; Treatment IV- 0.2% MOS;
Treatment V - 0.2% mannose; Treatment VI- 0.1% mannose.
Values are expressed as means of 6 replicates±SE.
[ab] Means with in a row with different letters (a, b) differ significantly ($P < 0.05$)

The level of immunoglobulin A and G in intestinal content was found to be significantly increased in 10% DDP diet and 0.2% MOS diet fed-broilers when compared to other treatments.

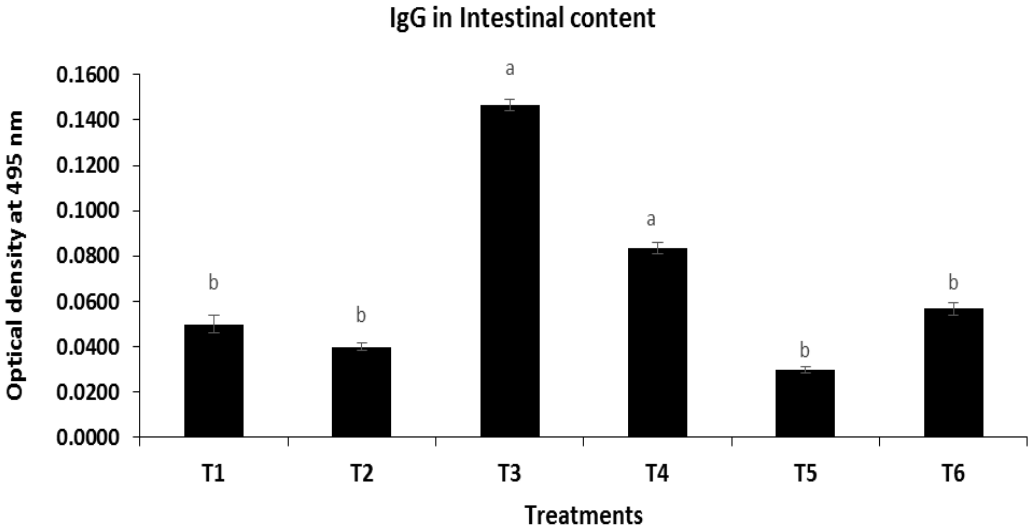

Figure 61: Effect of different dietary treatments on immunoglobulin-G in intestinal content

Treatment I - Control corn-soy; Treatment II - Control + antibiotic; Treatment III - 10% DDP; Treatment IV- 0.2% MOS; Treatment V - 0.2% mannose; Treatment VI- 0.1% mannose. Values are expressed as means of 6 replicates±SE.
[ab]Means with in a row with different letters (a, b) differ significantly ($P < 0.05$)

7.4.3 Effect of Different Dietary Treatments on Mucin-2 Expression in Jejunum

The effect of different dietary treatments on the expression of mucin-2 was studied and results are presented in Figure 62. The relative mRNA expression of mucin-2 was found to be significantly up regulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers on 42th day of experiment when compared to other treatments except for treatment given 0.1% mannose. The mRNA expression of mucin-2 was found to be similar in 0.2% mannose diet fed-broilers to other treatments.

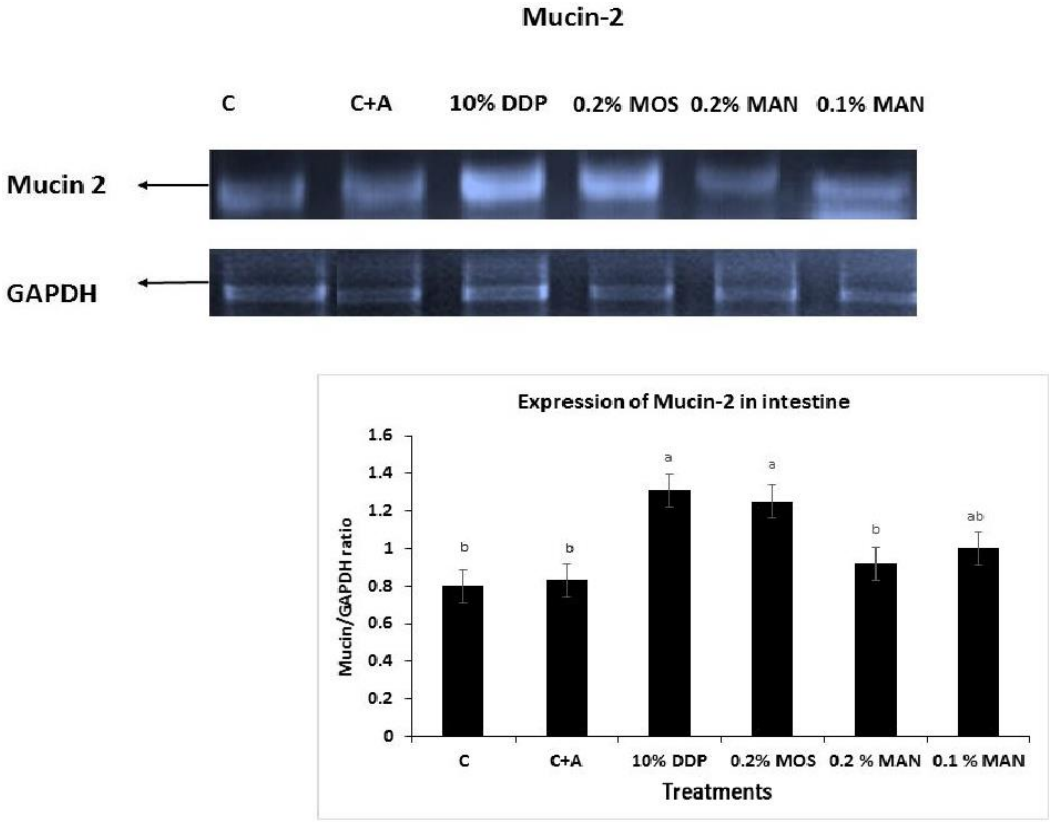

Figure 62: Effect of different dietary treatments on the mRNA expression of Mucin-2 on jejunum

C - Control corn-soy; C+A - Control + Antibiotic; 10% DDP- 10% DDP; 0.2% MOS- 0.2% MOS; 0.2% MAN- 0.2% Mannose; 0.1% MAN - 0.1% Mannose.
Values expressed as mean of quadruplicate experiments±SE.
[ab]Means with in a row with different letters (a, b) differ significantly (P <0.05).

7.4.4 Effect of Different Dietary Treatments on Cathelicidin-1 Expression in Jejunum

Figure 63 shows the generated results. The relative mRNA expression of cathelicidin-1 was found to be significantly up regulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared with other treatments except for 0.1% mannose. These others treatments did not significant different from each other.

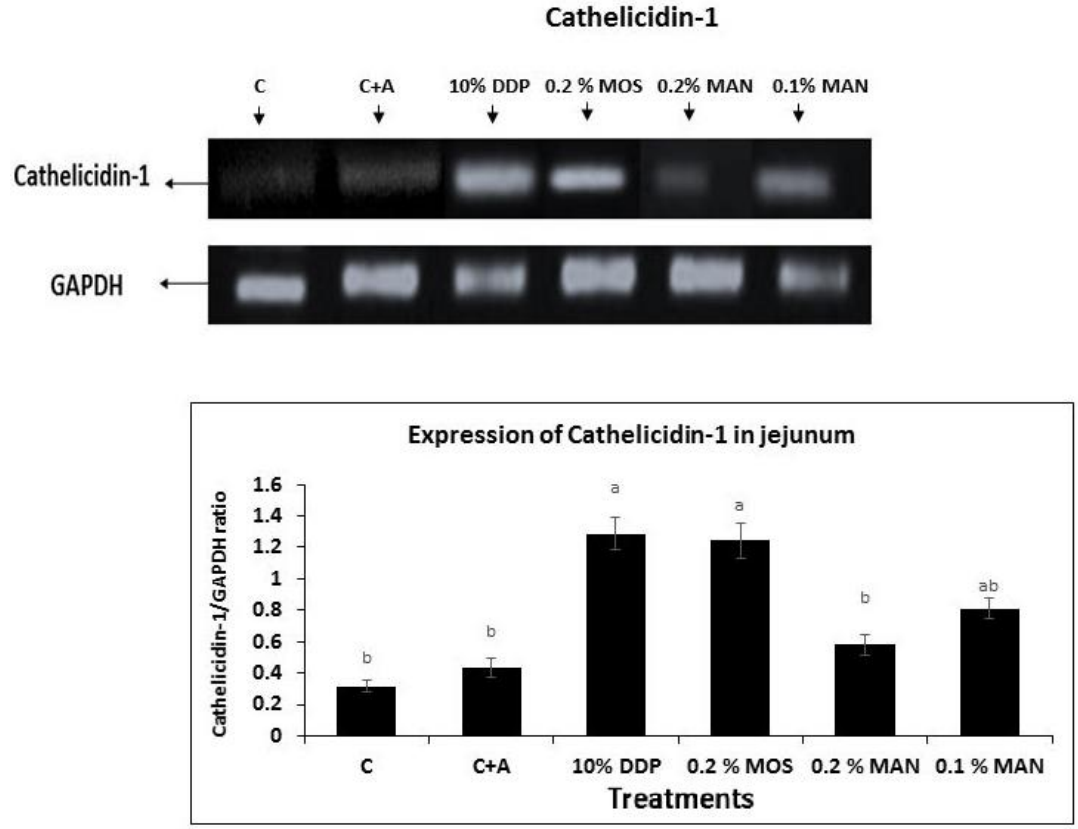

Figure 63: Effect of different dietary treatments on the mRNA expression of Cathelicidin-1 on jejunum

C - Control corn-soy; C+A - Control + Antibiotic; 10% DDP- 10% DDP; 0.2% MOS- 0.2% MOS; 0.2% MAN- 0.2% Mannose; 0.1% MAN - 0.1% Mannose.
Values expressed as mean of quadruplicate experiments±SE.
[ab]Means with in a row with different letters (a, b) differ significantly (P <0.05).

7.4.5 Effect of Different Dietary Treatments on Beta Defensins Expression in Jejunum

Figure 64 shows the obtained results. The relative mRNA expression of beta defensins was found to be significantly up regulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other groups. The other treatments did not significant different from each other.

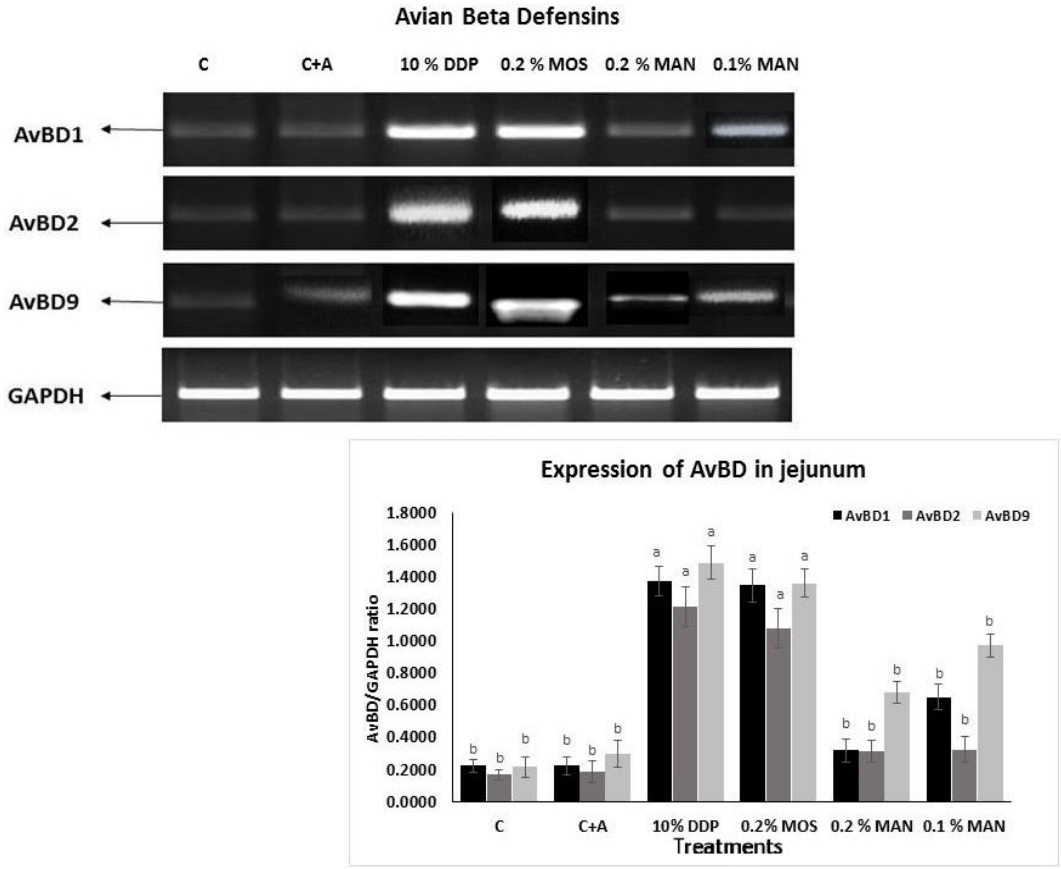

Figure 64: Effect of different dietary treatments on the mRNA expression of beta defensins on jejunum

C - Control corn-soy; C+A - Control + Antibiotic; 10% DDP- 10% DDP; 0.2% MOS- 0.2% MOS; 0.2% MAN- 0.2% Mannose; 0.1% MAN - 0.1% Mannose.
Values expressed as mean of quadruplicate experiments±SE.
[ab]Means with in a row with different letters (a, b) differ significantly (P <0.05).

7.4.6 Effect of Different Dietary Treatments on the LEAP-2 Expression in Jejunum

Figure 65 shows the generated results. The relative mRNA expression of LEAP-2 was found to be significantly up regulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to other treatments. The other treatments were not significantly different from each other.

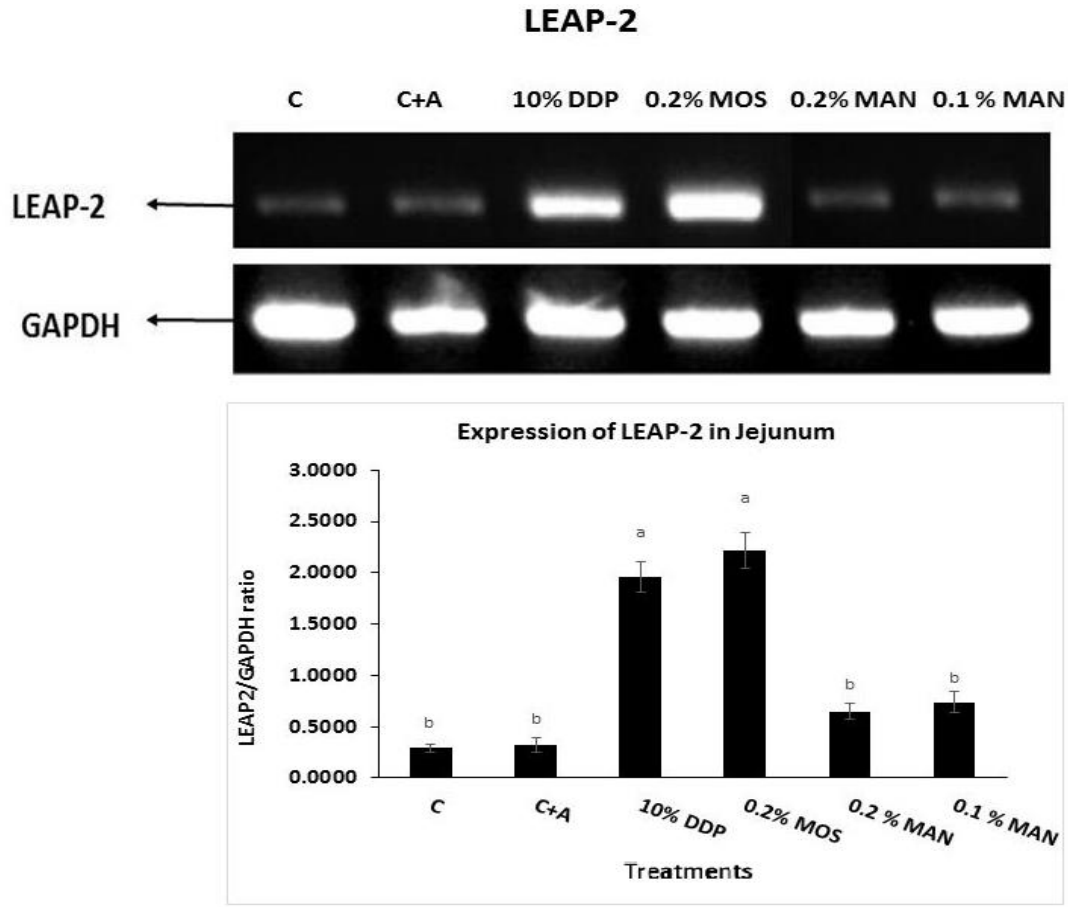

Figure 65: Effect of different dietary treatments on the mRNA expression of LEAP-2 on jejunum

C - Control corn-soy; C+A - Control + Antibiotic; 10% DDP- 10% DDP; 0.2% MOS- 0.2% MOS; 0.2% MAN- 0.2% Mannose; 0.1% MAN - 0.1% Mannose.
Values expressed as mean of quadruplicate experiments±SE.
[ab]Means with in a row with different letters (a, b) differ significantly (P <0.05).

7.5 Discussion

Colonisation of poultry intestine by commensal bacteria is a continuous process, which starts directly post hatching, and the microbiota of the small intestine starts by the second week-post-hatch. Commensal bacteria related to the *Lactobacillus* spp. are located all the time in the small intestine of young chicken, while anaerobes like Bifidobacterium spp., are found in the ceca of elder poultry (Macpherson et al., 2000). Commensal bacteria in the poultry intestine are near the cells of the gut-associated immune system. the reaction between the host cells and the bacteria or their structural

constituents may lead to the amendment to the T- or B-cell-mediated immune responses, either internally or systemically (Jiang et al., 2004). The presence of lymphoid tissues in the mucosa of the gastro intestinal tract enhances the circulation of activated B cells in the plasma through lymphatic or blood vessels.

The circulation of immune cells increases the secretion of specific IgA, thereby preventing the attack of more toxins or pathogens (Fukuyama et al., 2012). In the present study, the level of immunoglobulin were increased in both serum and intestinal contents of 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers which indicates the role of DDP in regulating the immunity of body through immunoglobulin secretion. Even though IgA regulates immunity through neutralizing the pathogens in their natural route of infection, serum IgG is the most prevalent immunoglobulin to analyze infections or immune status in humans and domestic animals (Kuttappan et al., 2012).

The presence of immunoglobulins especially IgA and IgG in chicken sera enhances the immunity of the birds and these antibodies are reactive to self or foreign antigens (Lammers et al., 2004; Rhee et al., 2004). Dietary supplementation of phenyl lactic acid, a natural feed additive increases the immune characteristics and the number of lactic acid bacteria and decreases the number of coliform bacteria in broiler chicks (Kim et al., 2014). Dietary yeast β-glucans supplementation in birds infected with necrotic enteritis enhances intensity of both serum specific IgG and intestine specific IgA antibodies against C. *perfringens* antigens which suggest that prebiotics like β-glucans have beneficial effects on humoral immunity in NE-infected broilers (Tian et al., 2016).

In animals, yeast β-glucans improved systemic humoral immunity and enhanced the IgA production by mediating the intestinal immune system in chickens (Shao et al., 2013b). In our study, results showed a reduction in the total bacterial count in the intestine and increase in immune globulin level in serum and intestinal content of 10% DDP and 0.2% MOS diet fed-broilers, suggesting an enhancement in the immune response with similar mode of action of DDP and MOS. These results are in agreement with (Corthesy, 2013) who suggested that the elevated levels in IgA levels might be due to increase in immune cells like B and T lymphocytes and a reduced level of pro-inflammatory cytokine in intestine which results in enhanced gut integrity in carbohydrate-based prebiotics like glucan-supplemented birds. Therefore, our results suggested that dietary supplementation of 10% DDP and 0.2% MOS stimulates humoral immunity of broiler chickens.

In broilers, the intestinal tract epithelium is protected by mucus layer composed of mucin glycoproteins. Goblet cells are the major area for the synthesis and secretion of mucin glycoproteins. The major role of mucin in mucus layer is to protect the gut against digestive enzymes, acidic chyme and from pathogens. Mucin mediates nutrition transport between the epithelial cells and the luminal contents and also acts as lubricant (Montagne et al., 2004). There are three classes of mucins: secretory gel-forming (MUC2, MUC5AC, MUC5B, MUC6), secretory soluble (MUC7), and membrane-bound (MUC1, MUC3, MUC4 and MUC12). Secretory gel-forming mucins are huge in size, have a high carbohydrate quantity and show both viscous and elastic features (Demouveaux et al., 2018). These mucins have at any rate one repeating domain which has high rates of threonine, proline, serine and also a cysteine rich domain. The dominant gel-forming mucin in the intestine is MUC2. Membrane-bound mucins, or receptor mucins, are engaged in signal clarification, oncogenic

actions and gel production (Loeffler, 2014). In 10% DDP and 0.2% MOS diet fed-broilers the mucin-2 gene expression was found to be significantly increased on the 42th day when compared to corn-soybean meals diets fed-broilers and mannose diet fed-broilers. Former research reports reported that adhesion of *Lactobacillus* strains and other bacterial strains to intestinal mucin 2 through competition between the harmful and beneficial bacteria (Lee and Puong, 2002). Examinations have been also found that diet supplemented with probiotic and prebiotic significantly increased mucin-2 gene expression (Smirnov et al., 2005). Carbohydrates, proteins, and specific amino acids such as threonine have the ability to change mucin excretion and may react immediately with goblet cells or with the enteric nervous system to cause changes in mucin excretion (Moghaddam et al., 2011). Mucin 2 gene expression increased after exposing the chicks to hunger (Smirnov et al., 2004).

Dietary factors like fiber, phytate, and protein may have an impact on both the creation and excretion of mucin from the goblet cells and the retrieval of mucin in digesta (Montagne et al., 2003). The expression type of the mucin 2 gene in chickens fed antibiotic growth promoter or a probiotic product were greater than that in controls (Smirnov et al., 2005). Supplementation of phyto-additives like turmeric, thyme and cinnamon to both basal diets enhanced the expression of mucin 2 mRNA in jejunum of poultry (Sadeghi et al., 2012). Cytokines, growth factors and bacterial products that influence the distinction of goblet cells can also alter mucin 2 gene expression. Our results showed up regulation of mucin 2 gene expression in DDP and MOS diet fed-broilers which indicates the role of 10% DDP and 0.2% MOS in regulating the mucin secretion in intestinal epithelial cells and enhancing the protective barrier of intestine against harmful pathogens.

Host Defence Peptides (HDPs), like cathelicidins and defensins are substantial effector molecules of the innate immune system. These peptides have several functions as they show both broad-spectrum antimicrobial activity and also have many immunomodulatory actions. Two of the main classes of HDPs are cathelicidins and defensins. In mammals, these peptides are believed to be very important in neonatal defence (Kai-Larsen et al., 2014). In the chicken, four cathelicidins and 14 β-defensins have been described. Expression analysis of chicken HDPs showed clear differences over the course of development. Cathelicidin-1, -2 and -3 showed a strongly increased expression in the cecum and tonsils at 4 weeks post-hatch (Achanta et al., 2012). Intestinal expression of the β-defensins 1, 2, 4 and 6 decreased in the first week of post-hatch, but increased again in the second week (Crhanova et al., 2011).

In our study cathelicidin expression was found to be increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers. These results show the expression of cathelicidin in the jejunum supports the previous studies that reported cathelicidin are expressed in the mucosal tissues of the digestive and respiratory of chickens (Goitsuka et al., 2007). The synthesized cathelicidins in the digestive tract may play roles in defence against pathogens since they have a broad spectrum of antimicrobial activities (Xiao et al., 2006). Cathelicidin gene expression are regulated by microbial contagion or by inflammatory cytokines (Akbari et al., 2008). Summers (2012) reported that jejunal Cath-3 mRNA expression is remarkably declined in broilers infected with bacteria. Higher expressions of cathelicidin gene in the jejunum, indicates the antibacterial effect of 10% DDP and 0.2% MOS. Broilers supplemented with yeast β-glucans showed decreased *C. perfringens* inhabitants in the cecum pointing that yeast β-glucans supplementation prevented *C. perfringens* colonization, by activating the intestinal cathelicidin gene expression (Tian et al., 2016).

Modification of defensin expression might be from the ways to enhance the health of the animal as well as to lower the zoonotic diseases. Defensins are little, cationic, and amphipathic cysteine-rich antibiotic peptides available in plants, insects, mammals and birds. Several β-defensins have been isolated from bird's heterophils. Moreover, β-defensins were found to be constituently or inducible expressed at mucosal surfaces of the respiratory, intestinal and urogenital tracts.

In the present study, 10% DDP and 0.2% MOS significantly up-regulated AvBD-1, 2 and 9 gene expressions in the jejunum of broilers. Studies have shown that avian β-defensins exhibit strong bactericidal and coccidiocidal action against pathogens, such as *Salmonella* (Ramasamy et al., 2012), as well as *E. maxima and C. perfringens* in poultry (Hong et al., 2012). Enhanced expression of defensin genes is associated with increased innate immune response and host resistance to infection (Crhanova et al., 2011). This suggested that dietary inclusion of 10% DDP and 0.2% MOS in broiler diets stimulates the production of avian β- defensins to exert immunity and bactericidal effects against harmful bacteria and thus improve animal health.

Chicken LEAP-2 (cLEAP-2), is a 76 amino acid peptide highly expressed in a number of chicken epithelial tissues. In the chicken genome cLEAP-2 is located on chromosome 13 (Brogden et al., 2003). The main role of cLEAP-2 is protecting intestinal epithelial tissues from pathogenic microbes through preventing the attachment of invading pathogens (Michailidis, 2010). In bacterial infected broilers the expression level of cLEAP-2 was found to be down regulated in broilers in their intestine (Summers, 2012). In the present study, supplementation of 10% DDP and 0.2% MOS activates the expression of cLEAP-2 in the jejunum of broilers. cLEAP-2 expression profiles proposed that encouraged cLEAP-2 gene expression represented

enhanced resistance to bacterial infection. Dietary yeast β-glucans supplementation also significantly increased cLEAP-2 gene expression compared with the non-supplemented birds with supressed populations of intestinal *C. perfringens* in the glucan-supplemented chicks (Tian et al., 2016).

7.6 Conclusion

The level of immunoglobulin A and G in serum and intestinal content was found to be increased in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers when compared to corn soy antibiotic diet fed-broilers, corn-soy antibiotic fed-broilers and mannose diet fed-broilers. Immunoglobulins level in serum and intestinal content was found to be decreased in corn-soy diet fed-broilers and the level was similar to corn-soy with antibiotic diet fed-broilers and mannose fed broilers as well. The relative mRNA expression of mucin-2, Cathelicidin, beta defensins (AvBD1, AvBD2 and AvBD9) and CLEAP-2 were found to be significantly up regulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers on 42th day of experiment when compared to corn-soybean meal with or without antibiotic diet fed-broilers and mannose fed broilers as well. In mannose diet fed-broilers in which 0.1% mannose diet fed-broilers the gene expression of mucin-2, Cathelicidin, beta defensins (AvBD1, AvBD2 and AvBD9) and CLEAP-2 was similar to 0.2% mannose diet fed-broilers and control diet fed-broilers.

Chapter 8: General Conclusions and Recommendations

Antimicrobial additives are widely used as growth enhancers in poultry nutrition. There is increasing demand for the public health about the effect of the extensive use of antimicrobial classical additives in poultry production. Addition of antibiotic growth promoters (AGPs) to poultry feed enhances digestion, nutrient absorption and maintains a balance in the microbial population with reduced intestinal disorders in birds. In recent years, the inclusion of these substances has been banned due to the harmful effects of antibiotic residues in animals and development of antibiotic-resistant strains of microorganisms.

Solid State Degradation (SSD) has enhanced the Neutral Detergent Fiber (NDF) and reduced Acid Detergent Fiber (ADF). Monosaccharide composition of fiber was significantly increased in *T. reesei* degraded date pits. Pectin, soluble polysaccharide and MOS content were also increased in which galactose and mannose were the major neutral sugars. When compared DP, the protein content of the DDP was higher by up to 20%. Over seventeen types of amino acids were observed and identified. Among the detected minerals, K, Ca, Mg, S, and P were preponderant. The phenolics and flavonoids content of DDP significantly increased. DDP also significantly improved in antioxidant activity.

The time interval study on growth performance in broilers showed the optimum time period of using DDP as a broiler chicken feed additive is between five weeks. This feeding period of DDP gave a higher growth performance on broilers and lower bacterial count, suggesting that DDP for at least five weeks is recommended. When DDP was used for six weeks, the growth performance results of the broiler chickens was comparable with that of MOS, and with AGPs. The only difference was that the

number of beneficial bacteria in the small intestine (*Lactobacillus* spp. and *Bifidobacterium* spp.) were significantly higher in both the DDP and MOS treatments in comparison with the other treatment used AGPs.

The activity of anti-oxidant enzymes in serum, liver and intestine of broilers fed diets with 10% DDP and 0.2% MOS were significantly surged. Whilst, the MDA content was significantly slumped. Dietary inclusion of DDP did not affect most of blood biochemical and parameters and they were in the normal ranges in the dietary treatments. DDP and MOS supplementation has elevated the immune response in broilers as evidenced by the results of MCH parameters, which might confirm the non-toxicity of, and the immune stimulate ability of DDP feed additive in broiler chickens.

The effectiveness of digestive enzymes amylase and lipase were upregulated in 10% DDP diet fed-broilers and 0.2% MOS diet fed-broilers. Which stimulates the secretory cells, enhances enzyme activity in the intestine and thereby providing a healthy gut for the digestion and absorption of consumed nutrients, therefore benefit the feed efficiency in broilers chickens. DDP and MOS influenced the weight of both proximal and distal portion of intestinal segments like duodenum, jejunum and ileum of broilers. That helps to improve the development of intestine thus improve the gut health. Supplementation of DDP gave similar results as the MOS supplement, showed beneficial effects on intestinal morphology and structure. Higher villus/crypt ratio and lower crypt depth helps efficient nutrient absorption of nutrients, increased villus height all over the intestinal segments of broilers, improved the resistance to diseases and decreased the secretion of the gut, which means better overall gut performance.

DDP upregulated the level of immunoglobulin A and G in serum and intestinal contents, which indicates the enhancement immunity of the birds throughout the

immunoglobulin secretion. DDP and MOS increased the expression patterns of Mucin-2, host defence peptides like cathelicidins especially cathelicidin-1 and beta-defensins like AvBD1, AvBD2 and AVBD9, cLEAP-2Anti-bacterial genes present in intestinal epithelium which explains the reduction of the microbial population and enhancement of the growth performance. Both DDP and MOS treatments elevated mRNA expression of genes in jejunum of broilers, which suggested that dietary DDP supplementation stimulates production of antibacterial peptide like genes and thereby exerts immunity and bactericidal effects against bacteria.

The good proximate composition, enhanced amounts of minerals and antioxidants suggested the utilization of DDP as a prebiotic. Results of the present study demonstrated that normal growth performance, and safe microbial count in broilers fed DDP, which recommends dietary inclusion of DDP in broiler diets, which acts as a prebiotic.

This study suggests the utilization of a new by-product from date palm in the form of DDP in poultry production. DDP can be included in broiler diets for to replace 10% of the corn in poultry diets and the use of oxytetracycline growth promoter. Also to replace 0.2% MOS a commonly used prebiotic from broiler diet, as it showed similar growth performance and antibacterial property with degraded date pits especially in the Middle East, where an plenty of date palm by-product is available. Exploitation of this by-product could reduce the expense of feed in the poultry production business as well as, reducing the consequents of antibiotic resistance in human pathogenic bacteria. Future research is important for explaining the mechanism, and the innovative capability of the commercial use of DDP as a viable source of energy and as prebiotic for the animal feed industry. In addition, DDP diet additive may be applied

to other animals such as ruminants and in the same time decreased the environmental pollution resulted from accumulation of unused agriculture by-products.

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- Alyileili, S., El-Tarabily, K. A., and Ibrahim, W., Effect of *T. reesei* degraded date pits on the growth performance and gut bacterial flora in broilers chickens. Annual Conference of the Association for General 2019 and Applied Microbiology 2019. BTP076. P 99.

- Alyileili, S., El-Tarabily, K. A., and Ibrahim, W., Phytochemical Composition and Antioxidant Activity of *Trichoderma reesei* Degraded Date (*Phoenix dactylifera* L.) Pits. Current Bioactive Compounds 2019; DOI : 10.2174/1573407215666190207093046

- Alyileili, S., El-Tarabily, K. A., and Ibrahim, W. (2019). Effect of dietary inclusion of degraded date (*Phoenix dactylifera* L.) pits on intestinal development and morphology of broilers. The fifth UAE Graduate Students Research Conference, Zayed University, Abu Dhabi, UAE on 16-4-2019.

Patents

- Hussein, Ahmed Soliman, Ibrahim Hassan Belal, Salem Ali Rashed and Khalid Abas El-Tarabily, “Date Pit Composition for Treatment of Animals” The European Union Patent and Trademark Office, Publication No. EP2586318 (A1). The EU Patent Office granted the patent on April 16, 2014.

- Hussein, Ahmed Soliman, Ibrahim Hassan Belal, Salem Ali Rashed and Khalid Abas El-Tarabily, “Date Pit Composition for Treatment of Animals” The United States Patent and Trademark Office. US - Patent Publication No. US 8,968,729 B2. The US Patent Office granted the patent on March 3, 2015.

- Hussein, Ahmed Soliman, Ibrahim Hassan Belal, Salem Ali Rashed and Khalid Abas El-Tarabily, “Date Pit Composition for Treatment of Animals” The Hong Kong Patent and Trademark Office Patent Publication No. HK patent No. HK1184642 B. The Hong Kong Patent Office granted the patent on January 9, 2015.

- Hussein, Ahmed Soliman, Ibrahim Hassan Belal, Salem Ali Rashed and Khalid Abas El-Tarabily, “Date Pit Composition for Treatment of Animals” The United States Patent and Trademark Office. US - Patent Publication No. US 10,265,368 B2. The US Patent Office granted the patent on April 23, 2019.