Sudan University of Science and Technology

College of Graduate Studies

Response of Broiler Chicks to Various Levels of Multi-enzymes and Feeding Durations

إستجابة الدجاج اللاحم لمستويات مختلفة من خليط الإنزيمات و فترات التغذية

A Thesis submitted in fulfillment of the Requirements for the Ph.D Degree in Animal Production Philosophy

Submitted by: Faisal Sayed Abdalgalil Nasr

B.Vet.Sc, Faculty of Veterinary Science, University of Khartoum (1994).
M.SC., Animal Production, Faculty of Natural Resources, University of Juba (1998).

Supervised by:
Prof. Dr .Mukhtar Ahmed Mukhtar

Co- supervised by:
Prof. Dr .Kamal Abd Elbagi Mohammed

2022

Sudan University of Science and

Technology College of Graduate Studies

Response of Broiler Chicks to Various Levels of Multi-enzymes and Feeding Durations

إستجابة الدجاج اللاحم لمستويات مختلفة من خليط الإنزيمات و فترات التغذية

A Thesis submitted in fulfillment of the Requirements for the Ph.D. Degree in Animal Production Philosophy

Submitted by: Faisal Sayed Abdalgalil Nasr

B.Vet.Sc., Faculty of Veterinary Science, University of Khartoum (1994).
M.Sc., Animal Production, Faculty of Natural Resources, University of Juba (1998).

Supervised by: Prof. Dr .Mukhtar Ahmed Mukhtar Ali

Department of Animal Production, College of Agricultural Studies,

Sudan University of Science and Technology

Co- supervised by: Prof. Dr .Kamal Abd Elbagi Mohammed

Department of Animal production, College of Agricultural Studies,

Sudan University of Science and Technology

Date of Examination: 20/06/2022

ألآيـّة

بسـم الله الرحمن الرحـيم

قال تعالـى:

(وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِراً نُخْرِجُ مِنْهُ حَبّاً مُتَرَاكِباً وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهاً وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لايَاتٍ لِقَوْمٍ يُؤْمِنُونَ).

صـدق الله العـظـيم

سورة الأنعام (٩٩)

DEDICATION

To My Parents,
To My Brothers and Sister,
To My boys Marwan and Mazin,
And To My Wife,
With LOVE,
To all people in working in poultry,
Production in Sudan,
With great gratitude,

Faisal

Declaration

The work described in this thesis has not been submitted for any other degree or diploma for this or any other examining body, except where acknowledgement is made by reference, the research described herein was the unaided effort of the author.

ACKNOWLEDGEMENT

Thanks to my merciful "**ALLAH**" who granted me a continuous help during my work to successfully start and finish this study, and gave me persistent in all my life.

I wish to express my deepest sincere gratitude and great thanks to my major advisor **Prof. Mukhtar Ahmed Mukhtar**, Professor of Poultry Nutrition, Department of animal production, College of Agricultural Sciences, Sudan University of Science and Technology, for his untiring assistance, keen guidance and his time to give continuous advice and valuable supervision through this study. I was very lucky to have him as my advisor, I appreciate his support with suggestions and planning for the work, revising and correcting the manuscripts and all his patience during the course, it would be very difficult to finish this work without his encouragement, and cordiality.

I would also acknowledge **Prof. Kamal Abd Albagi Mohamed**, Professor of Poultry Nutrition, Department of animal production, College of Agricultural Sciences, Sudan University of Science and Technology for his helpful advice, assistance, continuous interest and encouragement for which no words can describe.

I would like to thank all staff members and colleagues at Department of Animal Production, College of Agricultural Sciences, Sudan University of Science and Technology for their help, efforts and support in carcass and sensory evaluation work, that cannot be forgotten, I am most grateful to my parents, brothers, and sister, for their moral support, patience, warm love and encouragement.

Extending deep gratitude is to my wife and my sons **Marwan** and **Mazin,** I am eternally grateful for their love, last but not least, thanks for any one assisted me by any of means, my deepest appreciation to those whom I forgot to mention their names, may God bless you all.

LIST OF CONTENTS

List of Tables

List of Figures

List of Appendix

List of Abbreviations

ALT	Alanine aminotransferase.
ANOVA	Analysis of Variance.
AOAC	Association of Official Analytical Chemists.
AST	Aspartate aminotransferase.
BW	Body Weight.
BWG	Body Weight Gain.
Ca	Calcium.
CF	Crude Fiber.
CP	Crude Protein.
DM	Dry Matter.
EE	Ether Extract.
EPEF	European Production Efficiency Factor.
EU	European Union.
FCR	Feed Conversion Ratio.
FI	Feed Intake.
GLM	General Linear Model.
Hb	Hemoglobin.
HDL	High Density Lipid.
IU	International Units.
LDL	Low Density Lipid.
ME	Metabolizable Energy.
Me Cal	Mega Calorie.
MT	Metric Ton.
NRC	National Research Council.
NSPs	Non Starch Polysaccharides.
SDG	Sudanese Pound.

ABSTRACT

These studies were carried out to investigate the effect of using different levels of the commercial multi-enzymes NutriKEM Extend, when added to sorghum-ground nut cake basal diets, fed during different phases (starter or finisher only, or through the whole period), on growth performance, carcass characteristics, blood profile and economic efficiency of broiler chicks.

NutriKEM Extend Dry, a multi-enzymes product developed for use in total vegetable diets, enzymes present have hydrolytic activity towards pentosans and other Non-Starch Polysaccharides (NSP), in addition to NSP-degrading enzymes, NutriKEM Extend Dry also contains amylase, lipase and protease activity, lysophospholipids are also included in NutriKEM Extend, to provide a more complete mode of action for fat and nutrient digestion in animal nutrition.

A completely randomized design (CRD) experiment, of a (4×3) factorial arrangement was used, four graded inclusion levels of multi-enzymes (250,500,750 and 1,000 g / MT of complete feed), offered during three different feeding regimen.

A total number of 455 day old, unsexed broiler chicks, Cobb 500 strain were allocated into thirteen experimental groups, the control group fed on basal diet without multi-enzymes added, and each treatment diet was offered to one of the remainder twelve groups, each treatment had five replicates, with seven birds each.

Studies lasted for six weeks, and the experiments parameters covered growth performance, carcass and non- carcass values, serum constituents, and serum enzyme activities. Meat chemical composition was analyzed, and sensory evaluation was done to evaluate meat quality. Economical appraisal was performed to assess the production costs and returns of 1 Kg of broiler meat. All

data were statistically analyzed using GLM model, the one-way and multi-factorial ANOVA.

Results indicated that, feed intake, body weight gain, FCR and final body weight (BW) were significantly affected by different feeding times of enzyme supplemented diet, data showed an improvement of body weight with increased multi-enzymes feeding durations, good results for (BW) of 1,845g, was achieved when feeding diets with added multi-enzymes at 1,000 g / MT of feed for the whole fattening period (42 days).

Economic efficiency, was not affected by both variable factors (multi-enzymes levels or feeding durations), however birds fed diet with multi-enzymes level of 1,000g/1MT feed during the starter period only consumed the highest feed amount (4,158.80g).

Enzymes levels had no influence on the FCR, best FCR value (2.30) at the end of the whole experimental period was obtained for birds fed diet with multi-enzymes level of 500g / 1 MT of feed, offered during the finisher period only, both addition of different levels of added multi-enzymes or feeding durations, showed no significant interactive effect on bird's viability or EPEF, however, EPEF was significantly affected by feeding durations at p-value ≤ 0.10.

Feeding durations of added multi-enzymes NutriKEM Extend on broiler performance in terms of body weight gain and prospectively slaughter weight, were high significantly affected, compared to birds fed the control diet, (p-value = 0.0052 and 0.0068 respectively), moreover, feed conversion ratio was significantly affected by multi-enzymes diet feeding durations (p-value = 0.241), supplementation of multi-enzymes did not affect carcass parameters, however, results explained that, diet supplemented with multi-enzymes improved the liver size and the level of deposited abdominal fat, multi-enzymes supplementation did not induced any effect on broiler meat chemical composition, except for protein

content, addition of multi-enzymes did not significantly improved the sensory properties of meat, and had no significant influence on carcass cuts, nevertheless an apparent positive effect on breast meat percentage was observed, blood and serum parameters, were not affected by multi-enzymes supplemented to the experimental diets, but variation in values of serum constitutes were clearly observed.

The production cost of 1 Kg broiler meat, was not significantly affected by multi-enzymes inclusion, however the total feed cost was reduced by 10.10 %, when birds consumed a diet supplemented with multi-enzyme at level of 500 g /1MT of feed, offered during the finisher period only.

As a whole, NutriKEM Extend multi-enzymes supplementation improved broiler chick's performance, it may had positive effect on fat and energy utilization, and increasing the net returns per bird.

ARABIC ABSTRACT

أثر إضافة خليط الإنزيمات بمستويات مختلفة لعلائق الدجاج اللاحم و فترة التغذية علي الأداء الإنتاجي و خصائص الذبيحة

ملخص الدراسة

أُجريت هذه التجارب في حظائر الدجاج اللاحم بقسم الإنتاج الحيواني بكلية الدراسات الزراعية - شمبات- جامعة السودان للعلوم و التكنولوجيا خلال الفترة ما بين 5 يناير 2020 و حتي 9 فبراير 2020 ، بهدف دراسة و تقييّم تأثير إضافة و تضمين مخلوط الإنزيمات لعلائق الدجاج اللاحم المركبة من المواد الخام المحلية كالفتريتة و كسب الفول السوداني علي الأداء الإنتاجي و جودة اللحم و التحاليل الكيميائية لبعض مكونات الدم و الجدوي الإقتصادية عند إضافة هذه الإنزيمات بمستويات مختلفة أو تقديمها علي فترات تغذية مختلفة.

تم إستخدام عدد 455 كتكوت لاحم عمر يوم غير مجنسة من سلالة Cobb 500 تم توزيعها بإستخدام التحليل الإحصائي العاملي Factorial (3 X 4) و الذي يتكون من 3 فترات تغذية (خلال فترة البادي فقط ، فترة الناهي فقط أو خلال كل فترة التربية) و أربعة مستويات مختلفة من جرعة الإنزيمات المضافة (250،500،750،1,000) مل جرام لكل كيلوجرام من العلف الأساسي ، زائدا المجموعة الضابطة (الكنترول) و التي تمت تغذيتها علي عليقة بدون إضافة أي إنزيمات، تم التوزيع بشكل التصميم العشوائي الكامل (CRD) علي 13 معاملة كل معاملة تتكون من 5 مكررات او أقفاص و كل قفص به عدد 7 كتاكيت.

إستمرت التجارب لمدة 5 أسابيع تم خلالها تقديم الماء و العلف حتي الشبع و تم تسجيل بيانات العلف المستهلك وزيادة معدلات النمو و الوزن الحي عند نهاية كل أسبوع و كذلك معامل تحويل الغذاء و النفوق وصفات الذبيحة والأوزان النسبية لبعض الأحشاء الداخلية و قطع الذبيحة التجارية و كذلك تم أخذ عينات الدم لتحليل صفات بلازما الدم و المحتوي الدموي ومكونات الدم الأساسية و فحص إختبارات وظائف الكلي و الكبد و كذلك تم أخذ عينات اللحم لفحص التحليل الكيميائي و جودة و صفاته الحسية حيث تم إختيار طائر واحد للذبح من كل قفص بحيث يقترب وزنه مع متوسط وزن الوحدة التجريبية ، كذلك تم حساب معامل الإنتاج والكفاءة الإقتصادية للإنتاج في نهاية فترة التجارب. تم تحليل البيانات بإستخدام تحليل التباين (ANOVA) الأحادي one way و المعاملي factorial .

و يمكن تخليص النتائج كما يلي:-

أ – فترة تغذية الإنزيمات:

1- أظهرت بيانات العلف المستهلك و معدل الزيادة الوزنية و معامل التحويل الغذائي و وزن الذبيح ، تأثرا معنويا بفترة التغذية علي العلف المدعّم بمخلوط الإنزيمات مقارنة بمجموعة الكنترول. كان هنالك تحسّن معنوي ملحوظ في زيادة الوزن مصاحِبا للزيادة في فترة التغذية علي العلف المحتوي علي مخلوط الأنزيمات.

2- أظهرت فترة التغذية علي العلف المحتوي علي مخلوط الإنزيمات تأثيرا معنويا علي معامل كفاءة الإنتاج الأوروبي.

ب – جرعة الإنزيمات المضافة:

1- لم تظهر النتائج تأثير معنوي لمعدل إضافة مخلوط الإنزيمات علي معامل التحويل الغذائي أو التحليل الكيميائي و الخصائص الحسيّة للحوم أو الوزن النسبي للقطع التجارية أو محتوي الدم و التحليل البيوكيميائي للبلازما.

2- أظهرت البيانات تحسّن في وزن الكبد النسبي و محتوي دهون البطن في الدجاج المغذّي علي علف يحتوي مخلوط الإنزيمات.

ج – التفاعلات بين جرعة الإنزيمات و مدة التغذية:

1- أعلي وزن حي تم تحقيقه بإضافة مخلوط الإنزيمات بمعدل 1 جم لكل 1 كيوجرام علف خلال فترتي التربية (البادي و الناهي) لمدة 42 يوما.

2- لم يكن هنالك تأثير لأي من العاملين المتغريين علي خصائص الذبيحة و الكفاءة الإقتصادية.

الخلاصة:

أظهرت النتائج أنّ إضافة مخلوط الإنزيمات لعلائق الدجاج اللاحم المكوّنة من المدخلات المحلية ، أدي الي تحسين الأداء الإنتاجي و زيادة الكفاءة الإقتصادية ، بصورة عامة بغض النظر عن مدة التغذية أو جرعة الإنزيمات المضافة ، و ذلك بسبب التأثير ألإيجابي علي هضميّة المكوّنات الغذائية للعلف ، خاصة الدهون و محتوي الطاقة .

CHAPTER ONE
INTRODUCTION

Sudanese integrated companies of massive production scale in poultry sector and the rapid development of modern technological progresses, particularly in feed manufacturing, has resulted in a remarkable decrease in production cost and increase marketing of poultry products.

Poultry industry in Sudan commenced in 1926, when a British veterinarian entered a parent stock of Wyandotte Chicken from British, to improve the local breeds, followed by an experimental central organized model poultry farm in Khartoum North (Bahri) in 1951, Sudanese Kuwaiti in 1979, and Sudanese Arab in 1982 poultry companies, started the commercial poultry production of different European breeds, (Abdelbasit, 2016a).

Poultry industry in Sudan saw considerable development only in the last 10 years, with production increasing from 5 million broilers in 2006 to close to 90 million in 2017, to circa 170 million by 2021, integrations and modern sector (companies)produced about 60 % of the total broiler production, traditional sector (small farms), with ranging size from 10,000 to 100,000 birds, produced the rest,(Nabil Shuman, 2017), more than 85% of the broiler production is located in the Khartoum State, and around 70% of sales are in the Khartoum urban area. Consistent with discipline survey in 2005, poultry farms in Khartoum state was about 500, (Abdelbasit, 2016a), large companies were about ten, with broilers production potential of 30,000 birds / house, poultry meat production was estimated as 90 thousand tons per year whereas, consumption in 2005 was (0.75) kg/Capita, increased to 1kg/Capita in 2015, and reached 4 Kg/Capita in 2019,Khartoum State produce 90% of

Sudan production, companies with modern technology of closed system, are mainly found in Khartoum State (Mohamed, 2014).

Nutrition, environment and health are the most influential restricting factors affecting poultry production, feedstuff prices continue to be prohibitively high, this negatively affected consumer price and manufacturer profitability, it is well known that feed cost is about 65 -75% of overall production cost, so any small saving in feed cost, without jeopardizing chick performance, will highly increase the net profit, especially for high scale integrated poultry projects in which the net profit is usually below 10 %, the best strategy to reduce feed costs, is to formulate diets using locally available alternative ingredients, (Gabriel *et al*., 2007).

Dietary protein supplements constitute the largest component of poultry diets, protein sources of plant origin supplies most of dietary protein required for poultry feed formulation, in form of oilseed industry by-product, and however, vegetable protein sources can be introduced in poultry diets as raw materials or legumes such as dry beans.

Agro-industrial by products were used as feed ingredients, however many of these byproducts contains anti-nutritional factors (ANFs) such as phytate, variety of fibers or Non-Starch Polysaccharides (NSP),proteins and starches, which decrease growth rate,feed consumption and utilization by increasing gut viscosity and thus reducing the availability of nutrients for digestion and absorption (Choct and Annison,1992). Ingested feed will be digested in stomach and intestine where a wide range of enzymes are produced by the animal body itself or by the beneficial microbes present in their gastro intestinal tract, digestive enzymes are required to digest feed in all animals to break down starch, protein and lipids to their simple components to be subsequently absorbed, moreover, use of antibiotics has been prohibited, the

most widely alternatives include probiotics, prebiotics phytogenics and exogenous enzymes (Lee *et al.,* 2011, 2013).

Animals are not capable to digest about 15-25% of the feed they eat, because of some indigestible components and body lack of specific enzyme required for the digestion of those specific feed nutrients (Konietzny and Greiner, 2002), undigested feed passes to the hind gut where it is used by the microflora for their growth, whereas everything there is later excreted in the environment.

The application of feed enzymes to poultry diets for the enhancement of nutrient availability had been reported since 1926. Previously, studies conducted on feed enzymes in poultry nutrition targeted on non-starch polysaccharide (NSP) degrading enzymes, particularly xylanase and β-glucanase, in diets containing wheat, rye and barley (Choct, 2006). The use of unconventional feedstuff for poultry production is however restricted due to their fibrousness and inability of birds to possess the cellulase enzyme that can digest the fiber (Adebiyi *et al*., 2010), commercial digestive enzymes are being utilized in animal feeds since 1980s due to their economic, environmental and health associated benefits. The most commonly used enzyme, in animal feed is phytase, which is used worldwide and accounts for 50% of all the enzymes used in feed industry. Other enzymes, mainly polysaccharides degrading enzymes (non-starch) accounts the rest (Selle and Ravindran, 2007).

Anti-nutritional factors are problematic for normal feed digestion, results in low meat and egg production causes low feed efficiency and digestive upsets. Feed enzymes work to make the nutrient (starch, protein, amino acids and minerals, etc.) available from the feed ingredients. Feed enzymes also assist to mitigate the negative impact of animal nutrition over

environment by reducing the animal waste production, enzymes are proteins, ultimately digested or excreted by the animal, leaving no residues in meat or eggs (Greiner and Konietzny, 2006).

The principle rationale for the use of enzyme technology is to improve the nutritive value of feed stuffs. All animals use enzymes in the digestion of food, those produced either by the animal itself or by the microbes present in the digestive tract.

However, the digestive process is nowhere near 100% efficient, poultry are unable to digest 15-25% of the food they ingest, and supplementation of animal feed with appropriate enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestion process (Pariza and Cook, 2010). In many animal production systems, feed is the biggest single cost and profitability depends on the relative cost and nutritive value of the feeds available.

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades. The role of exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley has been elucidated by (Bedford and Schulze, 1998).

Digestion efficiency can be as low as 30% in the case of phytate (vegetable) phosphorus, and of course, crude fiber is virtually non-digestible, and this is a clear loss of an energy source for non-ruminants, thus enzyme supplementation in the feed plays an important role in increasing the availability of nutrients and retarding the adverse effect of anti-nutritional factors present in the feed components.

The purposes of the present studies were, therefore to investigate and determine the:-

1 - The effect of adding different levels (250,500,750 and 1,000 g / MT complete feed) of commercial multi-enzymes (NutriKEM Extend) to broiler diets, based on locally available feedstuffs of sorghum and ground nut cake, on growth performance, carcass characteristics and blood profiles.

2 - The effect of broiler diets supplemented with commercial multi-enzymes (NutriKEM Extend) to when offered at different feeding stages (starter or finisher period only or during the whole period) on growth performance, carcass characteristics and blood profiles.

3 - Economical efficacy of exogenous enzymes inclusion to broiler diets at different levels and different stages of broiler chick feeding.

CHAPTER TWO
LITERATURE REVIEW

2.1 Historical background:

The word ‘enzyme’ is derived from the Greek words en, meaning ‘within’ and zume , meaning ‘yeast’, was firstly used by the German physiologist Wilhelm Kühne in 1878, when he was describing the ability of yeast to produce alcohol from sugars, Robinson, (2015), recently enzyme application has been extensive and efforts in research intensified, early studies used crude amylase and protease preparations (Jensen *et al*., 1957; Fry *et al*., 1958; Burnett, 1962) which were later shown to contain *ß*-glucanase activity (Rickes *et al*., 1962).

Rőhm established the first commercial enzyme company in 1907, positive effects of feed enzymes were known by the 1920’s, breakthrough research in Washington State in 1950-60’s, launch of *ß*-glucanase supplemented barley-based feeds in Finland was in 1984 (Hadden, 2018), in the late 1980s, phytase was introduced to help animal producers, principally in the Netherlands, reduce the excretion of harmful indigestible phosphorus , Dutch animal producers also enjoyed the benefits of improved absorption of phosphorus, to less reliance on inorganic phosphorus sources, glycanases (e.g.xylanase, *ß*-glucanase) that cleaves the non-starch polysaccharides (NSP) in ‘viscous’ cereals (e.g. wheat, barley and triticale) were successfully added to the feed enzyme portfolio, xylanase was introduced in commercial UK wheat-based broiler feeds in 1989, (Peter, 2013).

In the 1990s the animal feed industry acknowledge the importance of enzyme use in diets containing a range of different raw materials, moreover, the century saw several technical innovations, including the introduction of

more bio-efficient *E.Coli* phytases, and the launch of more thermo-stable enzyme products, the increasing response to phosphorus (P) pollution from animal waste, and the replacing inorganic phosphorus sources after ban on the use of meat and bone meal, a major source of P, is another factor that has accelerated the use of microbial phytase, today, more than 70% of global poultry and eggs are produced from animals fed diets containing phytase, (Peter, 2013).

In the late 19th and early 20th century, advances were made in the extraction, characterization and commercial exploitation of many enzymes, in the 1920s enzymes were crystallized, revealing that enzyme catalytic activity is associated with protein molecules, for the next 60 years is was believed that all enzymes were proteins, but in the 1980s it was found that some ribonucleic acid (RNA, ribozymes) molecules are also able to exert catalytic effects, in the same decade, biochemists also developed the technology to generate antibodies 'abzymes' that possess catalytic properties (Robinson, 2015).

The effects of climate change with prolonged drought will drastically affect grain production, the depletion of non-renewable fossil energy reserves (oil and gas) will pressure the production of cereal ethanol, there will be an unprecedented competition between humans and animals for food resources (Corn, wheat, sorghum, soy, etc.), (Joaquín and Paulino, 2019), the producers will be forced to increase the use of fibrous by-products and low digestibility (DDGS, Wheat bran, rice bran, rice husk, soybean, sugar beet shell, bagasse cane, seaweed flour, even post-harvest ground residues) in animals and aquaculture feeds.

Enzyme industry started to search for highly efficacious products for poultry diets based on non-viscous cereal grains, such as sorghum and corn,

the next phase is the application of enzymes to non-cereal grain components of the diet (vegetable protein sources high in NSP), that their molecular structures are poorly characterized (Choct, 2006), furthermore the industry has not been able to produce commercially viable products that consistently improve the digestibility of vegetable proteins.

The enzyme industry today is constantly searching for new areas of application, future research and development will continue to be supported on an ever increasing level by industry in an ever widening field, development of enzyme technology needs to go hand in hand with better characterization of substrate structures, the gut microflora, and the immune system, (Choct, 2006).

2.2. Market trends, future opportunities and challenges:

Today, the use of microbial enzymes as feed additives is a well-established practice and the majority of poultry intensive production feed contain enzymes, especially of phytase (Vibe *et al*., 2015), but many other enzyme significantly improve the utilization of feed such as xylanases, ß-glucanases, pectinases, amylases and proteases.

According to the United Nations, by 2050 there will be a world population of 9,700 million inhabitants, more than 30 percent higher than today Annual cereal production will need to rise to about 3 billion tons from 2.5 billion today and annual meat production will need to rise by almost 130 million tons to reach 470 million tons, (FAO, 2020).

Over the past 15 years, world poultry meat production rose nearly 48.9 million metric tons from 2005 to its 2020 projection level, it was forecasted to reach 133 million metric tons in 2021, which is 6.0 % more than almost 125

million metric tons in 2019 (OECD-FAO, 2019), according to projections from the Food and Agriculture Organization (FAO), this again accounts for the majority of meat both produced and traded, the overall slowdown in growth in meat production is attributed by FAO to animal diseases, COVID-19-related market disruptions and lingering droughts.

According to (OECD-FAO, 2019), global meat trades (excluding live animals and processed products) are projected to be nearly 12% higher in 2029 than in the base period, meat trade remains important in securing global food supply and nutritional resources for both importing and exporting countries, over the next decade, overall global meat production is projected by the Organization for Economic Cooperation and Development (OECD) and Food and Agriculture Organization (FAO) to expand by nearly 40 million metric tons, reaching 366 million metric tons by 2029, 80% of this total will be driven by developing nations, poultry will remain a main contributor, with world poultry meat production at more than 125 million metric tons averaged from the overall meat production.

The replacement of expensive fish meal with feed enzymes, will provide lucrative opportunities in the feed enzymes market in the next five years, the increasing cost of fishmeal has encouraged feed manufacturers to search to cheaper alternative protein sources, such as plant proteins. This is one of the major opportunity in the feed enzymes market.

On the basis of type, the feed enzymes market can be broadly divided into phytase (approximately 60%), and non-phytase (40%) enzymes i.e. protease and carbohydrases (xylanase, amylase, cellulase, â-glucanase, and others), Adeola and Cowieson, (2011) assigned the largest market accounted for phytase, the market for protease in feed enzymes is projected to be the

fastest-growing during the forecast period, protease help farmers save on feed costs, as the use of protease can contribute significantly to the current efforts focused on reducing nitrogen emissions during livestock production, hence, protease is an emerging type of feed enzymes, which has been gaining popularity in the years, (Felton, 2017).

Regarding insight of livestock, the feed enzymes market has been segmented as**,** ruminants, swine, poultry, aquatic animals and others (equine and pets), poultry segment accounted for the largest market in global feed enzymes market, and held the highest share, constituting 43.9 % of the overall industry, (AllAboutFeed, 2018) these products help in increasing the energy value of feed, minimizes the content of anti-nutritive components, promotes weight gain, and also strength the immune system.

On the basis of source, the feed enzymes market has been segmented as, micro-organism (bacteria and fungi), plant and animal, microorganisms dominated the feed enzymes market by source, it is projected to grow at the highest CAGR from 2017 to 2022, microorganisms are economical to use, are more stable in extreme conditions, and can be easily manipulated in the laboratory, the latest molecular techniques are used to discover microbial enzymes, such benefits provided by the microorganisms over the other sources, and make it more popular and easy to use in animal feed, (Expert Market Research, 2019).

Regarding form insight, the feed enzymes market has been segmented as, liquid and dry, the liquid segment was larger by form, and is projected to be grow at a higher rate. The reasons for its increasing popularity are their higher suitability, cost-efficiency, and effective means of mixing enzymes in feed. Such advantages make this the more widely used form of feed enzymes,

over dry enzymes, among animal farmers, on the basis of Region, the feed enzymes market has been segmented as**,** North America, Europe, Asia-Pacific and Rest of the World.

The current generation of enzymes, the miro-organisms enzymes, have been found to be highly beneficial, but enzymes from other alternate sources (recombinant enzymes) will have the following properties: (i) high activities (ii) high level of resistance to inactivation by heat treatment, low pH and proteolytic enzymes; (iii) inexpensive to produce; (iv) long shelf-life under ambient storage conditions, (Marquardt and Brufau, 1997).

The major restraint in the feed enzymes market includes the regulatory structure and interventions, due to the stringent rules and protocols regarding animal welfare and food safety, which impacts the trade and posing challenges to the feed enzyme industry players.

Some of the future areas of emphasis will be: (i) improvements in the quality and efficacy of current enzymes that are available on the market with regard to cost, thermal stability, resistance to digestion and enhanced activity in the target section of the gastrointestinal tract (ii) an expanded range of use of enzymes in the diets of poultry and domestic livestock including classes of poultry other than chickens, fish, and exotic animals (iii) an expanded availability of different enzymes such as lipases, proteases, amylases, etc. as produced by the biotechnology industry (iv) alternate sources of genetically engineered enzymes that have been selected and/or designed for the particular target substrate and animal (v) an expanded number of feedstuffs that respond to enzyme treatments (vi) the development and standardization of procedures to evaluate different enzyme product: (vii) further research into the mode by which enzymes produce their beneficial effects: (viii) development of models

to predict response to enzymes in any class of livestock and with any feedstuff so as to facilitate cost-benefits studies; (ix) greater emphasis on other benefits of enzymes such as their effect on reducing pollution, partitioning of nutrients and altering the endocrine response and health status of the animal. Enzyme research will not only continue at a brisk pace but will undoubtedly be accelerated with many benefits being achieved. This exciting field of research will be a focus on animal nutrition research and development in the future, (Marquardt and Brufau, 1997).

2.3. Enzyme definition:

Enzymes are natural macromolecular biological catalysts (also known as biocatalysts) proteins or RNA molecules (ribozymes), synthesized and secreted (endogenous) by all living organisms and animals to speed up the biochemical reaction process of digestion (Zargi, 2018), but not themselves altered or being consumed during the reaction, centigrade for short time, they also exhibit low toxicity and act over wide pH and temperature ranges, they are stable at 80-85 °C.

Biochemists also developed the technology to generate antibodies that possess catalytic properties, these so-called ‘abzymes’ have significant potential both as novel industrial catalysts and in therapeutics, enzymes are too specific towards their substrates to which they react and there by their action will also be so specific the rate of an enzyme catalyzed reaction increases with increasing substrate concentration, to the point where there is no further response (saturation), (Acamovic and McCleary, 1996).

Enzymes are the catalysts of biochemical reactions, catalysis is defined as the acceleration of a chemical reaction by some substance which itself undergoes no permanent chemical change, they are not used during the

reaction, or appear as reaction products (Charles *et al.*, 2019), enzymes are responsible for bringing about almost all of the chemical reactions in living organisms,

Enzymes maintain their activity outside living cells, so they can be extracted from cells (exogenous) and then used to catalyze a wide range of commercially important processes. For example, production of sweetening agents, modification of antibiotics, washing powders and various cleaning products, and they play a key role in analytical clinical, forensic and environmental devices (Robinson, 2015).

2.4. **Enzyme activity:**

Enzyme activity describes the ability of an enzyme to convert a certain amount of substrate under defined conditions per time unit, this dependent on external factors such as substrate concentration, pH value, the most favorable pH value is the point where the enzyme is most active, is known as the optimum pH, an enzyme-catalyzed reaction increases as the temperature is raised (Charles *et al.*, 2019). A ten degree centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. The reaction rate increases with temperature to a maximum level, which is the optimum temperature, then abruptly declines because most animal enzymes rapidly become denatured at temperatures above 40°C.

David, (2017), defined three areas to be investigated by nutritionist when considering enzyme evaluation:

1. Activity Units

It is important to compare enzyme activity values of different products using the same unit of measurement.

2. Temperature and pH of the assay system:

Activity values are reported under the conditions where activity is found to be maximal. 20 to 50 percent change in activity when the pH or the temperature changes. Thus, care should be taken when comparing enzyme activity values obtained under laboratory conditions.

3. Substrate selection:

Normally, an isolated and defined substrate is used to measure activity in the laboratory, while in nature the enzyme substrate exists within a mix of complex feed components, such as protein starch, non-starch polysaccharides (e.g., cellulose, arabinoxylan, and beta-glucan). Laboratory-derived enzyme activity levels cannot accurately reflect real production conditions.

The environment in which an enzyme works inside the animal is quite complex, (pH 2.0 - 6.0), enzyme manufacturers and academic research institutions must invest in conducting trials to accurately evaluate enzyme performance. To ensure a complete understanding of an enzyme's efficacy, it is highly recommended to investigate multiple trials conducted under similar conditions and diets to those used by the producer. The environment of a broiler house (light, temperature, etc.), dietary formulation, feed quality and farm management methodologies dietary formulation, feed quality and farm management methodologies has influence on enzyme performance within a bird vary widely across production facilities, an enzyme product's real impact on animal performance can only be accurately measured by testing the product in the actual producer conditions (*in vivo* enzyme activity).

Enzymes as being capable of catalyzing the conversion of substrate molecules into product molecules as follows:

Enzyme

Substrate →→ Product

The catalytic activity of enzymes can perhaps best be expressed by a constant, k_{cat}, referred to as the turnover rate, turnover frequency or turnover number, this constant represents the number of substrate molecules that can be converted to product by a single enzyme molecule per unit time, usually per minute or per second (Robinson, 2015).

Enzymes as feed additives are classically characterized by activity units, the standard enzyme unit (U) is defined as the amount of enzyme that converts 1 μmol of substrate per minute (μmol/min), under standard conditions. An enzyme activity is generally given in units/gram (U/g) or units/kilogram (U/kg), representing the units of enzyme activity present in a gram of an enzyme product (Balint, 2019), Specific enzyme activity is the number of enzyme units per ml divided by the concentration of protein in mg/ml, specific activity values are therefore quoted as units/mg or nmol/min/mg, it is an important measure of enzyme purity (Innova, 2014).

Below are activity units of enzymes of NutriKEM Extend: Appendix (1):

1 U of **Endo-1, 3(4)-beta-glucanase** is the amount of enzyme which liberates 0.0056 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 7.5 and 30 °C.

1 U of **Endo-1, 4-beta-glucanase** is the amount of enzyme which liberates 0.0056 micromoles of reducing sugars (glucose equivalents) from carboxymethylcellulose per minute at pH 4.8 and 50 °C.

1 U of **Alfa-amylase** is the amount of enzyme which hydrolyses 1 micromole of glucosidic linkages from water insoluble cross-linked starch polymer per minute at pH 7.5 and 37 °C.

1 U of **Bacillolysin**is the amount of enzyme which makes 1 microgram of azo-casein soluble in trichoracetic acid per minute at pH 7, 5 and 37 °C.

1 U of **Endo-1, 4-beta-xylanase** is the amount of enzyme which liberates 0.0067 micromoles of reducing sugars (xylose equivalents) from birchwoodxylan per minute at pH 5.3 and 50 °C.

2.5. Enzyme matrix:

One of the economic benefits of the use of feed enzymes in birds' nutrition is the observed increase in apparent metabolizable energy (AME) concentration of the feed, this depends the type and quality of the diet ingredients and the type of enzymes used, the response is usually in the region of 50-150 Kcal/Kg feed (Adeola and Cowieson, 2011).

Emmans, (1994), proposed a new energy concept that considers not only AME *per se* but also the proportional contribution of protein, lipid and carbohydrate to a given AME and the modifying influence of metabolic heat production of these macro-nutrients, the effect of various enzymes on amino acid can be accommodated into least cost formulation by either application of a general amino acid matrix (nutrient-equivalent values assigned to enzyme products in least cost formulation) or assigning individual amino acid uplift response in each raw material (Cowieson, 2014).

Enzymes may be accommodated in feed formulation in various ways, firstly, enzymes may simply be added on by using over-the-top basis, this is common for carbohydrases, when re-formulation of diets is complicated, this

will increase the diet cost, but improvements in bird performance is expected to cover the extra cost. Secondly, enzymes can be included at a fixed dose with nutrient matrix and diet re-formulation, this way of application will reduce the added fat and an increase the inclusion of the primary cereal with a reduction in the primary protein source (Cowieson *et al.*, 2004). Finally, enzymes may be applied with ingredient-specific improvement values, effects of the enzyme are assigned to the ingredients (substrate) and released nutrients this approach results in a new set of raw materials (ingredient vs. ingredient + enzyme) that have enriched nutritional characteristics. The consequences of each inclusion strategy differ and the reasons for selecting one strategy over another are varied.

Cowieson *et al.*, (2004), conducted a series of formulation scenarios and showed 4-6% feed cost savings, on a global basis, the use of carbohydrases, phytases, and proteases saves the animal feed industry more than $8bn per annum in nutritional input costs and contributes to environmental sustainability regarding phosphorus and nitrogen emissions, enzyme suppliers must respond with clear and transparent recommendations for the use of these products, matrices may need modification if the enzymes specific substrate is limited.

Feed enzymes are global success story, its market continues to grow because of existing technologies and strongly of new developed technologies. To maintain this trajectory, it is important that feed enzyme suppliers ensure their recommendations (Bedford and Cowieson, 2019) and keep pace with the growing demand for transparency and precision by nutritionists and livestock production professionals.

With various second-tier ingredients such as sorghum, ground nut cake and sunflower oilcake meal were made available in the formulations, intensive researches are needed to investigate the changes brought about by the addition of feed enzymes: these local ingredients were favored over soybean meal and oil, expected results in feed saving of between 5 and 6 % when digestibility improvements were created with the use of feed enzymes (Cowieson *et al*, 2004).

2.6. Enzymes structure and chemical nature:

Enzymes are organic catalysts, usually of high molecular weight proteins (exception- *Ribozymes* or RNA enzymes).All enzymes contain a protein backbone, and they have unique three-dimensional shapes that fit the shapes of reactants. In some enzymes this is the only component in the structure, however there are additional non-protein moieties usually present which may or may not participate in the catalytic activity of the enzyme.

Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as holoenzyme (active enzyme), which is often made up of apoenzyme (the protein part) and a coenzyme (non-protein organic part).

$$\text{Holoenzyme} \rightarrow \text{Apoenzyme} + \text{Coenzyme}$$

The term prosthetic group is used when the non-protein moiety, coenzyme (if it is a vitamin derived organic compound) or cofactor (if it is a metal ion) tightly or covalently binds with the apoenzyme and remains attached throughout the course of a catalytic reaction, cofactors are either one or more inorganic ions, (Robert, 2000), whereas coenzymes are low molecular weight complex organic or metallo-organic molecules, some enzymes require

both organic molecules (coenzymes), both the protein and cofactor components may be directly involved in the catalytic processes taking place.

Enzymes increase and catalyze nearly all the chemical reactions taking place in the cells of the body the rate of reaction by lowering the energy of activation, enzymes may recognize and catalyze: a single substrate, group of similar substrates or particular type of bond, iso-enzymes catalyze the same reaction in different tissues in the body for e.g lactate dehydrogenase, which converts lactate to pyruvate, consists of five iso-enzymes (Charles *et al.*, 2019).

Enzymes are most active at an optimum temperature (usually 37°C in humans), they show little activity at low temperatures and lose activity at high temperatures as denaturation occurs, also they are most active at optimum pH, enzymes lose activity in low or high pH as tertiary structure is disrupted, the rate of reaction increases as enzyme concentration increases (at constant substrate concentration),at higher enzyme concentrations, more substrate binds with enzyme, maximum activity occurs when the enzyme is saturated (Robinson, 2015).

2.7. Enzyme mode of action:

For enzymes used in animal feeds, the degrading site of the substrate plays a major role in the effect to be achieved, there is a fundamental difference between exo- (external) and endo- (internal) enzymes, exo-enzymes only break down the terminal structural building blocks of the molecular strand whilst endo-enzymes degrade bonds within the molecular strand. Endo-enzymes are thus able to effectively break down large and long-chain molecules into smaller fragments (Buhler and Limper, 2004).

The limiting factor when formulating rations, is the animal's ability to digest different constituent parts of the feed raw material differently, particularly the fiber (Sheppy, 2003). Despite recent advances, the potential nutritional value of feedstuffs is not achieved at the animal level, this inefficiency in the utilization of nutrients can result in an extra cost to the farmer, the food company and the environment.

The basis of action of NSP hydrolyzing enzymes is the partial degradation of soluble and insoluble NSP in the upper digestive tract. Since soluble NSP produces viscosity in aqueous solutions, the degradation of this fraction leads to a decrease in digesta viscosity, this effect was observed in almost all experiments with broilers, the same enzymes hydrolyze partially insoluble NSP, which are primarily contained in cell walls, and transfer them into a soluble form (Pettersson and Aman, 1989). The "locking in" of nutrients in the cell lumen by NSP of the cell walls is sometimes called "cage effect", the mode of action seems to be rather complex, this is mainly significant for the effect on the digesta viscosity through enzymes which break down non-starch polysaccharides (Simon, 1998).

The active site is a groove or pocket region formed by the folding pattern of the enzyme protein that fits the shape of substrates and align or bind the substrate and later releases products when the reaction is complete, the active site consist of amino acids residues that form temporary bonds with the substrate (binding site) and residues that catalyze a reaction of that substrate (catalytic site), the active site is only 10 -20 % of the volume of an enzyme (Bugg, 2004), it is the most important part as it directly catalyzes the biochemical reaction.

The mechanism of enzyme catalyzed reactions steps are:

1-The proper fit of a substrate (S) in an active site forms an enzyme-substrate (ES) complex.

$$E + S = ES$$

2-Within the ES complex, the reaction occurs to convert substrate to product (P).

$$ES = E + P$$

3-The products, which are no longer attracted to the active site, are released, then substrate is converted to product.

$$E + S\ (ES) = E + P$$

As catalysts, enzymes are only required in very low concentrations, and they speed up reactions without themselves being consumed during the reaction, enzymes are usually being capable of catalyzing the conversion of substrate molecules into product molecules.

As well as being highly potent catalysts, enzymes also possess remarkable specificity in that they generally catalyze the conversion of only one type (or at most a range of similar types) of substrate molecule into product molecules. Some enzymes demonstrate group specificity (Trevor and Philip, 2007).

Other enzymes demonstrate much higher specificity, which is described as absolute specificity, for example, glucose oxidase shows almost total specificity for its substrate, β-glucose, and virtually no activity with any other mono-saccharides.

2.8. Enzymes role and benefits in poultry rations:

The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades. Bedford and Schulze, (1998) elucidated the role of exogenous enzymes capable of degrading non starch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley.

Benefits of using feed enzymes in poultry diets are improved feed intake, weight gain and feed conversion ratio, reduced water intake, reduced excreta moisture, improved apparent metabolizable energy, decreased size of gastrointestinal tract, reduced beak impaction and vent plugging (Khattak *et al.,* 2006; Mutaz, 2019).

The aim of adding enzymes is to improve bird performance and profitability by enhancing feed intake and utilization of dietary components (protein, amino acids, starch, lipids, and energy), however, there are many other reasons for the wider acceptance of feed enzymes and these will become more relevant in future production systems:

1. Increase in the range of feedstuffs that can be used or removing the constraint on the inclusion limit of poorly digested ingredients and reduce excreta moisture content (Collett, 2012).

2. Improved immune function, gut health and intestinal morphology because of improved digestion (Bedford and Cowieson, 2012; Yegani and Korver, 2008).

3. Decreased excreta nitrogen and phosphorus levels as a result of better utilization of these nutrients and the environmental effect of these benefits is relevant.

4. Better uniformity by uplifting the growth of poorly performing animals.

Feed enzymes have different modes of action, Bedford and Partridge, (2011); Bedford, and Schulze, (1998) demonstrated mechanisms responsible for the observed benefits:

1. Degradation of specific ingredients that are not usually hydrolyzed by endogenous digestive enzymes.

2. Degradation of anti-nutritional factors that directly limit nutrient digestion and increase intestinal viscosity.

3. Release of nutrients that are bound to or entrapped by the cell wall.

4. Reductions in endogenous secretions and protein losses from the gut resulting in reduced maintenance requirements (Cowieson and Ravindran, 2007).

5. Changes in the microflora profile in the small intestine (Apajalahti *et al.*, 2004; Vahjen *et al.*, 1998).

6. Feed enzymes also help to reduce the negative impact of animal production over environment by reducing the animal waste production, these Enzymes are proteins that are ultimately digested or excreted by the animal, leaving no residues in meat or eggs (Greiner and Konietzny, 2006).

7. Augmentation of endogenous digestive enzymes, especially for young birds with immature digestive system.

Generally, enzymes may increase the profitability of poultry production, by enhancing the apparent digestibility of nutrients, or by reducing nutrient requirements of the animal (Cowieson, 2010). It is broadly accepted that feed enzymes improve starch, amino acid, fat and mineral digestibility. However, enzymes also alter digestive physiology, and gross parameters, (Cowieson *et al*, 2009), enzyme supplementation has also been shown to influence the absorption of fats and fatty acids as well as fat-soluble micro-constituents contained (Acamovic, 2001), these changes may not be detected using conventional digestibility assays, moreover performance trials may miss such effects if the nutrient content has been formulated inappropriately or if the birds are not taken through to processing for carcass analysis.

Other than animal feed industry or animal nutrition, enzymes are applied in various other fields, including technical use, food manufacturing, cosmetics, medication, and as tools for research and development (Shuang *et al*, 2012), about 158 enzymes were used in food industry, 64 enzymes in technical application and 57 enzymes in feedstuff (Austrian Federal Environment Agency 2002), almost 75% of all industrial enzymes are hydrolytic enzymes i.e. carbohydrases, proteases and lipases.

Pharmaceutical Enzymes are applied for therapy, synthesis of antimicrobials and of amino acids, as digestive aids, for treatment of infectious diseases and cancer (Carlos and Vikas, 2018).

2.9. Enzyme nomenclature and classification:

In the 1950s, the number of known enzymes was increasing rapidly, there was no guiding authority, the same enzymes became known by several different names, and sometimes the same name was given to different

enzymes, the situation was chaotic, names often conveyed little or no idea of the nature of the reactions catalyzed, catalase was also known as equilase, caperase and optidase (Tipton *et al*, 2000), rapid growth in rate of discovery of enzymes led to development of nomenclature rules (from 600 enzymes in 1958 to 3,000 enzymes in1992), appendix (11) shows the EC numbers held in ExplorEnz.

Enzymes are assigned two names, and classification number (4 numbers that uniquely categorize, each enzymatic reaction), first name is the recommended or original name (Dixon and Webb, 1964), everyday use, often previous trivial name, named by appending -ase to either name of a substrate or type of catalytic reaction.

The second name is the systematic name, consists of two parts, substrate(s) and name of reaction catalyzed (group classification) with -ase suffix, e.g Lactate dehydrogenase (Tipton, 1993).The Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), recommendations of the nomenclature and classification of enzymes by the reactions they catalyze, this a functional system is based solely on the substrates transformed and products formed by an enzyme (McDonald *et a*l., 2009).

Enzymes were identified by EC (Enzyme Commission) in 1961 (with the latest update having occurred in 1992), EC is a numerical classification scheme for enzymes, hence all enzymes are assigned an "EC" number, the EC numbers represent enzymes and enzyme genes (genomic information), but they are also utilized as identifiers of enzymatic reactions (chemical information).EC numbers do not specify enzymes, but enzyme-catalyzed reactions (Hu *et al*., 2012), they were divided into 6 major classes and a

seventh, the translocases, was added in 2018 (Tipton, 2018), these are shown in appendices (12) and (13).

The EC number is made up of four digits known as the Enzyme Commission separated by full stops. for example a.b.c.and d ,a identifies the class of reaction catalyzed, b is the subclass, b and c generally contains information about the type of compound or group involved and reaction description, while the "d" digit is used to distinguish between different enzymes of the same function based on the actual substrate in the reaction.

For the oxidoreductases, the subclass indicates the type of group in the donor that undergoes oxidation or reduction (e.g., 1.1. acts on the CHOH group of donors whereas 1.4. acts on the CH-NH2 group of donors), EC numbers do not specify enzymes but enzyme-catalyzed reactions .If different enzymes (for instance from different organisms) catalyze the same reaction, then they receive the same EC number, new subclasses may be created as new information or interpretations become available, e.g., isomerases altering macromolecular conformation' has recently been added to the isomerases class.

The third number, the sub-subclass, further specifies the type of reaction involved, for instance, EC 1.x.1.- indicates that NAD+ or NADP+ is the acceptor, while 1.x.2.- has a cytochrome as the acceptor, etc. The fourth is a serial number that is used to identify the individual enzyme within a sub-subclass (Enzyme Nomenclature, 1992).

Appendex (15), illustrates the use of this system for the hydrolases with EC 3.1 expanded to show the complete sub-subclasses.

2.10. NutriKEM® Extend dry:

The commercial NutriKEM Extend dry product is a blend of exogenous and endogenous enzymes embedded in a bio surfactant matrix emulsifier that improve enzyme efficacy by facilitating the enzyme-substrate complex formation, feed digestion is improved through a faster enzymatic hydrolysis and nutrient absorption is enhanced through micelle formation and increased cell membrane permeability (Stefaan, 2009).

As a result more energy and more amino acids are released from the feed and are more readily absorbed, a unique digestive feed enzyme system, from fungal and bacterial fermentations, containing multiple enzymes activities, the combination of multi-enzymes was based on technical know-how and practical experience.

Enzymes in NutriKEM, has a particular broad pH range that can work in the entire animals gastrointestinal tract. NutriKEM also contains phospholipids, formulated for cereal based poultry diets, and contain β glucanase, cellulase, α amylase, protease and xylanase, these enzymes have been combined with a specific relative concentrations, carefully selected for their heat tolerance, long term stability, wide pH range and hydrolytic activity towards pentosans and other NSPs, in addition to NSP-degrading enzymes, it also contains amylase and protease activity, that enhance the action of the endogenous digestive enzyme which is very important during early rearing, this approach will fully capture nutritional value by degrading all anti-nutritional factors in all circumstances, and make the poultry feed nutrients more digestible, absorbable and available (Kemin Europa, N.,V., 2017).

Using products derived from separate microbial fermentations allows the flexibility to develop optimal, synergistic enzyme mixtures, moreover,

each fermentation product that is used as a raw material for NutriKEM is controlled separately to assure that the declared activity is consistently present from batch to batch, there is a major benefit from using multiple, molecular forms of specific enzymes, for instance different types of xylanase will increase the spectrum of activity against a specific substrate, which may vary subtly but continuously in nature. These benefits can be demonstrated in several aspects that are directly related to the performance of commercial enzyme products in animal nutrition (Kemin Europa N.,V., 2016).

NutriKEM Extend has been optimally developed for complex diets with varying inclusions of cereals and oil seeds. Taking also into account the natural variations in agricultural crops, a balanced blend from multiple fermentation products is exceptionally suitable to retrieve the highest and most consistent nutritional value, not just from a single ingredients, but from the feed as a whole.

The enzymes used in the formulation of NutriKEM are from widely varying microbial origins, each contributing a whole range of side activities. Considering that each activity is due to several different enzymes on a molecular and genetic level, NutriKEM is an extremely complex blend of enzymes. This complexity translates into, a wide functionality covering pH range, selectivity for different forms of a specific NSP fraction, synergistic action of multiple enzymes and the resistance against natural enzyme inhibitors.

2.10.1 Higher affinity for the substrate:

Although several enzymes may attack the same substrate, relative differences in selectivity result in different features in vivo. The xylanase from Aspergillusaculeatus is only active on water-extractable arabinoxylan, which

is an excellent feature when targeting viscosity reduction (David, 2016). However, breakdown of water-unextractable arabinoxylan is also required to release nutrients from the feed matrix. Xylanases from Trichoderma longi brachiatum are active both on the soluble and insoluble arabinoxylan. Nutrikem Extend combines both xylanase sources to assure that both types of arabinoxylan are efficiently targeted (Kemin Europa, N.,V., 2016).

2.10.2 Synergetic action:

Coordinated action of several enzymes is required for degradation of carbohydrates in plant materials which are very complex by nature, when acting together on the same substrate, enzymes with different specificities work synergistically (David, 2016), a classic example is the cellulase complex from *Trichoderma longi brachiatum*: while cellulase alone is active only on amorphous cellulose, the combined structure of cellulase, exoglucanase and β-glucosidase is also active on crystalline cellulose fibrils, which are the main structural cell wall component in plants (Kemin Europa N.,V., 2016).

2.10.3 Wider pH range:

Each enzyme has a characteristic optimal pH for activity. When two xylanases such as Xyn1 and Xyn2 from *Trichoderma longi brachiatum* are combined, each enzyme is having its own optimal pH then the overall xylanase activity will have a much wider pH range than the range observed for the individual components. Considering that the pH conditions during feed digestion vary widely between pH 2 and pH 8, the action of many enzymes is required that cover as much as possible of this pH range (David, 2016). As NutriKEM Extend contains both fungal enzymes, generally having optimal activity in acidic conditions, and bacterial enzymes, typically active in neutral

pH conditions, its activities cover the pH conditions of the whole gastrointestinal tract.

2.10.4 Enzyme inhibitors:

In almost all major feed raw materials, enzyme inhibitors have been identified, enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, reduce the performance of exogenous enzymes to a major extent, or in some cases, stop catalysis, there are common types of enzyme inhibition, competitive (substrate and a substance resembling the substrate are both added to the enzyme), non-competitive, (substances which alter the enzyme and no longer accept the substrate) and substrate inhibition (presence of excessive amounts of substrate (Charles *et al.,* 2019), uncompetitive inhibitors (the inhibitor binds to the enzyme and substrate after they have bound to each other, the products leave the active site less easily, and the reaction is slowed down), irreversible inhibitors (an irreversible inhibitor binds to an enzyme and permanently inactivates it), (Tim, 2018), many enzymes have been reported to remove or reduce mycotoxin contamination both in vitro and in real matrices, nonetheless, their application in feed is very limited (Martina *et al.,* 2017).

The types and levels of inhibitors vary hugely with crop variety and culture conditions. However, the sensitivity of enzymes to these natural inhibitors is predominantly determined by the microbial origin of the enzyme, xylanase inhibitors from wheat strongly inhibit xylanase from Bacillus species (Nuyens *et al.,* 2007) whereas the inhibition of Trichodermaxylanases is only partial and the xylanase from *Aspergillusaculeatus* is not inhibited at all. NutriKEM being is a multi-enzymes complex produced by multi-fermentation that can cope with the natural variations of inhibitors in feed raw materials.

2.11. Lysophospholipids:

Animal fats and vegetable oils are added in broiler chicken diets to increase the energy concentration, for improvement of growth performance of birds, According to (Noy and Sklan, 1995), lipase secretion in young birds is insufficient to digest fats and oils properly, Al-Marzooqi and Leeson, (1999), reported that exogenous lipase had no effect on digestion of fats and birds' growth performance, Sebastian *et al.*, (2015) and Zhang *et al.*, (2011) demonstrated that a supplementation of lysophosphatidylcholine can improve fat digestion of broilers during the first 21st day of experiment. On the other hand, some researchers reported that the addition of emulsifiers had no positive effects on birds' growth performance (Soares and Lopez-Bote, 2002; Azman *et al.*, 2004).

The well-known effect of lysophospholipids on all three steps in the fat digestion process (emulsification, hydrolysis and absorption) has been thoroughly researched and proven (Jansen, 2015). The specific additional effect of lysophospholipids on the absorption of different nutrients has been highlighted in recent studies (Hodallah *et al*., 2013), Lundbeakn and Anderson, (1994) explained this effect by the increasing permeability of the cell membrane, and was further confirmed in multiple animal trials.

Phospholipids are added to the feed mitigate the effect of high fat diets and low bile acid excretion, this requires phospholipids with a higher hydrophilic-lipophilic balance (HLB value), therefore these phospholipids must be hydrolyzed to lysophospholipids. The phospholipids in lecithin have an amphiphilic character, i.e. the molecule has both hydrophobic and hydrophilic characteristics (Olga, 2017).

Amphiphilic character can be explained by the molecular structure, which consists of two hydrophobic fatty acid tails and a hydrophilic phosphate head, joined together by a glycerol molecule. These so called native lecithins are by-products from the oil refinery and usually applied in animal feed as a relatively cheap energy source.

Fat emulsifiers are reported to cause a significant decrease in blood cholesterol. Phospholipid and bile acid fat emulsifiers are also reported to cause a significant increase in the serum high density lipo-proteins content which are considered as good cholesterol and are considered as a good indicator of chicken meat quality for human consumption.

Murata *et al.*, (1983) previous studies demonstrated that emulsifier as well as multi-enzymes in different energy density diet on growth performance, blood profiles and relative organ weight in broiler chickens, also (Cho *et al.*, 2012) demonstrated that the improvements that can be made with lysolecithin supplementation are highly dependent on the fat incorporated in broiler feeds.

Lysolecithins have a lower critical micelle concentration than bile salts and are therefore more powerful bio-surfactants. They improve adsorption of many products from enzymatic digestion. Lysolecithins increases the permeability of the epithelium cells and enhances an easier flow of nutrients.

It could be assumed that addition of bile salts or exogenous emulsifiers to broiler diets could affect fat emulsification and, consequently, absorption positively. Available research data regarding effectiveness of exogenous emulsifiers are limited and inconsistent. Polin *et al.*, (1980) showed that dietary supplementation of bile salts improves emulsification, micelle formation and, consequently, fat digestibility. Also, results of (Zhang *et al.*, 2011) demonstrated that a supplementation of lysophosphatidylcholine can

improve fat digestion of broilers during the first 21 d of experiment. On the other hand, some researchers reported that the addition of emulsifiers had no positive effects on birds' growth performance (Soares and Lopez, 2002; Azman *et al,* 2004).

Glyceryl polyethylene glycol ricinoleate (GPR) is an emulsifying agent that may also be used to enhance dietary fat availability. According to (Amitava *et al*., 2010), a dietary addition of GPR at 1% may improve chickens' live weight by up to 5% and, in this way, improves feed conversion, aforementioned authors showed that the GPR effect on fat utilization was evidenced from improvements in apparent total tract digestibility of fat and overall fat metabolizability.

Dietary NSPs, also can affect protein, fat and emulsified lipids utilization (Choct, 2001) revealed that the combination of lysophospholipids along with exogenous enzymes complex have positive effects on nutrients digestibility and absorption which is reflected on feed efficiency and production performance, supplementation of exogenous enzymes complex in combination with lysophospholipids is beneficial in terms of increased production and decreased relative feeding cost economics (Pandian *et al*, 2014).

2.12. Enzyme combination:

Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases.

Today, enzyme suppliers are promoting the additive benefits of combining enzyme for further drive down costs of production, each type of enzyme is targeting different anti-nutrients in the diet, combination of the enzyme activities, will result in release of more energy, amino acids and minerals, cocktail enzymes are usually referred to as blends of several enzymes from different origins, examples are amylase, protease, lipase and xylanase, produced by separate organisms , by nature, enzymes of heterogeneous origin may not possess the same enzymatic properties such as their optimum pH and temperature in spite of some overlapping (Liu, 2006), the use of various inclusion levels an enzyme cocktail, consisting of xylanase and β-glucanase to poultry diet containing rye and wheat, has been investigated by (Pettersson and Aman, 1989), and proved to be beneficial in terms of poultry growth increment.

Exogenous carbohydrases hydrolyze NSPs in the diet, Meng *et al*., (2005), thereby enhance access of endogenous enzymes in the gut to their substrates, phytase and, exogenous proteases mimic the action of endogenous proteases, would improve dietary protein hydrolysis and digestibility, phytases help release phytate phosphorus, thus improving phosphorus digestion and reducing phosphorus excretion in the environment by thirty percent or more (Angel *et al*., 2006; Applegate *et al*., 2003), this collective hydrolytic actions of multi-enzymes mixture would enhance growth performance and nutrient utilization (Samuel *et al*., 2014).

Samuel *et al*., (2014), compared different enzyme activities, results imply that, multi-enzymes mixtures acted differently, thus demonstrating the need to constitute enzyme blends and activities based on the substrate present in the feed ingredients, the high activity of xylanase and glucanase possibly

influenced hydrolysis of arabinoxylan and *β*-glucan, respectively, in diets that is rich in water soluble arabinoxylan and *β*-glucan (Knudsen, 1997).

In addition, phytate from different plants sources, differently resist phytase activity (Han, 1988), furthermore, phytate may alter configuration of digestive enzymes protein (Singh and Krikorian, 1982), leading to decreased digestion of dietary protein, by inhibiting proteolysis.

Generally, carbohydrases are known to hydrolyze complex polysaccharides of cell wall, hence, releasing starch, proteins and fatty acids and thereby improving energy content of the diets (Meng and Slominski, 2005; Rutherfurd *et al*., 2007), Romero *et al*., (2013); (2014), studies indicated that a mixture of xylanase, amylase and protease can induce greater improvements in AMEn, nutrient utilization and hydrolysis of NSP, Olukosi *et al*., (2015), compared to proteases or NSP-hydrolyzing enzymes when given alone in corn- and/or wheat-based poultry diets (Wealleans *et al.*, 2017).

The immune-modulatory effect of supplementing poultry feed with multi-enzymes has been well-documented in the literature (Attia *et al*, 2020), Hosseindoust *et al*., (2019) study showed mannanase ability to improve broiler gut health, growth performance and nutrients retention. Liu *et al.*, (2017) investigated the effect of multi-enzymes containing phytase, protease, and xylanase, authors showed that multi-enzymes significantly improved the whole broiler performance, gut health and immunity.

Feed enzymes and the gastro-intestinal tract microbiome relationship can understood from two points of view, on one hand are the effects of substrates on the gastro-intestinal tract physiology and biochemical characteristics of digesta, on the other hand is the modification of these effects by feed enzymes to the extent that the substrates are degraded or modified in

the gastro-intestinal tract (Attia *et al*, 2020). Probiotics, also known as Direct-fed microbials (DFMs), can offer an additional positive means that influence the health and performance of poultry, especially after global restriction of the use of antimicrobials, their mode of action is basically different to that of enzymes (Wealleans *et al*., 2017), they alter the gut environment, activate the immune system, promote colonization of beneficial microorganisms and inhibit colonization of potential pathogens, studies of a commercial poultry probiotic based on 3 *Bacillus* strains, reveal a positive effects on gut morphology, reduced inflammatory markers (Lee *et al*., 2010), lower mortality, and improved the whole broiler performance parameters (Dersjant-Li *et al*., 2014).

Xylanase showed improved ileal digestibility and retention of components, consequent improvements in apparent metabolizable energy (AMEn) and growth performance (Kiarie *et al.*, 2013), amylase improved the starch digestibility and caused release of more energy which can be utilized by the bird (Gracia *et al*., 2003).

Proteases are perhaps less well utilized in poultry production, and their mode of action in the gastrointestinal tract is less clear than for other enzymes (Adeola and Cowieson, 2011). Nevertheless, research indicates that they can be effective in mediating the hydrolysis of proteins in the feed which both improves protein digestibility and reduces the presence of indigestible protein substrates for pathogenic bacteria in the gut.

More energy is consequently released from the feed resulting in an energy sparing effect and more amino acids become available, resulting in an amino acids sparing effect, enzyme use in poultry diets has been predominately related to hydrolysis of fiber or NSP fractions (Cowieson,

2010; Perazzo *et al*., 2015 and Pessoa *et al.*, 2016), exogenous enzymes improve nutrient energy digestibility of commonly feed offered to broilers (Yang *et al*., 2010; Hahn and Purdum, 2014), hence improve poultry performance (Ravindran, 2013).

Use of phytase and carbohydrases and their combinations, is popular in wheat- barley based diets, however data on the advantage of multi-enzymes in maize-sorghum based diets is scarce (Gidado *et al*., 2020). Growth performance response, feed digestibility and serum biochemical values could serve for comparison of nutrient utilization and efficiency.

Combinations of xylanase and phytase have been of great interest in wheat-based diets, enhanced apparent metabolizable energy (AME) and improved protein digestibility in wheat-based diets supplemented with xylanase and phytase have been reported (Ravindran *et al.*, 1995).

2.13. Poultry feed in Sudan:

Local poultry feed industry or manufacturing can be divided into the three categories of animal feed industry defined by (FAO, 2004), firstly the commercial feed production for sale, these are usually large feed mills of fully computerized control, secondly is the integrated operations where large poultry producers make their own feedstuff, finally is the cooperative operations where a group of farmers own the feed mill that produce the feed they use (Izeldin, 2015), diets are formulated by least cost software, the ingredients are selected to fulfill nutrients requirements at the lowest cost, compound feeds can be used after mixing as meal form (mash) or marketed as pelleted feed.

Cereals are the most important economic food product worldwide, in most parts of the world, cereal-based diets are adopted as caloric and protein

source (Carlos and Vikas, 2018), wheat, maize, rice, barley, sorghum and millet cereal grains provide 56% of the food energy and 50% of the protein (Cordain, 1999).

The most frequent locally used plant ingredients in poultry diets are, sorghum and corn (when available), which are considered the main source of energy, sorghum (*Sorghum bicolor*) is one of the important food and fodder crop that sustain rural livelihoods in most of the Asian and African countries (Rao *et al*., 2003), Sorghum is an important cereal crop and plays a key role in animal feed (Kaufman *et al*., 2013). In regard to the nutritive value, cost and availability, sorghum grain is probably the next alternative to maize in poultry feed (Hancock, 2000), sorghum has frequently substituted for corn in feed rations in many regions of the world.

2.13.1. Sorghum:

The high growth rate (10 %) of Sudanese poultry industry demands large requirement of this cereal, maize availability is not in tune with the demand for poultry feed, there is a shortfall of maize for poultry feed formulations, sorghum grain new varieties are a good source of protein and energy for broilers and egg layers, it be used at up to 70% in a broiler and layer rations replacing all of the corn, when its price is competitive (Scott, 2011), sorghum nutrients will complement protein sources normally used in poultry rations, very similar to corn, amino acid digestibility, especially of newer sorghum varieties compares favorably with corn, fat and energy of grain sorghum value for poultry is slightly lower when compared to corn, this difference can be balanced with other sources of energy, such as vegetable oils.

Sorghum contains little amounts of yellow xanthophylls, so where consumers prefer white chicken meat, sorghum will has marketing advantages, however

for egg yolks, other sources of pigments like synthetic compounds can be added to layer feed.

Proximate analysis of several of sorghum varieties were compared to corn by (Kriegshauser *et al.,* 2006), researchers found that sorghum had higher values of protein, and slightly lower energy or fat content, amino acid profile of sorghums compared well to corn, although the average lysine content of sorghum was slightly lower, results of indicated that the nutritional value of sorghum is similar to corn in many nutrient values (Gidado *et al.*, 2020), Cocht, (2006) reported lesser NSP in sorghum than maize (4.8% *vs* 8.1%), biotin, pantothenic acid, vitamins and some of the trace minerals including selenium, manganese, copper were higher in sorghum than maize (NRC, 1994).

Table (1): effect of exogenous NSP-enzymes on performance.

(Adeola and Cowieson, 2011)**.**

Reference	Species	Ingredients	Majoractivity	Observations
Chauynarong *et al*., (2007)	Pullets	Corn, wheat products	Five carbohydrase activities	Higher ovary weight
Cowieson and Ravindran, (2008)	Broilers	Corn	Xylanase, amylase and protease	6% improvement in weight gain
Farrell and Martin, (1998)	Ducks	Rice bran, wheat, sorghum	Xylanase, and amylase	No effects on performance
Mathlouthi *et al*., (2002)	Broilers	Corn-or rye-based	Xylanase and β-glucanase	58% improve in weight gain
Mathlouthi *et al*., (2003)	Turkey	Wheat, barley	Xylanase, and β-glucanase	5% improvement in weight gain
Olukosi and Adeola, (2008)	Broilers	Wheat and wheat midds	Xylanase	No effect on weight gain
Olukosi *et al.*, (2007a)	Broilers	Wheat and rye	Xylanase	18% improve in weight gain
Olukosi *et al*., (2007b)	Broilers	Corn	Xylanase, amylase and protease	No effect on weight gain
Roberts and Choct, (2006)	Layers	Barley, wheat, triticale	Five carbohydrase activities	Effects differ with cereal grains
Olukosi *et al.*, (2008)	Broilers	Corn and wheat	Xylanase, amylase and protease	No effect on weight gain
Troche *et al.,* (2007)	Turkey	Corn, wheat	Xylanase, amylase and protease	No effects on performance
Ai *et al.,* (2007)	JSB	SBM,RSM, PNM	Xylanase, glucanase , pentosanase, cellulase	9% increased growth rate

JSB= Japanese sea bass SBM =Soya bean Meal, RSB= Rapeseed Meal, PNM=Peanut Meal

2.13.2. Oil seedcakes and meals:

Sesame, groundnut, cotton seed and sunflower are the most commonly produced oilseed in Sudan, their cakes and meals are produced in large amount as by-product of oil industry and are considered as the main source of protein (Babiker, 2012; Babiker *et al*, 2009), these agro-industrial components, with cereals are likely to constitute about 85-90% of the final formulated diet.

The peanut meal amino acid profile is deficient in lysine, threonine and methionine, part of the sub-optimal results obtained with peanut meal are due to unbalanced diets, and it can be used efficiently at levels of 5-15% in diets (El-Boushy and Ratherink, 1989). However, better results were obtained when peanut meal is used in mixture with other protein sources. Peanut meal also gives lower performance than other protein sources such as cottonseed meal or sunflower meal (Diaw *et al*., 2010; Singh and Pasal, 1979). Low protein diets should be avoided with peanut meal (Olomu and Offiong, 1980). The problem is linked to amino acid content, but can remain even with amino acid (threonine) supplementation (Costa *et al*., 2001), the recommendation is to use peanut meal in combination with other protein sources in broiler diets, and to take a great careof amino acid balance in formulation, taking digestibility into account especially in processed / detoxified peanut meal.

Groundnut meal is commercially used as main protein source for poultry diets (Babiker, 2012), and must be supplemented with methionine and lysine when used as main source of protein (Daghir, 2008), it has anti-nutritional factors such as trypsin inhibitor and susceptibility for myctoxins infestation (Ali *et al*., 2011), sesame seed, oil and meal are used as animal feed for long time, but because of its low lysine content, sesame cannot be used as main source of protein in Sudan poultry feed, it can be used in

combination with peanut meal, cotton seed meal can be an alternative protein source for poultry feed (Lordelo *et al*., 2008), but its high gossypol levels limits its use.

2.13.3 Other poultry feed ingredients:

Other included ingredients used at low rates are, 5% imported protein super concentrates, that are the base to be mixed with the raw materials (Nabil,2017), synthetic methionine and lysine, to correct possible amino acid deficiencies (Nuha, 2000), 1.5-3.0 % vegetable oils, mainly raw sunflower oil, Di-Calcium Phosphate, limestone, mycotoxin binders, sodium chloride and vitamin-minerals premixes. It is simply clear that there is a very limited range of ingredients, especially, when considering components of plant origin.

Table (2): Analysis of some local feed resources for poultry, modified from (Babiker, 2012):

Composition	Groundnut cake	Sesame cake	Sunflower cake	Cotton seed cake
Crude protein (%)	53.44	44.42	31.57	24.79
Crude fibre (%)	8.55	8.75	27.34	29.60
Fat (%)	7.47	13.11	11.20	8.91
Ash (%)	5.27	14.15	5.32	6.27
Nitrogen free extract (%)	20.54	14.48	20.94	25.46
Metabolizable energy (MJ/kg)	11.80	11.53	10.02	9.29
Calcium (%)	0.08	1.93	0.47	0.28
Potassium (%)	1.11	1.10	0.13	1.34
Magnesium (%)	0.34	0.65	0.43	0.46
Phosphorus (%)	0.65	1.17	0.77	0.52
Boron (mg/kg)	27.50	26.55	69.54	59.51
Cooper (mg/kg)	15.85	45.51	34.08	18.40
Iron (mg/kg)	215.70	304.92	0.31	0.40
Manganese (mg/kg)	52.17	71.96	32.17	37.65
Molybdenum (mg/kg)	1.07	1.89	1.22	0.58
Zinc (mg/kg)	68.54	136.45	93.82	55.16

2.14. Sources of feed enzymes:

Enzymes are mainly produced with the help of micro-organisms, using submerged liquid fermentation (SLF) technologies, an alternative fermentation method for enzyme production is solid state fermentation (Filer, 2003), commercially available feed enzymes are obtained from optimized fermentation systems relying on the use of genetically modified bacteria or fungi (Adeola and Cowieson, 2011), and this source is economical when compared to isolating enzymes from plant or animal materials. Moreover, micro-organisms are able to synthesize a very broad spectrum of hydrolytic enzymes, in addition micro-organisms enzymes are adapted to cope with extreme temperature, pH, and osmolality (Buhler, 2004). Microbial are economically preferred, and there have no ethical problems associated with animals use. Furthermore, microbial enzymes extraction and purification is simple, because enzymes are secreted extra-cellularly (Robinson, 2015). However enzymes from plant are considered to be free from the problems of toxicity and contamination that were associated with enzymes of microbial origin Microorganisms are also very amenable to genetic modification to produce novel or altered enzymes compared to enzymes from plant or animal sources. The exploitation of microbial enzymes at industrial level started 100 years ago with the patenting of a process for the production of alpha amylase from the fungus *Aspergillus oryzae* (Pariza and Cook, 2010).

Now hundreds of Enzyme products are marketed for livestock, they are derived primarily from only four bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *L. plantarum* and *Streptococcus faecium*) and three fungal (*Aspergillus oryzae*, *Trichoderma reesei* and *Saccharomyces cerevisiae*) species (Muirhead, 1996). Other fungal species, including *Humicola insolens*

and *Thermomyces anuginosus*, are being marketed to a lesser extent (Pendleton, 1996).

The majority of enzyme products to date have been derived from mesophilic and, to a lesser extent, alkaliphilic microorganisms, other microbial like extremophiles, are rapidly gaining attention (Adams and Kelly, 1998), extremophiles are organisms that reside in extreme environments and they include thermophiles (> 70°C and often in excess of 100°C), psychrophiles (< 20°C), barophiles (> 1 atmospheric pressure) and halophiles (high salt), (Bedford and Partridge, 2011).

2.14.1. Enzyme extraction:

Fermentation and Submerged Fermentation, combined with other biotechnological aspects, are the major methods for enzyme extraction which have (Sujani and Seresinhe, 2015), a series of extraction and purification steps are followed after fermentation to remove undesirable fermentation residues to increase the specific enzyme activity, then active enzyme protein is formulated for stability by using compounds such as sorbitol and NaCl to improve storage stability and general product characteristics (Schuster and Schmoll, 2010). Various coating techniques are used to improve product thermal tolerance, feed enzymes are often offered to end users in a variety of concentrations either as liquid or dry products.

2.14.2 Sources of phytase:

The greatest potential source of phytase are miro-organisms followed by plant, there are four possible sources of phytase, plant phytase, microbial phytase (Bacteria and fungi comprise the most important sources of phytases), phytases are generated by the small intestinal mucosa, and gut-associated microflora (Carlos and Vicaks, 2018). Most of plant phytases are of type 6 phytase, however soyabean phytase is of type 3 (Phillippy and Bland, 1988),

pelleting during the manufacture of commercial feeds results in substantial losses of intrinsic native phytase activity.

Currently, there are four commercially available microbial phytases, two obtained by fermentation of a genetically modified *Aspergillus*, and the other two are obtained by extraction of media with *Aspergillus*, most products are available in a powder, granular or as a liquid form.

2.14.3. Enzymes biotechnology:

Recent innovations in biotechnology is enzyme or protein engineering, it is the process through which the sequence of amino acids is changed by recombinant DNA mutation to design novel enzymes by the manipulation of microbes at the genetic level. This is done to modify the catalytic activity of single enzymes (Shuang *et al*, 2012), advancements in enzyme engineering led to exploitation of new enzymes of improved properties, higher hydrolysis activity towards anti-nutritional factors and improved production efficiency, better strains can be obtained through recombinant DNA (rDNA) techniques, chiefly to better the quality or characteristics of the enzyme and also to obtain higher yields (Alexander *et al.*, 2009).

2.15. Methods of enzyme supplementation:

There are various forms of provided enzymes for animal diets inclusion, they can be added as powders or granules before mixing and pelleting, this method allow the enzymes to mix with the dietary ingredients and react effectively with their substrates (Acamovic, 2001), however, feed processing at high temperatures may reduce thermo labile enzymes activity (Silversides and Bedford, 1999). Analytical proof of this, however, is not easily confirmed.

Recently enzymes are added as liquids after pelleting, to avoid problems with high pelleting, the enzyme being coated onto the surface of the pellets, this do not allow enzymes to have intimate contact with most of the

components of the pellet, reducing pre-ingestion enzymatic action compared with application of the enzyme before pellet formation. However, enzymes liquids form enzymes application for pellets exert positive effect on diet nutritional value and on performance (Kluntner *et al.*, 1995; Best and Gill, 1999).

Recent innovation technology, involves the inserting foreign genes into the relevant plant DNA to enable it to synthesize novel enzymes such as phytases, these are then present in the GMO plant product (e.g. soybean meal) when it is incorporated into poultry diets (Beudeker and Pen, 1995; Denbow *et al.*, 1998).

2.16. Uses of fibrolytic enzymes in ruminant nutrition:

Enzyme utilization in ruminant industries, cattle (both dairy and beef), goat, sheep and less with buffalo, sustained by the widest range of exogenous enzyme products availability, developed methods of enzyme activity evaluation, revised knowledge on rumen functions and recent advances of biotechnology (McAllister *et al*, 2001), enzymes as feed additives for ruminants, are used to catalyse the degradative digestion reactions, in turn, these reaction products are used for cell growth, either by ruminal microorganisms or by the host animal.

Cellulose and hemicellulose are the most important structural carbohydrates present in ruminant diets (Khattab *et al*., 2011), so enzyme preparations for ruminants are basically marketed on their capacity to degrade plant cell walls, these are often referred to as cellulases or xylanases, rumen micro-organisms produce enzymes that catalyze their hydrolysis, but the structural complexity of carbohydrates and lignin hinder and restrict digestibility and efficient utilization, so fiber degradation in the rumen is not optimal (Ana *et al*, 2015).

Beauchemin *et al*., (2003) reported that exogenous enzymes are more effective when applied to high moisture feeds (such as silages) compared to dry feeds, applying fibrolytic exogenous enzymes in a liquid form to the feeds prior to consumption can have a positive effect on animal performance (Yang *et al.,* 2000), however enzymes can be applied to total mixed rations (TMR), hay, ensiled forages, concentrate, supplement or premix.

Modes of action of exogenous fibrolytic enzymes can be pre consumption effects, when applied in liquid form onto dry feed prior to ingestion, this may partially digest feed and weaken cell wall barriers, and furthermore rises in reducing sugars increase available carbohydrates in the rumen (Yang *et al*., 2000).

In ruminal effects mode of action, McAllister *et al.*,(2001) reported that rumen enzymes may hydrolyze feed directly or work synergistically with ruminal microbes to enhance feed digestion, post ruminal effects also reported enzyme synergistic work with microbes even in the large intestine (Beauchemin *et al*., 2003).

Deli Nazmin, *et al.,* (2018) concluded, ruminant supplementation with exogenous fibrolytic enzymes thought to sustain the productive performance of ruminants (dry matter intake, average daily weight gain, FCR, ADF and NDF digestibility, volatile fatty acids, milk yield, milk fat and protein content) by enhancing ruminal fermentation and forage degradability, and there is potentiality of reducing feed costs, however, the underlying interactions are unknown and the obtained effects are highly variable, (Reddy *et al.*,2016) attributed inconsistent results to product formulation, supplemented enzyme activity, inappropriate providing method, and the productivity level of the tested animals.

Yilkal and Getachew (2015) recommended using of enzymes that exhibit high activity under ruminal pH and temperature conditions, preparation of proper enzyme-substrate specificity for hydrolysis of the fractions in the investigated feed or diet, uniformly application total mixed ration rather than to individual components at feeding. New products designed for specific types of ruminant feed, better understanding of mode of enzyme action and application techniques of commercial non starch polysaccharides, finally is researches to determine the optimum level of exogenous enzymes supplementation to different ruminant animals, and evaluation of results until clearer animal performance and cost: benefit ratios emerge.

2.17. Anti-nutritional Factors:

Since the animal proteins have been banned, poultry diets were formulated from totally vegetable origin, vegetable feeds are characterized by a variable content in anti-nutritional Factors (ANF's) and non-available carbohydrates, these comprise non-digestible oligosaccharides and non-starch polysaccharides (NSP's)with content ranging from 8.3 to 9.8% (Slominski *et al*., 2000).

Anti-nutritional factors are problematic for normal feed digestion, results in low meat and egg production and causes low feed efficiency and digestive upsets, they limits the availability of nutrients and causing adverse effects on animal performance, and hence the use of these feedstuffs in animal diets is limited (Huisman and Tolman,1992), Choct, (2006) reported lesser NSP in sorghum than maize (4.8% *vs* 8.1%), they cause increased digesta viscosity, impairs digestion or absorption in poultry (Reddy *et al.*, 2006), feed enzymes work to make the nutrient like starch, protein, amino acids and minerals available from the feed ingredients.

2.17.1. Classification of feed anti-nutritional factors:

Classification of feed anti-nutritional factors is based on their effects on the nutritional value and animal biological : (i) factors with negative effect on protein digestion and utilization (protease inhibitors, lectins, phenolic compounds, saponins); (ii) factors with depressive effect on the digestion of carbohydrates (amylase inhibitors, phenolic compounds, flatulence factors); (iii) factors with a negative effect on utilization of minerals and vitamins (glucosinolates, phytic acid, oxalic acid, gossypol); (iv)factors that cause a damaging hypersensitivity reaction (antigenic proteins); and (v) factors that have a toxic effect e.g. lectins, cyanide-containing compounds (Bedford and Partridge, 2011).

Tannins, phytate and non-starch polysaccharides are generally classed and widely accepted as being anti-nutrients (Acamovic, 2001), cellulose is insoluble whereas the other types of NSPs are soluble or partly soluble, soluble NSPs slow down the diffusion of digestive enzymes and the absorption of nutrients, these lead to anti-nutritive effects in monogastric animals (Kumar *et al*, 2012), while others such as starch and protein are less obvious contributors in performance reduction (Morrison and Karkalas, 1990).

2.17.2. Sorghum tannin:

Sorghum is unique among major cereals because some cultivars produce polymeric phenols known as tannins, tannins have antioxidant properties considered as neutraceuticals (Waniska *et al*, 1992). The agronomic practices and plant breeding protocols have significantly reduced the tannin content. Sorghum cultivars reported by (Tulasi *et al.*, 2000) contained very less tannins, particularly condensed tannins.

Tannin is still a limiting factor when used for chicken rations in regions where varieties of grain sorghum that contain significant quantities of tannin are still grown, in contrast, others consider it as a historical problem, today varieties grown for animal feeding are low tannin, or 99 percent free of tannin (Scott, 2011) new cultivars were specifically selected for minimal or zero tannin levels (Rooney *et al*., 2005), tannin is another factor causing reduction in the nutritional value of sorghum, mainly due to a decrease in the use of protein and a reduction in the activity of digestive enzymes (Haslam, 1981), tannins affect the nutritional value of grain by binding proteins, render them less available for metabolism and therefor impedes digestion, suppress growth and performance of adolescent and adult all poultry types (Tahirou and John, 2006), so tannin levels are important rations for chicks, especially in starter phase.

Even though the ability of tannins to chelate trace minerals is related to negative effects in some circumstances (especially in poor varied diets and in condition of minerals deficiency) it can result beneficial in others , recent studies demonstrated their role as antioxidant, scavengers of free radicals, prevention of pathologies and cancer (Ilaria, 2012).

2.17.3. Sorghum phytate:

Sorghum grains contain high levels of phytate or phytic acid (Doherty *et al*., 1982), the majority of phosphorus (P) occurs in the form of these salts of phytic acid, it represented 60-70% of total phosphorus in all feedstuffs used in poultry diets (Humer *et al*, 2014), in addition to chelating minerals, phytate binds with protein through binary and ternary complexes and binds with starch directly or indirectly through starch granule-associated protein. Due to this relationship, the enzymatic degradation of phytate increases availability of starch and protein in the sorghum (Mabelebele *et al*., 2013).

Health benefits of phytate include, lowering blood glucose, has antioxidant, anticancer, hypocholesterolemic and hypolipidemic effects. In animal studies, phytic acid showed a protective action in carcinogenesis, this could be explained by its mineral chelating potential (Mahmoud, 2012), phytate may reduce inflammation (Greiner, 2002).

Sorghum-based diets are associated with inferior broiler performance in comparison to maize and wheat based diets (Selle *et al*., 2010). According to (Kornegay, 2001), phytate percent in corn, wheat, sorghum, wheat bran, soybean, peanut meal and sunflower are 0.24, 0.27, 0.24, 0.92, 0.39, 0.48, and 0.89 respectively, while sesame phytate content is 1.03 (NCR, 1994), Cadogan *et al.*, (2005) tested phytase enzyme preparations on sorghum based diets and determined that the enzyme improved weight gain, amino acids digestibility, and starch digestibility and broiler performance.

2.17.4. Sorghum kafirin:

Kafirin, which is the dominant protein in sorghum, comprises 70 to 80% of total sorghum protein (Hamaker *et al*., 1995), is considered one of the limitations that affect the nutritional value of sorghum in non-ruminant species (Paulis and Wall, 1979). Kafirin proteins are unique because of their high content of cysteine and histidine, these disulfide amino acids create a matrix which encapsulates the starch and making it indigestible. Proteases, which break down disulfide bonds could be used to degrade kafirins and release the starch located within the protein complex making it readily absorbed (Selle *et al.*, 2013).

2.17.5. Sorghum mycotoxins and parasite threats:

Common mycotoxins in sorghum are fumonisins, aflatoxins, T2 and ochratoxins, these toxigenic fungal strains grow when moisture content exceeds 12 percent, fumonisins and aflatoxins were very low in certain

sorghum cultivars, sorghum is relatively less susceptible to mycotoxins, compared to other cereals due to hard seed coat and phenolic compounds, and moreover acotinic acid in sorghum is believed to be mycotoxin preventive agent (Hodges *et al*, 2000).

Finally, sorghum is subject to parasite threats such as *Striga*, ergot, and *Fusarium* in Africa. *Striga* is a plant parasite that attaches to sorghum roots from where it takes nutrients and inhibits plant and seed yield (Salissou, 2009).

2.17.6. Groundnut cake anti-nutritional factors:

Like other legume seeds, peanuts contain substances with potential anti-nutritional effects, such as tannins, (Sanders, 1979), lectins and trypsin inhibitors (Ahmed *et al*., 1988), anti-nutritional factors in peanut seem less deleterious, compared to other legumes like soybean, even though lectin concentration and anti-trypsic activity were similar in both seeds (Sitren *et al*., 1985). Peanut lectins can be fully inactivated by moist heat (Ahmed, 1986), so regular conditions involved in peanut seeds and meal processing are enough to make the products safe for animal feeding (Heuze *et al*, 2016). Still, tannins may be a contributing factor for low protein digestibility of peanut meals (Chiba, 2001).

2.17.7. Non Starch Polysaccharides (NSPs):

Substrates to be broken down by feed enzymes can be mainly divided into three main groups (Buhler and Limper, 2004):

a) Substrates for which monogastric animals synthesize suitable enzymes in their own digestive tract (starch, proteins and lipids).

b) Substrates for which enzymes are not produced by the animal's microflora and which have a very low digestibility (e.g. cellulose).

c) Substrates for which enzymes are not produced by the animal's organisms and additionally have anti-nutritive effects (e.g.β-glucans, pentosans and phytate).

Plant polysaccharides can be separated broadly into two distinct types, the storage polysaccharide starch (α-glucan) and the cell-wall polysaccharides (non-α-glucan) which may conveniently be called non-starch polysaccharides (NSPs).

Starch can be hydrolyzed by pancreatic α-amylase and may therefore be digested in the small intestine and absorbed as glucose, while non-starch polysaccharides (NSP) are not susceptible to pancreatic enzymes and can onlybe utilized through microbial fermentation (Englyst, 1989).

NSPs can be classified into various groups based on their physicochemical properties, e.g., viscosity, water-holding capacity, fermentation, and the capacity to bind organic and inorganic molecules, besides, based on the reaction with water, NSPs are classified as either soluble or insoluble (Carlos and Vikas, 2018), the most preferred and clear classification was proposed by (Bailey, 1973), in which non-starch polysaccharides, comprise 1-Cellulose polymers and 2-Non-cellulosic polymers, and 3- pecticpolysaccharides (galacturonans).

In cereal grains and their by-products, the non-cellulosic polysaccharides consist of pentosans (arabino-xylans and xylans) and β-glucans, whereas in soybean arabinans, arabinogalactans, galactans, galactomannans, mannans are predominate (Slominski, 2011; Choct, 1997), the predominant NSP in the cotyledon of legumes are pectic polysaccharides.

Arabinoxylan chains are the main NSP in sorghum, wheat and corn, reaching up to 6.5%, 7.3 and 4.7% dry matter, respectively (Bach Knudsen, 2014).

2.17.8. Other anti-nutritional factors:

Other than the NSPs, polyphenolic compounds (lignin and tannins) and phytic acid, anti-nutritive factors include lectins, alkaloids, protease inhibitors, haemagglutinin, glucosinolates, cyanogen, saponins, phytoestrogens, gossypol, antivitamins, amylase, invertase, cholinesterase and arginase inhibitors, dihydroxyphenylalanine, mimosine and cyclopropenoic acids (Tadele, 2015). Out of those, the impact of protease inhibitors is of great importance because they can affect the proteases' enzymatic activity, and they are usually found in legume seeds like soybean, kidney beans, Huo *et al.*, (1993) demonstrated that trypsin inhibitors in soybean were completely deactivated by a protease within 80 minutes *in vitro*.

α-amylase inhibitors, saponins, allergens, and toxic amino acids have been known to exhibit anti-vitamin and anti-hormonal activity (Dalgetty and Baik, 2003). Oxalic acid is mainly affecting monogastrics in a way very similar to the phytic acid (Theofilos, 2019).

2.18. Feed Enzyme Categorization:

Enzymes are sometimes considered under two broad categories:

(a) Intracellular enzymes - They are functional within cells where they are synthesized.

(b) Extracellular enzymes - These enzymes are active outside the cell, all the digestive enzymes belong to this group.

There are four distinct commercial categories of enzyme products currently available for use by the feed industry: (1) microbial phytases, (2) glycanases targeting viscous cereals (e.g. Wheat, barley), (3) enzymes targeting non-viscous cereals (e.g., corn, sorghum), and (4) enzymes targeting non-cereals (e.g., soybean meal, grain legumes and oil seed cakes), (Ravindran, 2013), according to the purpose of application, feed enzymes are

divided in two groups (Dida, 2016), the first group included the enzymes which supplement monogastric animals the endogenous enzymes, such as amylases, proteases, and lipases, the second group included the enzymes which are not produced by mono gastric animals like cellulases, β-glucanases, pentosanases and phytases.

Based on the targeted substrate, feed enzymes can be classified into five types: NSP-degrading enzymes, phytate-degrading enzymes, protein-degrading enzymes, starch-degrading enzymes and lipid-degrading enzymes (Mutaz, 2019), enzymes commonly used in feed industry include: amylases, pectinases, glucanases, arabinoxylanases, cellulases, hemicellulases, proteases (acid and alkaline proteases) other enzymes include phytases, esterases and lipases (Acamovic, 2001).

2.18.1. Phytate hydrolyzing enzymess:

Phytases (myo-inositol hexaphosphate phosphohydrolase) are a large family of hydrolyases, have been classified based on :(i) the pH of activity (acidic phytases, pH optimum: 3.0–5.5, and alkaline phytases, pH optimum: 7.0–8.0) and (ii) the position of hydrolysis onset, 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26), Humer *et al*, (2014) and Carlos and Vikas, (2018), phytase is known to increase the utilization of phytate phosphorus by catalyzing the stepwise hydrolysis of phytate to inorganic phosphate, and decreases the need to add inorganic P to poultry diets (Choct, 2001).

While the 3-phytases are primarily of microbial origin, the 6-phytases are mainly isolated from plant sources (Cosgrove and Irving, 1980).

Phytase is a standard inclusion in sorghum-based feeds, sorghum phytate is reported to be more resistance to phytase degradation than phytate in other grains, reasons for this are not clear but kafirin and phenols may impede access of phytase to its substrate.

2.18.2 Amylase:

The main classes of amylases act on starch which is the main source of energy from cereal grains (Cowieson *et al*., 2010), addition of amylase in poultry diets complements endogenous enzymes in young animals, amylase can degrade cereal intracellular stored starch to sugars, therefore using exogenous NSPs enzymes to degrade the cell wall, activity of endogenous amylase can also increase. Broiler diets supplemented with these enzymes may allow the access of pancreatic enzymes to nutrients trapped within the cell, thereby improving energy availability (Cowieson, 2005).

2.18.3. Protease:

Protease works by hydrolyzing proteins or peptides, and thus improving protein digestibility (Doskovic *et al*., 2015), also have a major function in cellular protein turnover of the immune system (Barrett, 1994), exopeptidases (cleave the peptide bond proximal to the amino orcarboxyl termini of the substrate) and endopeptidases (cleave internal peptide bonds) are a complex group of enzymes capable of hydrolyzing the protein molecule peptide bond, based on the functional group present at the active site, proteases are classified into four groups: serine (EC 3.4.21), cysteine (EC 3.4.22), aspartic (EC 3.4.23), and metalloprotease (EC 3.4.24).

Proteases have been reported to increase protein digestibility of grain protein, including sorghum. Because of the relatively low digestibility of sorghum protein, the potential for protease response is greater than with corn. However, kafirin with its disulphide cross-linking may be resistant to exogenous proteases (Walker, 2018).

2.18.4. Lipase:

Lipases (EC 3.1.13) are typically used to catalyze the hydrolysis of ester bonds, the products of this enzymatic hydrolysis are mono- and

diacylglycerols and free fatty acids, lipase can be extracted from plants, animals, bacteria, and fungi, lipases are stable over the wide range of pH; however, the majority of lipase is stable at or close to neutral pH (Ghosh *et al*, 1996), use of Lipid-degrading enzymes (lipases) in broiler diets containing animal and vegetable fats can help the birds to hydrolyze fats into smaller molecules called fatty acids and glycerol, thus, lipase can improve fat digestibility and enhance energy utilization in birds (Al-Marzooqi and Leeson, 2000).

Lipid digestion and absorption is affected by, composition of the diets, source and type of lipids, condition and age of birds (Zhao and Kim, 2017), and degree of saturation, unsaturated fatty acids had a better digestion and absorption rates than saturated fats (Danicke, 2001).

2.18.5. Cellulosic hydrolyzing enzymes:

Cellulase is an NSP-enzyme that degrades cellulose, being a principal component of plant cell walls, cellulose is the most abundant carbohydrate in nature (Pe´reza and Samain, 2010), comprises of about 33% of all vegetable materials, it cannot be degraded by humans and most animals because of the absence of cellulase enzyme, and therefore, does not contribute directly to the nutrition of these animals, Utilization of cellulose as a nutrient source requires the enzyme cellulase that cleaves β-1,4-glycosidic bonds in the polymer to release glucose units.

Cellulolytic bacteria and fungi developed cellulase systems which actively convert insoluble cellulosic substrates into soluble saccharides (Carlos and Vikas, 2018), direct-fed cellulases as are generally not very successful in improving monogastric animals' performance, compared other microbial enzymes such as phytases or xylanases in poultry (Saleh *et al*, 2005), reasons can be attributed for the very short duration contact time of the

enzyme with the digesta in the gastrointestinal tract(in monogastric species, the hydrolysis process with the available cellulases take at least three to five times longer than that of digesta flow), gastrointestinal pH, particularly in the stomach, gastrointestinal denaturation and proteolytic activity,

2.18.6. Non-cellulosic NSPs hydrolyzing enzymes:

Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed linked β-glucans and arabinoxylans (Parsippany, 2008). NSP-degrading carbohydrases, hydrolyze NSP into oligo-saccharides and monosaccharides, some of the enzymes that have been used over the past several years and have potential for use in the feed industry include xylanases and associated enzymes, and galactosidases. Most of the enzymes used in the feed industry have been applied for poultry to neutralize the effects of the viscous non starch polysaccharides in cereals

2.18.6.1. Xylanases:

Xylanases, have long been used in wheat-based diets for poultry (Bedford and Classen, 1992), endoxylanases help degrade arabinoxylan by hydrolyzing the xylan backbone. However, multiple arabinose substitutions reduce the efficiency of xylanases, especially in corn and associated by-products (Bach Knudsen, 2014). As described by (Bedford and Schulz, 1998), the mechanisms of xylanase include the degradation of the non-starch polysaccharides (NSP) in the cell wall matrix of the ingredients with the release of the encapsulated nutrients and lowered viscosity of digesta caused by soluble NSP and improved rate of diffusion between enzymes and digestion end products, phytate hydrolysis also increase protein and starch utilization (Selle *et al.,* 2010). Xylanase has been reported to improve broiler performance with sorghum (and corn) based feeds. The low soluble NSP

content of both grains indicates xylanase responses are not a result of digesta viscosity reduction (Walker, 2018).

Arabinofuranosidases can cleave arabinose from the xylose backbone and offer access to endo-xylanase activity (Dela Mare *et al*., 2013). Consequently, enriching a preparation with debranching enzymes represents an efficient way to increase the overall enzyme effect. Although the digestion of hydrolyzed arabinoxylan does not release a high amount of energy, it offers higher accessibility to the nutrients, which explains a large part of the observed digestibility improvement and mitigation of negative effects of NSP (McCracken *et al*., 2002).

2.18.6.2 Glucanases:

The use of glucanase enzymes to mitigate the negative effect of viscosity in wheat and barley-based diets are a good candidate for enzymes to improve feeding values, adding a commercial mixture of pectinases, a-glucanases and hemicellulases to sorghum-soybean feed rations for broilers increased ileal amino acid digestibility 3 % while the ME was increased by more than 6 % when used in rations that were marginal in nutrients (Dominguez *et al*, 2009), hence enzymes can be used to get more nutrients from sorghum. The digestion of cereal grains with β-glucanase leads to maximum release of the higher-quality protein and energy (Carlos and Vikas, 2018).

Glucanases, which are known to cleave non-starch polysaccharides (NSPs) into simpler form, thereby eliminating their ability to form viscous digesta and improving nutrient digestibility. The effects of glycanases are generally non-specific, except for their effect on fat, which is known to have a greater effect on saturated fat than on unsaturated fat.

Table (3): Relative NSPs % in some feed ingredients.

Feed ingredients	Arabinoxylans	Cellulose	Pectins	Betaglucans	Oligosaccharides	Total NSP
Corn	4.3	2.0	0.9	0.3	0.8	8.3
Wheat	7.1	1.8	0.4	0.6	0.1	10.0
Sorghum	3.7	1.1	0.4	0.1	0.2	5.5
Soybean	0.4	5.9	9.1	0.7	9.6	25.7
Corn DDGS	11.7	10.7	2.7	-	0.2	25.3

Adapted from Feedstuffs. Vol. 86, No. 04, January 27, 2014.

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods:

Four experiments were carried out to evaluate and determine the effect of multi-enzymes mixture NutriKEM Extend when added at different levels or different growing phases(starter or finisher only, or during the whole period), on broiler chick growth performance, blood chemistry and plasma constituents, carcass traits, meat quality and economical efficacy.

All procedures were conducted at Animal Production Department, Faculty of Agricultural Sciences, Sudan University of Science and Technology (SUST) from 05/01/2020 to 09/02/2020, chicks were fed the experimental diets *ad libitum* and given free access to water.

3.1. Experimental design, chick, housing, and supplement:

A total of 455 unsexed day-old Cobb500 broiler chicks were obtained from a commercial hatchery, broilers were housed and reared in a well-ventilated and illuminated semi-opened standard floor pens poultry house.

Their light schedule was 23 hours light up to 21 days of age, followed by 20 hours of light until slaughter, the average housing minimum and maximum temperature and relative humidity during the starter period were 17.2 and 20.2 °C and 15.7 and 18.7 % respectively.

Table (4): **Vaccination program.**

Age	Vaccine	Method
03 day	ND + IB	Spray
13 days	IBD	Drinking Water
17 days	ND	Drinking Water
20 days	IBD	Drinking Water
28 days	ND	Drinking Water

Chicks were vaccinated against the most common diseases, such as Newcastle disease (ND), infectious bursal disease (IBD), and infectious bronchitis (IB), under veterinary care.

After the 1st week of brooding in which a commercial pre starter crumbled complete feed was offered at 100 g /chick, broilers were used in a completely randomized design and distributed into thirteen groups(from A to M, each group with five replicates A1 , A2, A3, A4 and A5 and each replicate with seven chicks), keeping initial body weight similar (193.42 ± 8.70)g, in 65 floor pens (experimental unit of 1.00 m × 1.50 m) of seven chickens per pen equipped with 1 pan feeder, 1 bell water, and fresh pine shavings, experimental design is shown in table (5).

3.2. Experimental diets:

Commercial pre starter of 2mm pelleted diet was offered during the 1st week at 100 g / chick, the experimental diets were prepared at the experiment site feed mill, commercial multi-enzymes was added and mixed with basal diets at experiment site, all diets were formulated to meet the nutritional requirements of broilers stated by the Cobb 500 breed manual guide (Cobb500™, 2018).

Experimental diets were provided in three regimes: starter phase only (days 08 to 21), finisher phase only (days 22 to 42) or during the whole experimental period (days 08 to 42). All diets were fed in a mash form throughout the 5-week experimental period, all birds had *ad libitum* access to water and feed.

Water used was tap water recommended for human consumption offered clean and fresh on each morning. Water intake was not determined herein.

Table (5):Experimental design

Treatment	Period	Feeding Period	Group	Sub-groups	Enzyme Levels
1	Control	Day 08- 42	A	A1 to A5	No Enzyme was added
2	Whole Period	Day 08 - 42	B	B1 to B5	250 g per 1 ton of feed
			C	C1 to C5	500 g per 1 ton of feed
			D	D1 to D5	750 g per 1 ton of feed
			E	E1 to E5	1,000 g per 1 ton of feed
3	Starter Only	Day 08 - 21	F	F1 to F5	250 g per 1 ton of feed
			G	G1 to G5	500 g per 1 ton of feed
			H	H1 to G5	750 g per 1 ton of feed
			I	I1 to I5	1,000 g per 1 ton of feed
4	Finisher Only	Day 22 - 42	J	J1 to J5	250 g per 1 ton of feed
			K	K1 to K5	500 g per 1 ton of feed
			L	L1 to L5	750 g per 1 ton of feed
			M	M1 to M5	1,000 g per 1 ton of feed
Total			13	65	455 birds

Each treatment had 4 groups (except control, had one group), each group had 5 subgroups, a sub - group had seven birds (replicate), total birds were 455.

Table (6): Compositions of the basal starter and finisher diets (Ingredients %).

Item	Starter (days 07 to 21)	Grower (days 22 to 42)
Sorghum	65.00	75.00
Ground nut cake	27.50	17.50
Broiler 5% concentrate [1]	05.00	05.00
Limestone	01.10	1.20
Dicalcium phosphate	0.60	0.50
L-Lysine·HCl 98%	0.10	0.10
DL-Methionine 99%	0.10	0.10
Premix [2]	0.20	0.20
Mycotoxin binder	0.20	0.20
Anti-coccidial	0.10	0.10
Sodium chloride	0.10	0.10
Total	**100.00**	**100.00**

Calculated composition	**Starter**	**Finisher**
Metabolizable energy, KCal / kg	2,960	3,000
Analyzed composition %		
Crude protein	23.00	20.00
Lysine	1.35	1.23
Methionine	0.67	0.63
Methionine + cysteine	0.80	0.74
Calcium	0.94	0.94
Total phosphorus	0.53	0.51

[1] Analysis attached.

Vitamin and mineral premix includes the following per kilogram:

Vitamin A (vitamin A acetate)	6,000,000 IU	Vitamin D (cholecalciferol)	1,800,000 IU
Vitamin E (tocopherol acetate)	1,200 mg	Vitamin B1 (thiamine)	800 mg
Vitamin B2 (riboflavin)	2,000 mg	Vitamin B3 (niacine)	15,000 mg
Vitamin B5 (calcium pantothenate)	3,125 mg	Vitamin B6 (pyridoxine)	1,600 mg
Vitamin B7 (biotin)	30 mg	Vitamin B9 (folic acid)	600 mg
Vitamin B12 (cyanocobalamin)	15 mg		

Trace elements:

Manganese (manganousoxide)	30,000 mg	Zinc oxide	20,000 mg
Iron sulfate monohydrate	20,000 mg	Copper sulfate pentahydrate	4,500 mg
Iodine (calcium iodate)	100 mg	Selenium (sodium selenite)	100 mg

The ingredients inclusion rates and calculated chemical analysis of the formulated trial starter and finisher diets are shown in table (6), whereas laboratory chemical analysis results of formulated starter and finisher feed samples are shown in table (7).

Analyzed crude protein content of starter and finisher diets was 189.5 and 226.0 g / Kg respectively, calculated metabolizable energy was 3,136.36 Kcal / Kg and 3,097.22 Kcal / Kg respectively.

Crude protein content and calculated energy levels were within the standards recommended for Cobb 500 (Cobb 500 Nutrition Supplement, 2019).

Table (7): Determined chemical analysis of experimental diets.

Nutrient	Starter	Finisher
Crude Protein %	18.95 ± 0.29	22.60 ± 0.45
Crude Fat %	4.22 ± 0.14	4.98 ± 0.28
Moisture %	7.41 ± 0.17	7.76 ± 0.18
Ash %	7.03 ± 0.13	7.43 ± 0.36
Crude Fiber %	3.90 ± 0.35	4.17 ± 0.03
N.F.E %	58.49 ± 0.64	53.60 ± 0.28
ME Kcal / Kg	3,136.31 ± 4.56	3,097.22 ± 17.91

3.3. Experimental diet layout:

***Treatment 1:** Control, basal diet without enzyme used for whole period of starter and finisher phases.

***Treatment 2:** Starter phase only:

Basal diet + graded levels of 250, 500, 750 or 1,000 g enzyme per 1 ton of feed offered during starter phase only.

***Treatment 3:** Finisher phase only:

Basal diet + graded levels of 250, 500, 750 or 1,000 g enzyme per 1 ton of feed offered during finisher phase only.

***Treatment 4:** Starter and Finisher phases:

Basal diet + graded levels of 250 g, 500 g, and 750 g or 1,000 g enzyme per 1 ton of feed offered during starter and finisher phases.

The experimental diets were formulated to meet the nutrient requirements of broiler chickens (Cobb™, 2018), then NutriKEM Extend multi-enzymes and its matrix (nutrient-equivalent values assigned to enzyme products in least cost formulation) was added.

The ingredients and composition of the experimental basal diets fed during the two phases of broiler production (starter and growing periods) are shown in table (6).

All experimental groups were fed the same basal diet, and were given NutriKEM Extend multi-enzymes treatments as follows: the 1st group (control) did not receive enzyme supplementations; the 2nd, 3rd, and 4th groups were given multi-enzymes (NutriKEM Extend®) in starter or finisher phase only, or during the whole period respectively at 250, 500, 750 and 1,000 g / Ton of feed. The enzyme was added and mixed basal feed.

3.4. NutriKEM Extend Exogenous Multi-enzymes:

NutriKEM Extend Dry contains a multi-enzymes product manufactured by **Kemin Europa, N.V. © Kemin Industries, Inc** Belgium and developed for use in total vegetable diets. The enzymes present have been carefully selected for their hydrolytic activity towards pentosans and other Non-Starch Polysaccharides (NSP), by degrading these anti-nutritional factors, the enzymes in NutriKEM Extend Dry make the nutrients in an animal feed more available and digestible.

However, in addition to NSP-degrading enzymes, NutriKEM Extend Dry also contains amylase and protease activity, although these enzymes are also produced in the animal, it is useful to support the endogenous enzyme system.

Lysophospholipids in the form of lysolecithin as active ingredients, are also included in NutriKEM Extend, acts on all 3 key steps in fat digestion, i.e. emulsification, hydrolysis and absorption, to provide a more complete mode of action for fat and nutrient digestion in animal nutrition.

3.5. Data collection

3.5.1. Performance traits:

3.5.1.1. Live body weights (g):

Birds in each pen were weighed as a group at 7 and 14 days of age (starter period) and at 21, 35 and 42 d of age (finisher period), chicks were weighed in the morning after removing feeders and before offering new feed.

3.5.1.2. Body Weight Gain BWG (g):

BWG for each week of the 5-week experiment and for the whole experimental period were calculated by subtracting body weight at the begging, from that of the end of each week, using individual record for each replicate.

3.5.1.3. Feed Intake (FI) (g / bird / period):

Feed intake was recorded at the end of 2^{nd}, 3^{rd}, 4^{th}, 5^{th} , and 6^{th} weeks of age, according to the replicate-feeding system followed in the present work, each group was provided with enough pre weighed diet of its corresponding experimental diet. The remainder feed as well as the consumed feed was weekly estimated for each replicate and thereafter, the average feed consumption from 7-14, 15-21, 22-35 and 36-42 day of age and for the whole experimental period were calculated through dividing of group consumption by their chick numbers.

3.5.1.4. Feed Conversion Ratio (FCR) (Feed / Gain):

Consumed feed to weight gain ratio was calculated in the form of units of feed consumed required to produce one unit of live body weight gain (FCR) during each week, and for the whole experimental periods (07-42 day of age).

3.5.2. Mortality rate:

Number of dead birds was presented in each treatment during the whole experimental period divided by the initial number.

3.5.3. Slaughter procedure:

At the end of marketing age (42 days), one bird was taken randomly from each treatment group, in order to determine the carcass traits to represent all treatment replications, birds were identified, weighed and slaughtered manually, after being fasted overnight, with knife according to the Islamic method, and allowed to bleed for 5 minutes, a tag was fixed to leg shaft, the remaining carcass after bleed, was manually plucked, and the eviscerated carcass was weighed (dressed weight).

The carcass yield was calculated as a percentage of the pre slaughter body weights of broilers, after a complete bleeding, birds were scalded at 65 ^{0}C for 45 seconds, feather removed and manually eviscerated. Following evisceration, all carcasses were chilled in cold water for 15 minutes. Hot carcass, economical cuts, edible parts and organs were weighted and calculated as a percentage on basis of live body weight.

The whole carcass, heart, gizzard (full and empty), liver, abdominal fat, were separated and individually weighed. Intestinal weight and length was measured and expressed as relative weight to live body weight. The carcass parts were expressed as relative to live body weight.

The frozen bird carcass was split from front to back through the backbone and keel to produce two halves of approximately equal weight.

One half was proportioned into wing, back bone and commercial cuts (drumstick, thigh and breast), the carcass parts were weighed and expressed as relative to cold carcass weight, thigh and breast were washed and deboned, meat and bone were weighed, meat was store frozen for chemical analysis and panel taste.

3.5.4. Chemical composition of meat:

A sample of 50% of thigh meat + 50% breast meat was weighed and dried in an electric drying oven at 70 °C for 24 hours until constant weight. The dried flesh was finely ground through a suitable mixer pass through a sieve (1-mm2) and then carefully mixed, dried samples were kept into well tight glass container for subsequent analysis.

3.5.5. Chemical analysis:

The proximate composition were performed on diets and meat samples, dry matter analysis of samples was determined by drying the samples in a drying oven at 105°C for 24 hours, method 934.01, (AOAC, 2000).

Feed samples of the experimental diets (starter and finisher diets) as well as the flesh from experimental birds were chemically analyzed for Dry Matter, Ether Extract, Crude Protein, Crude Fiber and Ash according to the official methods of (AOAC, 1995).

3.5.6. Basic of Proximate Analysis:

The Proximate Analysis is the Estimation and determination of how much of the major feed components, exist in a given food, the proximate analyses therefore are:

1. Moisture. 2. Crude Fat.
3. Crude Protein. 4. CHO and Crude Fiber.

Total carbohydrate = 100-[moisture + crude fat + crude protein + ash].

3.5.6.1. Moisture Content determination:

The moisture content of the samples was determined using air oven method of (AOAC, 2010), samples were wrapped in a foil paper and weighed using sensitive weighing balance. The constant weighed samples were placed in the oven at 105 ^{o}C for 24 hours. After 24 hours, the samples were cooled in a desiccators and the weight taken.

Calculation

% Moisture content = 100 - % DM

$$\% \text{ Dry matter} = \frac{\text{Dry weight X 100}}{\text{Initial weigh}}$$

Moisture and low volatile materials are removed by heating at 95 -100°C under partial vacuum.

Procedure:

1. Accurately weigh a moisture dish of appropriate size.
2. Add approximately 10 g of the comminuted sample and reweigh.
3. Place the container in a vacuum oven at 100 ^{o}C and less than 100 mm Hg for approximately 5 hours.
4. Remove dish from the oven, cover, cool in desiccator, and weigh.
5. Redry 1 hour and repeat process until constant weight has been achieved, i.e., change in weight between successive dryings at 1 hour intervals is < 5 mg.

Calculate the percentage moisture (wet weight basis) as follows:

$$\text{percent moisture} = \frac{100\,(P-a)}{P}\ \%$$

P = weight in g of sample
a = weight in g of dried sample

3.5.6.2. Proximate analysis, Ash:

The ash fraction contains all the mineral elements jumbled together, it was determined according to the standard method of Association of Official Analytical Chemists (AOAC, 2010). Crucible was washed and oven dried. The sample was weighted into a known weight crucible and placed into a Bunsen burner in a fume cupboard to char the sample. Then, the charred sample was placed in a preheated muffle furnace at 550 ^{o}C until the color of the sample change to light grey ash. It was cooled in desiccators and weighed.

Procedure:

1. Accurately weight of 5 g of sample in a crucible which has been ignited and tarred (use 2.5 g of sample in the case of products which have a tendency to swell).
2. Place crucible in drying oven at 100 ^{o}C for 24 hours.
3. Transfer to cool muffle furnace and increase the temperature step wise to 550 ^{o}C ± 5 ^{o}C.
4. Maintain temperature for 8 hours or until a white ash is obtained.
5. If white ash is not obtained after 8 hours, moisten ash with distilled water, slowly dry on a hot plate, and re-ash at 550 ^{o}C to constant weight. Repeat if necessary.
6. Remove crucible to a desiccator and weight soon after cool, calculate the percentage ash content (wet weight basis) as follows:

$$\% \text{ Ash (wet)} = \frac{\text{(wt. crucible and ash - wt. crucible)}}{\text{(wt. crucible and sample - wt. crucible)}} \times 100$$

Calculation of ash content on dry basis (when moisture content is known):

$$\% \text{ Ash (dry)} = \frac{\% \text{ ash (wet)}}{(100 - \% \text{ moisture})} \times 100$$

3.5.6.3. Crude Protein Kjeldahl Procedure, EN ISO 5983-2 (AOAC, 2001:11)

FOSS-Kjeltec ™ 8000 series, (SAC, 2013):

Sampleweight 0.5-2g, theparticle size should be ≤ 1 mm.

Procedure:

a) Digestion**: -** The reaction between organic compounds and sulfuric acid produced ammonium sulfate solution:

i) Add sequentially15g of K_2SO_4, 0.9-1.2g of $CuSO_4$, and one or two salinized boiling granules (catalysts).
ii) Add 25 ml of conc. $H_2SO_4$to the flask, for 1g sample and 6-12 ml for each additional gram of sample, digest until solution is almost colorless or light green (two hours), then cool.
iii) Add water 250-350 ml, then add 100 ml 33% NaOH slowly down the side of the Kjeldahl flask so that it forms a layer underneath the digestion mixture. Steam distill until ≥150 ml of distillate

b) Distillation: Ammonium salts reacted with excess strong alkali, the ammonia gas produced in this stepwas distilled and dissolved in a standardized solution of hydrochloric acid or sulfuric acid.

c) Titration: - the solution was back titrated with Sodium hydroxide to indirectly measure nitrogen.

Receiver solution 25-30 ml of 4% H_3BO_3 solution + 1 ml Conway's indicator (1% Methyl Red solution with 0.1% Methylene Blue solution (in 50% ethanol), (pH change 5.4: Acid - Purple, Alakline - Green).

Titration with standard acid determines the amount of ammonia and therefore nitrogen in the sample.

4% H_2BO_3 solution →0.05M, 0.125 H_2SO_4

$$NH4^{+}:H_2BO_3^{-} + HCl \rightarrow NH_4Cl + H_3BO_3$$

Calculation of Nitrogen Content and % Protein:

$$\%N = \frac{(T-B) \times N \times 14.007 \times 10}{\text{Weight of sample in g}}$$

T = Volume of titrant used for Sample (ml)
B = Volume of titrant used for Blank (ml)
N = Normality of titrant (to 4 decimal places)
14.007 = Molecular weight of Nitrogen

Calculate nitrogen content on dry basis (when moisture content is known) as follows:

$$\%\text{ Nitrogen (dry)} = \frac{\%\text{ Nitrogen (wet)}}{(100 - \%\text{ moisture})} \times 100$$

Calculate the percentage protein (wet or dry basis) as follows:

%Protein=%N X factor % Protein = % nitrogen X 6.25

Where 6.25 is the protein-nitrogen conversion factor.

3.5.6.4. Crude Fiber:

Sample preparation: Homogeneous of particle size < 1 mm, if fat content is > 10% defatting prior to analysis is recommended.

Sample weight: An analytical balance accurate to 0.1 mg.

Crude Fiber analysis, Crude Fiber Weende Method, Fibertec M6, Cold Extraction Unit (AOAC, 4.6.01) .

Fiber Calculation: Residue Content

Crude fiber, residue after sequential treatment with acid and alkali:

Cellulose 50-80%
Hemicellulose ~20%
Lignin 10-50%

Detergent fiber:

1 - Neutral Detergent Fiber (NDF), residue after sequential treatment with Neutral Detergent Solution;

Cellulose 100%
Hemicellulose 100%
Lignin 100%

2 - Acid Detergent Fibre (ADF), residue after sequential treatment with Acid Detergent Solution;

Cellulose 100%
Lignin 100%

3 -Acid Detergent Lignin (ADL), residue after initial treatment with Acid Detergent Solution, followed by removal of cellulose fraction through extraction using 72% H_2SO_4:

Lignin 100%

Procedure:

1. Determine separately the sample moisture by heating in an oven at 105 °C to constant weight, then cool in a desiccator.
2. Weight accurately 1gof grinded sample (size <1mm) => W1
3. Add 1.25% sulfuric acid up to the 150 ml.
4. Add 3-5 drops of n-octanol as antifoam agent.
5. Boil for 30 minutes exactly from the onset of boiling.
6. Connect to vacuum for draining sulfuric acid.
7. Wash three times with 30 ml (crucible filled up to the top) of hot deionized water, connecting each time to compressed air for stirring the content of crucible.
8. After draining the last wash, add 150 ml of preheated potassium hydroxide (KOH) 1.25% and 3-5 drops of antifoam.
9. Boil for 30 minutes.
10. Filter and wash as point 7.
11. Perform a last washing with cold deionized water aimed to cool the crucibles and then wash three times the crucible content with 25 ml of acetone, stirring each time by compressed air.
12. Remove the crucibles and determine the dry weight after drying in an oven at 105 °C for an hour or up to constant weight, let cool in a desiccator, this weight (W2) represents the crude fiber plus ash content in comparison to initial weight.

Calculate the percentage crude fiber (wet weight basis) as follows:

$$\% \text{ Crude fiber (wet)} = \frac{(W2 - W1)}{W1} \times 100$$

3.5.6.5. Crude fat content, Soxtec system:

Crude fat is determined by a solvent extractione.g. Non-polar organic solvents such as hexanes and petroleum ether, total fat determination includes a preparatory acid hydrolysis step and a solvent extraction.

The fat that is bound to other non-solvent soluble as e.g. proteins are separated in hydrolysis step. Hydrolysis makes chemically or mechanically bound fats accessible to solvent extraction.

Sample Preparation:

The particle size should be equal to, or less than, 1 mm.

Procedure:

1- Boiling Step:
   Sample is immersed in boiling solvent, Provides rapid extraction of soluble materials, for most application, the boiling step is 15 to 25 minutes.
2- Rinsing Step:
   Sample is raised out of the boiling solvent, condensed solvent, drips through sample and rinses out residuals, usually 30 to 40 minutes.
3- Solvent Recovery Step:
   Condensed solvent is collected in the collection vessel, this step concentrates the extracted material in the extraction cup and saves solvent for reuse, usually 10 minutes.
4- Pre-drying Step:
   Shortened drying time through pre drying in instrument.

Calculation:

Percent Crude Fat (Ether Extract), DM basis:

$$\% \text{ Crude fat (wet)} = \frac{(\text{Wres} - \text{Wta})}{\text{weight (g) of sample}} \times \text{DM (\%)}$$

- Wta = tare weight of beaker in grams
- Wres = weight of beaker and fat residue in grams

3.5.7. Hematological and biochemical characteristics:

Before slaughter at 42 days of age, one blood sample (5 ml per sample) of each treatment was collected in clean non-heparinized tubes. Biochemical parameters of blood plasma and serum profile were photometrically determined using Cobass C 311 Analyzer (produced by Roche Diagnostics GmbH, Germany) and BC-30s Auto Hematology Analyzer, Shenzhen Mindray Bio-Medical Electronics Co., Ltd. China.

Plasma total protein (g/100ml) was measured according to (Henry *et al.*, 1974), albumin concentration (g/100ml) was determined according to (Doumas *et al.*, 1977). Globulin concentration (g/100ml) was calculated as the difference between total protein and albumin. Plasma glucose was determined according to (Trinder, 1969). Triglycerides, total cholesterol was measured according to (Watson, 1960).

The activities (μ/L) of the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were determined according to the method described by (Reitman and Frankel, 1957).Serum creatinine and urea were determined by (Fletcher, 2002). Hemoglobin concentration was determined by the cyanomethemoglobein method (Eilers, 1967).

3.5.8. Sensory Sample Preparation:

At the end of the experiment period (42nd day) and after slaughtering, one piece of chickens halves was chosen from each of the 15 groups and from each half, fifteen chilled samples of thigh and breast meat were transported to the laboratory facilities of the Department of Animal Production of the SUST and were heat-treated at 180 °C for 60 minutes and separately evaluated in sensory analysis.

Sensory evaluation of anonymous samples was performed by committee members for the self-assessment. Panelists evaluated color, juiciness, flavor and tenderness on 8 point hedonic scale where 1 (the worst) and 8 (the best) were the extremes of each characteristic (Appendix 4).

3.5.9. Sensory evaluation of meat:

Objective sensory evaluation of the samples (Stone *et al,* 1980; Lawless, 2013), was performed by 24 semi trained panelists, who were experienced in descriptive sensory profiling of poultry products. The sensory panel was selected according to ISO 8586-2012, Sensory analysis 1993, sensory evaluation was carried out in the laboratory, with well air exchange and standardized lighting, and each sample was evaluated for up to 10 min.

One cube of each sample was placed into relabeled Styrofoam. A new, random code was assigned to each treatment. Panelists had more than 3 years of food sensory panel experience. These parameters qualify the sensory panel as semi trained (Chambers *et al.*, 1981). Panelists were both men and women ranging in age from 22 to 65 years of age. The panel size required to perform a discriminative test by rating samples using differences or scalar from a control is variable, depending on product variability tested, but 10 is typical and a minimum of 5 is recommended (Institute of Food Technologists, Sensory Evaluation Division, 1981). Previous researchers comparing similar types of chicken meat

have used 10 experienced panelists (Jahan *et al*., 2005; Sandercock *et al*., 2009) and, for trained panels, 9 members (Castellini *et al*., 2002) or 15 to 17 members (Fanatico *et al*., 2007).

During the panel taste sessions, potential panelists were provided with evaluation sheet. The information sheet stated that the authors were evaluating the effects of different poultry feeding techniques on sensory quality of chicken breast and thigh. Potential panels were also provided with cooked chicken breast and thigh cubes in Styrofoam cups during the orientation sessions. They were then asked to taste the samples and review and make suggestions for changing the sensory evaluation sheet. This enabled the evaluators to correlate their actual observations of the samples with regard to the listed descriptors and overall format, providing a more accurate evaluation sheet (Chambers *et al*., 1981).

Other than the orientation sessions, panelists were not allowed to talk or ask questions during the actual evaluations. Panelists were also asked to identify sample preference and were provided with a comment section they could choose to use.

Each parameter was set up on a scale with a negative perception on the far low and a positive perception on the high. Panelists were asked to place a mark between these 2 perceptions to denote their evaluation.

3.5.10. Production index:

Evaluating broiler performance is a complex item. Over time, we have moved from a simple measurement of bird weight to weight for age, mortality, FCR and more recently to a European Production Efficiency Factor (EPEF).

The European Production Efficiency Factor (EPEF) was used to evaluate the growing performance of broilers as suggested by (Van, 2003; Marcu *et al*., 2013 and Aviagen, 2015). EPEF was calculated according to the following formula (Marcu *et al*., 2013).

TWG = Body weight (g) at the end - Body weight (g) at start;
ADG (g/chick/d) = TWG/ days of growth period;
FCR (kg feed/kg gain) = Cumulative feed intake (kg) / Total weight gain (kg);
Viability, % = 100 - Mortality, %

$$EPEF = \frac{\text{Viability (\%) x BW (kg)}}{\text{Age (d) x FCR (kg feed/kg gain)}} x100$$

3.5.11. Economic efficiency:

Feed ingredients prices were determined at experiment time, then the cost of each ingredient in 1 MT of feed was calculated, based on its inclusion rate, finally the cost of 1 kilogram of different feed formulas was determined. The cost of 1 kilogram weight gained was calculated by dividing the total cost of feed intake by the whole weight gain. A comparison has been done among cost results of treatments.

Economical evaluation for all experimental diets was made, then economic efficiency was calculated (during 08-42 days of age) using the following steps for growing trials, as described by (Zeweil, 1996):

1. Average carcass weight.
2. Price of 1 Kg chicken meat at time being of terminating of the experiment (210.00 SDG).
3. Total revenue /chick (SDG) = 1 x 2.
4. Total feed intake /chick (Kg).
5. Price/Kg feed (SDG).
6. Total feed cost/chick (SDG) = 4 x 5.
7. Fixed cost/chick (SDG).
8. Total cost/chick (SDG) = 6 +7.
9. Net revenue (SDG) = 3 – 8.
10. Economic efficiency (EE) = (Net revenue/ total costs) ×100.

3.5.12. Statistical Analysis

Pens were identified as an experimental unit, statistical analyses were performed using the GLM procedure of the software of Statgraphics, Minitab 19 and SPSS 11.5.

One-way ANOVA was used to test for the effects of four treatments, a contrast analysis was used after exclusion of the control group to compare intermittent (starter or finisher phase only) vs. continuous feeding (whole feeding period). The linear and non-linear effects of enzymes dose were tested.

In addition, a 3 X 4 factorial design analysis (three rearing phases, starter (7-21 days) or finisher (22-42 days) only, or for the whole period 1-37 days of age by four graded levels of enzymes treatments, was run after excluding the birds of control group fed on un-supplemented multi-enzymes treatment, to check for the result of multi-enzymes levels, feeding method, the experimental model also included the interaction analysis between the main factors.

Experimental data presented as mean ± standard errors of the mean, the significance of the differences among means has been determined by Duncan's multiple range tests (Duncans, 1955; Petrie and Watson, 1999). When a significant treatment effect was obtained by the analysis of variance, a probability level of ($P < 0.05$ or $P \leq 0.10$) was required for a statement of significance, the *p*-value between 0.05 and 0.10 was considered a trend.

CHAPTER FOUR

RESULTS

It should be noted that results are firstly presented according to the effect of feeding durations, then to the effect of various multi-enzymes inclusions levels, and finally the interaction effect of both variable factors (feeding durations and various multi-enzymes levels).

Data of growth performance of broiler chicks affected by dietary supplementation of multi-enzymes, namely NutriKEM Extend, at different graded levels, fed during starter (2^{nd} and 3^{rd} week) or finisher (4^{th},5^{th} and 6^{th} weeks) period only, or fed during the whole rearing period, are shown in tables (8),(9), and (10).

Results indicated that, final body weight (BW)) was not significantly affected by feeding durations phases, never the less, numerical data expressed an improvement of body weight with increased feeding duration.

Multi-enzymes supplementation at graded different levels has no effect on feed consumed during the starter (08-21 days) phase only, or finisher period (22-42 days) only, however a significant effect of feeding durations on cumulative consumed feed can be observed with p-value ≤ 0.10, the total feed intake of 4,043.80g recorded when birds offered multi-enzymes fed during starter period only, was greater, compared to feed consumed (3,821.85g) when multi-enzymes added feed was fed during the finisher period only.

BW of the 5^{th} week (day 28 – 35), was significantly (p-value 0.023) affected by feeding durations. Similarly, the (BWG) of the 3^{rd} week (day 22 – 28), was highly significantly affected by both feeding durations phases (p-value 0.0022) and supplemented multi-enzymes levels (p-value 0.0485).

4.1. Effect of added multi-enzymes NutriKEM Extend on broiler performance, when fed during starter period only.

4.1.1. Body weight (BW) and Body weight gain (BWG).

Diet with multi-enzymes level of 750 g / MT of feed fed during the starter phase only, resulted in the lowest final (BW) 1,722 g , which was lower than the weight obtained by control diet (1,735 g).

4.1.2. Feed intake (FI) and Feed Conversion Ratio (FCR).

The highest value of starter diet intake of 1,138.8g, was recorded with birds fed on diets containing 500 g multi-enzymes / MT feed, offered during the starter phase only.

Highest numerical finisher diet intake of 3,037g, was observed when adding 1,000 g multi-enzymes to 1MT of finished complete feed, fed during the starter period only.

Birds fed diet with multi-enzymes level of 1g/ Kg of feed, during the starter period only, consumed the highest feed amount (4,158.80g).

Feed consumption at the end of experimental period, was not affected by feeding durations.

FCR was significantly improved when feeding the multi-enzymes diet during starter period only compared to finisher period only, 2.59 and 2.36 respectively, the best FCR value of 1.85 for the starter diet was obtained for birds fed diet with multi-enzymes level of 500g/1MT of feed, fed during the whole starter and finisher period.

The worst FCR value (2.17) of the starter diet, was observed with birds fed diet with multi-enzymes level of 500g/1MT of feed, offered during the starter period only, FCR value (2.68) of the same period was observed with birds fed diet with multi-enzymes level of 750g/ 1MT of feed, offered during the starter period only.

4.2. Effect of added multi-enzymes NutriKEM Extend on broiler performance, when fed during finisher period only.

4.2.1. Body weight (BW) and Body weight gain (BWG).

Based on these studies, feeding durations of diets supplemented with various levels of multi-enzymes, showed no significant effect on BW and BWG.

4.2.2. Feed intake (FI) and Feed Conversion Ratio (FCR).

The lowest starter diet feed intake of (1,042.4g) for, was recorded with birds fed on diet supplemented with 250g multi-enzymes per 1MT of feed during the finisher period only.

The lowest finisher diet intake of (2,652.80g) was recorded when adding 500 g multi-enzymes to 1MT of feed, fed during the finisher period only.

Lowest cumulative consumption of (3,730.80g) was recorded for birds fed diet with multi-enzymes level of 0.5g/ Kg of feed, during the finisher period only.

The best FCR value (2.30) at the end of the whole experimental period was obtained for birds fed diet with multi-enzymes level of 500g/1MT of feed, offered during the finisher period only

4.3. Effect of added multi-enzymes NutriKEM Extend on broiler performance, when fed during the whole experimental period.

4.3.1. Body weight (BW) and Body weight gain (BWG).

The best numerical values of both (BW), 775.5g and (BWG), 576.4 g at the end of starter period (day 21), were obtained when feeding diets supplemented with multi-enzymes at level of 500 g / Ton of feed, during the whole period (from day 08 to day 42).

Lowest values of the same mentioned parameters for same period, were observed with control diet and diet with multi-enzymes level of 750 g/MT of feed (BW of 699g for both).

Good results offinal or slaughter weight (BW) of 1,845g, and hence body weight gain of finisher period (day 21-42) of 1,118 g, were achieved when feding diets with added multi-enzymes at 1,000 g / MT of feed for the whole fattening period (42 days).

4.3.2. Feed intake (FI) and Feed Conversion Ratio (FCR).

Multi-enzyme inclusion at 500mg / MT of complete feed, reduced feed intake at both starter and finisher stages.

According to the results obtained from current study, finisher stage is the best period for the enzyme inclusion.

Table (8): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on body weight and EPEF of broiler.

	Treatments	Body Weight (g)					EPEF
		Starter			Finisher		
		Day 08 - 14	Day 15 - 21	Day 22 - 28	Day 29 - 35	Day 36 - 42	
	Control	363	699	1,054	1,577	1,735	160.69
	Feeding Periods Effect						
1	Starter	383.80	734.50	1,114.60	1,526.3 [ab]	1,765.50	164.04
2	Finisher	377.95	738.25	1,104.20	1,566.95 [a]	1,815.05	185.34
3	Whole Period	389.45	741.95	1,069.65	1,464.4 [b]	1,824.65	180.24
	SEM	5.011	12.411	19.227	25.506	30.121	6.779
	p-value	0.2775	0.9140	0.2339	0.0227	0.3376	0.0785
	Sig	N.S	N.S	N.S	*	N.S	N.S
	Enzymes Levels Effect						
1	Enzyme 250 g	386.13	735.27	1,104.53	1,515.67	1,797.73	174.62
2	Enzyme 500 g	387.47	743.60	1,121.47	1,540.07	1,803.07	176.53
3	Enzyme 750 g	373.40	725.20	1,058.27	1,506.73	1,788.33	173.35
4	Enzyme 1,000 g	387.94	748.87	1,100.33	1,514.40	1,817.80	181.69
	SEM	5.786	14.331	22.202	29.452	34.780	7.827
	p-value	0.2442	0.6704	0.2363	0.8675	0.9446	0.8822
	Sig	N.S	N.S	N.S	N.S	N.S	N.S
	Feeding Periods X Enzymes Levels Interaction						
	SEM	10.021	24.822	38.454	51.012	60.241	13.558
	p-value	0.6476	0.4269	0.8177	0.4116	0.9912	0.9481
	Sig	N.S	N.S	N.S	N.S	N.S	N.S

a-b: Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).

*: Significant with (P≤0.05). N.S : Not significant.

Table (9): Effect graded levels of multi-enzymes NutriKEM Extend and feeding durations on body weight gain of broiler chickens.

	Treatment	Starter (g)				Finisher (g)			
		Day 08 - 14	Day 15 - 21	Starter	Day 22 - 28	Day 29 - 35	Day 36 - 42	Finisher	Whole Gain
	Control	172.00	336.00	508.00	346.00	449.00.00	242.00	1,037.00	1,545.00
	Feeding Periods Effect								
1	Starter	189.20	350.07	539.95	380.10 [b]	411.70	280.60	1,030.90	1,570.90
2	Finisher	187.55	360.40	547.85	366.00 [b]	462.70	273.75	1,076.65	1,624.55
3	Whole Period	193.50	352.45	545.85	327.70 [a]	394.85	272.25	1,082.85	1,628.60
	SEM	4.328	9.279	12.130	10.258	30.792	18.79	23.418	29.72
	p-value	0.6074	0.7346	0.8919	0.0022	0.2776	0.9455	0.2405	0.3178
	Sig	N.S	N.S	N.S	**	N.S	N.S	N.S	N.S
	Enzymes Levels Effect								
1	Enzyme 250 g	192.33	349.20	541.40	369.20	411.13	266.93	1,062.40	1,603.73
2	Enzyme 500 g	190.80	356.07	547.00	377.93	418.53	271.40	1,059.60	1,606.40
3	Enzyme750 g	181.87	351.87	533.60	333.00	448.47	290.93	1,062.93	1,596.73
4	Enzyme 1,000 g	195.33	360.93	556.20	351.60	414.20	272.87	1,068.93	1,625.20
	SEM	4.997	10.714	14.007	11.845	35.556	21.697	27.041	34.32
	p-value	0.2714	0.8757	0.7106	0.0485	0.8724	0.8698	0.9958	0.9446
	Sig	N.S	N.S	N.S	*	N.S	N.S	N.S	N.S
	Periods Effect X Enzymes Levels Interaction								
	SEM	8.656	18.557	24.260	20.517	61.548	37.580	46.836	59.45
	p-value	0.3110	0.1233	0.3657	0.0481	0.3928	0.8616	0.8683	0.9880
	Sig	N.S	N.S	N.S	*	N.S	N.S	N.S	N.S

a-b:Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).
**: Significant with ($p \leq 0.01$). *: Significant with ($p \leq 0.05$). N.S: Not significant.

Table (10): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on feed intake and FCR of broiler chickens.

Treatments	Feed Intake (g)								FCR	
	Starter			Finisher				Whole	Starter	Whole period
	Day 08 - 14	Day 15 - 21	Whole	Day 22 - 28	Day 29 - 35	Day 36 - 42	Whole	Period	Day 08 - 21	Day 08 - 42
Control	404.00	677.00	1,081.00	912.00	965.00	934.00	2,811.00	3,892.00	2.13	2.52
Feeding Periods Effect										
1 Starter	424.45	688.20 [a]	1,112.70 [a]	950.05 [a]	1,002.55	978.45 [a]	2,930.95	4,043.80	2.08	2.59 [a]
2 Finisher	401.90	659.30 [ab]	1,061.20 [b]	871.80 [b]	1,020.00	868.85 [b]	2,760.85	3,821.85	1.95	2.36 [b]
3 Whole period	424.75	639.55 [b]	1,064.35 [b]	942.20 [a]	1,061.35	898.75 [ab]	2,902.55	3,966.80	1.97	2.45 [ab]
SEM	10.02	12.84	15.94	20.31	23.43	30.89	63.49	71.60	0.050	0.063
p-value	0.1912	0.0339	0.0463	0.0162	0.2007	0.0429	0.1378	0.0947	0.1753	0.0446
Sig	N.S	*	*	*	N.S	*	N.S	N.S	N.S	*
Enzymes Levels Effect										
1 Enzyme 250g	412.47	664.60	1,077.20	913.60	1,027.93	909.20	2,850.67	3,927.80	2.01	2.46
2 Enzyme500g	408.27	684.20	1,092.53	922.07	1,021.20	912.20	2,855.73	3,948.20	2.01	2.47
3 Enzyme750g	432.00	640.40	1,072.27	932.87	1,033.07	917.73	2,883.33	3,955.47	2.02	2.49
4 Enzyme1,000g	415.40	660.20	1,075.67	916.87	1,029.67	922.60	2,869.40	3,945.13	1.96	2.45
SEM	11.56	14.82	18.41	23.46	27.06	35.66	73.20	82.68	0.058	0.073
p-value	0.4954	0.2345	0.8693	0.9423	0.9915	0.9938	0.9891	0.9961	0.8949	0.9884
Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Feeding Periods X Enzymes Level Interaction										
SEM	20.03	25.67	31.89	40.63	46.86	61.77	126.78	143.21	0.100	0.126
p-value	0.8387	0.6948	0.7821	0.1794	0.1147	0.9018	0.3243	0.3553	0.4186	0.7387
Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

a-b:Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).

** : Significant with ($p \leq 0.01$). * : Significant with ($p \leq 0.05$). N.S: Not significant.

Table (11): Broiler performance as affected by interactive action of feeding periods and or enzymes levels.

	Treatment	FI (g)		BWG (g)		BW (g)		FCR (g)	
		Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)
	CONTROL	1,081.00	2,811.00	508	1,037.00	699	1,735.00	2.13	2.52
	ENZYMES LEVELS EFFECT								
Starter period only									
1	**ENZYME 250 g**	1,090.80	2,694.60	545.80	1,020.40	745.80	1,766.60	2.00	2.43
2	**ENZYME 500 g**	1,138.80	2,999.80	531.20	1,057.20	728.20	1,785.00	2.16	2.62
3	**ENZYME 750 g**	1,099.60	2,992.40	507.40	1,022.60	699.00	1,722.00	2.17	2.68
4	**ENZYME 1,000 g**	1,121.60	3,037.00	575.40	1,023.40	765.00	1,788.40	1.98	2.63
Finisher period only									
5	**ENZYME 250 g**	1,042.40	2,841.00	532.00	1,073.00	721.80	1,794.80	1.97	2.43
6	**ENZYME 500 g**	1,078.40	2,652.80	533.40	1,092.00	727.20	1,819.20	2.02	2.30
7	**ENZYME 750 g**	1,057.20	2,787.20	563.80	1,076.20	749.60	1,826.20	1.88	2.35
8	**ENZYME 1,000 g**	1,066.80	2,762.40	562.20	1,065.40	754.40	1,820.00	1.92	2.38
Starter and Finisher									
9	**ENZYME 250 g**	1,098.40	3,016.40	546.40	1,093.80	738.20	1,831.80	2.06	2.51
10	**ENZYME 500 g**	1,060.40	2,914.60	576.40	1,029.60	775.40	1,805.00	1.85	2.49
11	**ENZYME 750 g**	1,060.00	2,870.40	529.60	1,090.00	727.00	1,816.80	2.01	2.44
12	**ENZYME 1,000 g**	1,038.60	2,808.80	531.00	1,118.00	727.20	1,845.00	1.98	2.35
	SEM	25.67	61.77	24.26	46.836	24.822	60.241	0.100	0.126
	***p*- value**	0.6948	0.9018	0.3657	0.8683	0.4269	0.9912	0.419	0.7387
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

N.S Not significant

4.4. Effect of various added levels of multi-enzymes NutriKEM Extend on performance and economic efficiency of broiler chickens.

Results indicated that, final body weight (BW)) was not significantly affected by different dietary treatments (graded multi-enzymes levels), never the less, numerical data expressed an improvement of body weight with the gradual increase of multi-enzymes supplementation levels.

Multiple enzymes mixture supplementation significantly improved broiler chicks performance at all levels compared to control group, the group fed on diet supplemented with the level of 1,000 g of enzyme / MT of feed, recorded the best performance.

All diets supplemented with multiple enzymes, recorded better profit and economic efficiency ratio compared to control group, however group fed on diet with 1,000mg per ton of feed achieved the highly economic efficiency, when fed during starter and finisher periods.

Only weight gain during 3^{th} week, explained a significant (*p*- value 0.0481) two way interaction effect between added multi-enzymes levels and feeding regime, however the feed consumed during the 2^{nd} week was affected (p-value ≤ 0.05) by feeding durations, feed intake during the starter period (08 – 21 days) was also significantly (p-value ≤ 0.0463) affected with feed durations, records revealed a decreased feed intake with increased feeding durations.

During the finisher period, consumed feed of 3^{rd} and 5^{th} weeks were significantly affected (p-value 0.0162 and 0.0429 respectively), but affection during 4^{th} week and during the whole finisher period, was not significant, the cumulative feed consumption was not affected by both added multi-enzymes levels and feeding durations.

None of the two trial variables factors, i.e. graded addition of multi-enzymes or feeding phases, had interactive effect on feed conversion ratio (FCR) of starter period (2

weeks), never the less, the final feed conversion at the end of experimental period (5 weeks), was significantly affected by feeding durations (*p*-value 0.0446)

The current studies, reflected that, the different experimental diets treatments, had no interaction impact on the broiler performance parameters, except for weight gain of 3rd week, the highest gain of this week (421.80g) was recorded for birds fed diet with multi-enzymes level of 500g/1MT feed during the starter period only.

Both addition of different levels of multi-enzymes or feeding durations, showed no significant effect on bird's viability and EPEF, however, EPEF was significantly affected by feeding durations at *p*-value ≤ 0.10.

Figure (1): Effect of feeding durations or the levels of included multi- enzymes and their interactive impact on 2nd week body weight (starter phase).

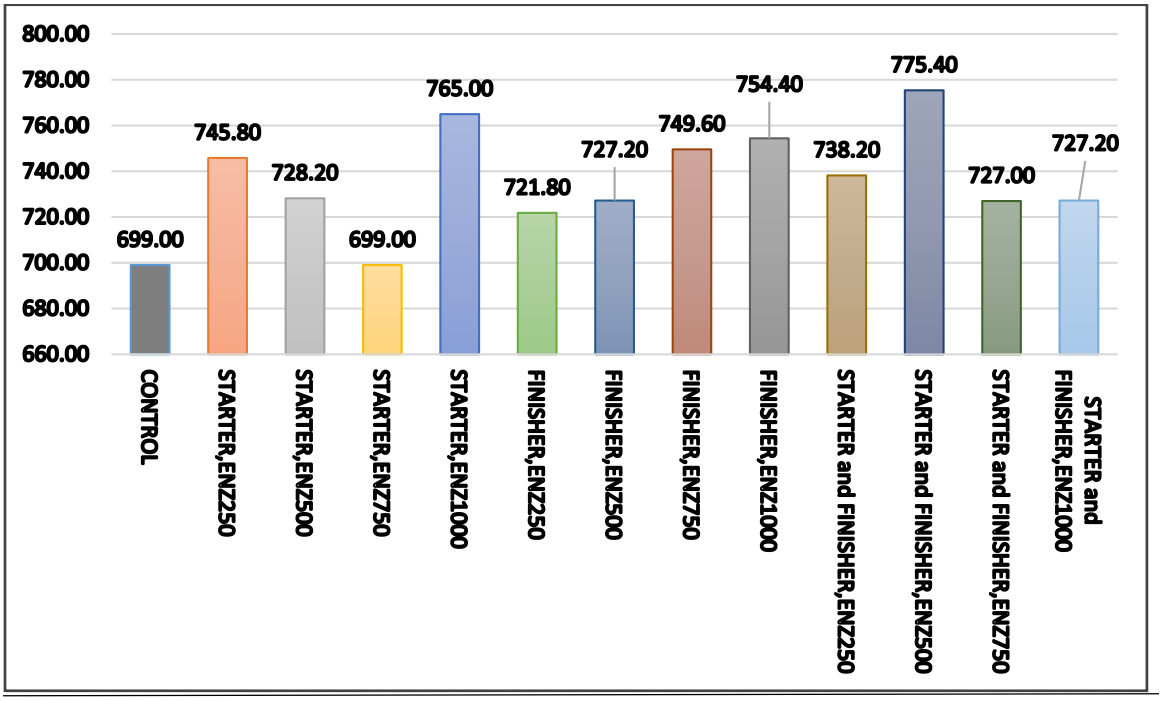


Figure (2): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 5th week body weight (slaughter weight).

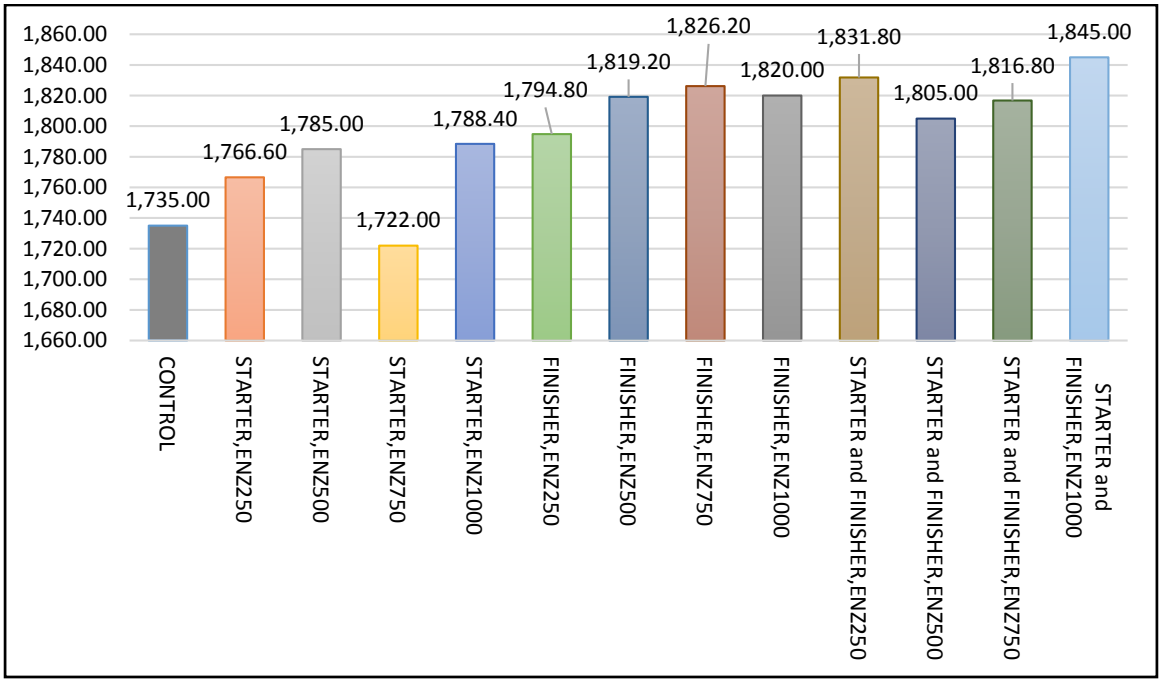


Figure (3): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on EPEF.

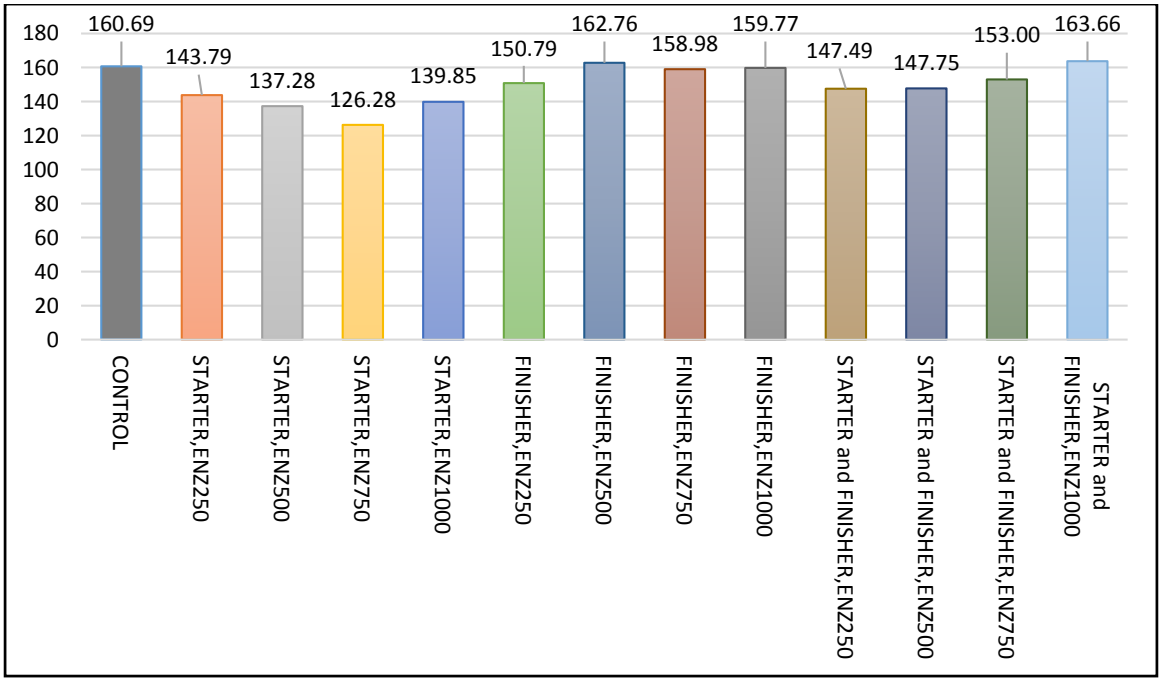


Figure (4): Effect of feeding durations or the levels of included multi- enzymes and their interactive impact on starter period body weight gain.

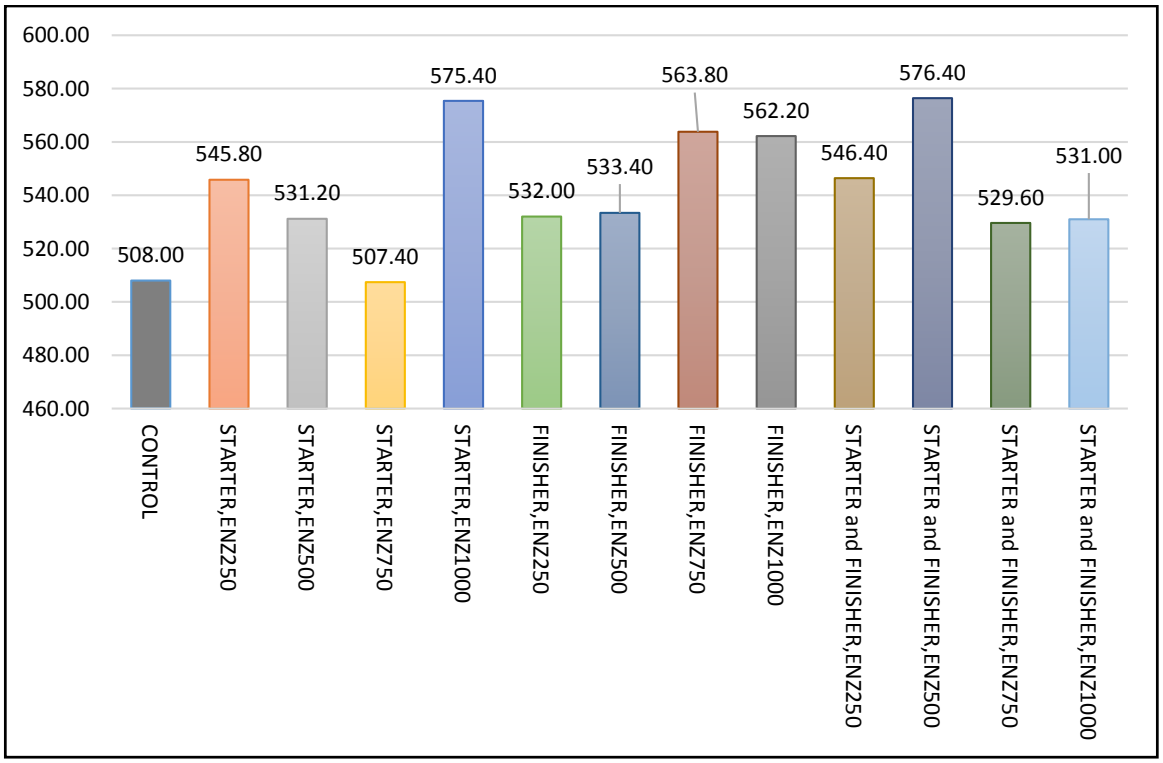


Figure (5): Effect of feeding durations or the levels of included multi- enzymes and their interactive impact on finisher period body weight gain.

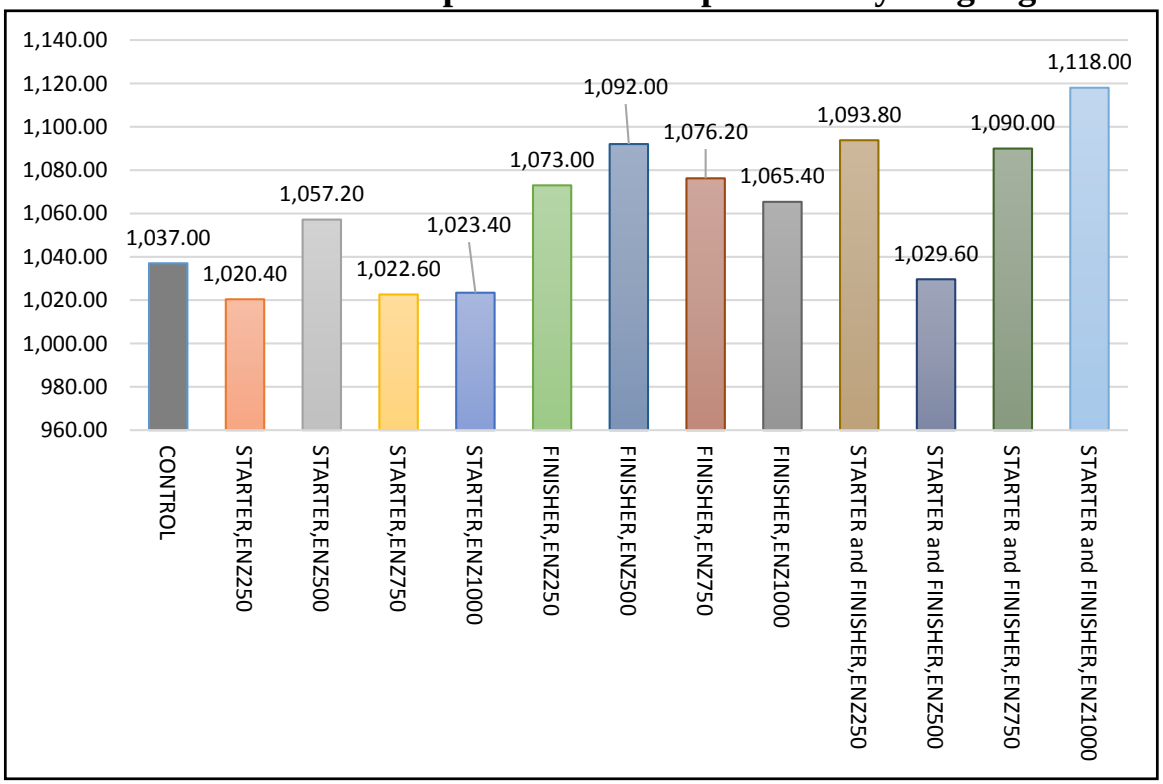


Figure (6): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on whole period body weight gain.

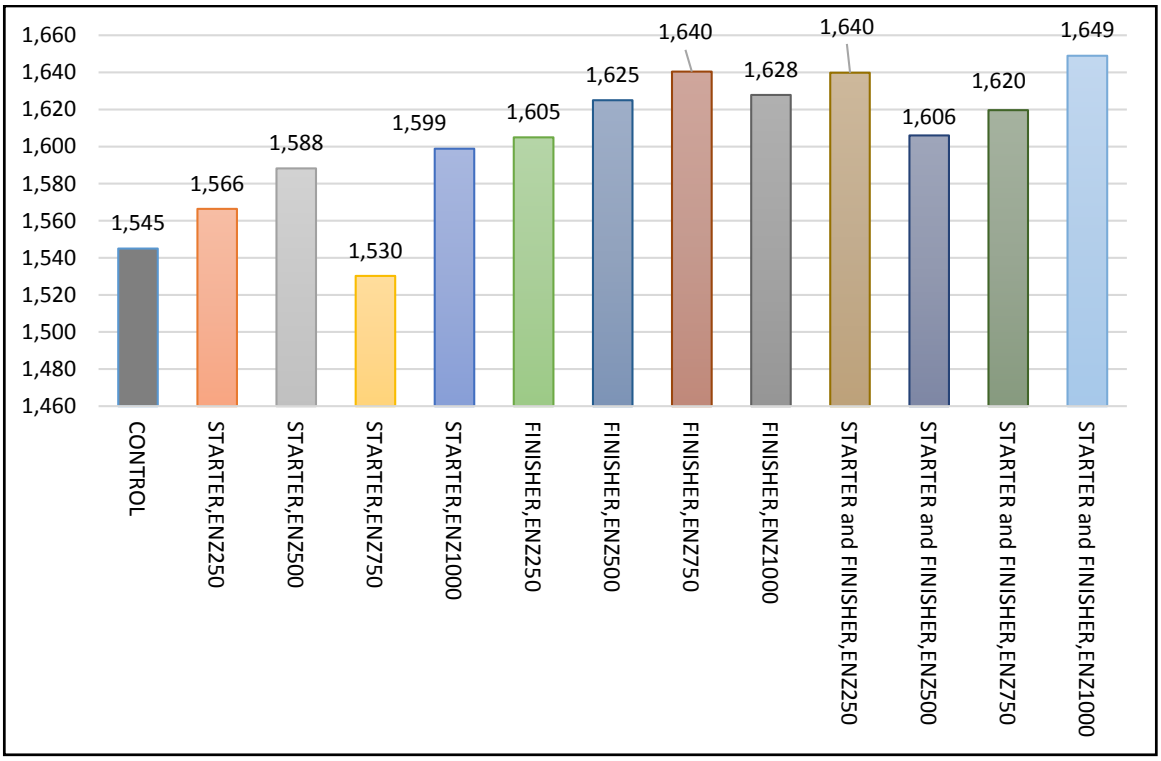


Figure (7): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on starter period feed intake.

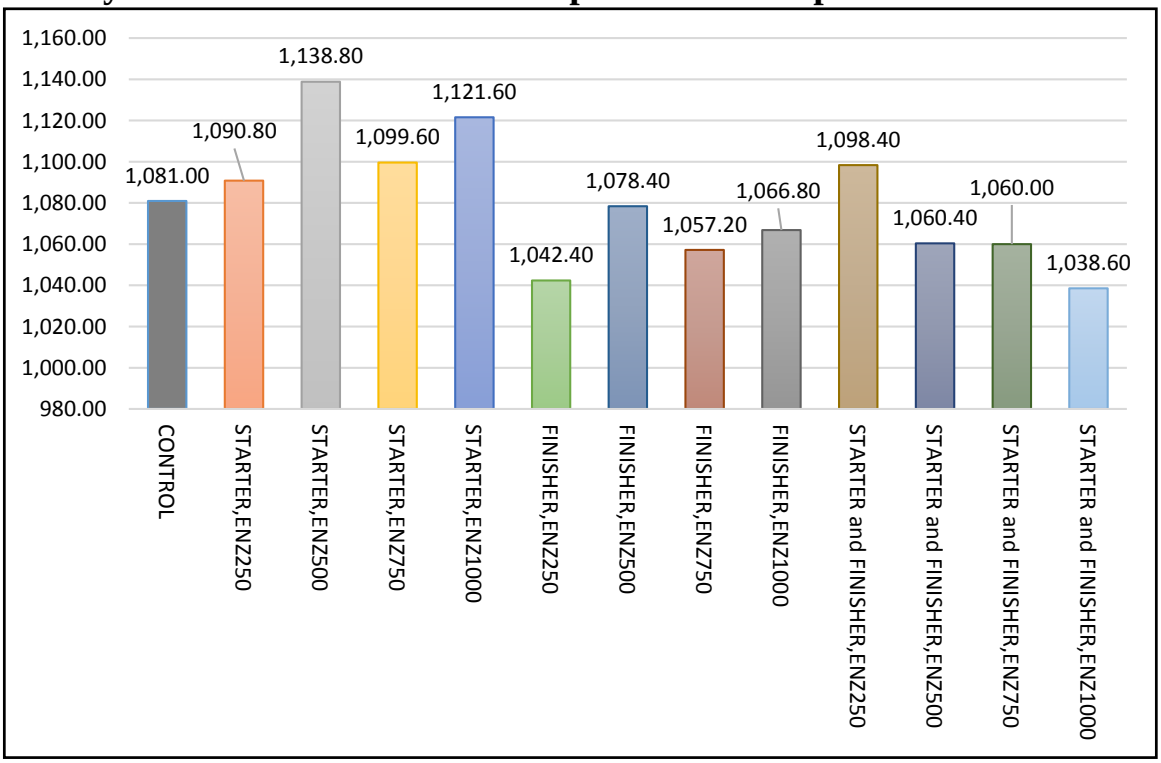


Figure (8): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on finisher period feed intake.

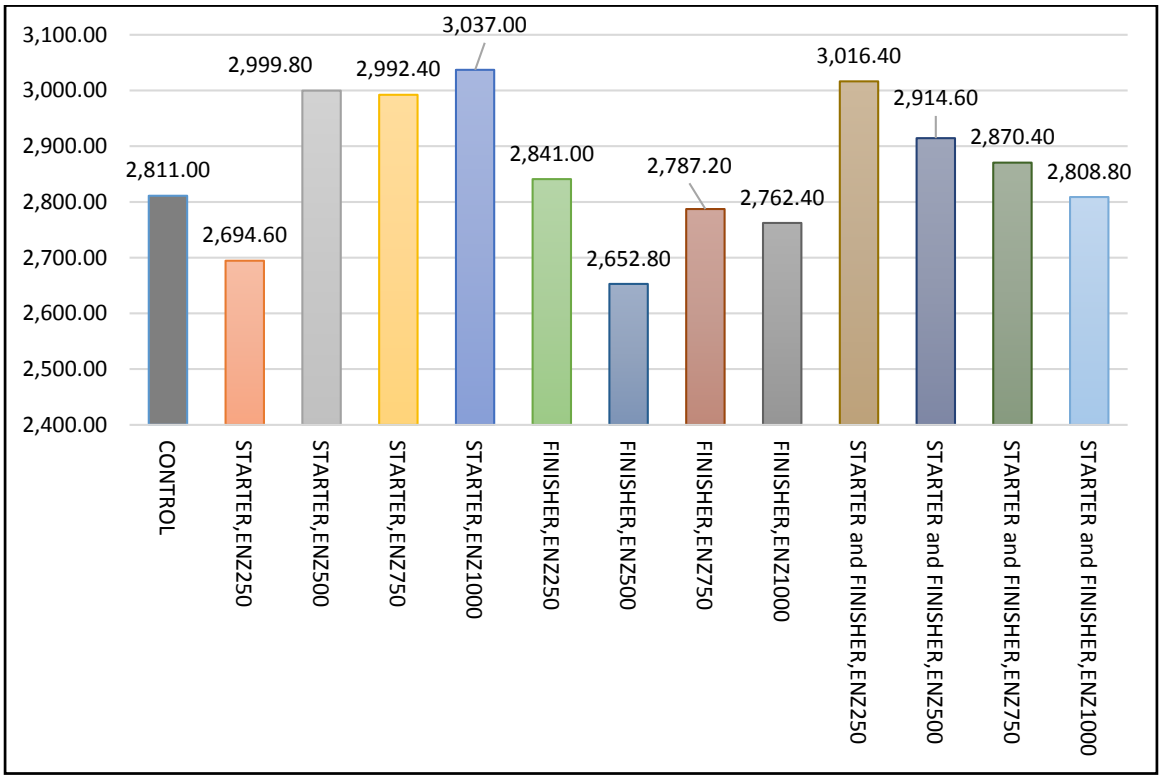


Figure (9): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on whole period feed intake.

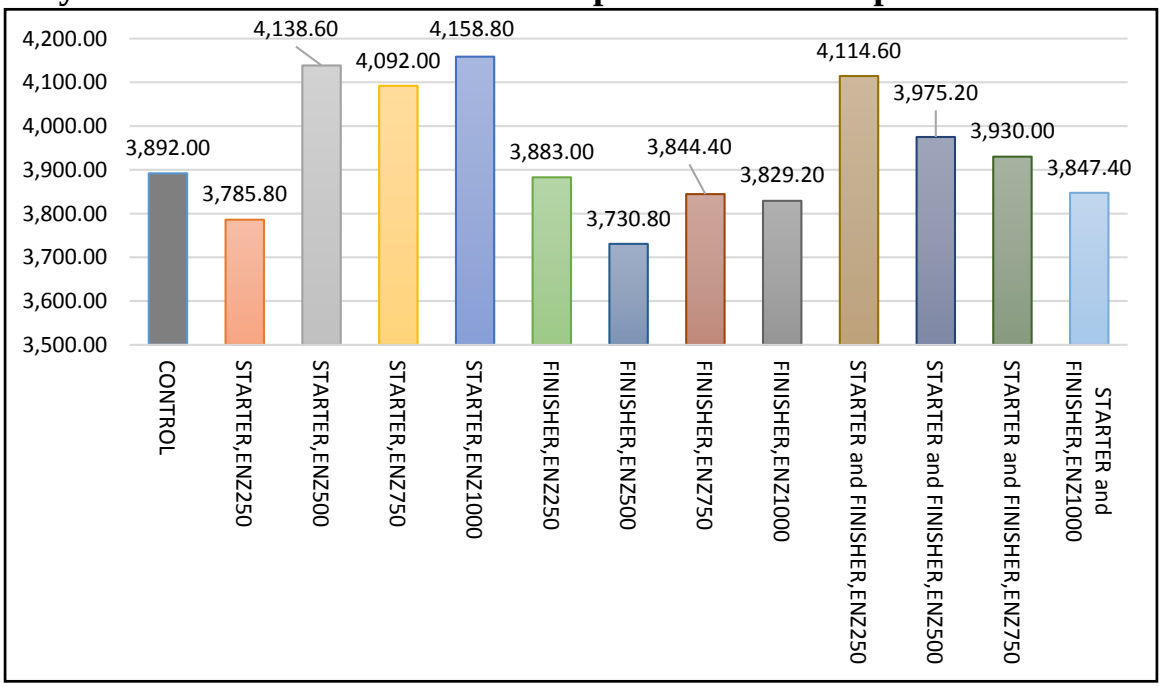


Figure (10): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on starter period FCR.

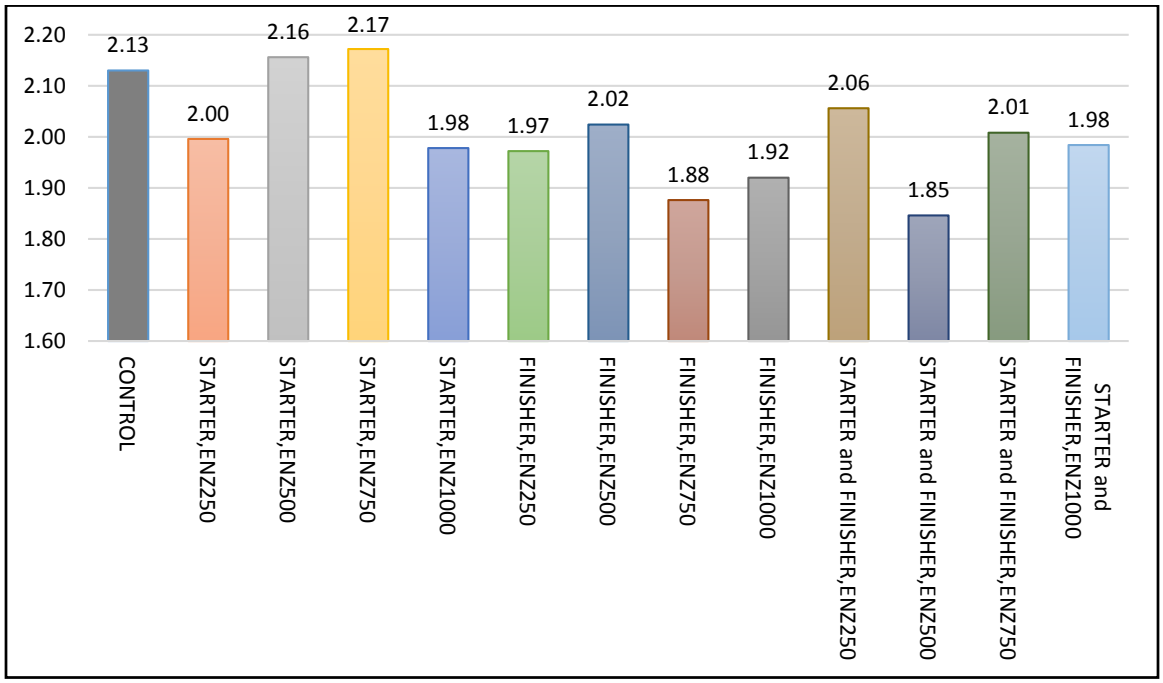
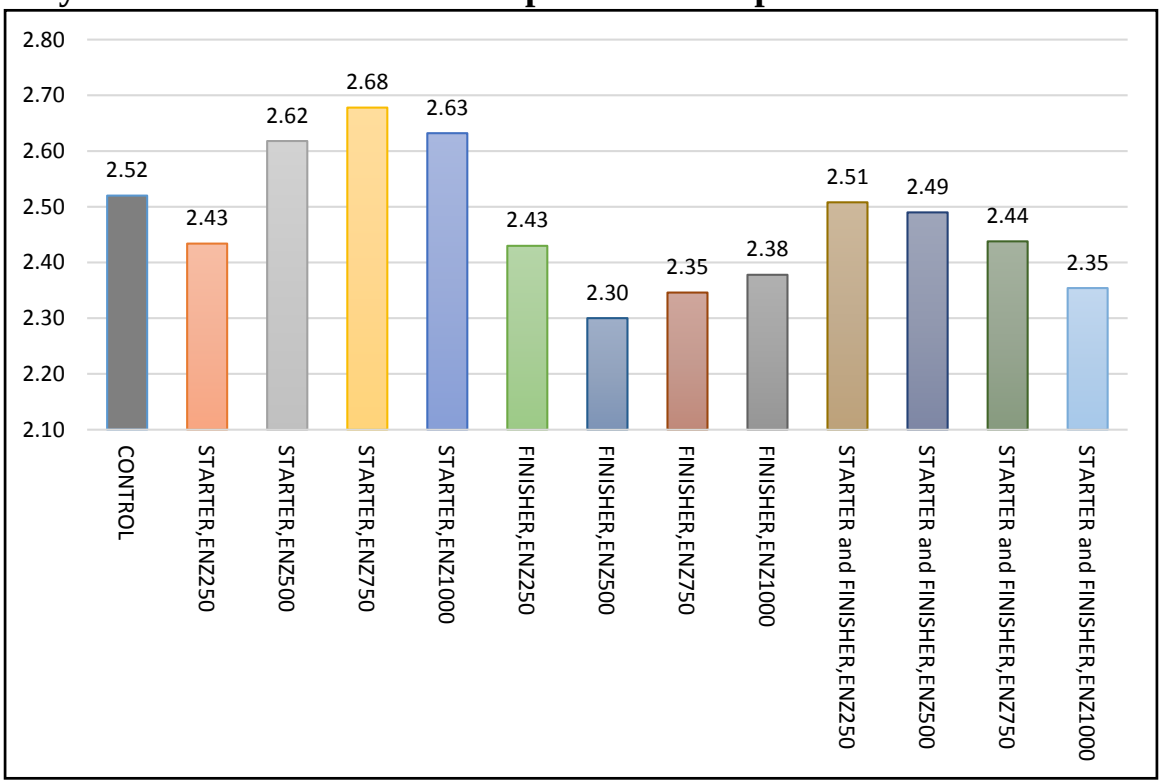


Figure (11): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on whole period FCR.



The EPEF value (185.35), observed for birds fed on diet containing multi-enzyme, offered during the finisher period only, was greater compared to value obtained when birds fed during the starter period only (164.04).The greatest numerical value (190.92) was scored by birds fed with multi-enzyme NutriKEM Extend at level of 1,000g / 1 MT of feed, when offered during the starter and finisher period (the whole five weeks), whereas lowest value (153.54) was recorded for birds fed on750g multi-enzymes / 1 MT feed when offered during the starter period only.

4.5. Effect of feeding durations of diets with added multi-enzymes NutriKEM Extend on broiler performance compared to control.

Table (12) and figure (1) showed the effect of feeding durations with of various added levels of multi-enzymes NutriKEM Extend on broiler performance, the effect of feeding durations only was studied, after the data for control diet were included, using one way ANOVA.

Feed consumption was significantly affected by feeding regimen, birds fed the multi-enzymes diets during the starter phase only, consumed more feed (4,044g) compared to birds offered the diet during the finisher phase only (3,821.75g)

Body weight gain and prospectively slaughter weight were highly affected by feeding duration (*p*-value = 0.0052 and 0.0068 respectively), increasing feeding duration showed a positive improvement in both weight gain, and hence the live body weight, when compared with control group.

Lowest values of gain (1,544g) and body weight (1,735 g) were recorded for the control birds fed on basal diets without multi-enzymes added, these two parameters showed gradual increase with increasing feeding duration, highest weight gain of1, 628.00g and heaviest birds of 1,824.75g were produced when feeding birds the diet fortified with multi-enzymes for the whole experimental period.

Feed conversion ratio was significantly affected by multi-enzymes diet feeding duration (*p*-value = 0.241), the best value of 2.35 was recorded for birds fed with added multi-enzymes diet during the finisher period only, whereas the worst ratio of 2.58 was shown by birds fed on diet with multi-enzymes inclusion during the starter period only.

Broiler feeding duration with diets supplemented with multi-enzymes NutriKEM Extend, did not affect the economic efficiency, however, the numerical value of 50.73% obtained from finisher period feeding, was higher than the value achieved by the control group (48.32).

Table (12): Effect of feeding durations of added multi-enzymes NutriKEM Extend diets on performance of broiler chickens.

Treatment	Feed Intake (g)	Body Weight (g)	B W (g)	FCR	E.E
Control	3,892.00 ^{ab}	1,735.00 ^{a}	1,544.00 ^{a}	2.52^{ab}	48.32
Feeding Periods					
Starter Period	4,044.00 ^{a}	1,765.50 ^{a}	1,570.75 ^{a}	2.58^{a}	43.91
Finisher Period	3,821.75 ^{b}	1,815.00 ^{b}	1,624.50 ^{b}	2.35^{b}	50.73
Whole Period	3,967.00 ^{ab}	1,824.75 ^{b}	1,628.00 ^{b}	2.44^{b}	46.76
Mean	3,928.00	1,782.00	1,589.20	2.47	47.48
SEM	59.3	11.50	11.10	0.0405	
St. D	244.60	47.30	45.80	0.1668	0.12
Coef. Var	6.23	2.65	2.88	6.75	24.78
p-value	0.0663	0.0052	0.0068	0.0241	0.8929
Sig	*	***	***	**	N.S

a-b: Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).
*: Significant with ($p \leq 0.10$). **: Significant with ($p \leq 0.05$). ***: Highly significant with ($p \leq 0.01$).

N.S: Not significant.

Figure (12): Effect of feeding durations of added multi-enzymes NutriKEM Extend diets on performance of broiler chickens.

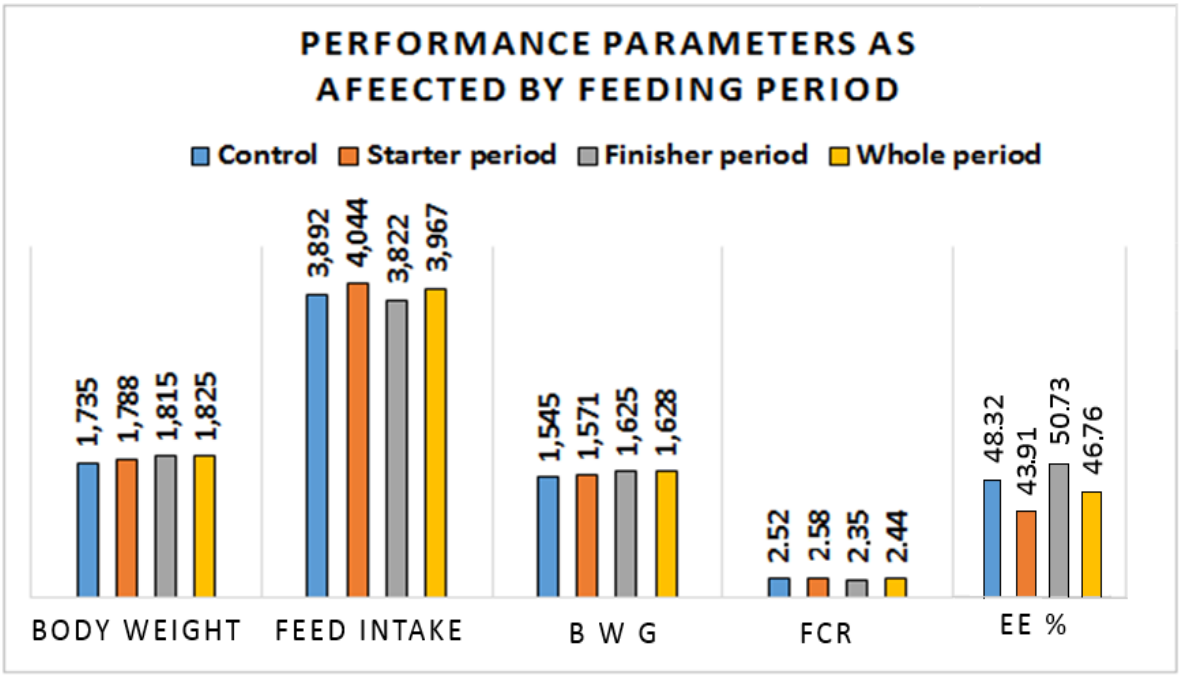


Table (13): Broiler performance as affected by feeding periods.

	Treatment	FI (g)		BWG (g)		BW (g)		FCR (g)	
		Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)
	CONTROL	1,081.00	2,811.00	508.00	1,037.00	699.00	1,735.00	2.13	2.52
					Feeding Periods Effect				
1	Starter only	688.20 ^{a}	978.45 ^{a}	539.95	1,030.90	734.50	1,765.50	2.08	2.59 ^{a}
2	Finisher only	659.30 ^{ab}	868.85 ^{b}	547.85	1,076.65	738.25	1,815.05	1.95	2.36 ^{b}
3	Whole Period	639.55 ^{b}	898.75 ^{ab}	545.85	1,082.85	741.95	1,824.65	1.97	2.45 ^{ab}
	SEM	12.84	30.89	12.130	23.418	12.411	30.121	0.050	0.063
	p- value	0.0339	0.0429	0.8919	0.2405	0.9140	0.3376	0.1753	0.0446
	Sig	*	*	N.S	N.S	N.S	N.S	N.S	*

a-b:Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).

****: Significant with ($p \leq 0.01$). *: Significant with ($p \leq 0.05$). N.S: Not significant.**

4.6. Effect of enzymes levels of diets with added multi-enzymes NutriKEM Extend on broiler performance compared to control.

Table (13) and figure (2) showed the effect of added levels of multi-enzymes NutriKEM Extend on broiler performance, the effect of enzyme levels only was studied, after data for control diet were included, using one way ANOVA.

Added levels of multi-enzymes NutriKEM Extend showed non-significant effect on values of feed consumption, FCR and economic efficiency, compared to control group.

Slaughter weight and body weight gain were significantly affected (at p-value $\leq$ 0.10) by the added levels of multi-enzymes NutriKEM Extend (p-value = 0.0832 and 0.0958 respectively), increasing the level of added multi-enzymes appeared to show a positive improvement in both weight gain, and hence the live body weight, when compared with control group.

Lowest values of gain (1,544g) and body weight (1,735 g) were obtained for the control birds fed on basal diets without added multi-enzymes, these two parameters showed gradual increase with increasing multi-enzymes levels, highest weight gain of 1,625.00g and heaviest birds of 1,817.00g were produced when chicks offered the diet fortified with multi-enzymes NutriKEM Extend at the level of 1g per Kg of complete feed.

Table (14): Effect of added levels of multi-enzymes NutriKEM Extend diets on performance of broiler chickens.

	Treatment	Feed Intake (g)	Live Body Weight (g)	B W Gain (g)	FCR	EE %
	Control	3,892.00	1,735.00 [a]	1,544.00 [a]	2.52	48.32
			Enzymes Levels			
1	Enzyme 250 g	3,928.00	1,798.00 [ab]	1,604.00[ab]	2.45	46.62
2	Enzyme 500 g	3,948.33	1,803.00 [b]	1,606.33[ab]	2.46	50.18
3	Enzyme 750 g	3,955.33	1,788.33 [ab]	1,595.67[ab]	2.48	41.30
4	Enzyme 1,000 g	3,945.33	1,817.67 [b]	1,625.00[b]	2.43	53.79
	Mean	3,928.82	1,782.24	1,589.24	2.47	48.07
	SEM	162.13	22.92	22.51	0.11	7.50
	St. Dev	244.60	47.30	45.83	0.17	11.93
	Coef. Var	6.23	2.65	2.88	6.75	24.82
	p-value	0.9974	0.0832	0.0958	0.9682	0.8194
	Sig	N.S	*	*	N.S	N.S

a-b: Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$). *: Significant with (P≤0.10). N.S: Not significant.

Figure (13): Effect of added levels of multi-enzymes NutriKEM Extend diets on performance of broiler chickens.

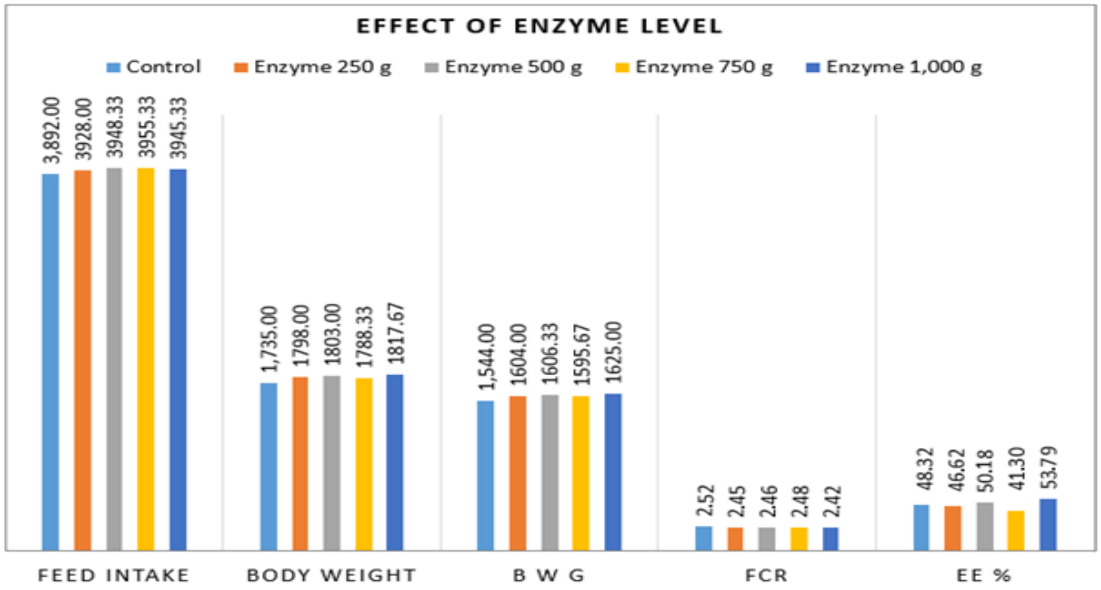


Table (15): Broiler performance as affected by enzymes levels.

	Treatment	FI (g)		BWG (g)		BW (g)		FCR	
		Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)	Starter (08-21)	Finisher (22-42)
	CONTROL	1,081.00g	2,811.00g	508.00g	1,037.00g	699.00g	1,735.00g	2.13	2.52
	EXPERIMENT (2):ENZYMES LEVELS EFFECT								
1	**ENZYME 250 g**	664.60	909.20	541.40	1,062.40	735.27	1,797.73	2.01	2.46
2	**ENZYME 500 g**	684.20	912.20	547.00	1,059.60	743.60	1,803.07	2.01	2.47
3	**ENZYME 750 g**	640.40	917.73	533.60	1,062.93	725.20	1,788.33	2.02	2.49
4	**ENZYME 1,000 g**	660.20	922.60	556.20	1,068.93	748.87	1,817.80	1.96	2.45
	SEM	14.82	35.66	14.007	27.041	14.331	34.780	0.058	0.073
	***p* -value**	0.2345	0.9938	0.7106	0.9958	0.6704	0.9446	0.8949	0.9884
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

N.S: Not significant.

4.7. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on carcass characteristics and organs relative weights.

Tables (16) and (17) showed the effect of dietary supplementation of different graded levels multi-enzymes, NutriKem Extend, fed during starter (2^{nd} and 3^{rd} week) or finisher (4^{th},5^{th} and 6^{th} weeks) period only, or fed during the whole rearing period, on carcass weight and relative organs weights.

Supplementation of multi-enzymes did not increased the carcass weight, or improved hot or cold dressing percentages, which were not significantly affected, furthermore, carcass characteristics were not significantly affected by feeding durations, and no interaction effect was observed between different multi-enzymes graded levels and feeding durations phases. Heaviest carcass weight yield of 1,329g, and the best hot dressing percentage (72.15%) was recorded for birds of control group, fed on basal diets without multi-enzymes added, whereas lowest weight of 1,160g was seen for birds fed on diet containing 750g of multi-enzymes per 1MT of feed, during the starter phase only, birds fed on diet containing 750g of multi-enzymes per 1MT of feed, during the finisher phase only, showed the lowest hot dressing percentage (68.11%).

Conformation of data recorded for birds live body weight, carcass yields and hot dressing percentages, was obviously clear and well observed in this study, the highest recorded cold dressing percentage of 72.79 %, was achieved by birds fed on diet containing 250g of multi-enzymes per 1MT of feed, during the whole experimental period, lowest value (67.44%) same parameter was recorded by birds fed on diet containing 250g of multi-enzymes per 1MT of feed, during the starter period only. Data of relative percentages of chicken internal and external edible or inedible parts of legs, head, neck, liver gizzard, heart, abdominal fat and intestinal length seen in table(17),

revealed that ,diets fortified with multi-enzymes NutriKEM Extend ,di d not significantly affect the chicken organ relative percentages.

The greatest relative weights of head (3.01%) and legs (4.06%) were observed for birds fed on diet containing 1,000g of multi-enzymes per 1MT of feed, during the whole experimental period, the heaviest neck weight (4.24%) was scored by birds fed with multi-enzymes NutriKEM Extend at level of 500g / 1 MT of feed, when offered during the finisher period only (last four weeks), whereas lightest weight (3.08%) was recorded for birds fed on multi-enzymes NutriKEM Extend at level of 750g / 1 MT of feed when offered during the finisher period only.

Results explained that, diet supplemented with multi-enzymes improved the liver size and the level of deposited abdominal fat, the groups of birds that consumed feed containing 250 g of NutriKEM Extend per 1MT of feed, yielded heaviest livers (2.27%), while the lowest live relative weight (1.97%), was recorded for the control group which was fed on basal diets without multi-enzymes added.

Diet with multi-enzymes added at level of 1,000g per 1MT of feed, resulted the in highest abdominal fat deposition level (2.15%), when fed during the finisher period only, whereas deposition of abdominal fat of control groups was the lowest (1.22%).

Heart relative weight and intestinal length were not significantly affected by both experimental factors, furthermore there was no interaction effect.

Table (16): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on Carcass Characteristics of broiler chickens.

	Treatments	Live weight (g)	Carcass (g)	Hot Dressing %	Cold dressing %
	Control	**1,843**	**1,329**	**72.16**	**69.36**
	Feeding Periods Effect				
1	**Starter**	**1,771**	**1,244**	**70.26%**	**69.34%**
2	**Finisher**	**1,834**	**1,266**	**68.99%**	**69.15%**
3	**Whole period**	**1,769**	**1,243**	**70.29%**	**70.36%**
	SEM	**41.41**	**31.29**	**0.006**	**0.006**
	***p*-value**	**0.4537**	**0.8405**	**0.1863**	**0.3362**
	Sig	**N.S**	**N.S**	**N.S**	**N.S**
	Enzymes Levels Effect				
1	**Enzyme 250 g**	**1,804**	**1,258**	**69.74%**	**69.76%**
2	**Enzyme 500 g**	**1,814**	**1,278**	**70.40%**	**69.71%**
3	**Enzyme 750 g**	**1,769**	**1,215**	**68.77%**	**69.03%**
4	**Enzyme 1,000 g**	**1,777**	**1,252**	**70.48%**	**69.97%**
	SEM	**47.81**	**36.13**	**0.007**	**0.007**
	***p*-value**	**0.8926**	**0.6642**	**0.2318**	**0.8073**
	Sig	**N.S**	**N.S**	**N.S**	**N.S**
	Feeding Periods X Enzymes Levels Interaction				
	SEM	**82.81**	**62.58**	**0.011**	**0.0124**
	***p*-value**	**0.6037**	**0.8278**	**0.6911**	**0.1695**
	Sig	**N.S**	**N.S**	**N.S**	**N.S**

Figure (14): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on live body weight.

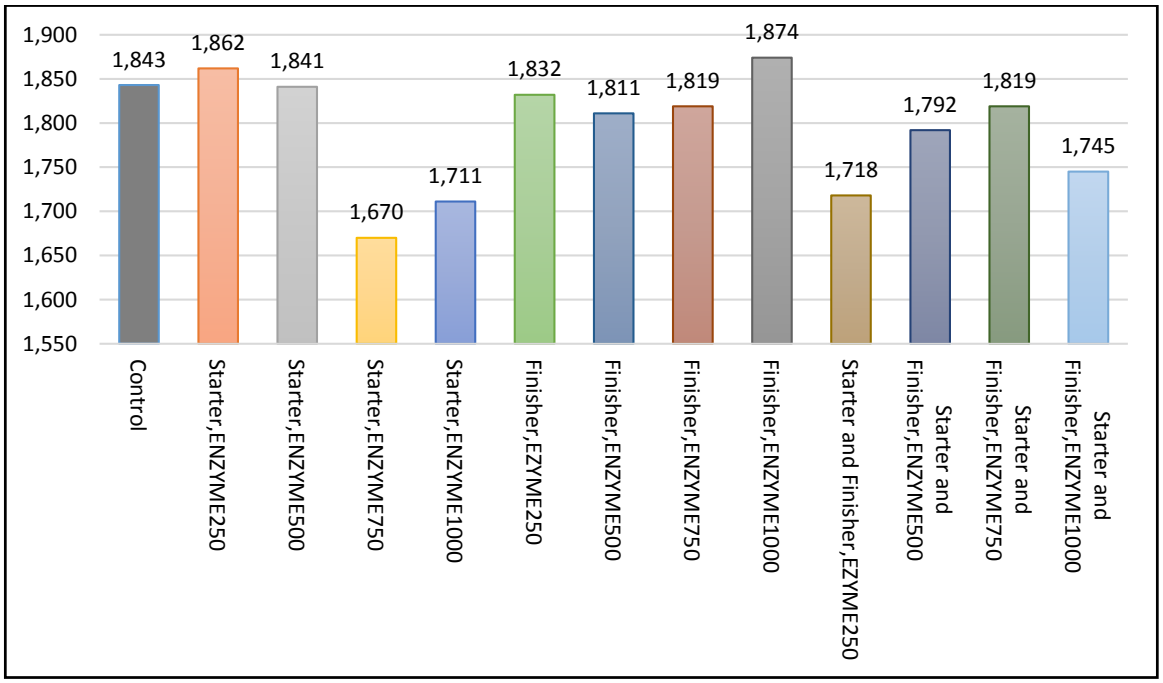


Figure (15): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on carcass weight.

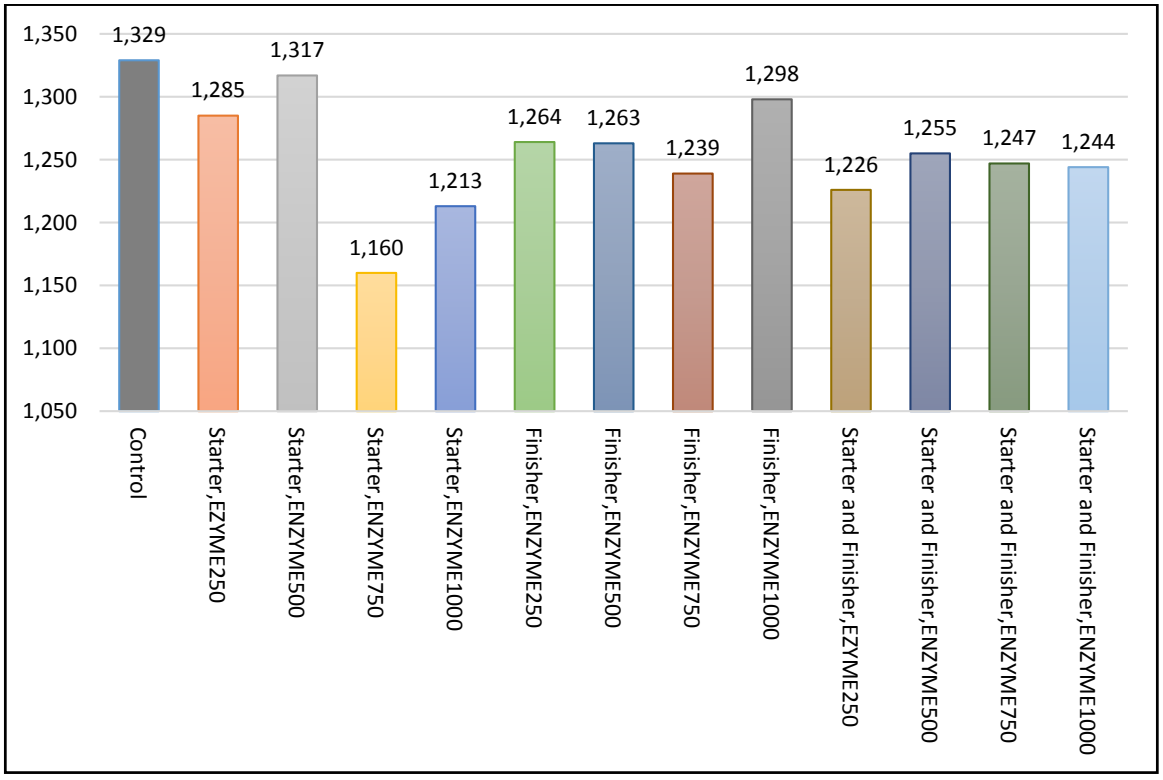


Figure (16): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on hot dressing percentage.

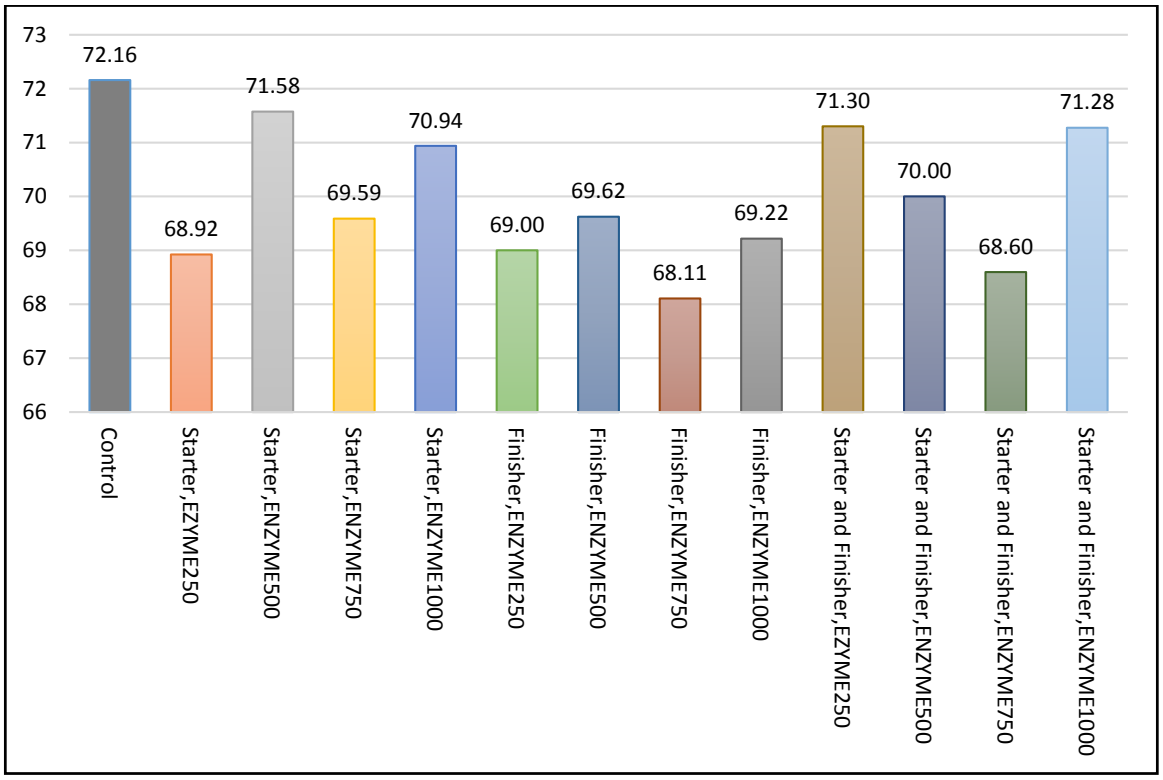


Figure (17): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on cold dressing percentage.

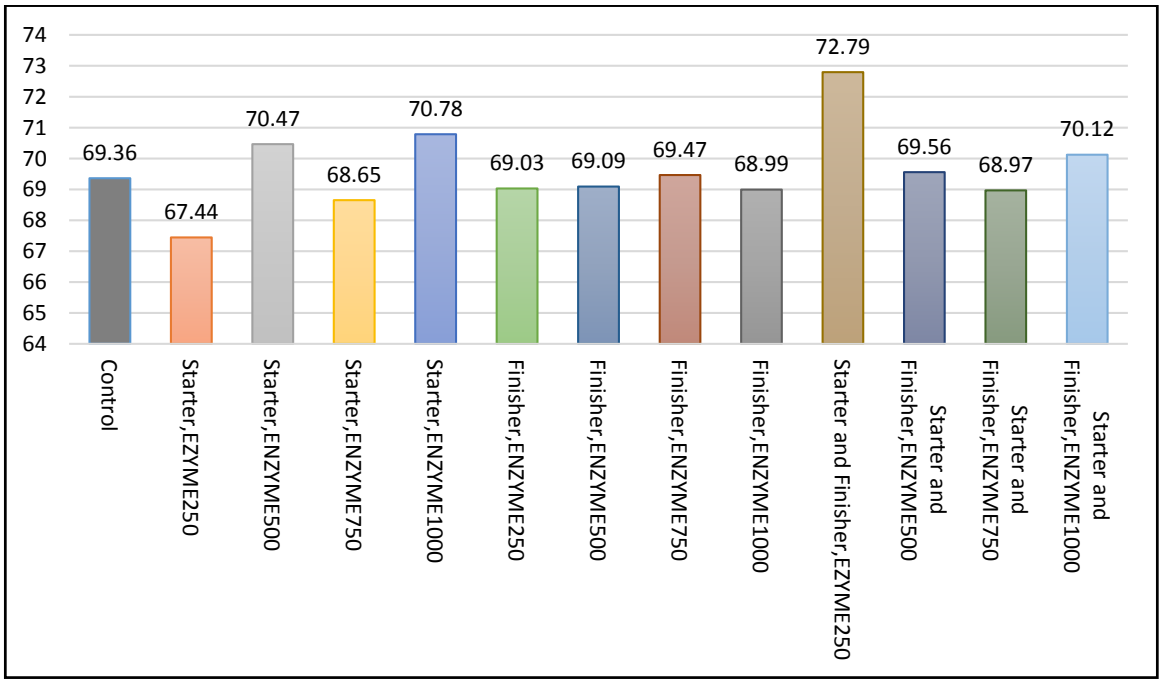


Table (17): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on organs relative weights of broiler chickens.

Treatments		Legs %	Head %	Neck %	Liver %	Gizzard %	Abdominal Fat %	Heart %	Intestinal length
Control		3.82	2.75	3.74	1.97	1.60	1.22	0.57	178
Feeding Periods Effect									
1	Starter	3.62	2.68	3.82	2.05	1.51	1.66	0.57	180
2	Finisher	3.88	2.61	3.78	2.08	1.40	1.92	0.61	165
3	Whole period	3.78	2.68	3.88	2.11	1.45	1.67	0.52	173
	SEM	0.099	0.066	0.145	0.074	0.100	0.154	0.038	7.421
	p-value	0.1685	0.7263	0.9215	0.8681	0.7731	0.4117	0.4883	0.3922
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Enzymes Levels Effect									
1	Enzyme 250 g	3.77	2.52	3.84	2.14	1.54	1.88	0.62	178.00
2	Enzyme 500 g	3.69	2.62	3.98	2.09	1.40	1.66	0.55	177.53
3	Enzyme 750 g	3.72	2.71	3.58	2.04	1.47	1.63	0.52	166.73
4	Enzyme 1,000 g	3.86	2.78	3.92	2.05	1.39	1.81	0.52	168.53
	SEM	0.011	0.077	0.170	0.086	0.116	0.178	0.044	8.569
	p-value	0.7472	0.1134	0.3671	0.8270	0.7965	0.7279	0.2620	0.7018
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Feeding Periods X Enzymes Levels Interaction									
	SEM	0.198	0.133	0.294	0.148	0.200	0.309	0.076	14.842
	p-value	0.3224	0.1853	0.3514	0.9732	0.9795	0.8662	0.349	0.6418
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

4.8. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on broiler meat quality, when fed during starter phase(2nd and 3rd weeks), finisher phase only (4th,5th and 6th weeks),or fed for the whole period :

The results for broiler meat quality as explained by chemical composition and physical characteristics evaluation of tenderness and sensory attributes, as affected by added graded multi-enzymes supplementation levels and feeding durations, are shown in table (18) and table (19) respectively.

Moisture content was affected by feeding durations (*p* value ≤ 0.10), it was significantly lower (68.77%) for chicks offered the experimental feed during the whole period, compared to chick those fed on diet during the starter period only (72.13%).Furthermore, meat ash percentage was significantly (*p* value 0.014) higher for birds fed during finisher period only (8.56%), than that of those fed during starter phase only (6.82%).

Meat protein, fat and fiber percentages were not significantly influenced by feeding durations, however the lowest numerical value of fiber (1.39%), and ash (6.42%) contents were recorded by the control chicks, fed on basal experimental diet that contains no multi-enzymes.

Multi-enzymes supplementation did not induced any effect on broiler meat chemical composition, except for protein content, which was significantly (*p* value 0.016) higher (81.84%) for meat produced by birds fed on diet with multi-enzymes added at level of 750g per 1MT of feed, compared to meat obtained from control group fed on diets not supplemented with multi-enzymes.

Highest water meat content of 73.01% was shown for birds fed on diets with added multi-enzymes level of 1,000 g per 1,000 Kg complete feed, when fed during starter period only, whereas the lowest value (66.22%) was seen for birds fed on diets with added multi-enzymes level of 500 g per 1,000 Kg complete feed when fed during finisher period only.

Feeding birds with diet of multi-enzymes added at level of 750g / 1MT of feed, during finisher phase, induced the highest meat protein production (84.79%) and ash (9.52%) contents. In addition, the lowest (6.42%) ash percentage was shown by birds of the control group. The greatest chicken meat fat percentage was shown by birds offered the multi-enzymes fortified diet with level of 1,000g when fed during the whole period. On the other hand, the lowest value (6.66%) of the same above mentioned parameter, was recorded for the birds fed on diet containing 250g of multi-enzymes added to 1,000 Kg of basal experimental diet fed during starter period only.

Addition of multi-enzymes NutriKEM Extend to basal broiler chicken diet, when fed during starter phase (2^{nd} and 3^{rd} weeks), finisher phase only (4^{th}, 5^{th} and 6^{th} weeks), or fed for the whole period, did not significantly improved the sensory properties of meat, however the best color score of 6.33, was observed for meat from birds fed on diet containing 750g of multi-enzymes added to 1,000 Kg of basal experimental diet fed during starter period only, furthermore, the same above mentioned group had produced the most tender meat ,the recorded score was (6.42).

Data showed that, the juiciest meat (scored 5.88), was obtained from chicken raised on diet supplemented with multi-enzymes added at level of 1,000g / 1MT of feed, offered during the starter and finisher phases, whereas the lowest juiciness score of 5.00, was obtained by chickens fed on diet with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole fattening period.

Figure (18): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat moisture.

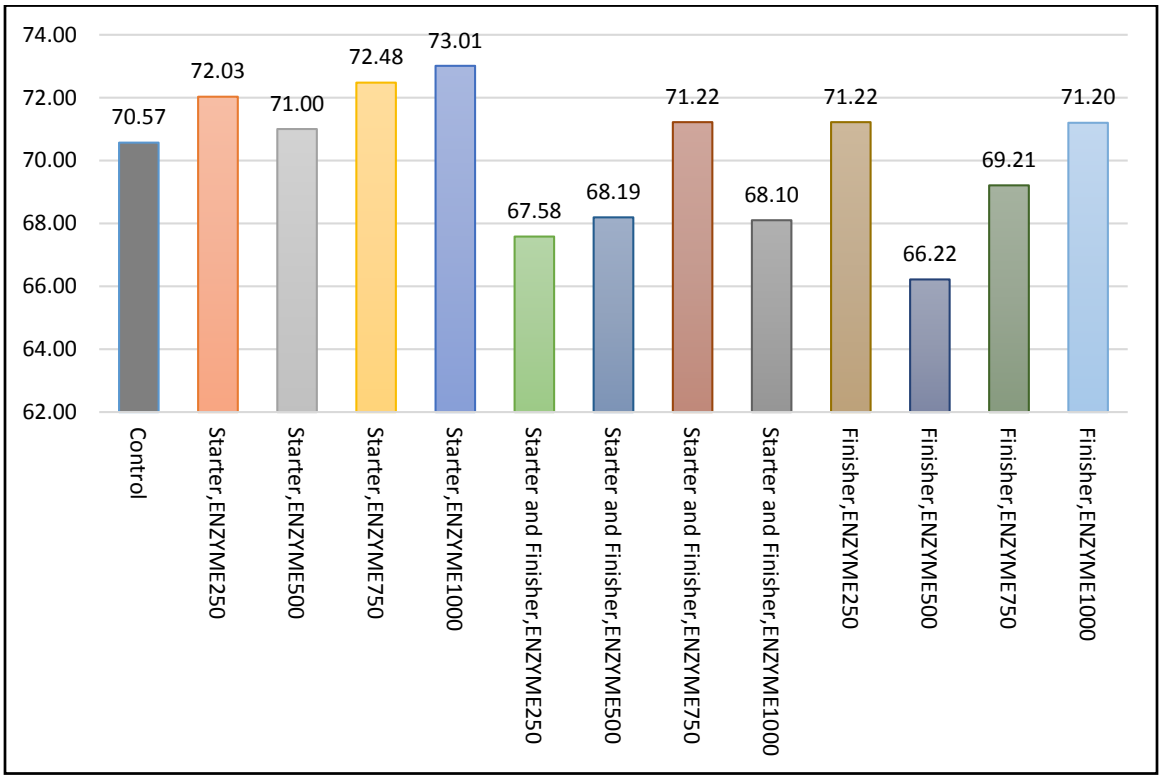


Figure (19): Effect of feeding durations or the levels of included multienzymes and their interactive impact on meat protein.

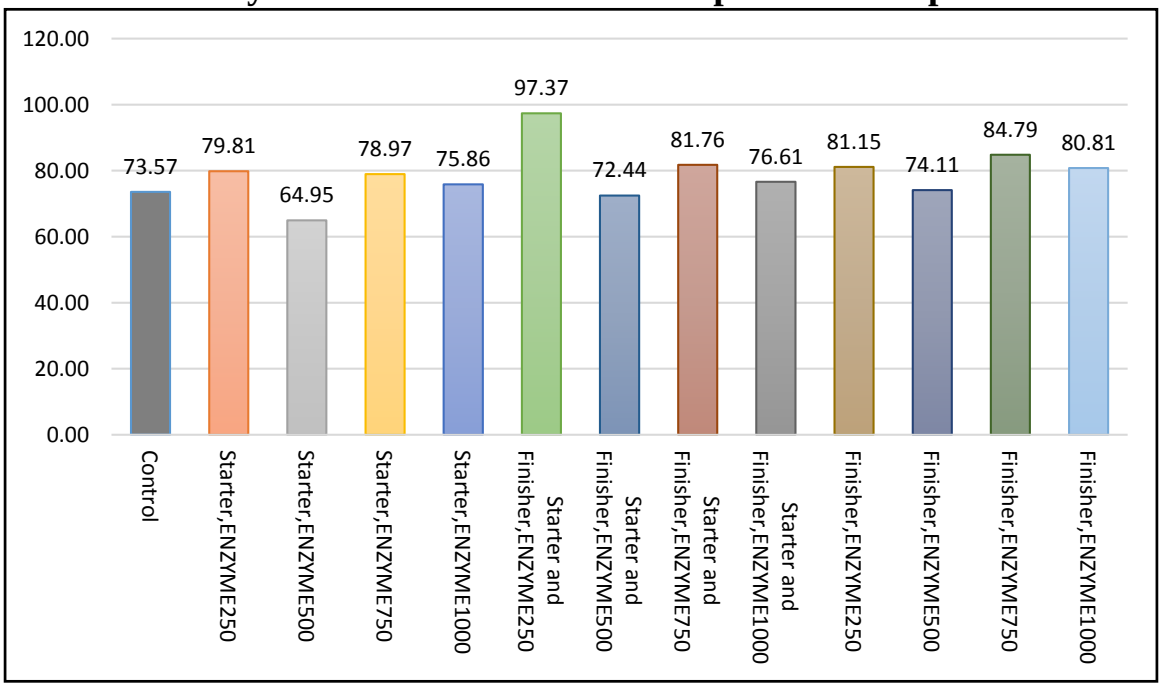


Table (18): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on meat chemical analysis of broiler chickens.

Treatments		Moisture %	Protein %	Fat %	Fiber %	Ash %
Control		70.57 ^{ab}	73.57	11.97	1.39	6.42 ^{a}
Feeding Periods Effect						
1	Starter	72.13 ^{b}	74.9	14.02	0.44	6.82 ^{ab}
2	Finisher	69.46 ^{ab}	80.22	11.47	2.49	8.56 ^{c}
3	Whole period	68.77 ^{a}	77.55	12.93	2.02	7.91 ^{ab}
	SEM	0.87	2.62	2.09	2.49	0.32
	p-value	0.1004	0.4957	0.8508	0.5397	0.014
	Sig	*	N.S	N.S	N.S	**
Enzymes Levels Effect						
1	Enzyme 250 g	70.28	80.11^{ab}	9.72	2.38	8.03
2	Enzyme 500 g	68.74	70.50 ^{b}	14.68	0.49	7.15
3	Enzyme 750 g	70.97	81.84 ^{b}	11.37	1.64	8.36
4	Enzyme 1,000 g	70.77	77.67 ^{b}	15.45	2.08	7.52
	SEM	1.30	1.83	2.09	1.28	0.55
	p-value	0.6827	0.0161	0.3548	0.8584	0.3941
	Sig	N.S	**	N.S	N.S	N.S

a-c:Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).
* : Significant with ($p \leq 0.10$). ** : Significant with ($p \leq 0.05$). N.S: Not significant.

Figure (20): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat fat.

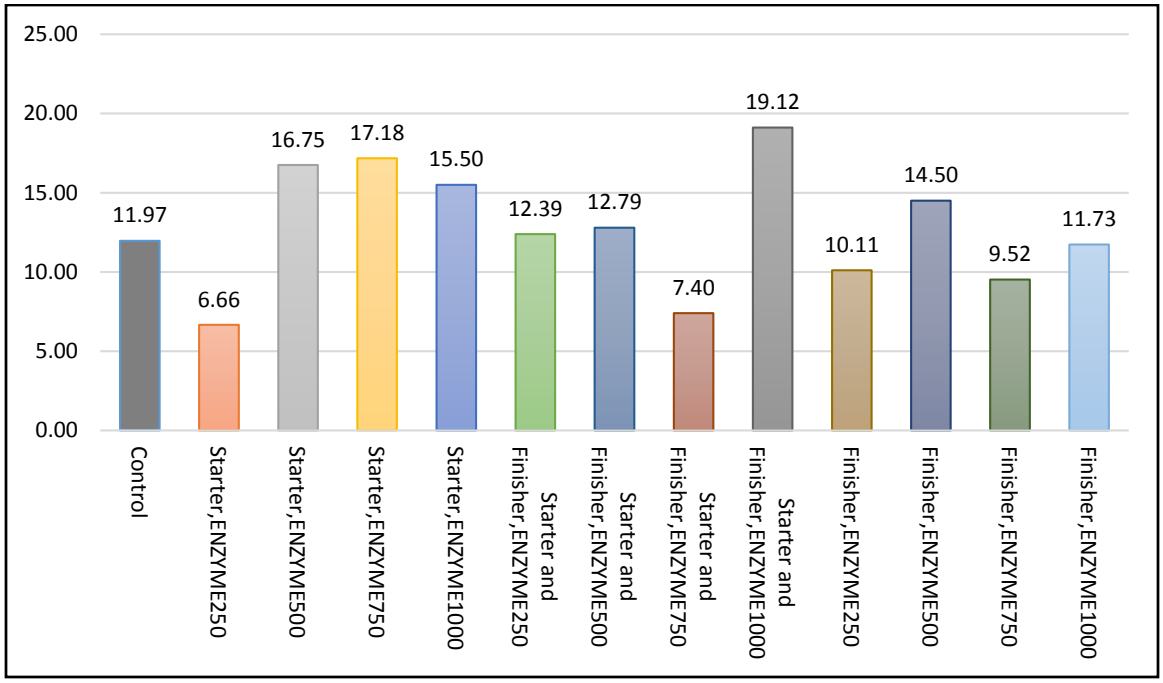


Figure (21): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat fiber.

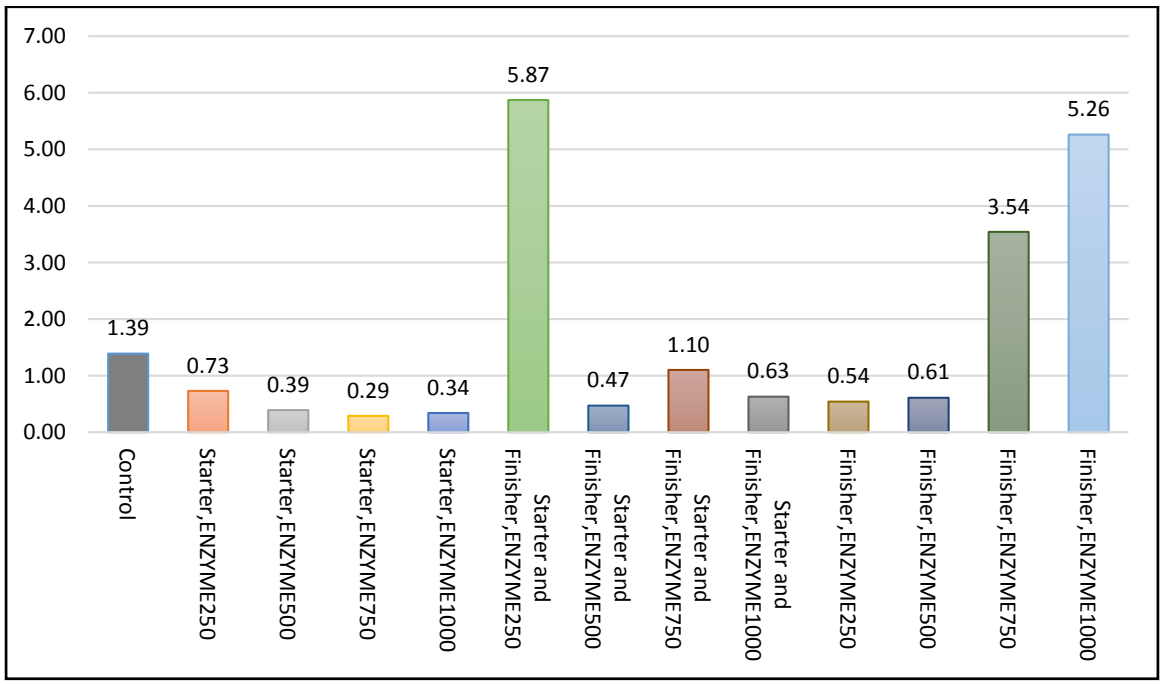


Figure (22): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat ash.

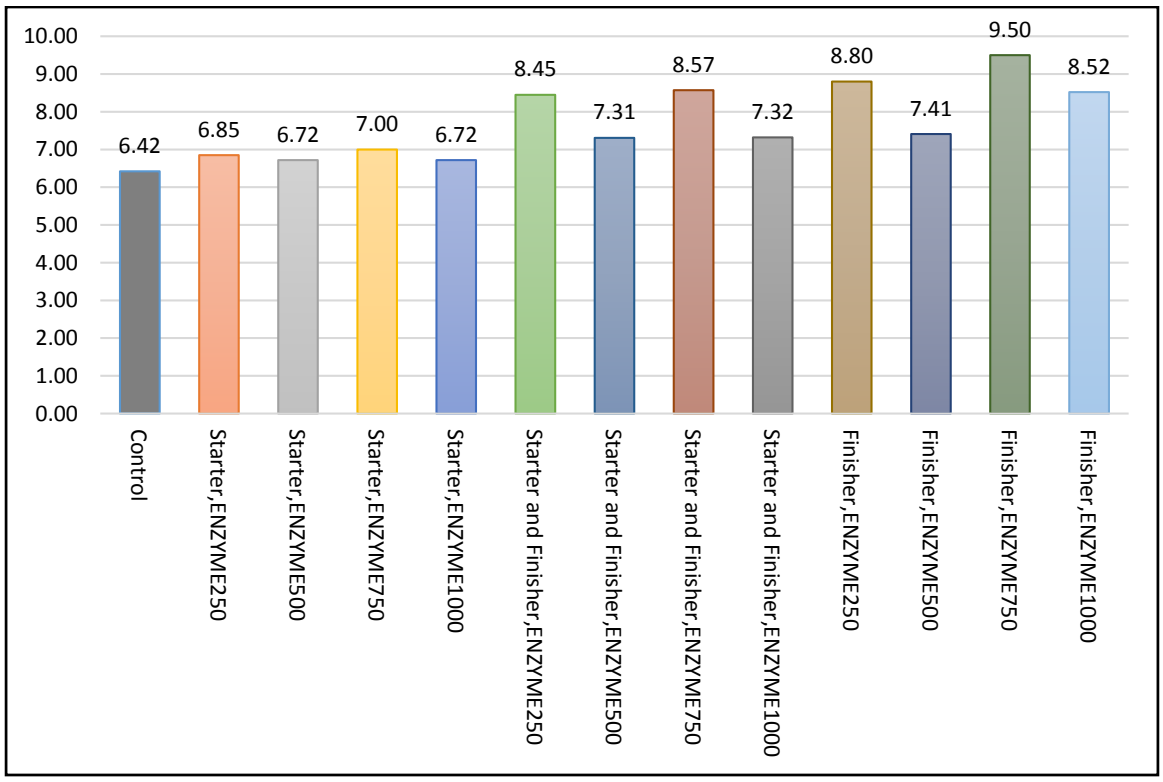


Figure (23): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat tenderness.

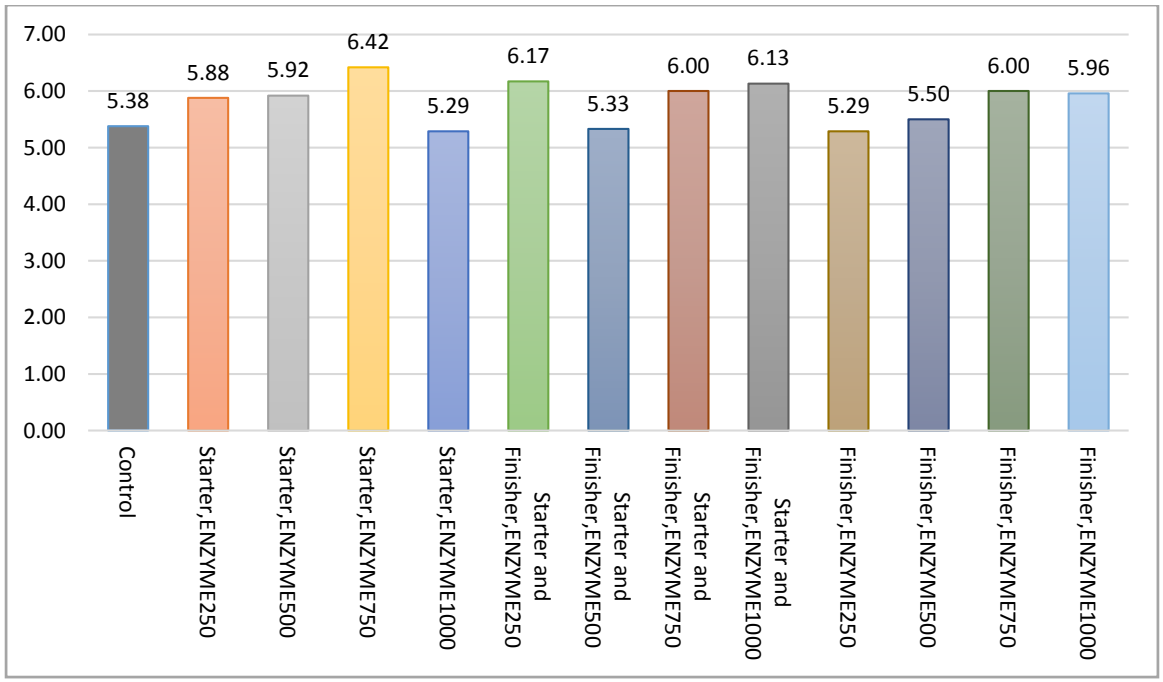


Figure (24): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat flavor.

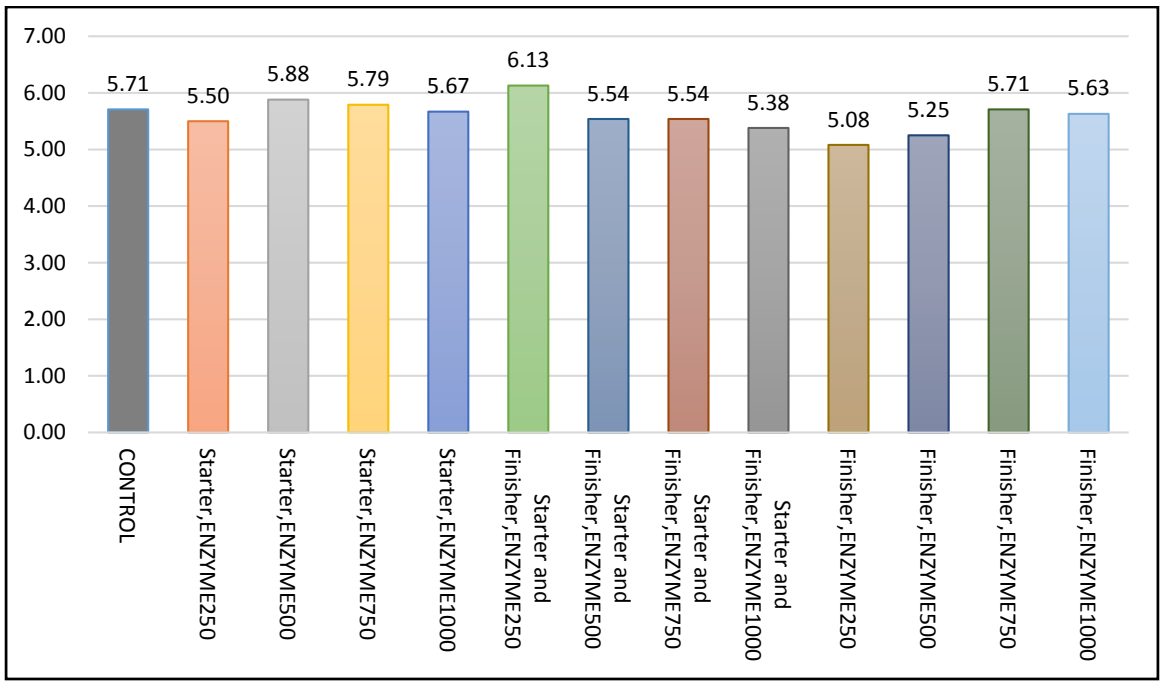


Figure (25): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat color.

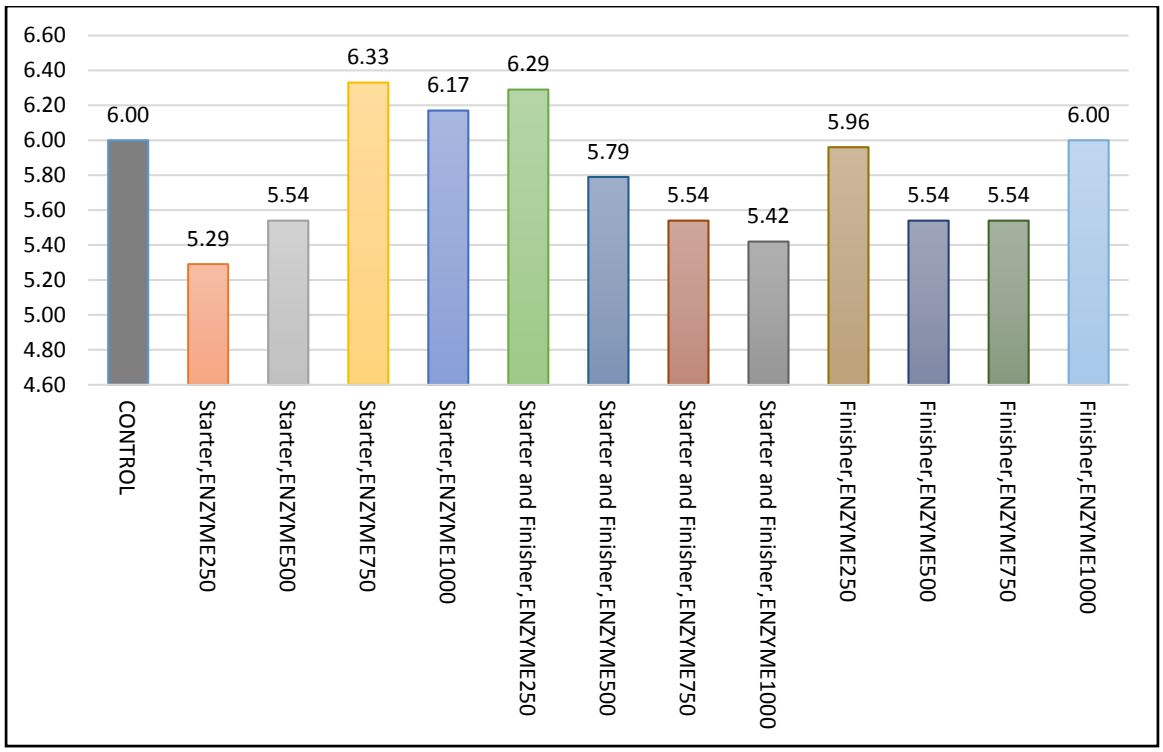
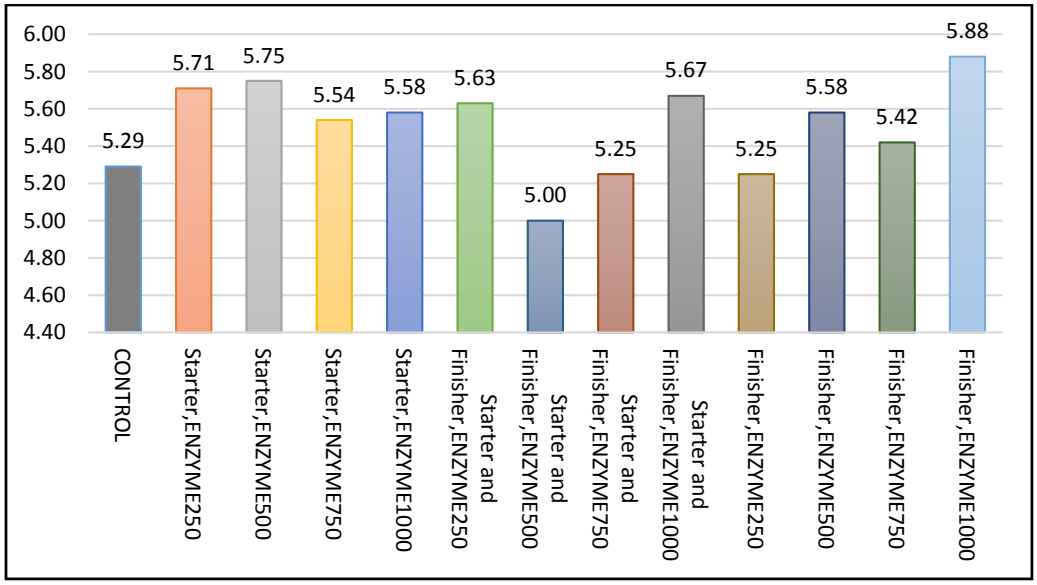


Table (19): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on Sensory Evaluation of broiler chickens.

Treatments		Tenderness	Flavor	Color	Juiciness
Control		5.38	5.71	6.00	5.29
Feeding Periods Effect					
1	Starter	5.88	5.70	5.83	5.65
2	Finisher	5.69	5.42	5.76	5.53
3	Whole period	5.91	5.65	5.76	5.39
	SEM	0.18	0.16	0.18	0.18
	p-value	0.65	0.41	0.94	0.59
	Sig	N.S	N.S	N.S	N.S
Enzymes Levels Effect					
1	Enzyme 250 g	5.78	5.57	5.85	5.53
2	Enzyme 500 g	5.58	5.56	5.63	5.44
3	Enzyme 750 g	6.14	5.68	5.81	5.40
4	Enzyme 1,000 g	5.79	5.56	5.86	5.71
	SEM	0.21	0.19	0.20	0.21
	p-value	0.30	0.96	0.84	0.74
	Sig	NS	NS	NS	NS
Period X Enzymes Levels Interaction					
	SEM	0.36	0.32	0.35	0.36
	p-value	0.29	0.42	0.12	0.82
	Sig	N.S	N.S	N.S	N.S

N.S Not significant

Figure (26): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on meat juiciness.



4.9. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on commercial cut parts and their relative weights, when fed during starter phase(2nd and 3rd weeks), finisher phase only (4th,5th and 6th weeks),or fed for the whole period :

Results of effects of added various levels of multi-enzymes and feeding durations on commercial cut parts and their relative weights, is shown in table (20).

Neither of the added multi-enzymes levels, nor the feeding durations had a significant influence on breast, thigh, drumstick or wing weights proportions. Never the less, numerical data values reflected an increased breast size with increased levels of the added multi-enzymes.

Drumstick proportion percentage showed a slight gradual increase with longer feeding durations,11.20% was recorded for birds fed on the multi-enzymes diet during starter period only,11.66% was recorded for birds fed the multi-enzymes diet during the finisher period only ,and 11.90% was recorded for birds fed the multi-enzymes diet during the whole period, in addition results for wing size showed similar graduated increase, in relation to feeding durations,10.14%,10.36%, and 10.56% for feeding multi-enzymes ration during starter period only, finisher period only or during the whole period

respectively, birds of the control group, fed on basal diets, recoded the lower most breast weight (34.49%),but highest drumstick size(13.03%), while the heaviest breast was yielded by birds fed on diet supplemented with multi-enzymes added at level of 250g / 1MT of feed, offered during the starter period(2nd and 3rd weeks during the trial.

Largest thigh proportion (15.06%) was observed with birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the finisher period (4th, 5th and 6th weeks of the trial), on the other hand, the smallest thigh proportion (12.61%) was observed with birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole period.

Table (20): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on commercial cuts proportions of broiler chickens.

Treatments		Breast %	Thigh %	Drumstick %	Wing %
Control		34.49	14.22	13.03	10.07
Feeding Periods Effect					
1	Starter	39.98	14.26	11.20	10.14
2	Finisher	38.97	14.12	11.66	10.36
3	Whole period	38.21	13.48	11.90	10.56
	SEM	0.95	0.42	0.003	0.19
	p-value	0.42	0.38	0.28	0.32
	Sig	N.S	N.S	N.S	N.S
Enzymes Levels Effect					
1	Enzyme 250 g	38.54	14.16	11.79	10.33
2	Enzyme 500 g	39.68	14.12	11.01	10.01
3	Enzyme 750 g	39.51	14.30	11.94	10.61
4	Enzyme 1,000 g	40.77	13.23	11.59	10.48
	SEM	1.10	0.48	0.003	0.22
	p-value	0.98	0.39	0.29	0.27
	Sig	NS	NS	NS	NS
Feeding Periods X Enzymes Levels Interaction					
	SEM	1.90	0.84	0.006	0.38
	p-value	0.79	0.65	0.79	0.44
	Sig	N.S	N.S	N.S	N.S

Figure (27): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on breast relative weight.

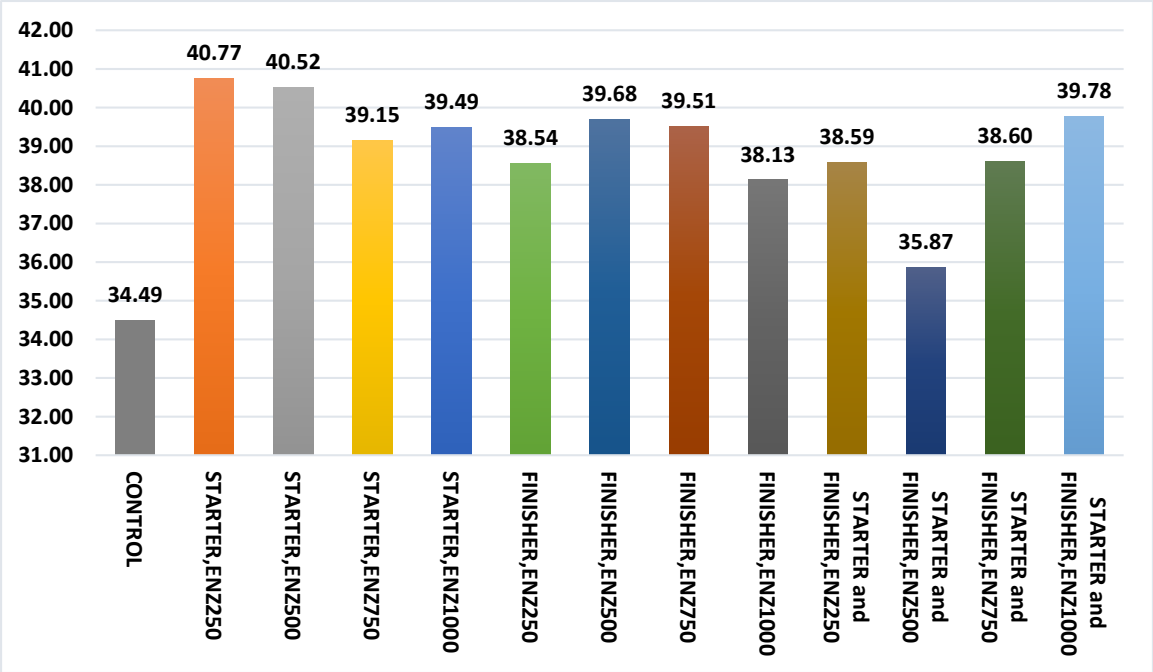


Figure (28): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on thigh relative weight.

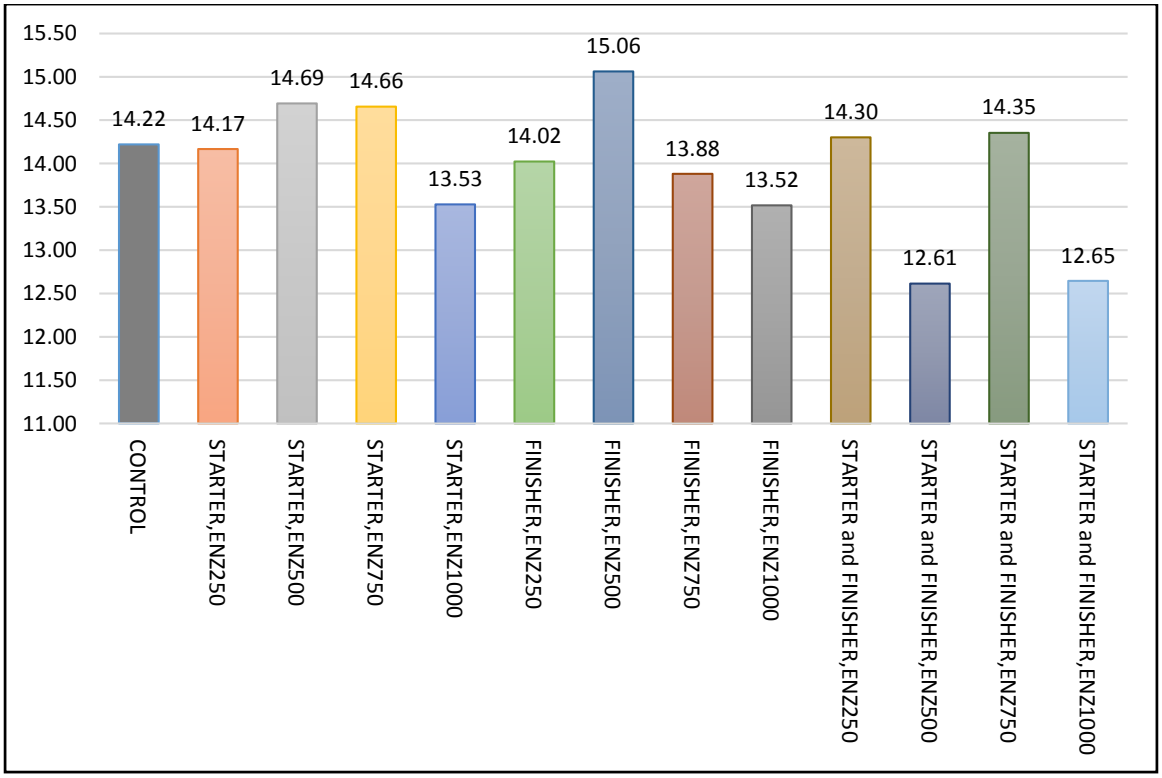


Figure (29): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on drumstick relative weight.

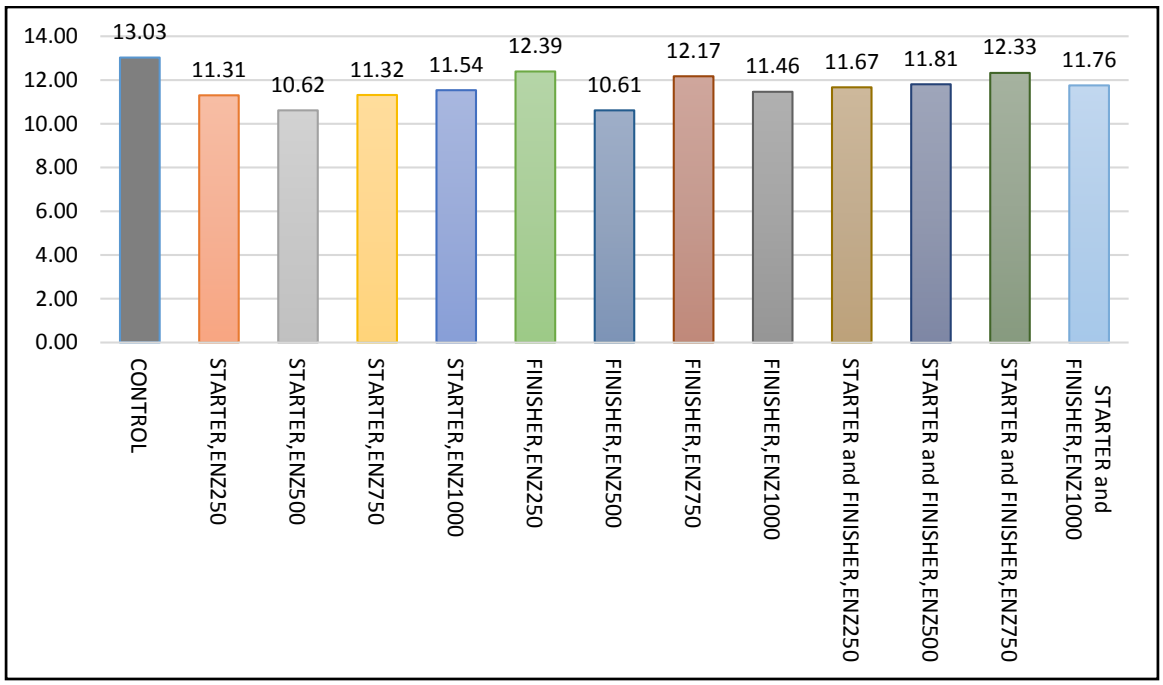
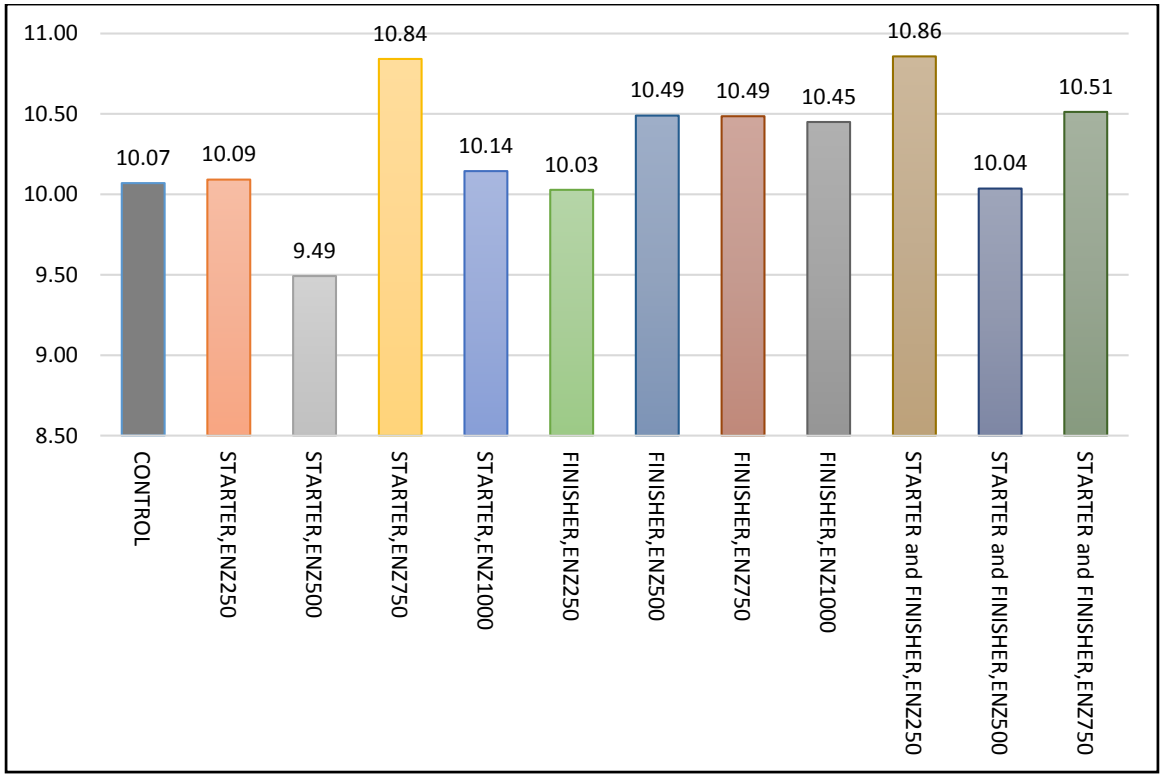


Figure (30): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on wing relative weight.



4.10. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on breast meat percentage and meat: bone ratio, when fed during starter phase, finisher phase only, or fed for the whole period:

Breast meat percentage and meat: bone ratio results obtained from deboned commercial breast cut, as affected by feeding different graded levels of added multi-enzymes and feeding durations or periods, are demonstrated in table (21).

Feeding durations phases showed a significant negative effect (p value ≤ 0.10) on meat breast amount, which was decreased with increased durations, more breast meat was yielded (34.49%) when multi-enzymes supplemented diet was fed during starter period only, compared to breast meat percentage(31.26%) obtained by birds afforded the multi-enzymes diet during both starter and finisher periods.

In contrast, different added multi-enzymes levels, resulted in an apparent positive effect on breast meat percentage, but the effect was not significant, 34.41% was recorded with the highest enzyme level of 1,000g, while the lowest level (250 g of multi-enzymes) resulted in 32.84% breast meat.

Neither of feeding durations or multi-enzymes supplementation, resulted in a significant impact on meat: bone ratio, the greatest numerical breast meat proportion (36.3%), was produced by birds fed on diet supplemented with multi-enzymes added at level of 1,000g / 1MT of feed, offered during the starter period only, whereas the smallest breast meat (28.28%) was recorded for birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole period.

Neither of feeding durations or multi-enzymes supplementation, resulted in a significant impact on meat: bone ratio, the greatest numerical breast meat proportion (36.3%), was produced by birds fed on diet supplemented with multi-enzymes added at level of 1,000g / 1MT of feed, offered during the starter period only, whereas the smallest breast meat (28.28%) was recorded for birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole period.

Table (21): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on breast meat% and meat: bone ratio of broiler chickens.

	Treatments	Breast Meat %	Meat: Bone
	Control	**29.24**	**7.41**
	Feeding Periods Effect		
1	**Starter**	**34.49 [a]**	**7.30**
2	**Finisher**	**32.90 [ab]**	**8.77**
3	**Whole period**	**31.26 [b]**	**7.56**
	SEM	**0.010**	**0.928**
	***p*-value**	**0.0945**	**0.4945**
	Sig	*****	**N.S**
	Enzymes Levels Effect		
1	**Enzyme 250 g**	**32.84**	**8.6**
2	**Enzyme 500 g**	**31.76**	**7.26**
3	**Enzyme 750 g**	**32.51**	**7.72**
4	**Enzyme 1,000 g**	**34.41**	**7.93**
	SEM	**0.012**	**1.072**
	***p*-value**	**0.4558**	**0.8485**
	Sig	**N.S**	**N.S**
	Feeding Periods X Enzyme Levels Interaction		
	SEM	**0.021**	**1.857**
	***p*-value**	**0.7040**	**0.9082**
	Sig	**N.S**	**N.S**

a-c: Means in a column and main effect with no common superscript differ significantly ($p \leq 0.05$).
*: Significant with ($p \leq 0.10$). N.S: Not significant.

Figure (31): Effect of feeding durations or the levels of included multi- enzymes and their interactive impact on breast deboned meat percent.

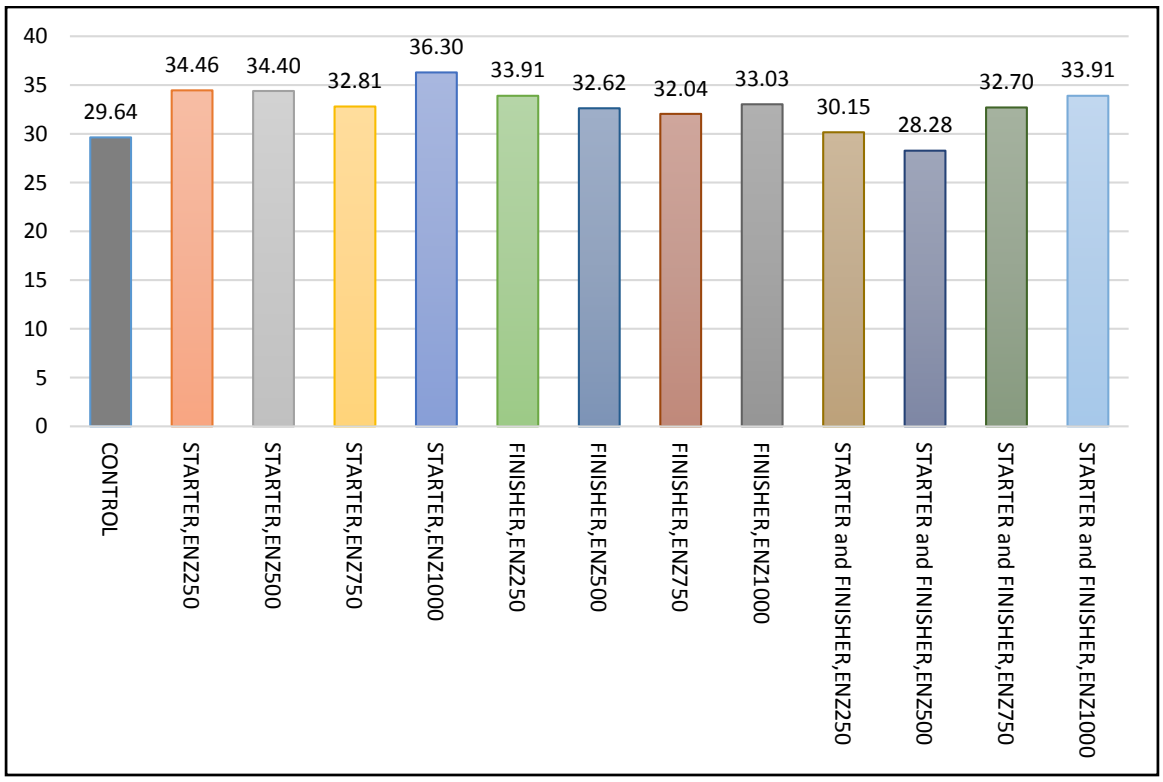
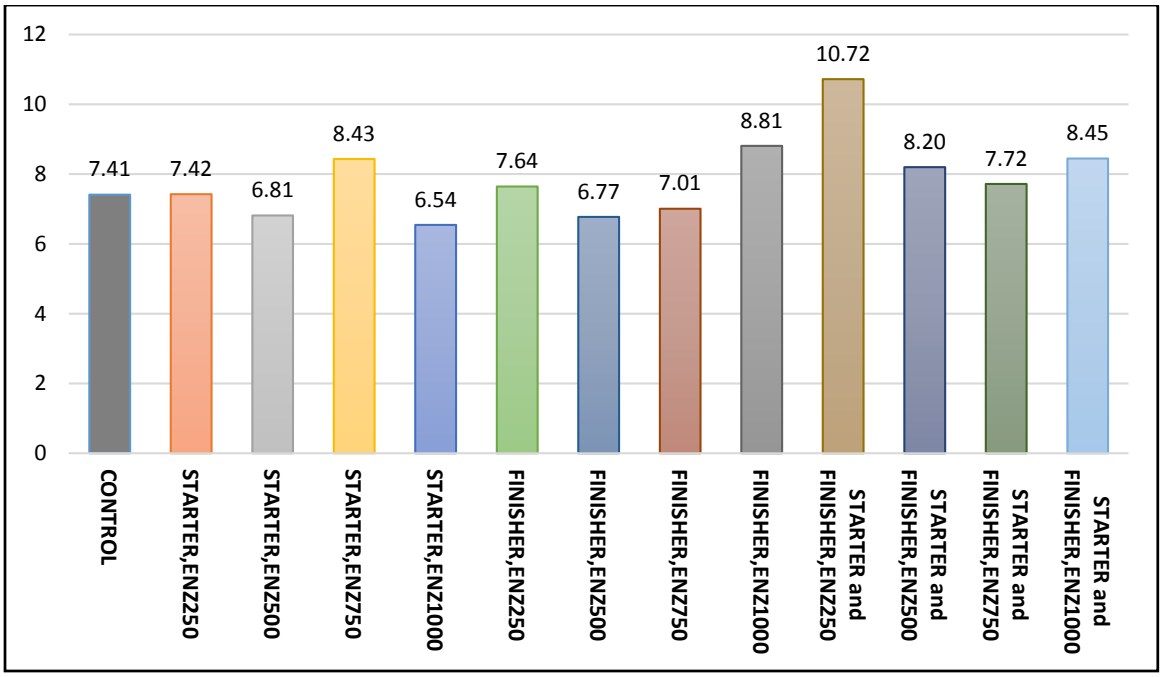


Figure (32): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on breast meat: bone ratio.



4.11. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on broiler blood plasma profile and serum biochemical constituents, when fed during starter phase(2^{nd} and 3^{rd} weeks), finisher phase only (4^{th},5^{th} and 6^{th} weeks),or fed for the whole period :

Results of blood plasma profile, kidney function tests and minerals of broiler chicken as affected by multi-enzymes supplementation and feeding durations, are displayed in table (22).

Although blood and serum parameters, were not significantly affected by multi-enzymes supplemented to the experimental diets, or by feeding durations, variation in values of serum constitutes were clearly observed among different treatments groups.

Highest blood Hb (16.10 g/dl) was recorded by birds fed on diet supplemented with multi-enzymes added at level of 750g / 1MT of feed, offered during the whole starter and finisher periods, while the lowest value (7.50 g/dl) was seen for birds fed on diet supplemented with multi-enzymes added at level of 250g / 1MT of feed, offered during the starter period only, blood glucose of birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the starter period only, showed the highest (235mg/dl) value, while the lowest glucose value (103 mg/dl) was recorded for the blood of birds fed on diet with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole trial period.

Results revealed that, the level of urea (1.975 mg/dl) is lower when birds fed on diets supplemented with multi-enzymes during starter period only, compared to those offered the multi-enzymes fortified diet during the whole period(2.825 mg/dl),furthermore, the lowest multi-enzymes inclusion level (250g/1,000Kg of feed),resulted in lowest serum urea (1.700 mg/dl), while highest multi-enzymes inclusion level (1,000g/1,000 Kg of feed), resulted in highest serum urea level (2.566 mg/dl).

Apparent interaction effect between feeding durations and added multi-enzymes level can be observed in case of serum urea level, the highest numerical value of urea

level (4.5 mg/dl), was recorded for birds fed on diet with multi-enzymes added at level of 1,000g / 1MT of feed, offered during the whole experimental period, the lowest value (0.6 mg/dl) was observed for birds fed on multi-enzymes diet added at level of 750g / 1MT of feed, offered during the finisher period only.

Although, the feeding durations did not explained a significant effect on uric acid, it was observed that, there was a gradual increase in the serum uric acid content, when feeding durations increase, these findings were clearly conformed to recorded serum urea levels.

Highest creatinine level of (0.20 mg/dl), was recorded for birds fed on diet with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole experimental period, while the lowest value (0.01 mg/dl) was observed for birds fed on multi-enzymes diet added at level of 500g / 1MT of feed, offered during the starter period only.

Highest calcium content (12.8 mg/dl) was recorded by birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the starter period only, while the lowest value (4.50 mg/dl) was seen for birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole experimental course.

The highest serum phosphorus (13.7 mg/dl) was recorded by birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the starter period only, while the lowest value (2.40 mg/dl) was seen for birds fed on diet supplemented with multi-enzymes added at level of 1,000g / 1MT of feed, offered only during the starter phase.

Table (22): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on kidney function tests and serum minerals of broiler chickens.

	Treatments	Hb	Glucose	Urea	Creatinine	Uric Acid	Phosphorus	Calcium
		g / dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
	Control	11.20	222.00	2.00	0.04	4.90	6.80	9.00
				Feeding Periods Effect				
1	Starter	10.200	177.250	1.975	0.098	3.300	6.350	7.250
2	Finisher	13.350	163.500	1.375	0.113	3.625	4.243	6.550
3	Whole period	11.725	171.250	2.825	0.108	4.450	5.483	7.675
	SEM	1.075	23.621	0.502	0.035	0.967	1.587	1.279
	p -value	0.292	0.743	0.304	0.825	0.789	0.783	0.796
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S
				Enzymes Levels Effect				
1	Enzyme 250 g	10.033	176.667	1.700	0.127	4.400	5.023	7.133
2	Enzyme 500 g	11.866	162.333	2.366	0.117	2.033	7.370	7.733
3	Enzyme 750 g	13.900	184.333	1.600	0.067	4.533	5.253	7.800
4	Enzyme1,000 g	11.233	159.333	2.566	0.113	4.200	4.120	6.866
	SEM	1.257	28.288	0.688	0.040	0.999	1.895	1.598
	p-value	0.362	0.814	0.824	0.712	0.403	0.833	0.963
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Multi-enzymes supplemented treatments diet revealed no significant effect on chicken blood serum cholesterol level, similar results are recorded for feeding durations effect on blood serum cholesterol. However, the obtained numerical values exhibited that, a beneficial non-significant interaction effect of lowering the cholesterol level, by increasing multi-enzymes inclusion levels and feeding durations.

The lowest serum cholesterol of 57.0 mg/dl was recorded by birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the entire fattening period, while the highest value (212.0 mg/dl) was seen for birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the starter phase only. Moreover, triglycerides results were identical to cholesterol records.

Results for blood serum proteins, lipids and liver function tests of broiler chicken as affected by multi-enzymes supplementation and / or feeding durations, are displayed in table (23).

Data of feeding durations of multi-enzymes added diet, reflected a non-significant effect on serum HDL, which increased with increased feeding period. The level of 78.75 mg\dl was recoded when feeding birds the experimental diets during the whole period, was higher compared the value (61.75 mg/dl) shown for birds fed on multi-enzymes fortified diet during the entire experimental course.

The highest value (100 mg/dl) of serum HDL, was recorded for birds fed on diet supplemented with multi-enzymes added at level of 750g / 1MT of feed, offered during the entire experimental period. Birds fed on diet supplemented with multi-enzymes added at level of 500g / 1MT of feed, offered during the whole experimental phases, recorded the lowest value of serum HDL (47 mg/dl) and LDL (13 mg/dl) in addition. The highest LDL values (45 mg/dl) was observed for birds fed on diet supplemented

with multi-enzymes added at level of 1,000g / 1MT of feed, offered during the finisher phase period alone.

Table (23): Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on liver function test and blood chemistry of broiler chickens.

	Treatments	Cholesterol	Triglycerides	LDL	HDL	Total Protein	Albumin	AST	ALT
		mg/dl	mg/dl	mg/dl	mg/dl	g/dl	g/dl	u/l	u/l
	Control	110.00	51.00	27.50	80.00	2.03	0.840	187.00	12.00
	Feeding Periods Effect								
1	Starter	115.38	39.5	18.50	61.75	2.02	0.793	276.00	4.90
2	Finisher	93.00	36.25	27.75	65.75	1.73	0.700	237.00	3.53
3	Whole period	109.75	42.75	25.25	78.75	1.79	0.695	285.25	5.08
	SEM	24.897	8.957	4.572	9.551	0.340	0.144	50.343	1.426
	p-value	0.9286	0.8838	0.5407	0.5839	0.9206	0.9317	0.7914	0.1389
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
	Enzyme Levels Effect								
1	Enzyme 250 g	95.50	41.33	22.33	71.00	1.61	0.647	217.670	4.10
2	Enzyme 500 g	112.67	35.67	15.33	53.33	2.04	0.763	234.000	5.90
3	Enzyme 750 g	110.33	39.33	28.33	78.00	1.99	0.827	279.300	3.73
4	Enzyme1Kg	105.67	41.67	29.33	72.67	1.75	0.680	333.333	4.27
	SEM	30.876	11.001	4.833	10.965	0.407	0.172	56.248	1.716
	p-value	0.9950	0.9666	0.3256	0.5602	0.9306	0.9311	0.5632	0.2413
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Serum total protein was not affected by adding multi-enzymes or by feeding durations, however values of albumin were decreased with increased feeding duration, multi-enzymes diet fed during starter phase only resulted in 793 mg/dl. In contrast, a value of 645 mg/dl was recorded when the diet was offered during the whole period.

Obtained results of both serum albumin and total proteins, suggested that, these two parameters were similarly affected by multi-enzymes supplemented feed and feeding durations, the highest recorded levels were 1.36 g/dl and 3.47 g/dl for albumin and total protein respectively, when birds fed on diet with multi-enzymes added at a level of 500 g / 1,000 Kg of complete feed during the starter period only.

The lowest recorded levels were 0.40 g/dl and 1.19 g/dl for albumin and total protein respectively, when birds fed on diet with multi-enzymes added at a level of 500 g / 1,000 Kg of complete feed during the whole trial period.

Results reported for AST and ALT enzymes serum level, showed that they were not significantly affected by feeding durations, however multi-enzymes gradual addition, increased the numerical values of AST, the value of 217.7 IU/dl, for birds fed on diet with 250g multi-enzymes added to 1MT of feed, was lower compared to the value (333.3 IU/dl) obtained when 1,000 g of multi-enzymes was added to 1MT of feed.

Birds raised on the control feed, a basal diet without multi-enzyme added, recorded the highest ALT value (12.0 IU/dl), while birds fed on diet with 1,000g multi-enzymes added to 1MT of feed offered during the starter period only, showed the lowest value(1.3 IU/dl). Highest AST value of 444 IU/dl was obtained for birds fed on added multi-enzymes diet at level of 1,000g /1MT of feed offered during the finisher period only, the lowest value (126 UI/dl) was recorded for birds those were raised on added multi-enzymes ration at level of 250g /1MT of feed applied at the finisher period only.

4.12. Effect of added graded levels of multi-enzymes NutriKEM Extend and feeding durations on economic efficiency, when fed during starter phase(2nd and 3rd weeks), finisher phase only (4th,5th and 6th weeks),or fed for the whole period :

Results of economical evaluation as affected by commercial multi-enzymes addition and / or feeding durations, is displayed in table (24), the cost of used feed ingredients and calculated cost of basal starter and finisher formulas and different multi-enzymes treated diets at the study date, are showed in appendix (14).

The cost of consumed starter feed with added multi-enzymes was significantly affected by feeding durations regimen (*p*-value =0.0169), the cost value of starter feed

fortified by multi-enzymes was lower (29.90 SDG), when offered during finisher period only, compared to the cost value (31.69 SDG when offered during starter period only.

The highest value (32.36 SDG/bird) of consumed starter feed, was recoded for birds fed on diet supplemented with multi-enzymes added at level of 500g per 1MT of feed, offered during the starter period only, whereas the lowest value (29.36 /bird SDG) was shown by birds fed on diet with multi-enzymes added at level of 250g / 1MT of feed, offered during the finisher period only.

The cost of consumed finisher feed with added multi-enzymes was not significantly affected by neither feeding durations or periods, and nor added levels of multi-enzymes, the highest value (82.66 SDG/bird) of consumed finisher feed, was assigned to birds consumed the diet with 1,000 g multi-enzymes added to 1,000 Kg of feed, offered during the starter period only, whereas the lowest value (72.83 SDG/bird) was shown by birds fed on diet with multi-enzymes added at level of 500g / 1MT of feed, offered during the finisher period only.

The cost of entire consumed feed, eventually obtained by the addition of both starter and finisher feed costs, reflected a similar figure pattern showed by finisher feed cost, highest total feed expenses of 114.80 SDG/bird, were paid for birds raised on diet fortified with 1,000 g multi-enzymes added to 1,000 Kg of complete feed when fed during the starter period alone.

The total feed cost was reduced by 10.10 %, when birds consumed a diet offered during the finisher period only and supplemented with multi-enzymes at level of 500 g /1MT of feed, these birds recorded the lowest feeding cost value of 103.21 SDG/bird.

Neither of feeding regimen, nor of multi-enzymes supplementation had a significant effect on total revenue, net profit or economic efficiency, the interaction figures of the three above mentioned values, explained a typical or similarity trend, the

best revenue of 272.58 SDG/bird was earned from birds fed on diet with 500 g multi-enzymes added to 1,000 Kg of complete feed when offered during the starter period only, this profit was impaired with a reduction rate of 11.6%, when the multi-enzymes was applied at the level of 750 g, and the feed was offered during the starter period only, in this case the recorded revenue value was 240.87 SDG/bird.

Birds fed on diet with 1,000 g multi-enzymes included in 1,000 Kg of complete feed when offered during the finisher period only, achieved the highest numerical values of both net profit value (96.76 SDG/bird), and economic efficiency score (55.92%), moreover an explained improvement estimated at a rate of 7.30 % was observed, compared to birds fed on control diet, without multi-enzymes added. The recorded values for control birds were 86.80 SDG and 48.32% score for net profit and economic efficiency respectively.

The bottom net profit value of 59.84 SDG/bird, accompanied with the worst economic efficiency score of 33.35%, were assigned to birds raised on feed with 750g of multi-enzymes added to 1MT of complete feed, fed the starter period alone.

Based on data obtained from the current study, the production cost of 1 Kg broiler meat, was not affected by multi-enzymes inclusion levels, or different feeding styles, nevertheless the affection was significant with p-value ≤ 0.10, production cost of 1 Kg chicken meat ,when multi-enzymes added diet was fed to birds during the finisher period only (84.00 SDG),was lower or more cheaper, compared to meat (71.99 SDG/Kg) produced by birds fed on diet supplemented with multi-enzymes added at level of 500g per 1MT of feed fed during the finisher period only, while the most expensive meat (90.17 SDG/Kg) was produced by birds fed on the basal control diet without multi-enzymes supplementation.

Table (24): Effect of added graded levels of multi-enzymes NutriKEM Extend on Economic Efficiency of broiler chickens (SDG).

	Treatments	Starter feed cost	Finisher feed cost	Total feed cost	Feed cost/ Kg meat	Total cost	Total revenue	Net profit	E %
	Control	32.14	75.66	113.79	90.17	182.00	268.80	86.80	48.32
				Feeding Periods Effect					
1	Starter	31.69 [a]	79.77	111.46	92.26	179.67	258.04	78.37	43.91
2	Finisher	29.90 [b]	75.96	105.86	84.00	174.07	266.44	92.37	53.24
3	Whole period	30.31 [b]	79.85	110.16	89.38	178.37	261.08	82.71	46.76
	SEM	0.453	1.736	1.972	2.722	1.972	6.666	6.805	3.992
	p-value	0.0196	0.2043	0.1211	0.1039	0.1211	0.6679	0.3384	0.2481
	Sig	*	N.S	N.S	N.S	N.S	N.S	N.S	N.S
				Enzyme Levels Effect					
1	Enzyme 250 g	30.44	77.82	108.25	86.76	176.46	263.97	87.51	49.69
2	Enzyme 500 g	30.96	78.16	109.12	87.37	177.33	265.72	88.39	50.19
3	Enzyme 750 g	30.47	79.15	109.62	90.89	177.83	256.62	78.79	44.66
4	Enzyme 1,000 g	30.66	78.98	109.64	89.18	177.85	261.10	83.25	47.34
	SEM	0.522	2.005	2.277	3.143	2.277	7.697	7.858	4.610
	p-value	0.8894	0.9587	0.9700	0.7872	0.9700	0.8491	0.8146	0.8243
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
				Feeding Periods X Enzyme Levels Interaction					
	SEM	0.905	3.472	3.944	5.443	3.944	13.332	13.610	7.984
	p-value	0.8149	0.3916	0.4154	0.6646	0.4154	0.8459	0.7936	0.7401
	Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

The Percentage improvements in performance traits of broiler are shown in table (25)**,** , the highest numerical percentage improvement of FCR (8.73 %) was obtained with multi-enzymes level of 500g Kg $^{-1}$ feed, when fed during finisher period only, best body weight gain improvement percentage of (6.73%) was recorded with multi-enzymes level of 1,000g Kg $^{-1}$ feed, when fed during the whole period, while the best net profit improvement percentage of (9.96%) was assigned to multi-enzymes level of 1,000g Kg $^{-1}$ feed, when fed during finisher period only.

Table (25): Percentage improvements in performance traits of broiler as affected by feeding durations, when fed diets supplemented with different multi-enzymes levels and interaction between them.

No.	Treatment	FCR	Body Weight	Net Profit
1	Control	0	0	0
2	Starter, Enzyme 250g	3.41%	1.39%	4.75%
3	Starter, Enzyme 500g	-	2.80%	3.58%
4	Starter, Enzyme 750g	-	-	-
5	Starter, Enzyme 1,000g	-	3.48%	-
6	Finisher, Enzyme 250g	3.57%	3.88%	3.62%
7	Finisher, Enzyme 500g	8.73%	5.18%	4.70%
8	Finisher, Enzyme 750g	6.90%	6.17%	4.00%
9	Finisher, Enzyme 1,000g	5.63%	5.36%	9.96%
10	Starter and Finisher, Enzyme 250g	0.48%	6.14%	-
11	Starter and Finisher, Enzyme 500g	1.19%	3.95%	-
12	Starter and Finisher, Enzyme 750g	3.25%	4.83%	-
13	Starter and Finisher, Enzyme 1,000g	6.59%	6.73%	-

Figure (33): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on starter feed cost.

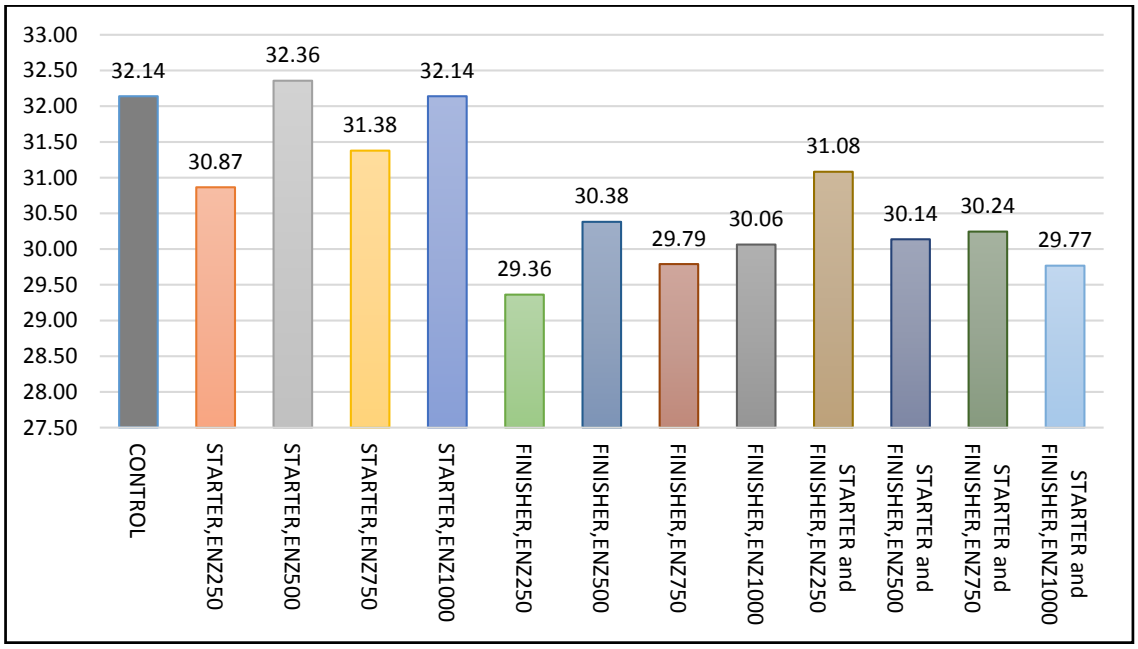


Figure (34): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on finisher feed cost.

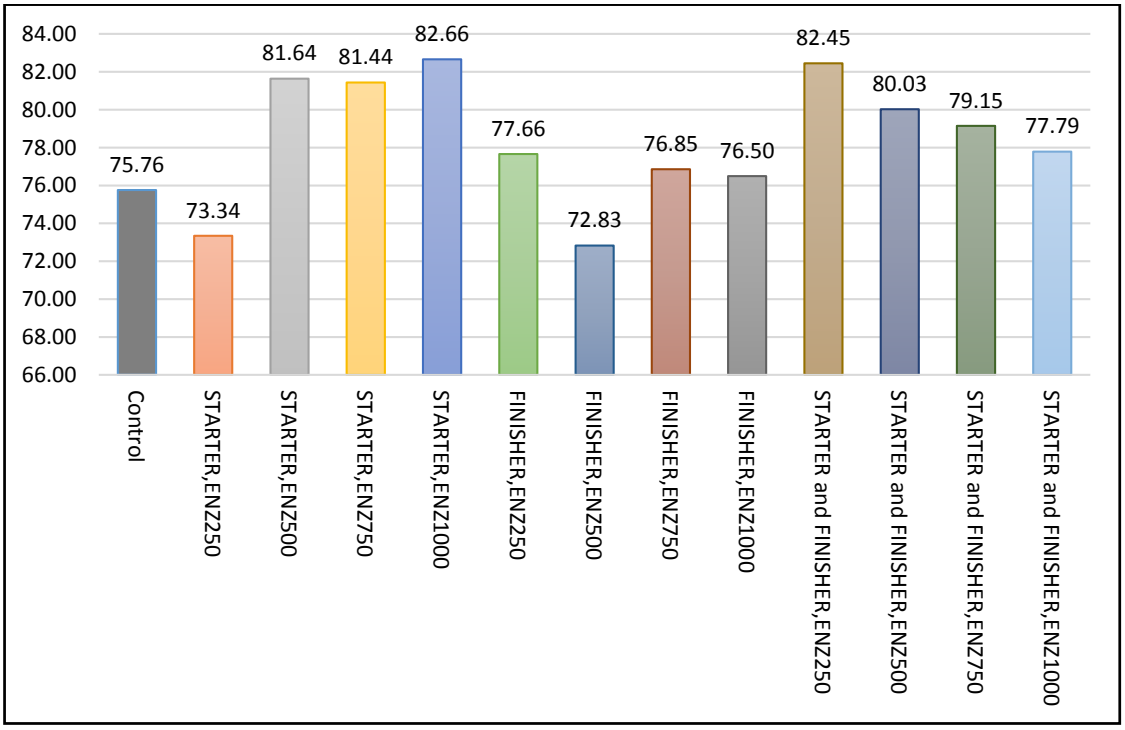


Figure (35): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on total feed cost.

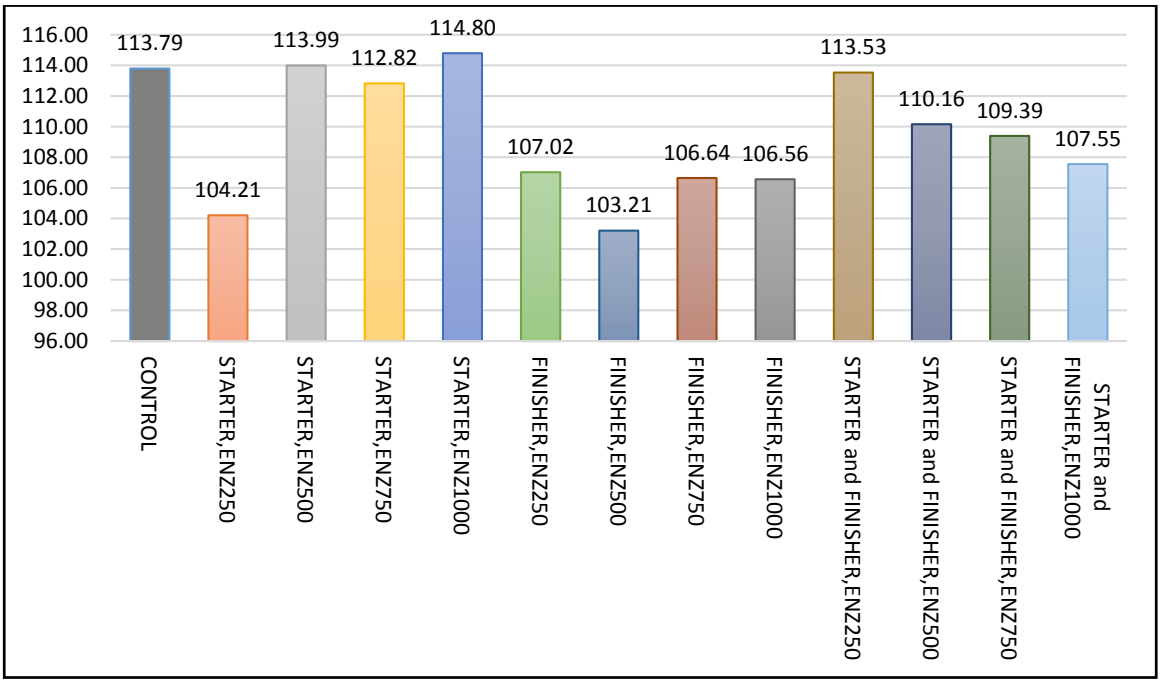


Figure (36): Effect of feeding durations or the levels of included multi- enzymes and their interactive impact on feed cost / 1 Kg broiler meat.

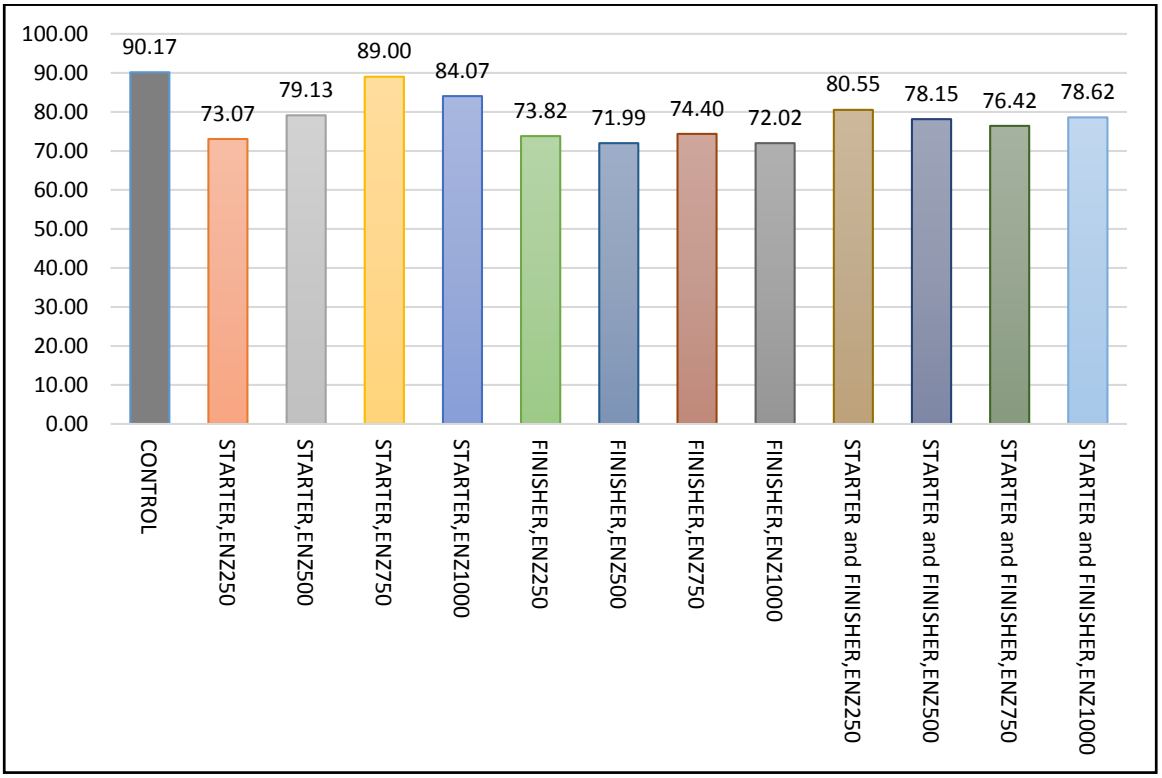


Figure (37): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on total cost.

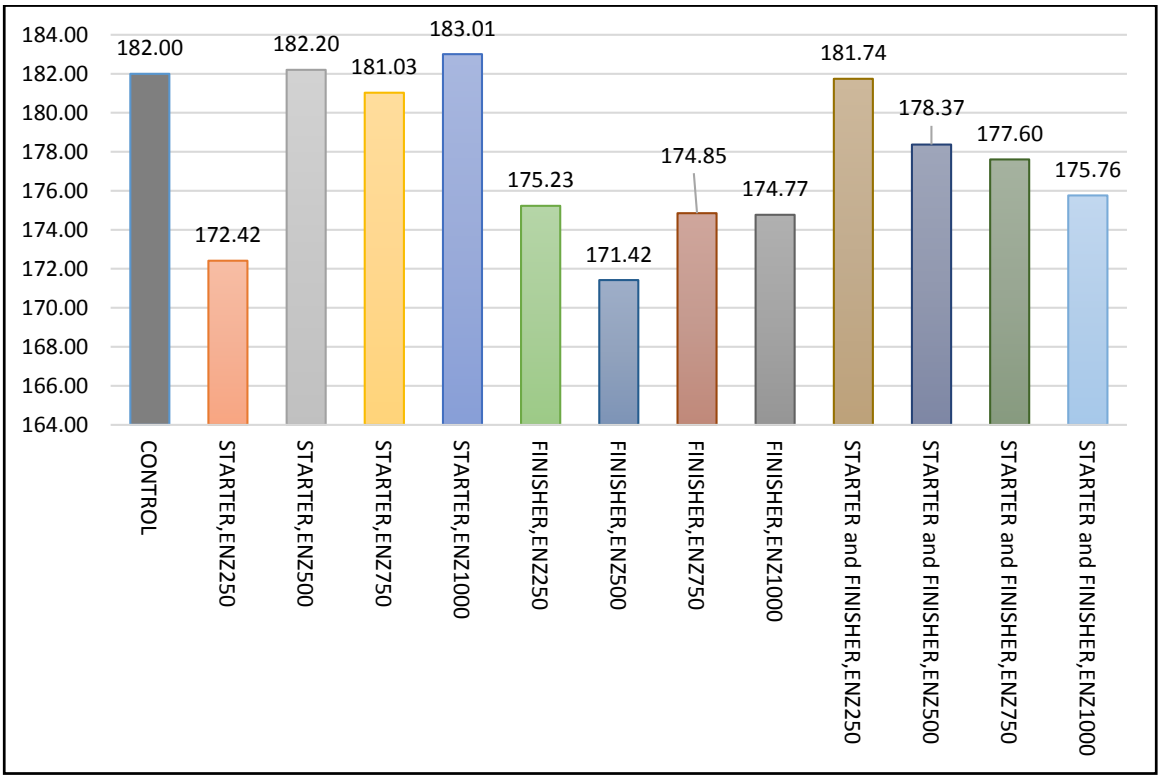


Figure (38): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on total revenue (SDG).

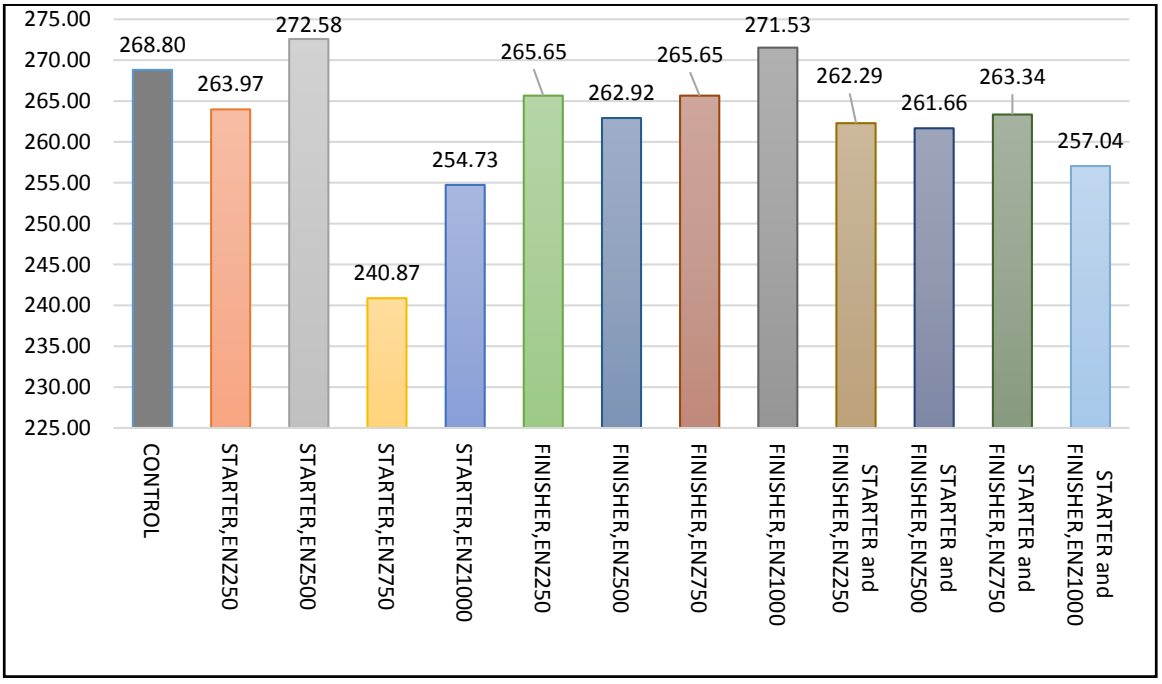


Figure (39): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on net profit (SDG).

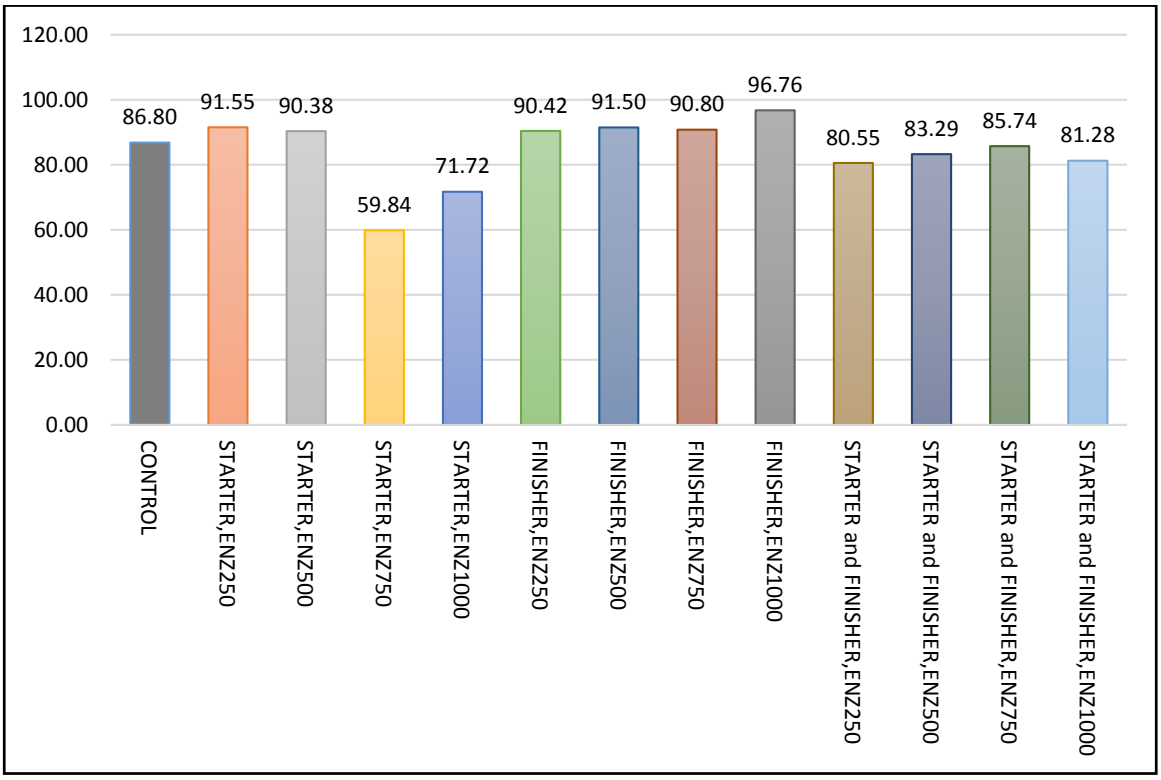
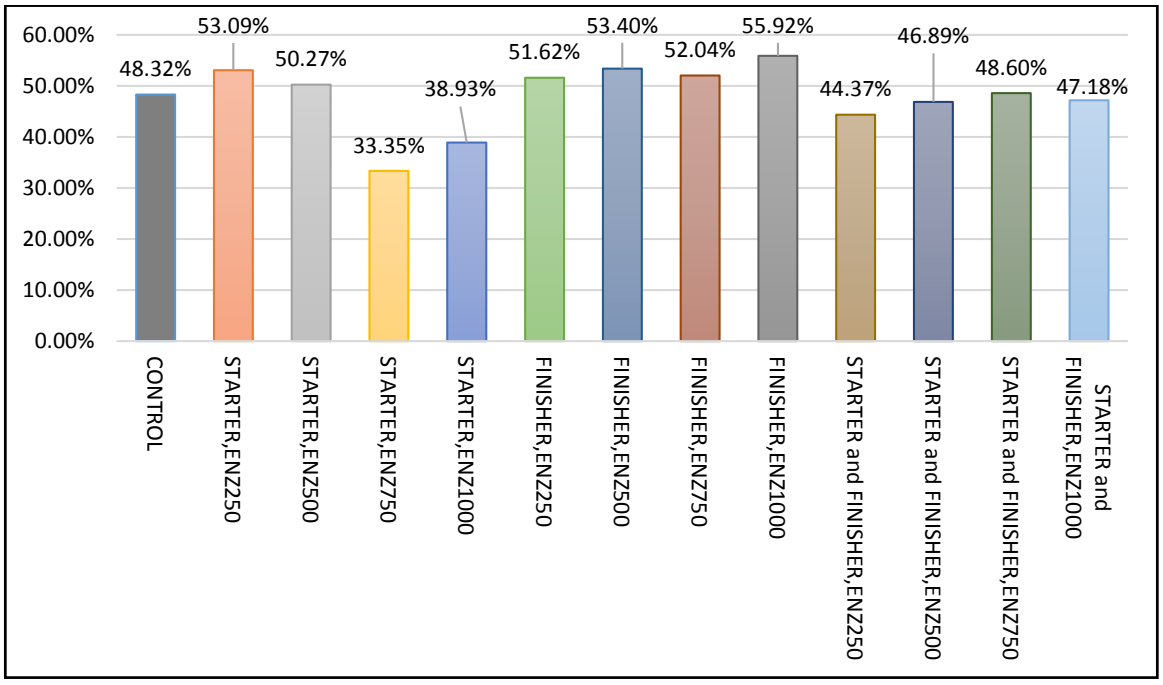


Figure (40): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on economic efficiency.



CHAPTER FIVE

DISCUSSION

5.1. Experimental diets chemical composition:

The slight differences of nutrient content, mainly crude protein and calculated ME, between the formulated diets (Table 6) and laboratory chemical analysis results (Table 7) is attributed to variations of chemical composition of different used local varieties of sorghum and ground nut cake, used in feed formulation, also the impact of mentioned raw materials quality from the expected.

5.2 Broiler performance:

Sorghum and peanut cake are the mainly used ingredients in locally produced broiler diets, due to their availability and comparatively lower prices, however most studies of exogenous multi-enzymes supplementation have investigated their effect on maize or wheat soybean-based diets, investigations into the inclusion of exogenous enzymes other than phytase in sorghum-based diets are limited (Liu, *et al*. 2017), furthermore enzymes supplementation time and their application frequency have received little attention other than enzymes dose and types (Youssif *et al*., 2020b).

Inclusion of exogenous enzymes in broiler diets may lead to different responses due to ingredient composition and their substrate content (Pasquali *et al.,* 2017), moreover, responses to phytase, protease, and xylanase supplementation may differ in broiler diets based on maize or sorghum, due to different phytate concentrations and localizations (Doherty *et al*., 1982), arabinoxylan content and crude protein content and digestibility (Rostagno *et al*., 2011), variation results and differences among various studies of broiler response to supplementing different levels of

multi-enzymes in term of improved carcass characteristics, could be attributed to the differences in dietary plant-based feed ingredients, as well as their interactions among constituents within feed ingredients and diets (Abdelrahim *et al*, 2018).

The purpose of the present study was to investigate and determine the effect and interaction of added different levels of multi-enzymes NutriKEM Extend to broiler diets during different growing periods, on growth performance, carcass characteristics, blood profile and economical efficacy.

Although the experimental and control diets were iso-caloric and iso-nitrogenous, the present study showed that, inclusion of exogenous multi-enzymes on sorghum peanut meal based diets significantly improved broiler chicks performance at all levels compared to control group, Bedford, (1996) reported that, enzymes supplementation actions may include improving the overall nutrient digestion and reducing endogenous amino acid losses, this agreed with (Youssef *et al.*, 2020b) who found that, enzymes supplementation significantly increased growth rate, feed intake European Production Index (EPI) and protein digestibility, serum albumin, and high-density lipoprotein (HDL) regardless of time or frequency of application.

Furthermore, this study generally revealed that, the out performance parameters were recorded when feeding multi-enzymes supplemented diets during both starter and finisher phases, Krogdahl and Sell, (1989) suggested that, early age birds have limited types and amounts of enzymes needed to utilize a high carbohydrate and vegetable protein diets, this will affect nutrient digestibility, Choct *et al.*,(1996) and (Sklan, 2002) reported 90% ileal digestibility of starch in 29-day old broilers fed on sorghum-based diets (Tony *et al.*, 2007), researchers who determined the changes in broiler digestive capability over the first 3 weeks, concluded that, the enzymes for

fat and starch digestion were secreted in adequate amounts, but may not be sufficient for proteolysis in early post hatch period (Ravinderan and Abdollahi, 2021).

Mabelebele *et al*., (2017) reported that, enzyme supplementation, apparently had a more pronounce defect during the early phase, when feeding different sorghum varieties supplemented with multi-enzymes, similarly (Selle *et al*., 2010) reported an increase in feed intake and weight gain when xylanase was added to sorghum-based broiler diets, but depressed feed efficiency, this may be due to the fact that sorghum is a 'non-viscous' grain with only 4% soluble NSP (Choct, 2006; Selle *et al*., 2010). Furthermore, there is a limited NSP-degrading enzymes disruption of insoluble NSP in sorghum, which is attributed to different composition of sorghum arabinoxylan, inclusion of the β-glucanase enzyme in sorghum-based diets significantly decreased total feed intake and significantly improved weight gain and the FCR of broiler chickens (Mabelebele *et al*., 2017).

Enzyme supplementation improved live body weight at the end of the experimental period by 6.73% for group fed on 1,000g multi-enzymes level during the whole period. Zanella *et al.*,(1998) reported an enzyme supplementation improved body weight and feed conversion ratio by 1.9 and 2.2%, respectively, Marsman *et al.*,(1997) suggested that an improvement in the nutritional value of soybeans could be achieved with protease and carbohydrase enzymes supplementation, this can be also true for other plant proteins sources or oil seeds legumes like peanut meal.

The combination of xylanase, amylase, beta-glucanase, cellulase and protease enzymes improve amino acid and energy utilization, this was

shown on performance, uniformity and beneficial impacted on intestinal microbial population (Douglas *et al.*, 2000).

Results obtained for FCR, body weight and body weight gain were similar to (Sanaa and Abdel-Wareth, 2014), who revealed that, addition of dietary enzyme significantly increase body weight ($p<0.001$), body weight gain (for both starter and finisher phases), profitability and improved feed conversion ratio ($p<0.001$) while, it had no significant effect on feed consumption, however, in the present study, feed intake was significantly affected by multi-enzymes supplementation, whereas economic efficiency showed no significant difference.

Current results were agreed with those of (Carvajal *et al*., 2010) who reported improved ($p<0.05$) body weight, body weight gain and feed conversion ratio during both starter and grower periods, when protease was supplemented at 500g MT-1 to sorghum based broiler diets. Amal *et al.* (2015) found contradict results and reported that addition of enzymes to the diet had no significant effect on the body weight gain, even though the diet supplemental with enzyme improved the body weight gain of broiler chicks in their study.

Balges *et al*., (2012) recommended an addition of 0.25g glucanase / Kg of broiler starter diet and indicated that, inclusion of the glucanase enzyme in sorghum based diet significantly decreased total feed intake, weight gain and feed conversion ratio were significantly improved, birds looked healthy throughout the experiments period and mortality was not affected by enzyme treated broiler diets, dietary enzyme significantly reduced starter (0 – 28 days) and total feed intake (0 – 42 days), reduction of feed intake was observed with increasing level of enzyme, likewise

Shakouri *et al*., (2009) found that multi-enzymes mixture did not improve growth rates of birds offered sorghum-based diets.

Results from this study was different from (Youssef *et al*., 2020b), who found that, the enzyme applications during days 1–37 of age yield the least response, and the application of multi-enzymes during the starter age yielded better BWG and FCR values than the use during the finisher ages and the whole period of growth, this may be because of digestive tract under development, it is clear from the literature that the efficacy of enzymes decreased with broiler age increase, this is correlated with maturation of gut in terms of capacity, endogenous enzyme secretion, and ecology.

Current observation of multi-enzymes supplementation for different feeding times, agreed with (El Boushy and Ratherink, 1989) results, who observed reductions in performance resulting from increasing peanut meal (PNM) percentage in the diet in young birds, older birds were better able to perform with groundnut meal, in addition to having lowered amino acid requirements, the older chicks may also be better able to digest the PNM or tolerate anti-nutritional or toxic factors present in the PNM (Costa, 2001). In addition least performance values obtained with diets based on sorghum-peanut meal during younger age could be attributed to high fiber content coupled with anti-nutritional factors (Oguntoye *et al.*, 2018).

Youssef *et al*, (2020a) studied the interaction between multi-enzymes supplementation level and time of application, results indicated that, higher doses yields superior effects on biological and economic value than the low dose of enzymes, low dosage could yield better economic benefits due to

low cost of supplementation, the level of enzyme supplemented at 1 ml/L water improved growth of chickens only during days 1–21 of age compared to 0.5 ml/L level, during days 22–35 and 1–35 of age, enzyme level at 1.5 mL resulted

in higher growth of broilers by 5.9% and 5.1%, respectively, compared to 0.5 ml. This indicates that enzymes supplemented continuously at 1.5 mL/L water of a corn-soybean diet at 22–35 days of age was adequate to improve growth and FCR, linear and non-linear components of enzymes concentration showed a weak linear effect on BWG during 1–21 days of age ($p < 0.089$) and FCR during 1–21 days of ($p = 0.076$), Abdelbasit *et al.*, (2016b) concluded that adding 1 kg of commercial xylanase enzyme in broiler chicks diets resulted in economic benefits, these results were similar to current study findings, where best performance was achieved with 1,000 g of multi-enzymes applied during the whole growing time, however the best recorded profit and economic efficiency was obtained with 1,000g multi-enzymes when applied during finisher period only.

Based on these studies records, the best net profit improvement percentage of (9.96%) was assigned to multi-enzymes level of 1,000 mg Kg^{-1} feed, when fed during finisher period only, this mentioned dose/time combination was also a companied with relatively better percentage of FCR improvement (5.63%) and BWG improvement percentage (5.63%).

5.3. Carcass characteristics and organs relative weights.

Sanaa and Abdel-Wareth, (2014) observed no significant effect of multi-enzymes (protease, amylase, β-glucanase, xylanase, pectinase, cellulose and phytase) when added at 0.02 % inclusion rate in sorghum-

soybean based broiler diets, on carcass criteria (dressing percentage, heart, gizzard and liver weights) or internal organs, except for abdominal fat, dressing percentage was not significantly ($P > 0.05$) affected by enzyme supplementation, however, the dressing percentage was slightly better with the enzyme treated feeds, diet with (0.25g enzyme) resulted in dressing percentage (74.64%), whereas (0.125 g enzyme level) gave slight result (72.97%) than control diet (0.0 g enzyme), these results agreed with current findings, moreover dietary enzyme increased liver size and abdominal fat deposition, this was attributed to the increased or improved fat digestibility (Balges, 2012) and also can be attributed to presence of lysophospholipids added to NutriKEM Extend.

Abdominal fat is very important parameter in assessment of broiler meat quality, excessive fat deposition is considered as a disadvantage trait in poultry industry, it reflects poor finishing which affects carcass quality, and it is not appreciated by every broiler consumers (Emmerson, 1997), nevertheless its organoleptic effects on the flavor of the meat is favorable (Mutaz, 2019), the impact of high abdominal fat deposition can be mitigated by applying multi-enzymes energy matrix during diet formulation, hence supplementation is done to lower energy content feeds instead of over on top application, this could be supported by Giotee *et al*, (2015), who declared that, adding enzymes to corn-soy-based diets allowed the reduction in the energy level of broiler diets without any negative effects on the performance of broiler chickens.

Guilherme *et al*., (2017) in their study, stated that, multi-enzymes complex consisting of protease, cellulase and amylase did not improve broiler performance, probably because it was added to diets composed of adequate nutritional and energetic levels and, that fulfilled requirements of

poultry, adding enzymes to low energy diets is an effective feeding strategy to improve the meat quality criteria and small intestine characteristics (Elsayed *et al*., 2019).

Balges, (2012) reported that, glucanase supplementation had no effect on dressing percentage, however, weight of abdominal fat and weight of the internal organs (liver, spleen, gizzard and intestine) were significantly ($P < 0.05$) decreased, this may explain the benefit of using multi-enzymes mixture having other enzymes rather than NSPs depredating ones.

Bin Baraik, (2010) found no effect of commercial enzymes, applied individually or in combinations, on carcass yield, dressing percent and weight of internal organs of broilers, Sayyazadeh *et al.*, (2006) concluded that abdominal fat and carcass yield of broiler chickens were not significantly influenced by supplementation of enzyme to wheat, maize and barley-based diets (Elsayed *et al*,, 2019).

Abdelasit *et al.*, (2016b) reported a significant ($P> 0.05$) difference values of carcass yield between enzymes treatment groups, chicks fed on a diet supplemented with (1) kg of xylam recorded significantly ($p < 0.05$) high percentage in carcass dressing, Mariam *et al.*, (2013) mentioned a similar reports and concluded that, addition of xylanase and phytase enzymes combinations at all levels to diets affect dressing percentages, carcass yield and internal organs (liver, heart and gizzard) percentages of the experimental chicks.

On the other hand, this result contradict with findings of Wang *et al*., (2005), who reported increased carcass yield by addition of enzymes in diet attributable to higher fat deposition in carcass and also for increased breast meat yield, in addition Hajati, (2010), concluded that, supplementation diets

with 500 mg kg−1 multi-enzymes in broiler chickens (Cobb 500) diets improved feed to gain ratio, carcass yield, but it had not significant effect on carcass composition.

Generally, the absence of effects of enzymes on most of carcass and organ traits is constant with our current study and many previous studies findings, authors indicated that broiler carcass parameters and body organs are not affected with application time or level of enzymes, except for liver percent that was reduced with multi-enzymes application (Youssef *et al*., 2020a).

5.4. Broiler meat chemical quality and physical properties.

The results of these experiments showed an apparent effect ($p < 0.10$) of multi-enzymes feeding time on meat moisture and a significant effect on ash contents ($p<0.05$) compared to control group, ash is an important indicator of the content of mineral substances in muscles, our study showed that, meat protein percentage was significantly affected by multi-enzymes supplementation level, data obtained by Abdelbasit *et al*., (2016b) reported no significant effect of diets supplemented with xylam on meat chemical composition (moisture, fat, and ash) values, these findings were in accordance with (Bin Baraik, 2010; Abdelrahim *et al*, 2018) who observed no significant effect in meat chemical composition or quality values in regard to subjective and objective quality values due to the use of commercial enzymes xylanase and phytase when applied individually or in combinations, similarly Hajati, (2010) results showed that enzyme supplementation to corn-soybean based diets, did not significantly affected Cobb 500 broilers carcass composition.

Results of current studies showed reduction in meat moisture content with increasing multi-enzymes feeding durations, this was in line with (Mahmoud, 2012) who demonstrated that, phytase plus multi-enzymes resulted in significantly lower meat moisture percentage but, significantly induced higher meat protein than those fed un-supplemented control group, this may be a result of comparatively greater Calorie: Protein ration of broiler diets supplemented with multi-enzymes compared to control group, the same researcher reported no significant influence of enzyme supplementations on meat lipid, ash and meat physical characteristics.

In these studies, dietary treatments with added multi-enzymes were unable to significantly affect ($P > 0.05$) meat sensory attributes of broiler meat, determined as meat color, juiciness, texture and tenderness, however the juiciest meat (scored 5.88), was obtained from chicken raised on diet supplemented with multi-enzymes added at level of 1,000 mg / 1Kg of feed, offered during the starter and finisher phases.

Food safety is an important aspect of food quality and efforts should be led to safety of new functional products from poultry meat (Burdock *et al*., 2006). Meat quality may be affected already by manipulation of animal feeding (Assi and King, 2007) or manipulation of carcass body, fat performs the primary role in sensory aspects as taste and juiciness of all meat products (Cofrades *et al*., 2000).

Quality assessment parameters of chicken meat, including sensory flavor and texture profiles, have been widely used in scientific studies to validate pre-processing treatments and postharvest processing technologies for chicken meat (Lyon *et al*., 2001), evaluations of properties as taste, smell, juiciness and tenderness, which are subject of sensory analysis, are

important factors that consumers will consider before making a decision to buy poultry (Liu *et al*., 2004).

Evaluated sensory properties are dependent on type of used feed mixture, content of intramuscular fat in meat, way of meat preparation, genetics and many others intra-vital and extra-vital factors (Hascik *et al*., 2004).

This experiment, examined the influence of multi-enzymes as feed additive applied in chicken nutrition on sensory properties of broiler meat, based on obtained results, no negative influence on sensory properties of breast muscles after application of multi-enzymes in Cobb 500 chicken nutrition.

5.5. Commercial cut parts and their relative weights.

Poultry producers, consumers and researchers are concerned about the yield of edible meat proportions, skin, bone and cooking losses. The cost of an individual serving, is kept in sharp economic focus. Increased merchandising of parts and further-processed poultry has increased the need for yield information on individual parts (breast, thigh, wings, etc.) and on components (meat, skin and bone) as a percentage of the ready-to-cook carcass (Preston and William, 1973).

Poultry meat is classified as either white (breast meat, has a high proportion of white fibers, high in glycogen and low in lipid content), or dark red (leg and thigh meat, has a high proportion of red fibers which are low in glycogen and high in lipid content) (Barbut, 2002).

Results reported by (Abdelbasit *et al.*, 2016b) revealed no significant differences between all groups in percentages of commercial carcass cuts (breast, drumstick, thigh and wing) when different levels of xylanase

enzyme was added to sorghum-groundnut cake based broiler diets, same finidings were confirmed by (Mariam *et al*., 2013)

Bin Baraik, (2010) and (Sayyazadeh *et al*., 2006) observed that there were no statistical differences in the percentage of commercial cuts (drumstick, breast, wing and thigh), these results also agreed with the recent results obtained by (Younis, 2013).

All above mentioned studies results, in addition to (Abdelbasit *et al*, 2016b) agreed with our experiment, which revealed no significant differences in commercial cuts (breast, drumstick, thigh and wing) percentage, moreover (Abdelrahim *et al*, 2018) found no significant differences ($p>0.05$) in the carcass parameters except for breast meat: bone ratio, which was higher ($p<0.05$) in diet with (750g added enzyme) compared to control and (500g added enzyme) to sorghum-Peanut meal based broiler feed, however the current study reported apparent significant ($p <0.10$) effect of feeding durations, heavier breast was yielded when added multi-enzymes was fed during the starter period only, thigh and drumstick showed slight numerical increase with increased enzyme level or feeding durations, this may be due to improved apparent metabolizable energy, and protein and NSP digestibility in birds fed on diets with supplementation of enzyme, which helped with better utilization of feedstuffs (Hosseini and Afshar, 2017).

On contrary, analysis of carcass characteristics data reported by (Giotee *et al*., 2015) showed that, only breast, thigh and liver were affected by ($P < 0.05$) the corn-soybean based dietary treatments of different metabolizable energy when supplemented by multi-enzymes, the maximum breast, thigh and liver weights were achieved with birds fed a diet containing 13.18 MJ/Kg ME and 500 mg multi-enzymes per kg of diet.

In conclusion, according to Tony *et al*., (2007), exogenous multi enzymes mixtures especially those containing protease could improve broiler performance, FCR, dressing weight and the breast muscle weight.

5.6. Plasma and serum constituents.

Hematological parameters reflects the influence of stress caused by environment, nutrition and pathological factors on animals health status (Afolabi, 2010), although enzymes' influence on blood constituents may explain the impact of enzymes on metabolic processes, contradictory results were found in the literature (Youssef *et al*., 2020b).

The different levels of multi-enzymes treated diets or their application during different broiler growing times in this experiment and their interactive action had no significant ($p>0.05$) effect on all blood determined hematological or serum biochemical parameters, these findings were in line with (Gidado *et al*., 2020) who reported that, enzyme supplemented to broiler diets based on corn-sorghum mixture had no impact on broiler blood profile, however, Hajati, (2010) study reported a significantly elevated concentration of blood glucose by enzyme supplemented corn-soybean based diet fed to Cobb 500, at 44 days of age, he also showed that adding multi-enzymes to broilers diet significantly increased the concentration of blood total cholesterol at 10, 28 and 44 days, whereas concentration of blood uric acid was reduced at 28 and 44 days ($p <0.05$), the enzyme preparation increased nutrient metabolism, particularly protein anabolism of birds, therefore, promoting the growth of chickens (Hajati, 2010).

Values of total serum Hb of broiler chicks obtained in this study, ranging from 7.50g/dl – 16.10g/dl, compared favorably with 7-13 g/dl reported by (Banerjee, 2009), current red blood cells haemoglobin

concentration indicates effective oxygen carrying capacity, thus experimental chicks were in a good health and nutritional status (Gidado *et al*., 2020), also normal blood Hb content suggested that, experimental diets contained adequate nutrients required for Hb synthesis, and supplementing of varying levels of multi-enzymes had no impact on Hb formation or nutrients transportation, total serum glucose ranging from 103g/dl-235g/dl on other hand reveal sufficient starch utilization and promoted glucose uptake in addition to amino acid and minerals.

The plasma glucose concentration was totally concurred with serum cholesterol content, birds fed in group, and closely concurred with increasing abdominal fat and meat lipid on the same mentioned experimental groups, this may explain the improved dietary lipids digestion and utilization of caused by multi-enzymes supplementation, lipids are the precursors for cholesterol and lipid enhanced utilization result in high cholesterol content in the blood

In general, our contrast analyses reported no significant effects from the level of applied multi-enzymes and/or application time frequency on the blood serum biochemistry and hematology, however HDL, LDL values showed a numerical increase with increased enzyme inclusion dose or prolonged feeding durations, while serum albumin revealed a reduced concentration when birds fed the multi-enzymes supplemented diet during the entire rearing period, compared to starter period only, these results corroborated with (Youssef *et al*, 2020b) reports (except for albumin) who found that, enzyme treatments increased the albumin, total lipids, HDL, compared to the un-supplemented control, but decreased serum globulin, furthermore differences between the enzymes' treatment and the un-supplemented control approached significant for total protein ($p < 0.054$).

Referring to (Youssef *et al*., 2020b), plasma total lipids were lower when enzymes were applied during days 22–37 of age than during the other times, the effect of time of application approached significant ($p < 0.063$) for serum triglycerides, with the highest being the 1–21 days group.

Based on (Dinani *et al*, 2017) study, dietary enzyme addition did not affect serum protein concentrations, continuous addition of enzymes resulted a decrease in plasma cholesterol, and there was also a decrease in LDL when enzymes were supplemented during days 1–21 of age, hence a beneficial effects due to multi-enzymes application on lipid metabolites were investigated (Youssef *et al*, 2020b), however, El-Katcha *et al*., (2014) found that, enzymes had no significant effect on of triglyceride and cholesterol concentrations compared with birds fed on the same diet without the addition of enzymes.

Normal serum creatinine, uric acid and urea concentrations reflects healthy liver and kidneys in terms of efficiency of protein retention (Swennen *et al*., 2005), moreover normal values indicate no toxic effect with feeding diets with added multi-enzymes, a balanced amino acid diets and consequently adequate energy utilization, high serum uric acid indicate excess amino-N and energy waste (Nworgu *et al.*, 2007), and moreover this has negative environmental impacts, creatinine, the biomarker of protein metabolism, its high level is associated with increased activity (Harr, 2002), creatinine is an excellent indicators of protein metabolism and kidney function (Ibrahim *et al*., 2012).

Values for serum calcium level was in agreement with values of 8-14 mg/dl reported previously by (Harr, 2002), and 3.3 - 3.8 mg/dl for serum phosphorous (Nazifi, 2011), were within normal ranges, which indicate adequate mineral absorption and retention, Pirgozliev *et al*., (2010)

confirmed a significantly enhanced retention of K, Mg, Mn, Na and S minerals, relative to the control diet, by feeding xylanase, but no significant changes in the daily retention of dietary Ca and P or the excretion of cations were detected.

Based on this study, liver leakage markers, alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were not significantly affected by multi-enzymes inclusion level or feeding durations, same results were cited by (Al-Harthi, 2017), however (Youssef *et al*., 2020b) and (Ahmed *et al*., 2007) recorded a significantly decreased (ALT) and (AST) with multi-enzymes supplementations at 0.5–1 mL/L drinking water, showing an improvement in liver leakage markers, normal AST and ALT serum values of broiler chicken, reflects healthy liver in terms of intact hepatocytes, this also reveals non-toxic or safety of using multi-enzymes supplemented diets for broiler.

5.7. Economic efficiency.

The objective of inclusion of exogenous enzymes in broiler diets, is to maximize nutrient utilization and reducing feed costs by improving dietary nutritional and energy levels, strategic use of phytases and xylanases can result in economic advantages if appropriate diet modification, with careful attention to the used feed ingredients is done, these advantages may be lost if matrix values (nutrient-equivalent values assigned to enzyme products in least cost formulation) are incorrectly assigned (Adeola and Cowieson, 2011).

In this study, the addition of exogenous multi-enzymes in the experimental feeds was done on top, without considering the nutritional contribution of activities of enzymes matrix.

Based on current study findings, the associated use of enzyme mixture addition, without joint application of nutritional matrices does not favor economic appraisal of broiler chicken, because of relatively high cost of commercial multi-enzymes, moreover, achieved performance improvements in terms of FI, BWG and FCR were not able to cover the extra cost of added multi-enzymes, however the starter feed cost was significantly reduced when supplanted multi-enzymes diets was fed during the whole growing period, this can be explained by the reduction in starter feed intake during the mentioned period.

A number of researches have reported positive responses to the addition of exogenous enzymes in terms of reduced diet cost, improved FCR, weight and egg weight (Mathlouthi *et al*., 2003; Saleh *et al*., 2005), these improvements in relative economic efficiency are due to reduced total feed costs for starter and grower diets and the high body weight gain of such treatments (Tony, 2007).

Mathlouthi *et al.,* (2002) stated that enzymes application maintain good performance on poorer quality feed, decreased formulation cost, widens range of raw materials and encourage better economic returns

Similar reports to our economic data were recorded by Ramesh and Devegowda, (2006), who found improved performance in terms of final body weight and feed conversion ratio after added commercial Allzyme which possess seven enzyme activities, on top of corn-soya bean diet fed to broilers, however the reduction in diet cost was obtained with diet reformulation by account for the equivalent energy(75 kcal/kg less of ME), and both available calcium and phosphorus content (reduced by 0.1 %), released by the multi-enzymes addition, the reduced diet cost did not affected either weight gain or feed efficiency.

In contrary to current data finding, Yohana, (2012) confirmed that, birds fed the Allzyme supplemented diet gave a better return compared to the control, while Nutrase supplemented diets causes a net loss, this loss according to (Yohana 2012), was due to low body weight gain, and low feed conversion ratio, during overall feeding phase, in addition, our experiment disagree with findings of (Kadam *et al.,* 1991) who achieved about 17 % more increased profit than control group, with groups fed on 100 mg/kg Roxazyme (glucanase, xylanase and cellulose and some traces of amylase, hemicellulases, pectinases and proteases) supplemented broiler feed, the better performance of Allzyme over Nutrase is due to its multiple enzymes content (β-glucanases, hemicellulases, amylases, proteases, lipase and pectinases) targeting different anti-nutritional factors (NSPs) of the feed ingredients broiler diets, while Nutrase Xylla is a single enzyme (Yohana, 2012).

More economic efficiency or profitability can be obtained by reformulation of basal diets with including the nutritional matrix of the product, with this application method, it is possible to reduce the inclusion of high-cost ingredients like the vegetable oils and soybean meal, this can allow a reduction in feed cost, with keeping the overall performance similar to that maintained with a standard diet (Guilherme *et al*., 2017), for example, metabolizable energy and digestible amino acids of soybean can be over-estimated by 5 to 9% when exogenous enzymes are included in diets for broilers (Garcia *et al*., 2000), however (Mahmoud, 2012) stated that, a positive response can be obtained by feeding birds a positive control diet supplemented with enzymes indicating that supplementation of xylanase, amylase, protease, and phytase over-the-top might be a nutritional and economical alternative to a reformulated approach.

Several studies reported an economic improvement of multi-enzymes supplementation to broiler feed, some of these studies were mentioned by (Mahmoud, 2012), Salem *et al.* (2008) observed 5% improved economic efficiency with addition of avizyme to the diet, compared to un-supplemented control diet, similar reports were demonstrated by El-Serwy *et al*., (2012), in addition, (Mutaz, 2019) advised to add exogenous enzymes to broiler diet after studying the effect of supplemented multi-enzymes to experimental sorghum based broiler diets with different levels of non-conventional plant protein sources to replace groundnut cake in his study, declared that, enhanced weight gain and improved FCR achieved by added multi-enzymes, were quite sufficient to cover the extra cost of enzyme supplementation, hence enzyme supplementation confirmed to be a positive and economical treatment with most of Sudanese local plant protein sources alternatives, so it will be.

Abou El–Wafa *et al*., (2005) reported an improved economic efficiency of avizyme added to corn-soybean broiler diets compared to the control group diet, also, Ghazalah *el al.*, (2005) found positive effect of dietary enzyme supplementation, in terms of reduced feed cost/Kg broiler meat and broiler chick economic efficiency, the degree of the economic effect depends considerably on the actual raw material and compound feed prices, as well as the sales per unit weight (Buhler and Limper, 2004).

CHAPTER SIX
Conclusion and Recommendations

6.1. Conclusion:

The effect of adding commercial multi-enzymes to broiler diet was studied, based on the hypothesis that, supplementation of feed with exogenous multi-enzymes, would improve performance, carcass characteristics, meat chemical quality, meat sensory parameters, and economic appraisal.

Bellow were the conclusions from this study:

1. The supplementation of broiler diets with NutriKEM Extend had beneficial effect on broiler performance, it improved the body weight and FCR and liver size, birds looked healthy and in a good condition.
2. It is also appeared that it had positive effect on fat and energy utilization.
3. Inclusion of NutriKEM Extend in broiler diets at various levels had no significant effects on carcass and carcass yield, subjective or objective meat quality, all scores being at above moderate values.
4. The supplementation of broiler diets with NutriKEM Extend decreased the total serum cholesterol, total protein and uric acid concentrations compared to control group, while the levels of calcium, phosphorus ALP and AST enzymes activities remained unchanged.
5. Inclusion of exogenous multi-enzymes improved the net returns per bird (96.76 SDG/bird), and economic efficiency score (55.92%), compared to control birds (86.80 SDG/bird net profit), and economic efficiency score (48.32%) fed on basal diets without added multi-enzymes.
6. Raw materials used to formulate broiler diets, would impact the choice of commercial exogenous multi-enzymes, in terms of enzymes included and their concentrations.

7. Based on current studies, NutriKEM Extend economically feasible dose was 1,000 g per 1,000 Kg feed when offered during the finisher period only, whereas the recommended manufacturer dose is 500 g per 1,000 Kg of feed to be fed during the whole period.

6.2. Recommendations:

6.2.1. Practical Implication:

Based on these studies findings, it is recommended:

1- To carry out more detailed investigations to determine types, percentages and structure of NSPs and anti-nutritional factors found in local ingredients, cereals and oil seed grains by-product used in feed.

2- Appropriate multi-enzymes combination blend and concentration of optimum cost can be formed to improve digestion and absorption of nutrients from commonly used feed ingredients in Sudan.

3- Expanded availability of other non NSPs enzymes such as lipases, proteases, amylases, etc., to optimize digestion and absorption of local feed ingredients, cereals and oil seed grains by-products used in Sudan.

4- Conducting more economical studies to evaluate if performance and meat quality improvement could cover the extra cost of added commercial multi-enzymes.

5- Conducting studies by reformulation of basal diets with including the nutritional matrix of the product, to investigate if more economic efficiency or profitability can be obtained.

6.2.2. Suggestions for future research:

Based on the finding of present study, further researches are needed to confirm these results in commercial grandparent, parent, breeders and layers to investigate the effects of multi-enzymes on flock health, fertility, hatchability, DOC quality, egg yield, egg quality and economic feasibility.

References:

Abdelbasit B. Habib (2016a). Development of poultry industry in Sudan, Scholars press, Germany.

Abdelbasit B. Habib, Abdelrahim A. Mohamed, Ahmed M. Eltrifi, Egbal S. Abu shulukh, and Abubaker A. Abubaker (2016b). Effect of feed Supplemented with Xylam Enzyme on Performance, Carcass Characteristics and Meat Quality of Broiler Chicks, *Journal of Applied Veterinary Sciences*, 1(1): 15-20.

Abdelrahim Abubakr Mohammed, Abdelbasit Basheer Habib, Ahmed Mukhtar. Eltrefi, Egbal Sulaiman Abu Shulukh and Abubaker Ahmed Abubaker (2018). Effect of different levels of multi-enzymes (Natuzyme Plus®) on growth performance, carcass traits and meat quality of broiler chicken. Asian J. Anim. Vet. Adv., 13.

Abou El-Wafa, S. El-Husseiny O.M., and Shabaan M. (2005). Influence of microbial phytase and energy levels on broiler performance fed low-phosphorus diets. 3rd International Poultry Conference 4-7 Apr. 2005 Hurghada - Egypt.

Acamovic T. (2001). Commercial application of enzyme technology for poultry production. World's Poultry Science Journal, 57:3: 225-242.

Acamovic T. and McCleary B.V. (1996). Optimizing the response. Feed Mix, 4.

Adams M.J. and Kelly R.M. (1998). Finding and using hyperthermophilic enzymes. *Trends inBiotechnology* 16, 329–332.

Adebiyi O.A., A.D. Ologhobo, O.A. Adu and T.O. Olasehinde (2010). Evaluation of the nutritional potentials of physically treated cowpea seed hulls in poultry feed. Emir. J. Food Agric. 22:232-239.

Adeola O., and Cowieson A.J. (2011). BOARD-INVITED REVIEW: opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. J. Anim. Sci. 89(10):3189-218.

Afolabi S.B. (2010). Hematological parameters of the Nigerian local grower chickens fed varying dietary level of palm kernel cake. Proceeding of the 35th annual conference of Nigerian society for Animal Production held at University of Ibadan Oyo State Nigeria. 247 - 252.

Ahmed E.M., and Applewhite J.A. (1988). Characterization of trypsin inhibitor in Florunner peanut seeds (*Arachis hypogaea*L.). Peanut Sci., 15 (2): 81-84.

Ahmed E.M. (1986). Lectin quantitation in peanut and soybean seeds. Peanut Sci., 13 (1): 4-7.

Ahmed S., Rashid M., Lucky N., Ahmad N., and Myenuddin M. (2007). Effect of enzyme and vitamin supplementation on physio-biochemical parameters in broiler chickens. Bangladesh J. Vet. Med. 5, 55–58.

Ai Q., K. Mai, W. Zhang, W. Xu, B. Tan, C. Zhang, and H. Li (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, Lateolabrax japonicas. Comp. Biochem. Physiol. A MolIntegr. Physiol. 147.

Alexander S.P.H., Mathie A., and Peters J.A. (2009). Guide to Receptors and Channels (GRAC), 4th Edition. *Br J Pharmacol* 158: S1–S239.

Al-Harthi M. (2017). The effect of olive cake, with or without enzymes supplementation, on growth performance, carcass characteristics, lymphoid organs and lipid metabolism of broiler chickens. Rev. Bras. Cienc. Avic.19, 83–90.

Ali S.A.M., H.O. Abdalla and M.A. Abasaid (2011). Sunflower meal as an alternative protein source to groundnut meal in laying hens rations. Egypt. Poult.Sci, 31: 745-753.

AllAboutFeed (2018). Feed enzymes to grow fastest in poultry News *27 Jun 2018*, Misset Uitgeverij B.V.

Al-Marzooqi and Leeson S. (1999). Evaluation of dietary supplements of lipase, detergent, and crude porcine pancreas on fat utilization by young broiler chicks. Poult Sci. 78:1561–1566.

Al-Marzooqi, and Leeson S. (2000). Effect of dietary lipase enzyme on gut morphology, gastric motility, and long-term performance of broiler chicks. *Poult Sci*, (79): 956–960.

Amal M., H. Mohamed, K.A., and Mukhtar A. (2015). Comparative study to evaluate nutritive value of maize and sorghum (feterita) with or without commercial enzyme (xylam) in broiler diets. Jornal of global biosciences 4, 5: 2296-2303.

Amitava R., Sudipto H., Souvik M., and Tapan K.G. (2010). Effects of supplemental exogenous emulsifier on performance, nutrient metabolism, and serum lipid profile in broiler chickens. Vet Med Int.2010:9.

Ana I., Mireles-Arriaga, Enrique Espinosa-Ayala, Pedro A., Hernández-García, and Ofelia Márquez-Molina (2015). Use of Exogenous Enzyme in Animal Feed**,** Life Science Journal 12 (2s).

Angel R., Saylor W.W., Mitchell A.D., Powers W., and Applegate T.J. (2006). Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poultry Science, 85: 1200-1211.

AOAC (1995). Association of Official Analytical Chemists. The official method of analysis. 16th Edition: Washington, DC.

AOAC (2000). Association of Official Analytical Chemists. The official method of analysis. 17th Edition: Gaithersburg, MD, USA.

AOAC (2010). Association of Official Analytical Chemists. The official method of analysis. 18th Edition: Washington, DC.

Apajalahti J., Kettunen A., and Graham H. (2004). Characteristics of the gastrointestinal microbial communities, with special reference to chickens. World's Poult. Sci. J. 60:223–232.

Applegate T.J., Joern B.C., Nussbaum-Wagler D.L., and Angel R. (2003). Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poultry Science, 82.

Assi J.A., and King A.J. (2007). Assessment of selected antioxidants in tomato pomace subsequent to treatment with the edible oyster mushroom, *Pleurotusostreatus*, under solid-state fermentation. *Journal of Agricultural and food chemistry.*

Attia Y.A., Al-Khalaifah H., Abd El-Hamid H.S., Al-Harthi M.A., and El-shafey A.A. (2020). Effect of Different Levels of Multi-enzymes on Immune Response, Blood Hematology and Biochemistry, Antioxidants Status and Organs Histology of Broiler Chicks Fed Standard and Low-Density Diets. Front. Vet. Sci. 6:510.

Australian Federal Environment Agency (2002). Collection of information on enzymes,

Aviagen (2015). Ross broiler pocket guide. http://eu.aviagen.com (Retrieved August 20, 2017).

Azman M.A., Konar V., and Seven P.T. (2004). Effects of different dietary fat sources on growth performances and carcass fatty acid composition of broiler chicken. Rev. Med. Vet. 5:278–286.

Babiker M.S. (2012). Chemical Composition of Some Non-Conventional and Local Feed Resources for Poultry in Sudan, International Journal of Poultry Science 11 (4): 283-287.

Babiker M.S., C. Kijora, S.A. Abbas and J. Danier (2009). Nutrient composition of main poultry feed ingredient used in Sudan and their variations from local standard table's values. Int. J. Poult. Sci., 8: 355-358.

Bach Knudsen K.E. (2014). Fiber and non-starch polysaccharide content and variation in common crops used in broiler diets. Poult. Sci. 93:1–14.

Bailey R.W. (1973). Structural carbohydrates. In: Butler, G., W., Bailey, R., W., editors. Chemistry and biochemistry of herbage, vol. 1. New York: Academic Press.

Balges Abd Allah Ibrahim (2012). The effect of β-glucanase inclusion in sorghum based diet on growth performance of broiler chicks. (Master's thesis and Dissertations Master). Omdurman Islamic University, Sudan.

Balint Földesi (2019). Guide to Enzyme Unit Definitions and Assay Design, https://www.biomol.com/resources/biomol-blog/guide-to-enzyme-unit-definitions-and-assay-design

Banerjee G.C. (2009). A Textbook of Animal Husbandry 8th ed. Oxford and IBH Publishing Co. PVT. Ltd. New Delhi India. 2009. 118 – 139.

Barbut S. (2002). Poultry Products Processing (An Industry Guide). CRC Press LLC, Boca Raton, FL.

Barrett A.J. (1994). Classification of peptidases. Methods of Enzymology 244.

Beauchemin K.A., Colombatto D., Morgavi D.P., and Yang W.Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81 (E. Suppl.), E37–E47.

Bedford M.R., and A.J. Cowieson (2019). Matrix values for exogenous enzymes and their application in the real world, Poultry Science Association Inc.

Bedford M.R., and A.J. Cowieson (2012). Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Technol. 173:76–85.

Bedford M.R., and Partridge G. (2011). Enzymes in Farm Animal Nutrition, 2nd edition. CAB International, Wallingford, Oxon, UK.

Bedford, M.R. and H.L. Classen (1992). Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentrations is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122:560–569.

Bedford M.R., and H. Schulze (1998). Exogenous enzymes in pigs and poultry. Nutr. Res. Rev. 11:91–114.

Bedford, M.R. (1996). Interaction between ingested feed and the digestive system in poultry. J.Appl. Poult. Res. 5: 86 – 95.

Best P., and Gill C. (1999). Post-pelleting micro-liquid application in Britain. *Feed International* 20:22-32.

Beudeker R.F. and Pen J. (1995). Development of plant seeds expressing phytase as a feed additive. In: *2nd European Symposium on Feed Enzymes, Noordwijkerhout,* pp. 225-235.

Bin Baraik B.S. (2010). Effect of adding xylanase and phytase enzymes to broiler diets on performance and carcass yield and quality, Ph.D. Thesis, Sudan University of Science and Technology, Khartoum, Sudan.

Bugg, T.D. (2004). Introduction to enzyme and coenzyme chemistry, 2nd edition, Blackwell Publishing.

Buhler and J. Limper (2004). Enzymes in animal nutrition, AWT (Arbeitsgemeinschaft für Wirkstoffe in der Tierernährung e. V.).

Burdock G.A., Carabin I.G., and Griffiths J.C. (2006). The importance of GRAS to the functional food and nutraceutical industries. *Toxicology*, vol. 221, no. 1.

Burnett G.S. (1962). The effect of damaged starch, amylolytic enzymes, and proteolytic enzymes on the utilization of cereals by chickens.Br. Poult. Sci.3.

Cadogan D.J., P.H. Selle, D. Creswell, and G. Partridge (2005). Phytate limits broiler performance and nutrient digestibility in sorghum based diets. Proc of the 17th Australian Poultry Science Symposium, Sydney, New South Wales, Australia.

Carlos Simões Nunes and Vikas Kumar (2018). Enzymes in Human and Animal Nutrition Principles and Perspectives, 1st edition, UK, Academic Press.

Carvajal J.G., K.A. Rincon and E.O. Oviedo-Rondon (2010). Protease improves performance and energy utilization in broilers fed diets with high tannin sorghum. Poult. Sci. 91:116-116.

Castellini C., Mugnai C., and Dal Bosco A. (2002). Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60, 219–225.

Chambers E., Jane A., and Bowers A. (1981). Statistical Designs and Panel Training / Experience for Sensory Analysis, Journal of Food Science Volume 46, Issue 6 p. 1902-1906.

Charles C., Worthington, Von Worthington, and Andrew Worthington (2019). Introduction to enzymes, Worthington Biochemical Corporation.

Chauynarong N., P. Iji, S. Isariyodom, M. Choct, and L. Mikkelsen (2007). Change in body weight and ovary weight of pre-lay pullets fed maize-soy based diet supplemented with an exogenous microbial enzyme. Kasetsart J. 41:49-55.

Chiba L.I. (2001). Proteins supplements. In: A.J. Lewis and L.L. Southern (eds.) Swine nutrition (second edition). p 35. CRC Press LLC, Boca Raton London New York Washington, D.C.

Cho J.H., P.Y. Zhao and I.H. Kim (2012). Effects of emulsifier and multi-enzyme in different energy density diet on growth performance, blood profiles and relative organ weight in broiler chickens. J. Agric. Sci.

Choct M. (1997). Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance, *Feed Milling International, June Issue.*

Choct M. (2001). Enzyme supplementation of poultry diets based on viscous cereals. In: *Enzymes in Farm Animal Nutrition* (Bedford, M., R., and Partridge, G., G, editors), pp. 145-160. CABI publishing, United Kingdom.

Choct M. (2006). Enzymes for the feed industry, past, present and future. *World poultry science journal*. 6:5-15.

Choct M. and G. Annison (1992). The inhibition of nutrient digestion by wheat pentosans. British Journal of Nutrition 76:123–132.

Choct M., Hughes R.J., Wang J., Bedford M.R., Morgan A.J., and Annison G. (1996). Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poultry Sci. 37.

Cobb500™ (2018). Broiler Performance and Nutrition Supplement. L-2114-08 EN:April 2018.

Cofrades S., Guerra M.A., Carballo J., Fernández-Martín F., and Jiménez-Colmenero F. (2000). Plasma protein and soy fiber content effect on Bologna sausage properties as influenced by fat level. *Journal of Food Science*, vol. 65.

Collett S.R. (2012). Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. 173:65–75.

Cordain L. (1999). Cereal grains: humanity's double edged sword. World Rev Nutr Diet; 84:19-73.

Cosgrove D.J., and Irving G.C.J. (1980). Inositol Phosphates: Their Chemistry, Biochemistry and Physiology. Elsevier Scientific Publication, Amsterdam.

Costa B.R., Miller G.M., Pesti R.I., Bakalli and H.P. Ewing (2001). Studies on Feeding Peanut Meal as a Protein Source for Broiler Chickens, Poultry Science 80:306–313.

Cowieson A. J. (2005). Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol., 119, pp. 293-305.

Cowieson A. J. (2010). Strategic selection of exogenous enzymes for corn/soy-based poultry diets. Poultry Science 47:1-7.

Cowieson A. J. (2014). The geometric framework: An in vivo approach, Journal of applied poultry research, V 23 (2), 295-300.

Cowieson A.J., Acamovic T., and Bedford M.R. (2004). The effect of phytase and phytate on endogenous losses from broiler chickens Br. Poult. Sci., 45, pp. 101-108

Cowieson A. J., and V. Ravindran (2007). Effect of phytic acid and microbial phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. Br. J. Nutr. 98.

Cowieson, A. J., and V. Ravindran (2008). Sensitivity of broiler starters to three doses of an enzyme cocktail in maize-based diets. Br. Poult. Sci. 49.346.

Cowieson, A.J., M.R. Bedford and V. Ravindran (2010). Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Brit. Poult. Sci. 51:246-257.

Cowieson A.J., M.R. Bedford, P.H. Selle, and V.Ravindran (2009). Phytate and microbial phytase: Implications for endogenous nitrogen losses and nutrient availability. World's Poult. Sci. J. 65:401–418.

Daghir N.J. (2008). Poultry production in hot climates. 2nd Edition. CAB international.

Dalgetty D.D., and Baik B.K. (2003). Isolation and Characterization of Cotyledon Fibers from Peas, Lentils, and Chickpeas. Cereal Chemistry, 80: 310-315.

Danicke S. (2001). Interaction between cereal identity and fat quality and content in response to feed enzymes in broilers. Bedford, M., R., and Partridge, G., G., editors. Enzymes in farm animal nutrition. Wallingford, UK: CABI, Pub.

David Gonzalez (2016). A feed determined multi-enzyme approach, Enzymes.

David Serene (2017). Are your enzymes really active?

De la Mare M., O. Guais, E. Bonnin, J. Weber, and J.M. Fran (2013). Molecular and biochemical characterization of three GH62 α-I- 383 arabinofuranosidases from the soil deuteromycete *Penicillium funiculosum*. Enz. Microb. Techno. 53:351–358.

Deli Nazmín Tirado-González, Luis Alberto Miranda-Romero, Agustín Ruíz-Flores, Sergio Ernesto Medina-Cuéllar, Rodolfo Ramírez-Valverde and Gustavo Tirado-Estrada (2018). Meta-analysis: effects of exogenous fibrolytic enzymes in ruminant diets, Journal of Applied Animal Research.

Denbow D.M., Grabau E.A., Lacy G.H., Kornegay E.T., Russell D.R., and Umbeck P.F. (1998). Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. *Poultry Science* 77.

Dersjant-Li Y., A. Awati, C. Kromm, and C. Evans (2014). A direct fed microbial containing a combination of 3 - strain *Bacillus* sp. Can be used as an alternative to feed antibiotic growth promoters in broiler production. J. Appl. Anim. Nutr.Vol.2:e11.

Diaw M.T., Dieng A., Mergeai G., Dotreppe O., Youssouf I., and Hornick J.L. (2010). Effect of groundnut cake substitution by glandless cottonseed kernels on

broilers production: animal performance, nutrient digestibility, carcass characteristics and fatty acid composition of muscle and fat. Int. J. Poult. Sci., 9 (5).

Dida M.F. (2016). Review paper on enzyme supplementation in poultry ration. *International Journal of Bioorganic Chemistry*, (1)1: 1-7.

Dinani O.P., Tyagi P.K., Mandal A.B., and Jaiswal S.K. (2017). Effect of feeding fermented guar meal vis-àvis toasted guar meal with or without enzyme application on immune response, caeca micro flora status and blood biochemical parameters of broiler quails international. *J. Pure App. Biosci*. *5*, 624–630.

Dixon M., and Webb E.C. (1964). Enzymes. 2nd edition, Academic Press, New York.

Doherty C., Faubion J.M., and Rooney L.W. (1982). Semi-automated determination of phytate in sorghum and sorghum products. *Cereal Chemistry* 59.

Dominguez A.D., A.C. Cuevas, B.F. Martinez, C.L. Coello, and A.E. Gonzalez (2009). Effect of supplementing an enzyme mixture in sorghum-soybean meal diets on apparent ileal amino acid and protein digestibility, metabolizable energy, and productivity in broilers. Tecnica Pecuaria en Mexico 47:15-29.

Doskovic V., Snezana B.B., Zlatica B., Zdenka S., Rakonjac S., and Petriceevic V. (2015). Effect of dietary protease supplementation on carcass weight and dressing percentage of broilers. Proceeding of the 4th International Congress 'New Perspectives and Challenges of Sustainable Livestock Production", October 7-9, Belgrade, 574-581.

Douglas M.W., Parsons C.M., and Bedford M.R. (2000). Effect of various soybean meal sources and Avizyme on chick growth performance and ileal digestible energy. J. Appl. Poultry Res.9: 74 – 80.

Doumas B.T., Watson D., and Biggs H.G. (1977). Albumin standers and the measurement of blood albumin with bromocisol green .clin. chem. Acta. 31:87.

Duncans D.B. (1955). Multiple ranges and multiple F-tests. Biometrics, (11): 1-42.

Eilers R.J. (1967). Notification of final adoption of an international method and standard solution for hemoglobinometry specifications for preparation of a standard solution. Am. J. Gun. Path. 47: 212-214.

El-Boushy A.R. and Ratherink R. (1989). Replacement of soybean meal by cottonseed meal and peanut meal or both in low energy diets for broilers. Poult. Sci., 68 (6): 799-804.

El-Katcha M.I., Soltan M. A., El-Kaney H.F. and Karwarie, E.R. (2014). Growth performance, blood parameters, immune response and carcass traits of broiler chicks fed on graded levels of wheat instead of corn without or with enzyme application. *Alex. J. Vet. Sci.40*, 95–111.

Elsayed O.S. Hussein, Gamaleldin M. Suliman, Alaeldein M. Abudabos, Abdullah N. Alowaimer, Shamseldein H. Ahmed, Mohamed E. Abd El-Hack, Mahmoud Alagawany, Ayman A. Swelum, Antonella Tinelli, Vincenzo Tufarelli, and Vito Laudadio (2019). Effect of a low-energy and enzyme-supplemented diet on broiler chicken growth, carcass traits and meat quality, Arch. Anim. Breed., 62, 297–304.

El-Serwy, Amina A., Shoeib M., and Fathey I.A. (2012). Performance of broiler chicks fed mash or pelleted diets containing corn-with-cobs meal with or without enzyme supplementation. J. Animal and poultry prod., Mansoura Univ., Vol.3 (3).

Emmans G.C. (1994). Effective energy: a concept of energy utilization applied across species. Br. J. Nutr., 71: 801-821.

Emmerson D.A. (1997). Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. *Poult Sci.* 76:1121–1125.

Englyst H. (1989). Classification and measurement of plant polysaccharides. Anim. Feed Sci. Technol. 23: 27-42.

Enzyme Nomenclature (1992). Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Academic Press, San Diego, CA.

Expert Market Research (2019). Global Feed Enzymes Market Report and Forecast 2019-2024.

Fanatico A.C., Pillai P.B., Emmert J.L., and Owens C.M. (2007). Meat quality of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 86.

FAO (2004). Protein sources for the animal feed industry. Bangkok: FAO Anim Prod and Health (Proc) Exp Consult and Workshops.

FAO (2009). How to Feed the World in 2050. Rome, Italy.

FAO (2020). World Food and Agriculture, Statistical Yearbook 2020. Rome.

Farrell D.J., and E.A. Martin (1998). Strategies to improve the nutritive value of rice bran in poultry diets. I. The addition of food enzymes to target the non-starch polysaccharide fractions in diets of chickens and ducks gave no response. Br. Poult. Sci. 39:549-554.

Feed Enzymes Market by Livestock, by Types & by Geography - Global Trends & Forecasts to 2019. Research and Markets, Dublin.

Felton (2017). Animal Feed Enzymes Market to Witness Enhanced Usage in Poultry and Ruminant Applications Till 2025, Million Insights.

Filer K. (2003). Industrial Production of Enzymes for the Feed Industry, New Horizons in Biotechnology.

Fletcher D.L. (2002). Poultry meat quality. Worlds Poult. Sci. J., 58, 131–145. *Food Chemistry*, vol. 55, no. 22, p. 9095-9098.

Fry R.E., Allred J.B., Jensen L.S., and McGinnis J. (1958). Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poult. Sci. 37:372-3'75.

Gabriel U.U., Akinrotimi O.A., Bekibele D.O., Onunkwo D.N., and Anyanwu P.E. (2007). Locally produced fish feed: potentials for aquaculture development in sub-Saharan Africa. *Afr. J. Agr. Res.*, 2(7).

Garcia E.R.M., Murakami A.E., Branco A.F., Furlan A.C., and Moreira I. (2000). Effect of enzyme supplementation in diets with soybean meal and extruded whole soybeans on nutrient digestibility, nutrient flow in ileal digesta and broiler performance. Brazilian Journal of Animal Science, 29(5), 1414-1426.

Ghazalah A.A., Abd EI-Gawad A.H., Soliman M.S., and Youssef Amany W. (2005). Effect of enzyme preparation on performance of broilers fed corn-soybean meal based diets. Egypt. Poult. Sci. J. 25: 295-316.

Ghosh P.K., Saxena R.K., Gupta R., Yadav R.P., Davidson S. (1996). Microbial lipases: production and applications. Sci Prog; 79:119-57.

Gidado A.S., Oguntoye M.A., and Akintunde A.R. (2020). Growth performance, nutrient digestibility and serum biochemical indices of finisher broiler chickens fed varying levels of sorghum in replacement of maize with the supplementation of exogenous enzymes, Nigerian *J. Anim. Sci. 2020 Vol 22 (2):81-89.*

Giotee A., H. Janmohammadi, A. Taghizadeh and S.A. Rafat (2015). Effects of a multi-enzyme on performance and carcass characteristics of broiler chickens fed corn-soybean meal basal diets with different metabolizable energy levels, Journal of Applied Animal Research, 43:3.

Gracia M.I., Aranibar M.J., Lazaro R., Medel P. and Mateos G.G. (2003). Alpha-amylase supplementation of broiler diets based on corn. Poult. Sci. 82:436–442.

Greiner R. (2002). Purification and characterization of three phytases from germinated lupin seeds (Lupinus albus var.amiga). J. Agric. Food Chem. 50.

Greiner R., and Konietzny U. (2006). Phytase for food applications. Food Technol. Biotechnol., 44(2): 125-140.

Guilherme Aguiar Mateus Pasquali, Raphael Fortes de Oliveira, Paula Andressa Bedim Aiello, Gustavo do Valle Polycarpo, Rafael Crivellari and Valquíria Cação da Cruz-PolycarpoActa (2017). Performance and economic viability of broiler chicken fed diets with multi-enzyme complexes Scientiarum. Animal Sciences, Maringá, v. 39, n. 1, p. 91-96.

Hadden (2018). A Technical update on the use of enzymes in animal feed, global services ABVISTA. Hahn-Didde D., Purdum S.E., The effects of an enzyme complex in moderate and low nutrient-dense diets with dried distiller's grains with soluble in laying hens. Journal of Applied Poultry Research 2014; 23:23–33.

Hahn-Didde D., and Purdum S.E. (2014). The effects of an enzyme complex in moderate and low nutrient-dense diets with dried distiller's grains with soluble in laying hens. Journal of Applied Poultry Research; 23:23–33.

Hajati H. (2010). Effects of Enzyme Supplementation on Performance, Carcass characteristics, Carcass Composition and Some Blood Parameters of Broiler Chicken. American Journal of Animal and Veterinary Sciences 5 (3): 221-227.

Hamaker B.R., A.A. Mohamed, J.E. Habben, C.P. Huang, and B.A. Larkins (1995). An efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method, Cereal Chemistry 72.

Han Y.W. (1988). Removal of phytic acid from soybean and cottonseed meals. Journal of Agricultural and Food Chemistry, 36: 1181-1183.

Hancock J.D. (2000). Value of sorghum and sorghum co-products in diets for livestock. In: sorghum origin, history, technology and production, Smith W., and R.A. Fredericksen (Eds.). Wiley series crop science, New York.

Harr K.E. (2002). Clinical chemistry of companion avian species: A review. Vet. Clin. Path, 31: 140-151.

Hascik P., Čuboň J., and Vagač V. (2004). Evaluation of sensory quality of poultry meat due to probiotic preparation IMB 52. *Maso*, vol. 40.

Haslam E. (1981). Vegetable tannins. The biochemistry of plants. Vol. 7, Academic Press, New York, pp. 527-544.

Henry R.J., D.C. Cannon, and J.W. Winkelman (1974). Clinical chemistry, principles and techniques Harper and Row, 2nd Edition. Br. Poult. Sci. 40:348- 352.

Heuzé V., Thiollet H., Tran G., Bastianelli D., and Lebas F. (2016). *Peanut meal*. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/699*Last updated on October 4, 2016, 10:25*

Hodallah H. Ahmed, N.S. El-Toukhey, K.A. Attia, and Salma I. El-Samannoudy (2013). Effect of Multi-enzymes and Absorption Enhancers on Productive Performance, Gut Morphology and Some Blood Biochemical and Hormonal Parameters in Broiler Chicks; Journal of Agricultural Science; Vol. 5, No. 12.

Hodges R.J., Hall A.J., Jayaraj K., Yoganand B., Jaiswal P., Potdar N., and Navi S.S. (2000). Quality changes in farm-stored grains grown in the wet on dry season I south India. Pages 57-60. In: *Proceedings of workshop on Sorghum Utilization on the livelihoods of the poor in India*, 4-5 Feburary 1999 (Hall, A.J and Yoganand, B. eds) International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.2000.

Hosseindoust A., Lee S., Gook Nho W., Song Y.H., Shin J.S., and Laxman Ingale S. (2019). A dose–response study to evaluate the effects of pH-stable β-mannanase derived from Trichoderma citrinoviride on growth performance, nutrient retention, and intestine morphology in broiler chickens. Ital J AnimSci. 18:147–54.

Hosseini, S.M., and Afshar M. (2017). Effects of feed form and xylanase supplementation on performance and ileal nutrients digestibility of heat-stressed broilers fed wheat-soybean diet, J. Appl. Poult. Res., 45, 550–556.

http://dx.doi.org/10.3382/ps/pex226

http://jas.fass.org/content/early/2011/04/21/jas.2010-3715

http://www.sbcs.qmul.ac.uk/iubmb/enzyme/

https://doi.org/10.4060/cb1329en

https://www.biomol.com/resources/biomol-blog/guide-to-enzyme-unit-definitions-and-assay-design

https://www.enzyme-database.org/

https://www.nutrispices.com/single-post/are-your-enzymes-really-active

https://www.poultryworld.net/Nutrition/Articles/2017/6/Lower-your-feeding-costs-with-lysolecithins-143775E/

Humer E., C. Schwarz, and K. Schedle (2014). Phytate in pig and poultry nutrition, Journal of Animal Physiology and Animal Nutrition

Hu Q.N, Zhu H., Li X., Zhang M., and Deng Z. (2012). Assignment of EC Numbers to Enzymatic Reactions with Reaction Difference Fingerprints. PLoS ONE 7(12).

Huisman J., and Tolman G.H. (1992). Anti-nutritional factors in the plant proteins of diets for non-ruminants. In: Garnsworthy, P.C., Haresign, W. and Cole, D.J.A. (eds) *Recent Advances in Animal Nutrition*. Butterworth-Heinemann, Oxford.

Huo G.C., Fowler V.R., Inborr J., and Bedford M.R. (1993). The use of enzymes to denature anti-nutritive factors in soybean. *Proceedings of the 2nd International Workshop on ANFs in Legume Seed,* p.60, Wageningen, The Netherlands.

Ibrahim Albokhadaim, Thnaian Althnaian, and S.M. El-Bahr (2012). Investigation of Selected Biochemical Parameters of Local Chickens with Different Age and Sex in Al-ahsa, Saudi Arabia, Pakistan Journal of Biological Sciences 15 (17): 827-832.

Ilaria Proietti (2012). Impact of food processing on tannins and phytic acid activities in sorghum porridge: integrated *in vitro* and analytical approaches, Ph.D thesis, Unversita Cattolica.

Innova (2014). Guide to enzyme unit definitions and assay design, DSM.

Institute of Food Technologists, Sensory Evaluation Division (1981). Sensory evaluation guide for testing food and beverage products.Food Technol. 35:50–59.

International Organization for Standardization (ISO8586:2012). Sensory analysis, General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors.

Izeldin A. Babiker (2015). Animal feed industry in Sudan, current status, problems and prospects, Journal of Dairy, Veterinary & Animal Research, Vol. 2 (3).

Jahan K., Paterson A., and Piggott J.R. (2005). Sensory quality in retailed organic, free range and corn-fed chicken breast. Food Res. Int. 38, 495–503.

Jansen M. (2015). Modes of action of lysophospholipids as feed additives on fat digestion in broilers. KU Leuven, Leuven.

Jensen L.S., Fry R.E., Allred J.B., and McGinnis J. (1957). Improvement in the nutritional value of barley for chicks by enzyme supplementation, Poult. Sci.36.

Joaquín I.A.Z., and A. Paulino (2019). Carbohydrases are the Enzymes of the Future in Animal Feed, Engormix.

Kadam A.S., Ranade A.S., Rajmane B.V., Dange S.H., and Patil S.S. (1991)**.** Effect of enzyme supplementation on the performance of broilers; *Poultry Advisor*. 24(11).

Kaufman R.C., Herald T.J., Bean S.R., Wilson J.D., and Tuinstra M.R. (2013). Variability in tannin content, chemistry and activity in a diverse group of tannin containing sorghum cultivars. *Journal of Science Food Agriculture*, 93: 1233-1241.

Kemin Europa N.V. (2016). Benefits of KEMZYME® PLUS as a multi-enzyme system derived from multiple microbial fermentations, TL-16-00032 ®™ Trademarks of Kemin Industries, Inc., U.S.A.

Kemin Europa N.V. (2017). Demonstration of the added value of the amylase and protease activity in NutriKEM ™ DRY.

Khattab H.M., Gado H.M., Kholif A.E., Mansour A.M., and Kholif A.M. (2011). The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Inter J Dairy Sci. 6:267-277.

Khattak F.M., Pasha T.N., Hayat Z., and Mahmud A. (2006). Enzymes in poultry nutrition. *J. Anim. Pl. Sci*, (16): 1-2.

Kiarie E.L., F. Romero, and C.M. Nyachoti (2013). The role of added feed enzymes in promoting gut health in swine and poultry. Nutr. Res. Rev. 26.

Kluntner A.M., Devaud A., and Wolker L. (1995). Influence of liquid feed enzymes on performance and nutrient retention of broiler chickens fed a cereal diet. In: *2nd European Symposium on Feed Enzymes, Noordwijkerhout*.

Knudsen K.E. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology, 67: 319-338.

Konietzny U., and Greiner R. (2002). Molecular and catalytic properties of phytate-degrading enzymes (phytases). Int. J. Food Sci. Technol., 37.

Kornegay E.T. (2001). Digestion of Phosphorus and Other Nutrients: The Role of Phytases and Factors Influencing their Activity.CAB International, London.

Kriegshauser T.D., M.R, Tuinstra and J.D., Hancock (2006). Variation in Nutritional Value of Sorghum Hybrids with Contrasting Seed Weight Characteristics and Comparisons with Maize in Broiler Chicks. Crop Sci 46.

Krogdahl A., and Sell J.L. (1989). Influence of age on lipase, amylase and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poultry Sci. 68: 1561 – 1568.

Kumar V., Sinha A.K., Makkar H.P., de Boeck G., and Becker K. (2012). Dietary roles of non-starch polysachharides in human nutrition: a review. Crit Rev Food Sci Nutr ; 52:899-935.

Lawless H.T., and Appendix A. (2013). Overview of sensory evaluation. In Quantitative Sensory Analysis: Psychophysics, Models and Intelligent Design; Wiley–Blackwell: Chichester, UK, pp. 361–376.

Lee K.W., Li G.X., Lillehoj H.S., Lee S.H., Jang S.I., Babu U.S., Lillehoj E.P., Neumann A.P., and Siragusa G.R. (2011). Bacillus subtitle's-based direct-fed microbials augment macrophage function in broiler chickens. Res. Vet. Sci. 91.

Lee K.W., Lillehoj H.S., Jang S.I., Lee S.H., Bautista D.A., and Siragusa G.R. (2013). Effect of Bacillus subtitle's-based direct-fed microbials on immune status in broiler chickens raised on fresh or used litter. Asian Australas J Anim Sci 26.

Lee K.W., S.H. Lee, H.S. Lillehoj, G.X. Li, S.I. Jang, U.S. Babu, M.S. Park, D.K. Kim, E.P. Lillehoj, A.P. Neumann, T.G. Rehberger, and G.R. Siragusa (2010). Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. Poult. Sci. 89:203–216.

Liu N., Wang J.Q., Gu K.T., Deng Q.Q., andWang J.P. (2017). Effects of dietary protein levels and multi-enzyme supplementation on growth performance and markers of gut health of broilers fed a miscellaneous meal based diet. Anim Feed Sci Technol. 234:110–7.

Liu Y.G. (2006). Critical evaluation of enzyme products, International Poultry Production — Volume 14 Number 7.Lucas A.M and Jamroz C., (1961). Atlas of Avian Hematology. Agriculture Monography 25.United States Department of Agriculture, Washington.

Liu Y., Lyon B.G., Windham W.R., Lyon C.E., and Savage E.M. (2004). Principal component analysis of physical, color and sensory characteristics of chicken breasts deboned at two, four, six and twenty-four hours postmortem. *Poultry Science*, vol. 83, no. 1, p. 101-108.

Lordelo M.M., S.A. Shaaban, N.M. Dale, M.C. Calhoun, P.F. Vendrel, and A.J. Davis (2008). Near infrared reflectance spectroscopy for the determination of free gossypol in cotton seed meal. J. Appl. Poult.Res., 17: 243-248.

Lundbeakn J.A., and Andersen O.S. (1994). Lysophospholipids modulate channel function by altering the mechanical properties of lipid bilayers. The Journal of General Physiology 104:645-673.

Lyon B.G., Windham W.R., Lyon C.E., Barton F.E. (2001). Sensory characteristics and near infrared spectroscopy of broiler breast meat from various chill-storage regimes. *Journal Food Qual*, vol. 24, p. 435-52.

Mabelebele R.M., Gous M., Siwela H.V.M., O'Neil and P.A. Iji (2017). Performance of broiler chickens fed South African sorghum-based diets with xylanase, South African Journal of Animal Science, 47 (No.5).

Mabelebele M., Siwela M., Gous R.M. and Iji P.A. (2013). Chemical composition and Nutritive Value of South African Sorghum Varieties as Feed for Broiler Chickens. *South African Journal of Animal Science*, 45: 206-2013.

Mahmoud I. M. (2012). Effect of feed form, pellet diameter and enzymes supplementation on productive and physiological performance of broiler chicks. Ph.D., thesis, Faculty of Agriculture (Damanhour), Alexandria University.

Marcu A., Vacaru I., Gabi D., Liliana P.C., Marioara N., Ioan P., Dorel D., Bartolomeu K., and Cosmin M. (2013). The Influence of Genetics on Economic

Efficiency of Broiler Chickens Growth. Anim. Sci. Biotech. 46, 339 346.Models and Intelligent Design; Wiley–Blackwell: Chichester, UK, 2013; pp. 361–376.

Mariam A. E. (2013).The utilization of (Mesquite) Prosopis juliflora pods with Xylanase and phytase enzymes in the Broilers diets. Ph.D. Thesis, Sudan University of science and Technology. Department of Animal production, College of Agriculture Studies. Sudan.

MarketsandMarkets
http://s151744727.t.en25.com/e/es?s=151744727&e=28569&elqTrackId=78D8A052C380BCBFF284D754BEBE9730&elq=19b241f19a7e4e1cb8b1a993b2b41a3f&elqaid=12895&elqat=1

Marquardt R.R., and Brufau J., (1997). Future of feed enzymes: Orientation and perspectives. In: Moran d-FehrP. (Ed.). *Feed manufacturing in Southern Europe: New challenges.* Zaragoza: CIHEAM, 1997. p. 181 -187 (Cahiers Options Méditerranéennes; n. 26).

Marsman G.J., Gruppen H., Van Der Poel A.F., Kwakkel R.P., Verstegen M.W., and Voragen A.G. (1997). The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibilities, and chyme characteristics in broiler chicks. PoultrySci. 76.

Martina Loi, Francesca Fanelli, Vania C. Liuzzi, Antonio F. Logrieco and Giuseppina Mulè (2017). Mycotoxin Biotransformation by Native and Commercial Enzymes: Present and Future Perspectives.

Mathlouthi N., H. Juin, and M. Larbier (2003). Effect of xylanase and ß-glucanase supplementation of wheat- or wheat- and barley-based diets on the performance of male turkeys. Br. Poult. Sci. 44:291-298.

Mathlouthi N., J.P. Lalles, P. Lepercq, C. Juste, and M. Larbier (2002). Xylanase and b-glucanase supplementation improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet. J. Anim Sci. 80:2773-2779.

McAllister T.A., Hristov A.N., Beauchemin K.A., Rode L.M., and Cheng K.J. (2001). Enzymes in Ruminant Diets, Michael R. B. and Gary G. P.(eds). Enzymes in Farm Animal Nutrition. CABI Publishing is a division of CAB International.

McCracken K. J., C.M. Preston, and C. Butler (2002). Effects of wheat variety and specific weight on dietary apparent metabolizable energy concentration and performance of broiler chicks. Br.Poult. Sci. 43:253–260.

Mc Donald A.G., Boyce S., and Tipton K.F. (2009). ExplorEnz: the primary source of the IUBMB enzyme list. Nucleic Acids Res 37, D593–D597.

Meng X., and Slominski B.A. (2005). Nutritive values of corn, soybean meal, canola meal, and peas for broiler chickens as affected by a multi-carbohydrase preparation of cell wall degrading enzymes. Poultry Science, 84: 1242-1251.

Mohamed Sirdar (2014).The Sudanese History Statistics and future Investment Challenges .University of Pretoria Department of Avian Studies, South Africa.

Morrison W.R., and Karkalas J. (1990). Methods in Plant Biochemistry. Volume 2. Academic Press, London, pp. 323-352.

Muataz Fadol (2019). Response of Broiler Chickens to Partial Substitution of Groundnut Cake by Non-conventional Protein Sources Fortified with Enzyme Supplementation. Faculty of Animal Production University of Khartoum Ph.D, thesis.

Muirhead S. (1996). Direct Fed Microbial, Enzyme and Forage Additive Compendium, 3rd edn.The Miller Publishing Company, Minetonka, Minnesota.

Murata M., K. Imaizumi, and M. Sugano (1983). Hepatic secretion of lipids and apolipoproteins in rats fed soybean phospholipid and soybean oil. J. Nutr., 113.

Mutaz Faul (2019). Response of Broiler Chickens to Partial Substitution of Groundnut Cake by Non-conventional Protein Sources Fortified with Enzyme Supplementation. PhD thesis, University of Gezira.

Nabil Shuman (2017). Overview and quick scan of the poultry sector In Sudan with focus on the eastern provinces.Report for: *Rijksdienst Voor Ondernemend Nederland*.

Nazifi S., Mosleh N., Ranjbar V.R., and Khordadmehr M. (2011). Reference Values of Serum Biochemical Parameters in Adult Male and Female Iranian Chukar Partridge (*Alectoris Chukar*). Australian Journal of Basic and Applied Sciences, 5(3): 252-256.

Noy Y., and Sklan D. (1995). Digestion and absorption in the young chick. Poult Sci. 74:366–373.

NRC (1994). Nutrient requirement of poultry. In: *Nutritional Research Council*, 9th edition, National Academy press, Washington DC.

Nuha Ahmed Ali (2000). The feeding value of groundnut and sesame meals in broiler diets, MSc thesis, U of K.

Nuyens F., André Meeusen, and Clifford Adams (2007). Benefits of Kemzyme® plus as a multi-enzyme system derived from multiple microbial fermentations. Kemin Internal Reference BB-07-00003.

Nworgu F. C., Ogungbenro S. A., and Solesi K. S. (2007). Performance and some blood chemistry indices of broiler chicken served fluted pumpkin (*Telfaria occidentalis*) leaves extract supplement. *Am-Euras. J. Agric. & Environ. Sci*., 2(1).

OECD-FAO (2019). Agricultural Outlook 2019-28.R.R.

Oguntoye M.A., Mafindi U.M., Ardo B.A., Adamu F., Daniel D.K., Marcel G., and Tangsom S.K. (2018). Effect of enzyme inclusion on growth performance, hematological and serum biochemical responses of finisher broiler chicks fed sorghum based diets. *J. Anim. Prod. Res. 30(1):134-143.*

Olga Dansen (2017). Lower-your-feeding-costs-with-lysolecithins.

Olomu J.M., and Offiong S.A. (1980). Effect of groundnut meal as the sole supplementary dietary-protein on the performance of broiler-chickens. Trop. Agric., 57 (2): 173-181.

Olukosi O.A., A.J. Cowieson, and O.Adeola (2007b). Age-Related Influence of a Cocktail of Xylanase, Amylase, and Protease or Phytase Individually or in Combination in Broilers. Poult. Sci. 86:77-86.

Olukosi O.A., and O. Adeola (2008). Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal based diets. J. Poult. Sci. 45:192-198.

Olukosi O.A., L.A. Beeson, K. Englyst, and L.F. Romero (2015). Effects of exogenous proteases without or with NSP-hydrolysing enzymes on nutrient digestibility and disappearance of non-starch polysaccharides in broiler chickens. Poult. Sci. 00:1–8.

Olukosi O.A., M.R. Bedford, and O.Adeola (2007a). Xylanase in diets for growing swine and broiler chicks. Can. J. Anim. Sci. 87:227-235.

Overland M., and Sundstol F. (1995). Effects of lecithin on fat utilization by weanling pigs. Livest Sci.41:217–224.

Pandian C., Omprakash A.V., Kumaravelu N., and Babu M. (2014). Effect of Supplementation of Exogenous Enzymes Complex in Combination with Lysophospholipids on Production Performance of White Leghorn Layers, International Journal of Multidisciplinary Research, Volume No. 1, Issue No. 12, February 2014.

Pariza M.W., and M. Cook (2010). Determining the safety of enzymes used in animal feed. Reg.Toxicol. Pharmacol. 56:332–342.

Parsippany N.J. (2008). Non-starch polysaccharide enzymes for poultry. Proceedings of the 6th MID Atlantic nutrition conference, University of Maryland.

Pasquali G.A., V.B. Fascina, A.L. Silva, M.M. Aoyagi, E.M. Muro, P.G. Serpa, D.A. Berto, E.S.P.B. Saldanha, and J.R. Sartori (2017). Maize replacement with sorghum and a combination of protease, xylanase, and phytase on performance, nutrient utilization, litter moisture, and digestive organ size in broiler chicken, Can. J. Anim. Sci. 97: 328–337.

Paulis J.W., and J.S. Wall (1979). Distribution and electrophoretic properties of alcohol-soluble proteins in normal and high-lysine sorghums. Cereal Chem. 56.

Pendleton B. (1996). The regulatory environment. In: Muirhead, S. (ed.) *Direct-FedMicrobial, Enzyme and Forage Additive Compendium*. The Miller Publishing Company, Minetonka, Minnesota, pp. 47–52.

Perazzo Costa, Fernando Lima, Matheus Ceccantini, Marcio Montanhini Neto, Roberto Goulart, Cláudia Oliveira, Cleber Vieira, Danilo Santos, and Clariana (2015). Exogenous enzyme complexes and linoleic acid to laying hens. The Journal of Applied Poultry Research. 24.

Pe´reza S., and Samain D. (2010). Structure and engineering of celluloses. Adv Carbohydr Chem Biochem; 64:25-116.

Pessôa G.B.S., Ribeiro Junior, V. Albino, L.F. Araújo, W.A. Silva, D.L. Hannas M.I., and Rostagno, H.S. (2016). Enzyme Complex Added to Broiler Diets: Effects

on Performance, Metabolizable Energy Content, and Nitrogen and Phosphorus Balance. Brazilian Journal of Poultry Science, 18(3).

Peter P. (2013). Developing enzymes to deliver current and future values, Danisco Animal Nutrition. All About Feed Vol.21 no 6.

Petrie A., and Watson P. (1999). Statistics for veterinary and animal science. *Blackwell Sci, Malen,* MA.

Pettersson D., Aman P. (1989). Enzyme supplementation of a poultry diet containing rye and wheat.Brit. J. Nutr. 62, 139-149.

Phillippy B.Q., and Bland J.M. (1988). Gradient ion chromatography of inositol phosphates. AnalBiochem; 175:162-6.

Pirgozliev, Acamovic and Bedford (2010). The effect of dietary xylanase on energy, amino acid and mineral metabolism and egg production and quality in laying hens. British Poultry Science, Taylor and Francis, 51 (05).

Polin D., Wing T.L., Ping K.I., Pell K.E. (1980). The effect of bile acids and lipase on absorption of tallow in young chicks. Poult Sci. 59:2738–2743.

Preston L. Hayse and William W. Mario (1973). Eviscerated Yield, Component Parts, and Meat, Skin and Bone Ratios in the Chicken Broiler, Poultry Science52.

Ramesh, K. R., and Devegowda, (2006). Effect of feeding varying levels of double zero rapeseed meal with and without enzyme supplementation on performance of broilers. In: Proceedings of 22nd World Poultry Congress, Istanbul, Turkey.

Rao B.D., Rana B.S., Jyothi S.H., Karthikeyan K., Bharathkumar K.A., and Seetharaman N. (2003). Importance and economics of sorghum and pearl millet production in Asia. In: *Alternate uses of Sorghum and Pearl millet in Asia*. Common Fund for Commodities technical bulletin, 34, pp. 14-41. International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India 2003.

Ravindran V., (2013). Feed enzymes: The science, practice, and metabolic realities. J Appl Poult Res 22:628–636.

Ravindran V., Bryden W.L., and Kornegay E.T. (1995). Phytates: occurrence, bioavailability and implications in poultry nutrition. *Avianand Poultry Biology Reviews,* (6): 125–143.

Ravindran V., and Abdollahi M.R. (2021). Nutrition and Digestive Physiology of the Broiler Chick: State of the Art and Outlook. Animals, 11, 2795**.**

Reddy A. R., Gowda C., Reddy V., Rai K., Farid W., Ashok S., and Reddy C. R. (2006). Enhanced Utilization Of Sorghum And Pearl Millet Grains In Poultry Feeds, An Indian Perspective.

Reddy A. R., Raju J.A., Nagarjuna Reddy, A. Ramadevi and P. Pandu Ranga Reddy (2016). Recent Trends in Supplementation of Exogenous Fibrolytic Enzymes in Ruminant Nutrition – A Review*Indian Journal Of Natural Sciences Vol.7 / Issue 38.*

Retiman S., and Frankel S. (1957). Calorimetric method for the determination of blood aminotransferase enzymatic activities. Am. Clin. Pathol. 28:56-63.

Rickes E.L., E.A. Ham, E. A. Moscatelli, and W.H. Ott (1962). The isolation and biological properties of a β-glucanase from *B. subtilis.* Arch. Biochem. Biophys. 69.

Robert A., and Copeland A. (2000). Enzymes. A Practical Introduction to Structure, Mechanism, and Data Analysis. Second Edition John Wiley and Sons, Inc., Publication. Wiley-VCH, New York.

Roberts J. R., and M. Choct (2006). Effects of commercial enzyme preparations on egg and eggshell quality in laying hens. Br. Poult. Sci. 47.

Robinson, P.K. (2015). Enzymes: principles and biotechnological applications. Essays in Biochemistry, 59:1 - 41.

Romero L.F., C.M. Parsons, P.L. Utterback, P.W. Plumstead, and V. Ravindran (2013). Comparative effects of dietary NSP-hydrolysing enzymes without or with protease on the ileal digestibility of energy and amino acids and AMEn in young broilers. Anim. Feed Sci. Tech. 181:35–44.

Romero L.F., J.S. Sands, S.E. Indrakumar, P.W. Plumstead, S. Dalsgaard, and V.Ravindran (2014). Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanases and amylase without or with protease. Poult. Sci. 93:1–13.

Rooney L., C. McDonough, and L. Dykes (2005). Les mythes du sorgho avec tannins, circular, Texas A and M University.

Rostagno H.S., Albino L.F.T., Donzele J.L., Gomes P.C., Oliveira R.F., Lopes D.C., Ferreira A.S., Barreto S.L.T., and Euclides R.F. (2011). Brazilian tables for poultry

and swine: feed composition and nutritional requirements. 3rd ed. Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Rutherfurd S.M., Chung T.K., and Moughan P.J. (2007). The effect of a commercial enzyme preparation on apparent metabolizable energy, the true ileal amino acid digestibility, and endogenous ileal lysine losses in broiler chickens. Poultry Science, 86:665-672.

SAC (2013). Basic of Proximate Analysis, Sithiporn Scientific and Technology Conference, Sithiporn Associates Co., Ltd.

Saleh F., Tahir M., Ohtsuka A., Hayashi K. (2005). A mixture of pure cellulase, emicellulose and pectinase improves broiler performance. Br Poult Sci; 46(5).

Salem, Amina A., Anwer, Enaiat M.M., Abo-Eita M., and Namra Eman M.M. (2008). Productive and physiological performance of golden montazah male chickens as affected by feed restriction and anizyme supplementation. Egypt Poult. Sci., 28:1137-1164.

Salissou Issa (2009). Nutritional value of sorghum for poultry feed in West Africa, Ph.D thesis, Kansan State University, Manhattan, Kansas.

Samuel M. Waititu, Anna Rogiewicz, Bogdan A. Slominski, Joyce G. Maina, James O. Ochanda1 and Charles M. Nyachoti (2014). Effect of Multi-Enzyme Mixtures on Performance and Nutrient Utilization in Broilers Fed Diets Containing Different Types of Cereals and Industrial By-Products, Japan Poultry Science Association.

Sanaa H.M. Elnagar and A.A.A. Abdel-Wareth (2014). Performance, carcass criteria and profitability of broiler chicks as affected by yellow corn replacement with sorghum grains and enzymes supplementation, Asian Journal of Poultry Science 8 (4): 123-130.

Sandercock D.A., Nute G.R., and Hocking P.M. (2009). Quantifying the effects of genetic selection and genetic variation for body size, carcass composition, and meat quality in the domestic fowl (*Gallus domesticus*). Poult. Sci. 88, 923–931.

Sanders T. H. (1979). Effect of variety, location and year on tannin content of peanut seed coats. Peanut Sci., 6 (1): 62-64.

Sayyazadeh H., Rahimi G., and Rezaei M. (2006). Influence of enzyme supplementation of maize, wheat and barley-based diets on the performance of broiler chickens, Pak. J. Biol. Sci., 9, 616–621.

Schuster A., and M. Schmoll (2010). Biology and biotechnology of *Trichoderma*. Appl. Microbiol. Biotech. 87:787-799.

Scott Beyer (2011). Sorghum in Poultry Production Feeding Guide, Kansas State University Manhattan, Kan. Available online at http://texassorghum.org/wp-content/uploads/2011/09/Poultry-Feeding-Guide.pdf

Sebastian A.K., Maciej B., Anne Cathrine S., and Andrzej R. (2015). Effects of glyceryl polyethylene glycol ricinoleate on nutrient utilization and performance of broiler chickens, Archives of Animal Nutrition, 69:4.

Selle P.H., and V. Ravindran. (2007). Microbial phytase in poultry nutrition. Anim. Feed. Sci.Technol. 135:1-41.

Selle P.H., S.Y. Liu, J.Cai, and A.J. Cowieson (2013). Steam-pelleting temperatures, grain variety, feed form and protease supplementation of medium-ground, sorghum-based broiler diets. Anim. Prod. Sci. 53: 378-387.

Selle P.H., Cadogan, D.G., Ru Y.J., and Partridge G.G. (2010). Impact of exogenous enzymes in sorghum-or wheat-based broiler diets on nutrient utilization and growth performance. *Journal of Poultry Science*, 9: 53-58.

Selle P.H., Gill R.J., and Downing J.A. (2010). The vulnerability of sorghum to 'moist-heat'. Proceedings APSS. 21: 68-71.

Shakouri M.D., A. lji, L.L. Mikkelsen, and A.J. Cowieson (2009). Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. Anim. Physiol. Anim. Nutr., 93: 647-658.

Sheppy C. (2003). The current feed enzyme market and likely trends. In: M. R. Bedford, and G. G. Partridge, (Eds.). pp. 1-10.

Shuang Li, Xiaofeng Yang, Shuai Yang, Muzi Zhu, and Xiaoning Wang (2012). Technology Prospecting on Enzymes: Application, Marketing and Engineering, CSBJ Volume No: 2, Issue: 3.

Silversides F.G., and Bedford M.R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poultry Science* 78: 1184-1190.

Simon O. (1998). The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract, Journal of Animal and Feed Sciences, 7, 115 – 123.

Singh M., and Krikorian A.D. (1982). Inhibition of trypsin activity in vitro by phytate. Journal of Agricultural and Food Chemistry, 30: 799-800.

Singh K.S., and Prasad C.M. (1979). Feeding value of sunflower and groundnut cakes for broilers. Anim. Feed Sci. Technol. 4 (2): 143–159.

Sitren H.S., Ahmed E.M., and George D.E. (1985). *In vivo* and *in vitro* assessment of anti-nutritional factors in peanut and soy. J. Food Sci., 50 (2).

Sklan D. (2002). Development of the digestive tract of poultry. World's Poultry Sci. 57 (4). 415 – 428.

Slominski B. A. (2011). Recent advances in research on enzymes in poultry diets. Poult. Sci. 90:2013–2023.

Slominski B.A., J. Gdala, D. Boros, L.D. Campbell, W. Guenter, and O. Jones (2000). Variability in chemical and nutritive composition of Canadian wheat and the potential for its minimization by enzyme use. In Proceedings of the XXI World Poultry Congress, Montreal, Canada.

Soares M., and Lopez-Bote C.J. (2002). Effects of dietary lecithin and fat unsaturation on nutrient utilization in weaned piglets. Anim Feed Sci Technol. 95:169–177.

Stefaan Van Dyck (2009). NutriKEM™ A branded Kemin nutricine that greatly enhances animal performance, © Kemin Industries, Inc. and its group of companies.

Stone H., Sidel J.L., and Bloomquist J. (1980). Quantitative descriptive analysis. Cereal Food World 25, 642–644.

Sujani, S., and Seresinhe, R.T. (2015). Exogenous Enzymes in Ruminant Nutrition: A Review. Asian Journal of Animal Sciences 9 (3): 85-99.

Swennen Q., G.P.J. Janssens, S. Millet., G. Vansant, E. Decuypere and J. Buyse (2005). Effect of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: endocrine functioning and intermediary metabolism. Poult. Sci., 84: 1051-1057.

Tadele Y. (2015). Important Anti-Nutritional Substances and Inherent Toxicants of Feeds, Food Science and Quality management .36, pp. 40–48.

Tahirou Abdoulaye and John Sanders (2006). Sorghum or maize in West African poultry rations, USAID, West Africa.

Theofilos Kempapidis (2019). Relationship between digestive enzymes, proteins and anti-nutritional factors in monogastric digestion, Ph.D. thesis, Faculty of Engineering, The University of Sheffield.

Tim Newman, (2018). Enzymes: How they work and what they do? (Online) https://www.medicalnewstoday.com/articles/319704#In-a-nutshell.

Tipton K. (2018). Translocases (2018:EC7). A new EC Class. Enzyme Nomenclature News, August 2018.

Tipton K.F., and Sin´ead Boyce (2000). History of the enzyme nomenclature system, Bioinformatics Vol. 16 no. 1 pages 34 – 40.

Tipton K.F. (1993). The naming of parts, Trends Biochem. Sci. 18.

Tony M.A., Meeusen A., and Abdellatif H.A. (2007). Effect of multi-enzyme systems with increasing levels of protease activity on broiler performance. In: Proceedings of 16th European Symposium on Poultry Nutrition, Strasbourg, France, 629-632.

Trevor P., and Philip L. (2007). Enzymes: Biochemistry, Biotechnology and Clinical Chemistry *Second Edition*, Wood head Publishing Limited, UK.

Trinder P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. L. I. N. Biochem. 6, 24.

Troche C., X. Sun, A.P. McElroy, J. Remus, and C.L. Novak (2007). Supplementation of Avizyme 1502 to corn-soybean meal-wheat diets fed to turkey tom poults: the first fifty-six days of age. Poult. Sci.86:496-502.

Tulasi S.L., Rajashekher A.R, Raghunadha Reddy G., Prasad V.L.K, Raju Mvln, Rao C.L.N, Belum V.S.R, Rao P.P, and Waniska, R.D. (2000). Structure, phenolic compounds and anti-fungal protein of sorghum caryopses. In: *Proceedings of International Conference on Technical and Institutional options for sorghum grain*, 18-19 May 2000 (Chandrashekhar, A., Bandyopadhyay, R. and Hall A.J. eds) pp 72-106. International Crops Research Institute for Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. 2000.

Vahjen W., K. Gläser, K. Schäfer, and O. Simon. (1998). Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. J. Agric. Sci. 130:489–500.

Van I. (2003). Growth and broilers industrialization. Ed. Ceres, Bucharest.

Vibe Glitsoe, Jean-Paul Ruckebusch, and Inge Knap (2015), Innovation in enzyme development, 36th Western Nutrition Conference. September 2015, Winnipeg, Manitoba, Canada.

Walker T., (2018). Australian sorghum for broilers: value and opportunity, available at:

https://www.aegic.org.au/wp-content/uploads/2021/04/BroilerFQC-2018_Sorghum-for-broilers_Tim-Walker-paper.pdf

Wang Z.R., S.Y. Qiao, W.Q. Lu, and D.F. Li (2005). Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci, 84: 875-881.

Waniska R. D., L.F. Hugo, and L.W. Rooney (1992). Practical methods to determine the presence of tannins in sorghum. J. Appl. Poultry Res. 1.

Watson D. (1960). Determination of cholesterol in blood. Clinical Chemistry Acta.

Wealleans A.L., M.C. Walsh, L.F. Romero, and V. Ravindran (2017). Comparative effects of two multi-enzyme combinations and a *Bacillus* probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides, and gut microflora in broiler chickens, Poultry Science 96.

Yang W.Z., Beauchemin K.A., and Rode L.M. (2000). A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *J Dairy Sci*; 83:2512–2520.

Yang Z.B., Yang W.R., Jiang S.Z., Zhang G.G., Zhang Q.Q., and Siow K.C. (2010). Effects of a thermo tolerant multi-enzyme product on nutrient and energy utilization of broilers fed mash or crumbled corn-soybean meal diets. Journal of Applied Poultry Research; 19:38–45.

Yegani M., and D.R. Korver. (2008). Factors affecting intestinal health in poultry. Poult. Sci. 87:2052–2063.

Yilkal Tadele and Getachew Animut (2015). Effect of Exogenous Enzymes on Ruminal degradation of Feed and Animal Performance: A review, Advances in Life Science and Technology Vol.28.

Yohana Mayol (2012). Effect of feed enzymes and energy level on broiler chicken *(Gallus domesticus) performance in* Kenya, Master degree thesis, University of Juba.

Younis M. A. E. (2013). The utilization of (mesquite) Prosopis juliflora pods with xylanase and phytase enzymes in the broilers diets, PhD Thesis, Sudan University of science and Technology, Department of Animal production, College of Agriculture Studies, Sudan.

Youssef A. Attia, Mohammed A. Al-Harthi and Ali S. El-Shafey (2020a). Impact of Multi-enzymes Dose Supplemented Continuously or Intermittently in Drinking Water on Growth Performance, Nutrient Digestibility, and Blood Constituents of Broiler Chickens, *Animals*, *10*, 375 MDPI.

Youssef A. Attia, Mohammed A. Al-Harthi and Ali S. El-Shafey (2020b). Influence of Different Time and Frequency of Multienzyme Application on the Efficiency of Broiler Chicken Rearing and Some Selected Metabolic Indicators, *Animals*, *10*, 450 MDPI.

Zargi H. (2018). Application of Xylanas and ß-Glucanase to Improve Nutrient Utilization in Poultry Fed Cereal Base Diets: Used of Enzymes in Poultry Diet. Insights Enzym Res., Vol. 2 No. 1:11.

Zanella I., Sakomura N., Silversides F., Pack M., Nogueira, and Figueiredo (1998). Effect of Enzyme Supplementation of Broiler Diets Based on Corn and Soybeans. Poultry Science. 78. 561-568.

Zeweil H.S. (1996). Enzyme supplements to diets of growing Japanese quails. Egypt. Poult. Sci. J., 16 (III): 535-557.

Zhang B., Haitao L., Zhao D., Guo Y., and Barri A. (2011). Effect of fat type and lysophosphatidylcholine addition to broiler diets on performance, apparent digestibility of fatty acids, and apparent metabolizable energy content. Anim Feed Sci Technol. 163:177–184.

Zhao P.Y., and Kim I. H. (2017). Effect of diets with different energy and lysophospholipids levels on performance, nutrient metabolism, and body composition in broilers. *Poult Sci*, (96).

Appendixes

Appendix (1): NutriKEM®Extend Product.

CERTIFICATEOFCOMPOSITION

PRODUCTNutriKEM®ExtenddryCODENUMBER 510320

Enzyme	IUBno.	Active substance level
Endo-1,3(4)-beta-glucanase (beta-glucanase)	3.2.1.6.	Min.1175U/g
Endo-1,4-beta-glucanase(cellulase)	3.2.1.4.	Min.9000U/g
Alpha-amylase	3.2.1.1.	Min.200U/g
Bacillolysin(protease)	3.4.24.28.	Min.850U/g
Endo-1,4-beta-xylanase(xylanase)	3.2.1.8.	Min.17500U/g
Lecithin(produced from GM soya)(E322)		150g/ Kg
Glyceryl polyethylene glycolricinoleate		2,5g/Kg
Mono-and diglycerides of fattyacids		25g/Kg

1U of **Endo-1,3(4)-beta-glucanase** is the amount of enzyme which liberates 0.0056 micromoles of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH7.5 and 30°C.

1U **Endo-1,4-beta-glucanase** is the amount of enzyme which liberates 0.0056 micromoles of reducing sugars (glucose equivalents) from carboxy methyl cellulose per minute at pH 4.8 and 50°C.

1U of **Alfa-amylase** is the amount of enzyme which hydrolyses1micromole of glucosidic linkages from water insoluble cross-linked starch polymer per minute at pH7.5 and 37°C.

1U of **Bacillolysin** is the amount of enzyme which makes1microgram of azo-casein soluble in trichoracetic acid per minute at pH7.5 and 37°C.

1U of **Endo-1,4-beta-xylanase** is the amount of enzyme which liberates 0,0067 micromoles of reducing sugars (xyloseequivalents) from birchwoodxylan pe rminute at pH5.3 and 50°C.

Ir.Chris Buyens

Intellectual Property & Regulatory Affairs Manager

Appendix (2): Suggested nutritional matrix NutriKEM®Extend Poultry

SuggestedNutritionalMatrix **NutriKEM®ExtendPoultry**

Nutrient	Unit	% of replacement @ 1,000 g/t	@ 500 g/t
Dig. Lysine po	%	62.4	0.031
Tot. Lysine po	%	70.0	0.035
Dig. Methionine po	%	25.5	0.013
Tot. Methionine po	%	27.7	0.014
Dig. Met+Cyst po	%	47.2	0.024
Tot. Met+Cyst po	%	53.7	0.027
Dig. Threonine po	%	41.8	0.021
Tot. Threonine po	%	47.7	0.024
Dig. Tryptophan po	%	9.8	0.005
Tot. Tryptophan po	%	11.4	0.006
Dig. Arginine po	%	66.7	0.033
Tot. Arginine po	%	74.3	0.037
Dig. Isoleucine po	%	42.3	0.021
Tot. Isoleucine po	%	48.4	0.024
Dig. Valine po	%	47.2	0.024
Tot. Valine po	%	54.2	0.027
Crude Protein	%	1000	0.500
MEpo	MJ/kg	921.1	0.377
MEpo	kcal/kg	220 000	110.00

Matrix is the nutrient-equivalent values assigned to enzyme products in least cost formulation.

Remarks: The use of the matrix should always be evaluated under the available raw materials quality and changes on the protein and energy sources and/or quality.

02/17 KEMIN EUROPA N.V. Kemin Europa N.V.Industriezone Wolfstee, Toekomstlaan 422200 Herentals

Belgium Tel: +32 142 86 200 **Fax: +3214283012** **www.kemin.com**

Appendix (3): 5% broiler concentrate.

Broiler concentrate 5% **Inclusion rate: 5.00%**

Specification	Unit	ProductTypical.
Crudeprotein	%	35.00
Crudefat	%	2.70
Crudefiber	%	3.30
MetEnergyPoultry	Kcal/Kg	2,000
Sodium	%	3.0
Calcium	%	6.10
PhosphorusAvailable	%	6.50
Lysine	%	14.00
Methionine	%	6.50
Methio+Cyst	%	6.80
Threonine	%	5.30
Magnesium	%	2.90
VitaminsandMineral	**Unit**	**Product**
VitaminA	IU/Kg	200,000.00
VitaminD3	IU/Kg	40,000.00
VitaminE	Mg/Kg	500.00
VitaminK3	Mg/Kg	40.00
VitaminB1/Thiamine	Mg/Kg	30.00
VitaminB2/Riboflavin	Mg/Kg	100.00
VitaminB3/Niacin	Mg/Kg	600.00
VitaminB5/Pantothenica	Mg/Kg	200.00
VitaminB6/Pyridoxine	Mg/Kg	40.00
VitaminB9/FolicAcid	Mg/Kg	20.00
VitaminB12/Cyanocobal	Mcg/Kg	500.00
Biotin	Mcg/Kg	2,000.00
CholineChloride	Mg/Kg	7,000.00
Iron	Mg/Kg	800.00
Copper	Mg/Kg	160.00
Zinc	Mg/Kg	1,000.00
Manganese	Mg/Kg	1,200.00
Iodine	Mg/Kg	8.00
Selenium	Mg/Kg	3.00
6 Phytase	FTY/Kg	30,000.00
Additives:-		
Antioxidants	MCFA	
Anti-molds	NSP enzymes	

Appendix (4) : ***SENSORY EVALUATION CARD***

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

1-Tenderness	2-Flavor	3-Color	4-Juiciness
8-Extremelytender	8-Extremelyintense	8-Extremelydesirable	8-Extremelyjuicy
7-Verytender	7-Veryintense	7-Verydesirable	7-Veryjuicy
6-Moderately	6-Moderatelyintense	6-Moderately	6-Moderatelyjuicy
5-Slightlytender	5-Slightlyintense	5-Slightlydesirable	5-Slightlyjuicy
4-Slightlytough	4-Slightlybland	4-Slightlyundesirable	4-Slightlydry
3-Moderatelytough	3-Moderatelybland	3-Moderatelyundesirable	3-Moderatelydry
2-Verytough	2-Verybland	2-Veryundesirable	2-Verydry
1-Extremelytough	1-Extremelybland	1-Extremelyundesirable	1-Extremelydry

Serial	Code	Tendernes	Flavor	Color	Juiciness	Comments
A	1					
B	2					
C	3					
D	4					
E	5					
F	6					
G	7					
H	8					
I	9					
J	10					
K	11					
L	12					
M	13					

Name……..…… Date…….

Appendix (5): Cobb 500.

Cobb 500 CARCASS CHARACTERISTICS

Live weight (g)	1,795.15	%
Hot carcass	1,256.92	70.02%
Cold carcass	1,249.46	69.60%
Blood and feather	143.54	8.00%
Legs	67.54	3.76%
Head	47.60	2.65%
Neck	68.28	3.80%
Liver	37.05	2.06%
Full gizzard	43.29	2.41%
Empty gizzard	26.03	2.07%
Gizzard content	17.26	1.38%
Heart	10.22	0.57%
Abdominal fat	30.65	1.71%
Lung	10.89	0.01
Kidneys	6.78	0.38%
Intestine	83.63	4.66%
Loss	6.03	0.37%

WHOLE CARCASS: -

MEAT 62 % BONE 22 % SKIN 14 %

CUTS

Cold carcass (g)		1,249.77
Wing	10.28%	66.55
Back bone	22.37%	144.78
Breast	38.71%	250.57
Thigh	13.94%	90.23
Drumstick	11.67%	75.52
Cutting Loss	3.02%	19.57
Commercial cuts	66.62 %	416.32
Meat	75.43%	314.02
Bone	16.31%	67.91
Deboning loss	8.26%	34.40
Thigh	21.63 %	90.23
Meat	71.56%	64.57
Bone	16.78%	15.14
Loss	11.66%	10.52
Drumstick	18.15 %	75.52
Meat	59.81%	45.17
Bone	29.56%	22.32
Loss	10.63%	8.03
Breast	60.22 %	250.57
Meat	81.53%	204.28
Bone	12.15%	30.45
Loss	6.32%	15.85
Whole cutting loss	11.29%	

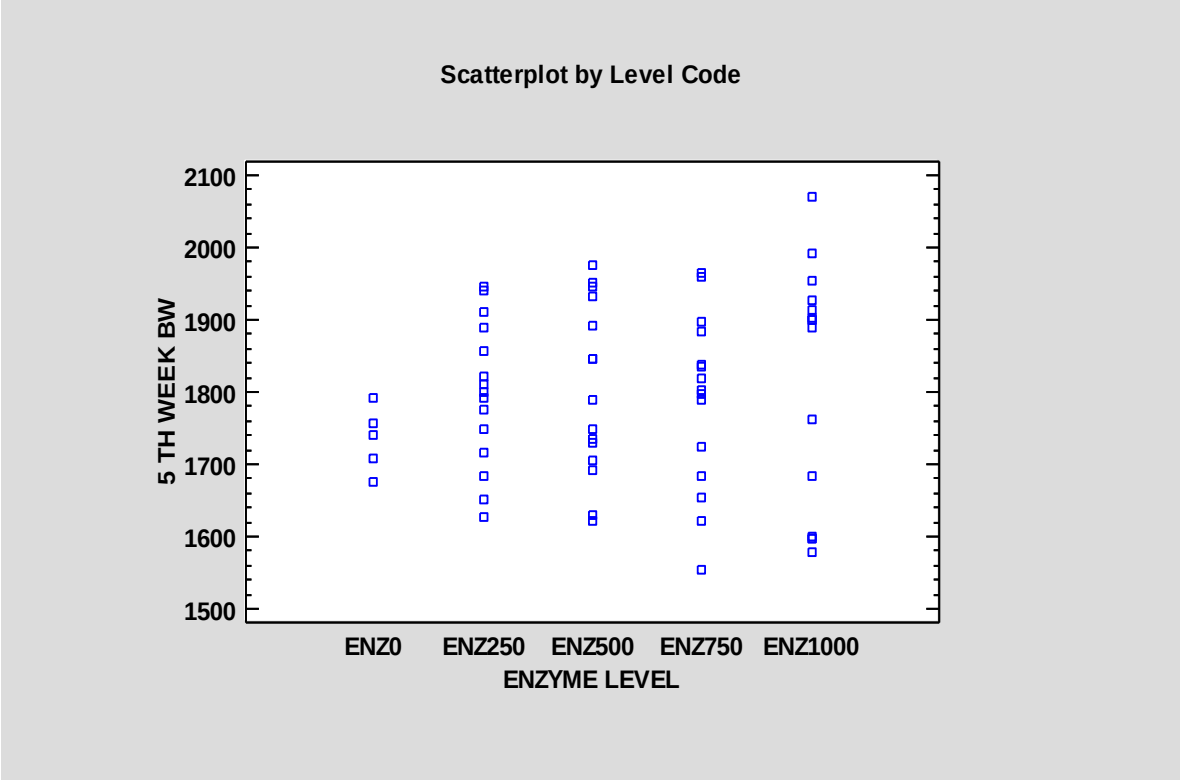
Appendix (6): **Broiler carcass, organs and commercial cuts parameters.**

Cold carcass		1,249.77
Wing	10.28%	66.55
Back bone	22.37%	144.78
Breast	38.71%	250.57
Thigh	13.94%	90.23
Drumstick	11.67%	75.52
Cutting Loss	3.02%	19.57
Commercial cuts	66.62 %	416.32
Meat	75.43%	314.02
Bone	16.31%	67.91
Deboning loss	8.26%	34.40
thigh	21.63 %	90.23
Meat	71.56%	64.57
Bone	16.78%	15.14
Loss	11.66%	10.52
Drumstick	18.15 %	75.52
Meat	59.81%	45.17
Bone	29.56%	22.32
Loss	10.63%	8.03
Breast	60.22 %	250.57
Meat	81.53%	204.28
Bone	12.15%	30.45
Loss	6.32%	15.85
Whole loss	11.29%	

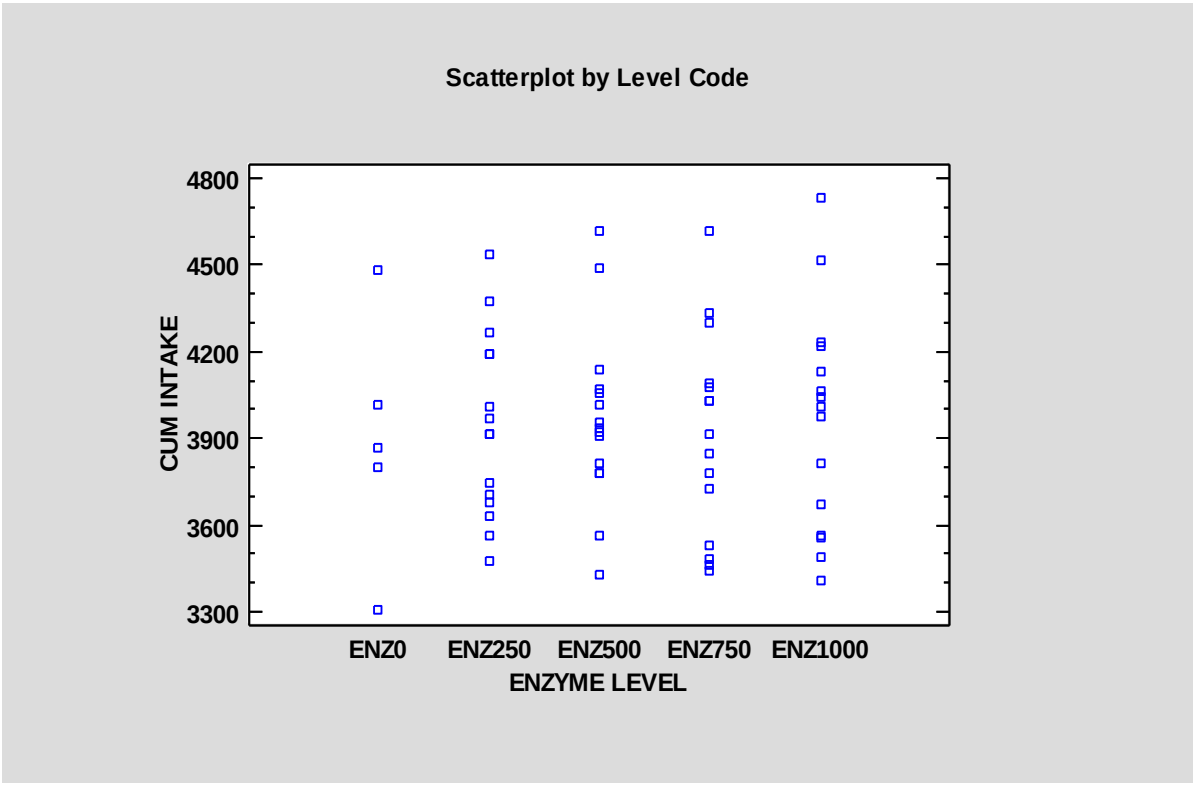
Appendix (7): Temperature and Humidity.

Week	Highest temp.	Lowest temp.	Humidity
1	21	17	15
2	23	18	16
3	27	21	15
4	24	20	19
5	22	19	17

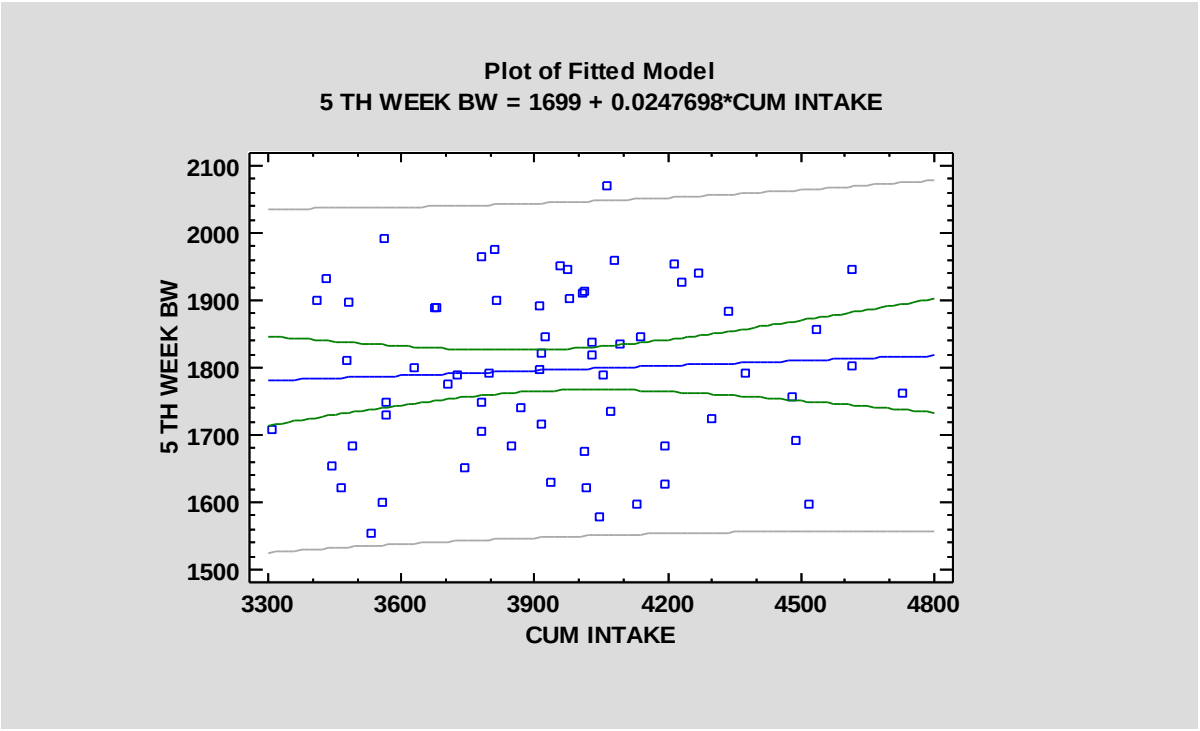
Appendix (8): Body weight versus Enzyme level scatter plot.



Appendix (9): Feed intake versus Enzyme level scatter plot.



Appendix (10): Body weight and feed intake correlation.



Appendix (11): Tallies of the EC numbers held in ExplorEnz.

	Class 1 (Oxidoreductases)	Class 2 (Transferases)	Class 3 (Hydrolases)	Class 4 (Lyases)	Class 5 (Isomerases)	Class 6 (Ligases)	All classes
Current	1119	1179	1127	371	165	141	4102
Transferred	146	51	276	64	3	2	542
Deleted	63	59	98	23	7	4	254
Total	1328	1289	1501	458	175	147	4898

The most recent version of these data can be viewed at http://www.enzyme-database.org/stats.php

Appendix (12): Six major classes of enzyme.

Six Major Classes of Enzymes and Examples of Their Subclasses

	Classification	Distinguishing Feature
1.	Oxidoreductases	$A_{red} + B_{ox} \rightarrow A_{ox} + B_{red}$
	Oxidases	Use oxygen as an electron acceptor but do not incorporate it into the substrate
	Dehydrogenases	Use molecules other than oxygen (e.g., NAD^+) as an electron acceptor
	Oxygenases	Directly incorporate oxygen into the substrate
	Peroxidases	Use H_2O_2 as an electron acceptor
2.	Transferases	A-B+C → A+B-C
	Methyltransferases	Transfer one-carbon units between substrates
	Aminotransferases	Transfer NH_2 from amino acids to keto acids
	Kinases	Transfer PO_3~ from ATP to a substrate
	Phosphorylases	Transfer PO_3~ from inorganic phosphate (P.) to a substrate
3.	Hydrolases	A-B+H_2O → A-H + B-OH
	Phosphatases	Remove PO_3~ from a substrate
	Phosphodiesterases	Cleave phosphodiester bonds such as those in nucleic acids
	Proteases	Cleave amide bonds such as those in proteins
4.	Lyases	A(XH)-B → A-X+B-H
	Decarboxylases	Produce CO_2 via elimination reactions
	Aldolases	Produce aldehydes via elimination reactions
	Synthases	Link two molecules without involvement of ATP
5.	Isomerases	A⇔Изо-A
	Racemases	Interconvert L and D stereoisomers
	Mutases	Transfer groups between atoms within a molecule
6.	Ligases	A+B+ATP → A-B+ADP+Pi
	Carboxylases	Use CO_2 as a substrate
	Synthetases	Link two molecules via an ATP-dependent reaction

Appendix (13): Enzyme classes (Tipton, 2018).

Enzyme classes

No.	Name	Reaction catalysed
1	Oxidoreductases	*$AH_2 + B = A + BH_2$
2	Transferases	$AX + B = BX + A$
3	Hydrolases	$A\text{-}B + H_2O = AH + BOH$
4	Lyases	A=B + X-Y = A-B | | X Y
5	Isomerases	A = B
6	Ligases	$^{+}A + B + NTP = A\text{-}B + NDP + P$ (or NMP + PP)
7	Translocases	AX + B || = A + X + || B (side 1) (side 2)

*Where nicotinamide-adenine dinucleotides are the acceptors, NAD^+ and $NADH + H^+$ are used, by convention.

$^{+}$NTP = nucleoside triphosphate.

Appendix (14): Cost of different experiment diets (SDG).

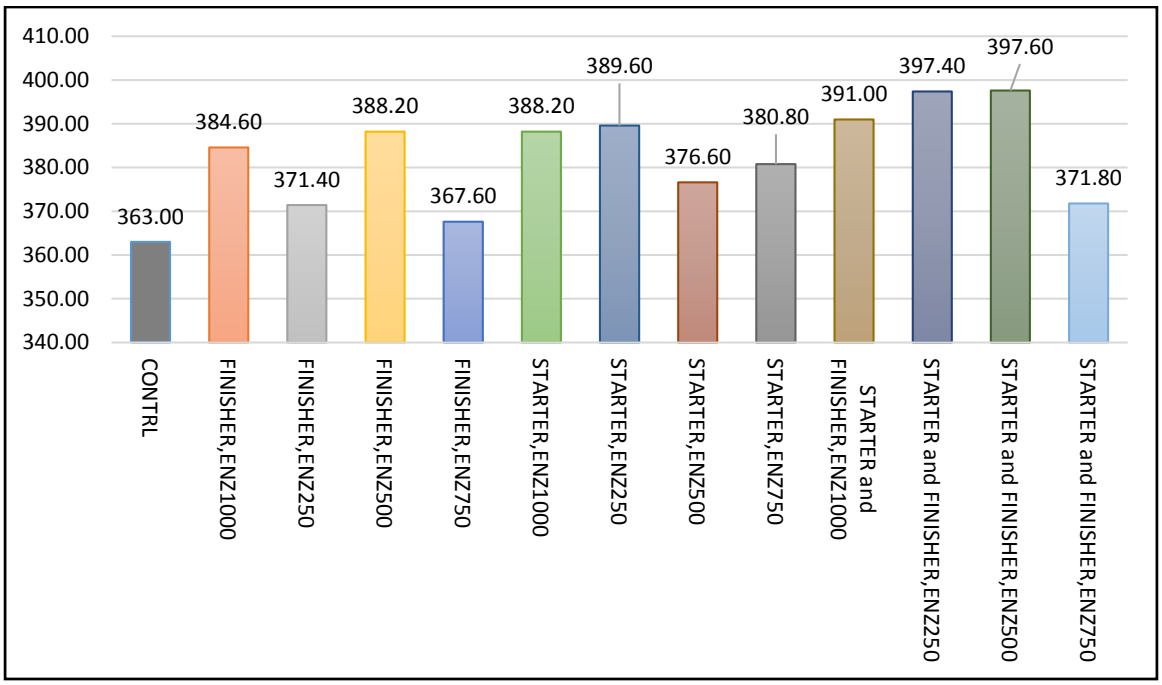
Cost of Experiment Diets (SDG)		
Diet	Starter	Finisher
Control	28,176.25	27,215.00
Enzyme250 gm / Ton	28,296.25	27,335.00
Enzyme500 gm / Ton	28,416.25	27,455.00
Enzyme750 gm / Ton	28,536.25	27,575.00
Enzyme1,000gm / Ton	28,656.25	27,695.00

Appendix (15): EC data Explorer of the IUBMB Enzyme Nomenclature list.

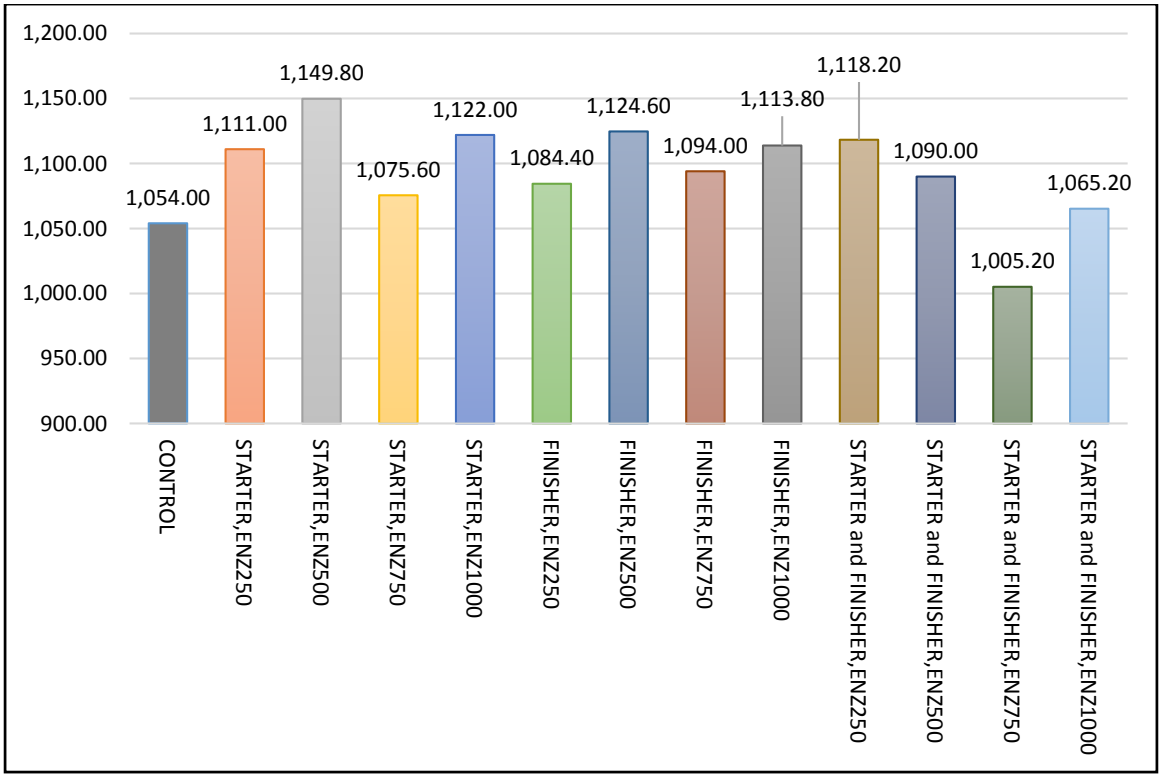
EC 1 [+] **Oxidoreductases**
EC 2 [+] **Transferases**
EC 3 [–] **Hydrolases**
EC 3.1 [–] Acting on ester bonds
EC 3.1.1 [+] Carboxylic-ester hydrolases
EC 3.1.2 [+] Thioester hydrolases
EC 3.1.3 [+] Phosphoric-monoester hydrolases
EC 3.1.4 [+] Phosphoric-diester hydrolases
EC 3.1.5 [+] Triphosphoric-monoester hydrolases
EC 3.1.6 [+] Sulfuric-ester hydrolases
EC 3.1.7 [+] Diphosphoric-monoester hydrolases
EC 3.1.8 [+] Phosphoric-triester hydrolases
EC 3.1.11 [+] Exodeoxyribonucleases producing 5′-phosphomonoesters
EC 3.1.12 [+] Exodeoxyribonucleases producing 3′-phosphomonoesters
EC 3.1.13 [+] Exoribonucleases producing 5′-phosphomonoesters
EC 3.1.14 [+] Exoribonucleases producing 3'-phosphomonoesters
EC 3.1.15 [+] Exonucleases that are active with either ribo or deoxyribonucleic acids and produce 5'phosphomonoesters
EC 3.1.16 [+] Exonucleases that are active with either ribo or deoxyribonucleic acids and produce 3'phosphomonoesters
EC 3.1.21 [+] Endodeoxyribonucleases producing 5'-phosphomonoesters
EC 3.1.22 [+] Endodeoxyribonucleases producing 3'-phosphomonoesters
EC 3.1.23 [+] Sitespecific endodeoxyribonucleases: cleavage is sequence specific (deleted subsubclass)
EC 3.1.24 [+] Site specific endodeoxyribonucleases: cleavage is not sequence specific (deleted sub-ubclass)
EC 3.1.25 [+] Site-specific endodeoxyribonucleases that are specific for altered bases
EC 3.2 [+] Glycosylases
EC 3.3 [+] Acting on ether bonds
EC 3.4 [+] Acting on peptide bonds (peptidases)
EC 3.5 [+] Acting on carbon-nitrogen bonds, other than peptide bonds
EC 3.6 [+] Acting on acid anhydrides
EC 3.7 [+] Acting on carbon-carbon bonds
EC 3.8 [+] Acting on halide bonds
EC 3.9 [+] Acting on phosphorus-nitrogen bonds
EC 3.10 [+] Acting on sulfur-nitrogen bonds
EC 3.11 [+] Acting on carbon-phosphorus bonds
EC 3.12 [+] Acting on sulfur-sulfur bonds
EC 3.13 [+] Acting on carbon-sulfur bonds
EC 4 [+] **Lyases**
EC 5 [+] **Isomerases**
EC 6 [+] **Ligases**
EC 7 [+] **Translocases**

(http://www.sbcs.qmul.ac.uk/iubmb/enzyme/)

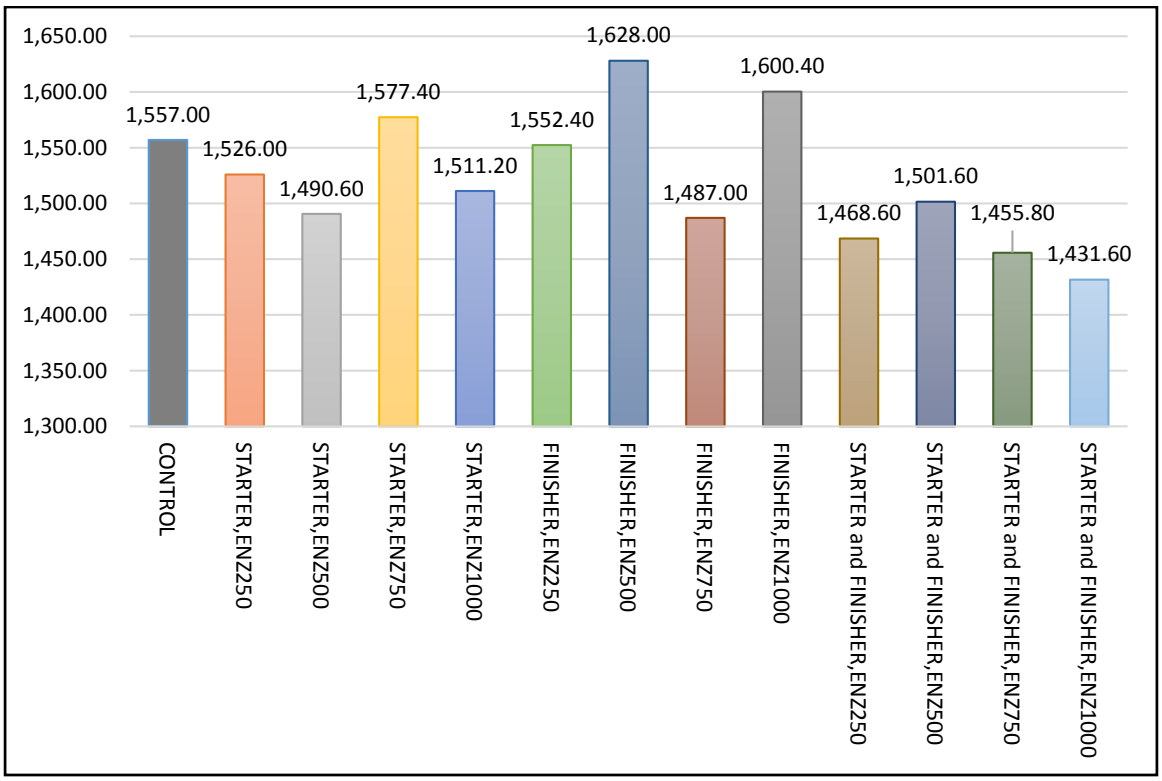
Appendix (16): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 1st week body weight.



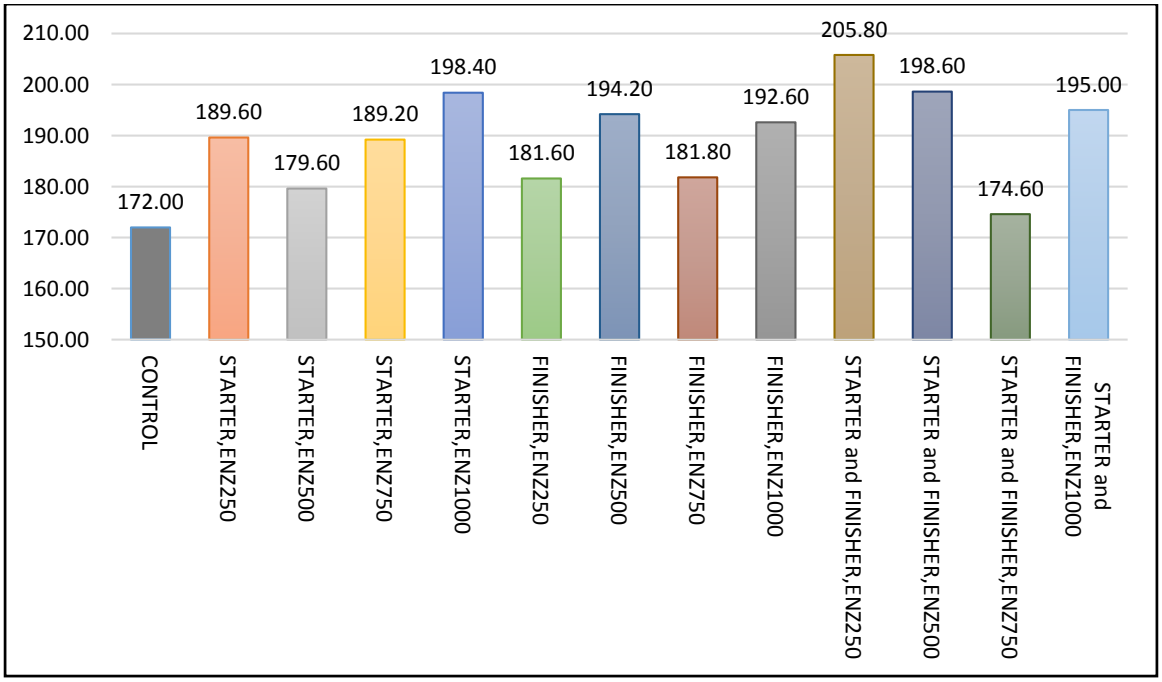
Appendix (17): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 3rdweek body weight.



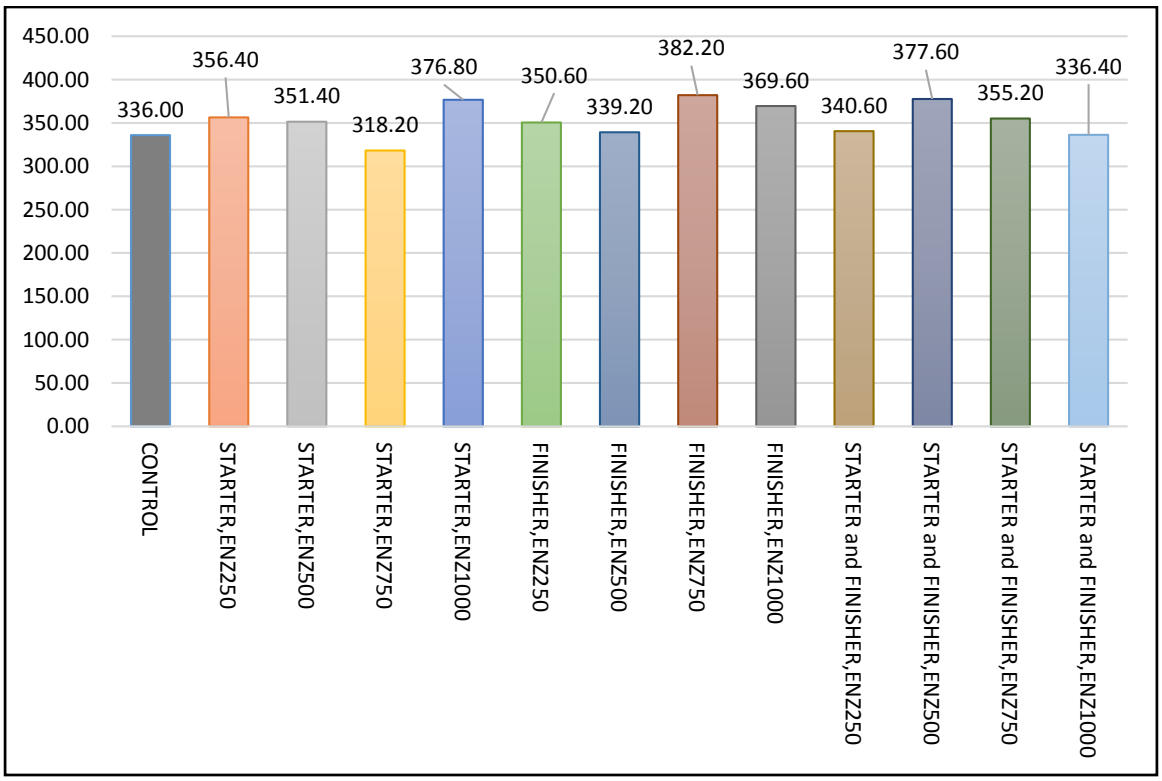
Appendix (18): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 4thweek body weight.



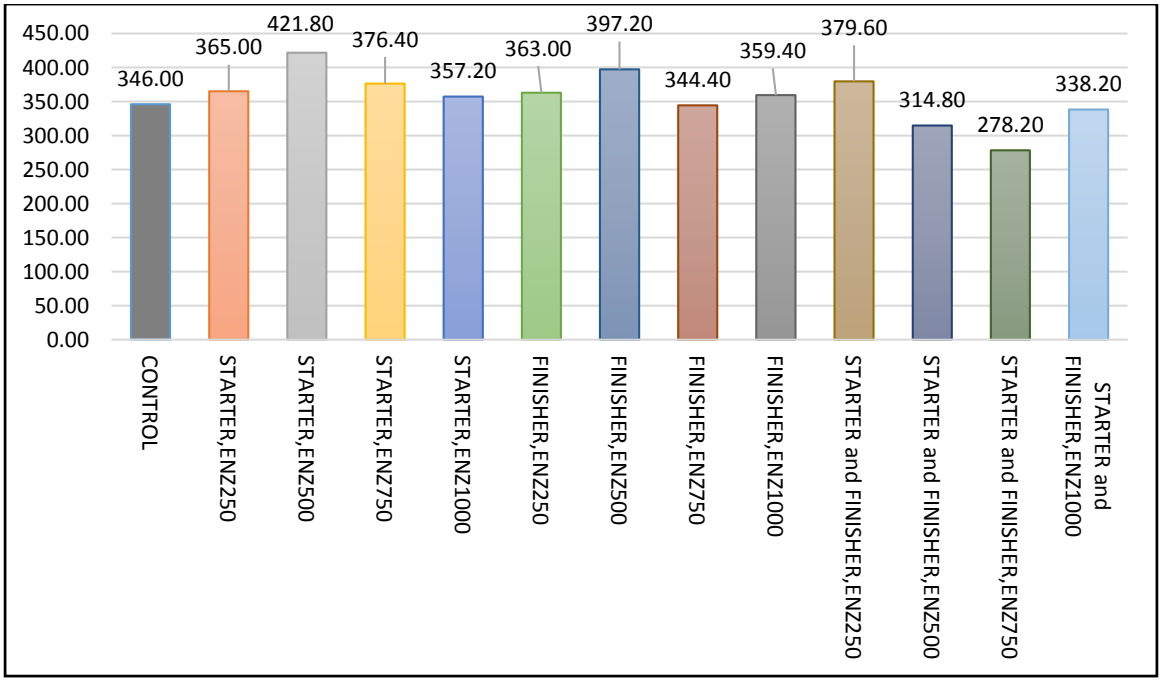
Appendix (19): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 1st week body weight gain.



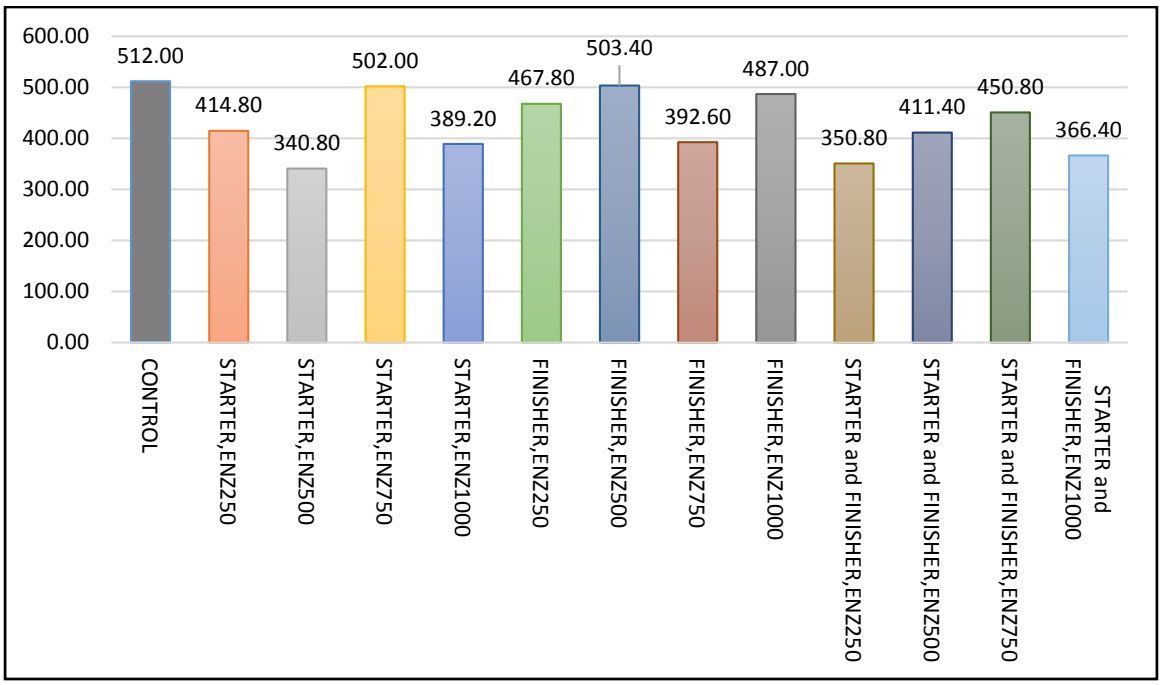
Appendix (20): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 2nd week body weight gain.



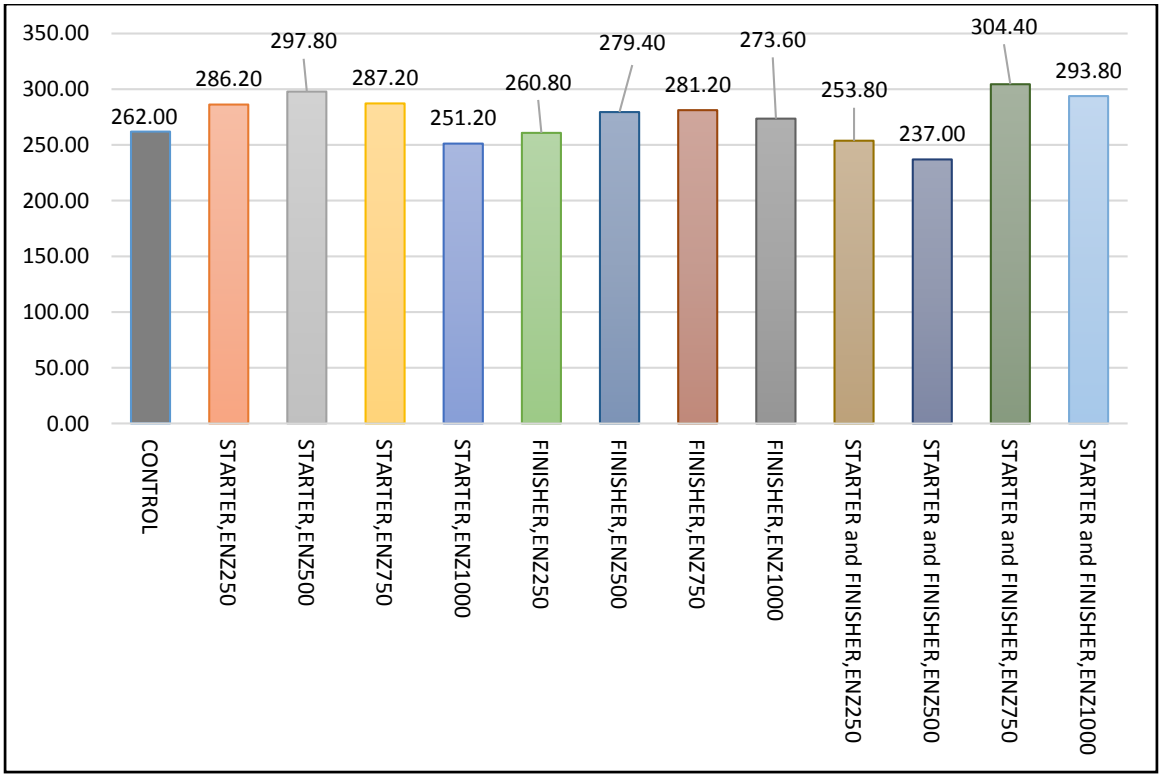
Appendix (21): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 3rd week body weight gain.



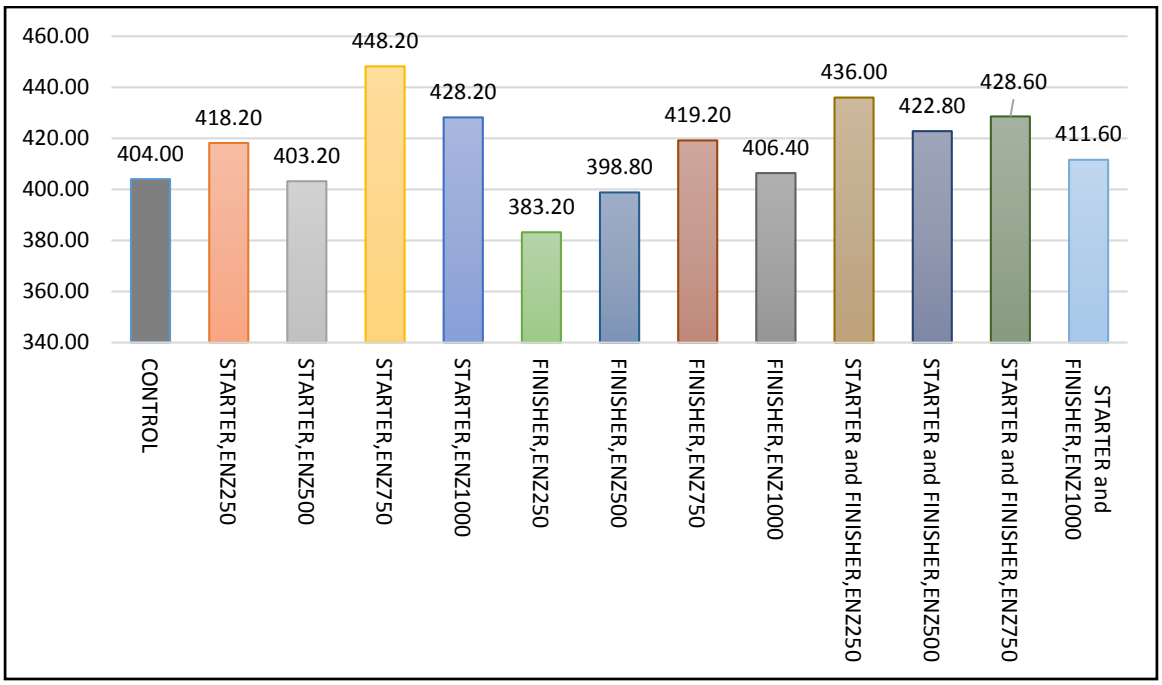
Appendix (22): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 4th week body weight gain.



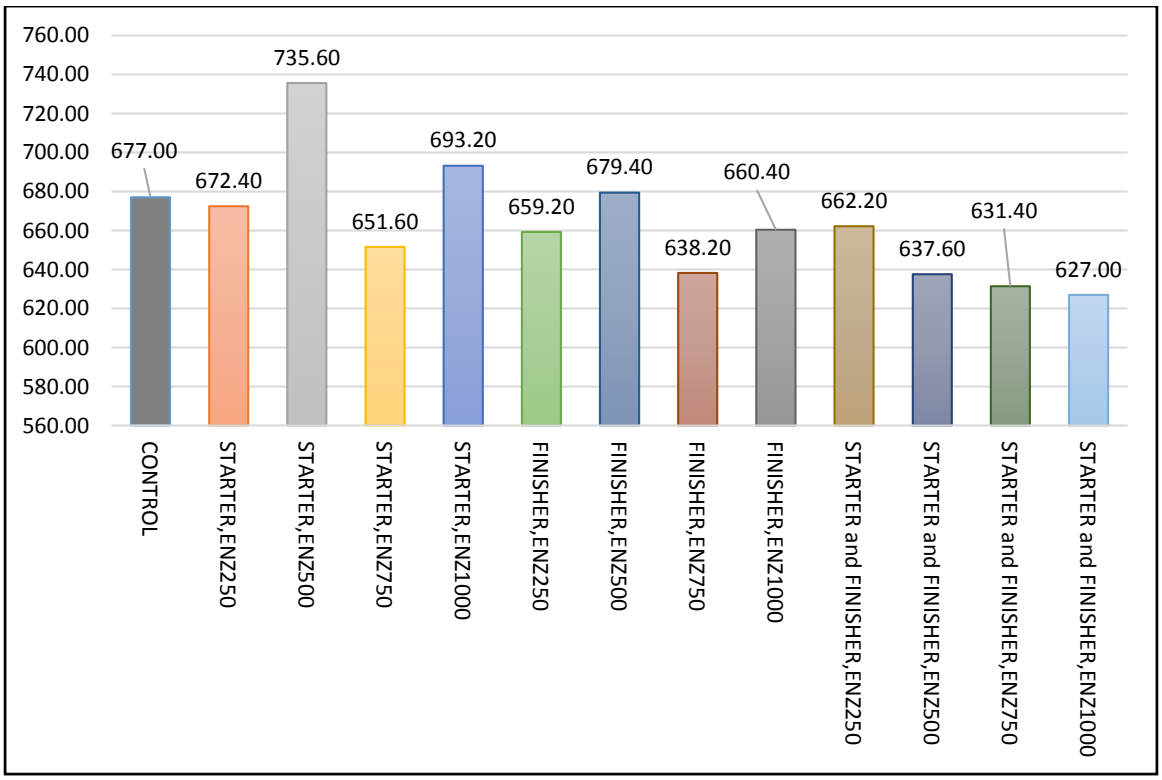
Appendix (23): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 5th week body weight gain.



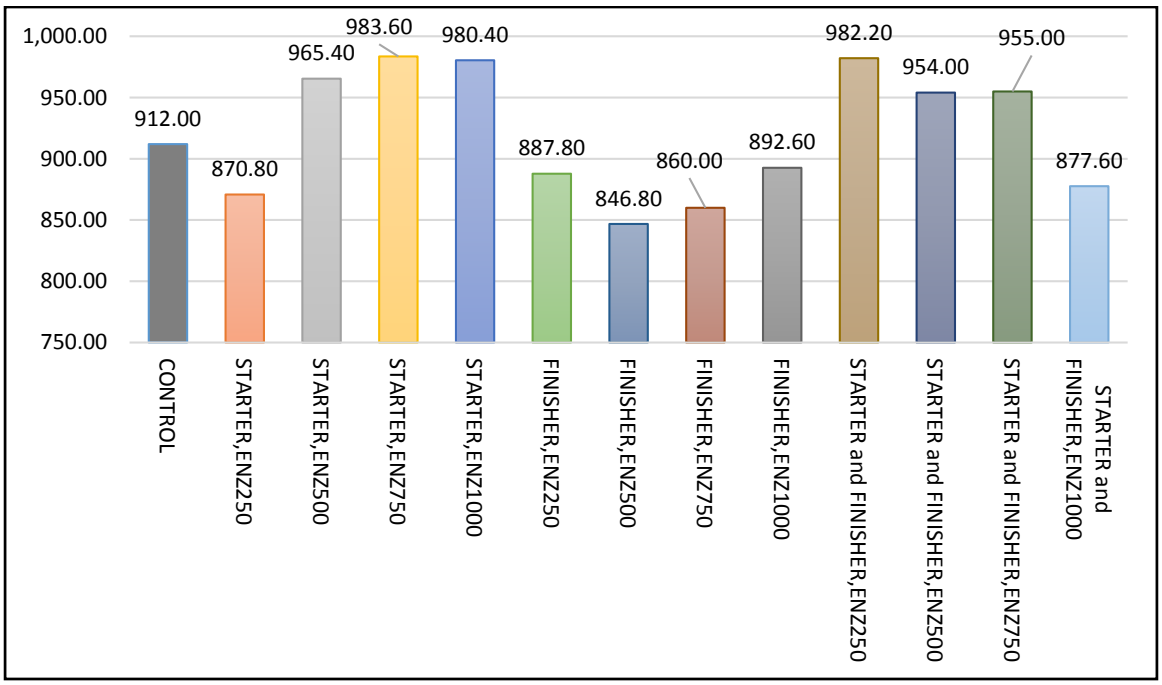
Appendix (24): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 1st week feed intake.



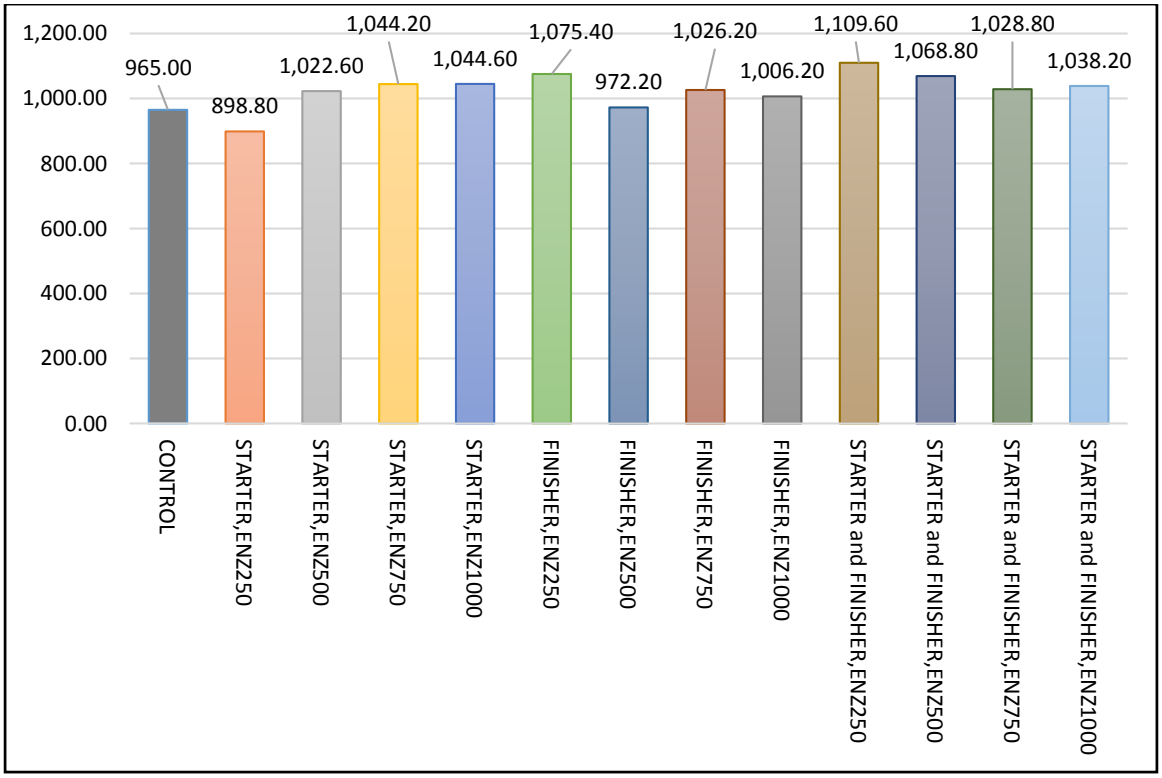
Appendix (25): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 2nd week feed intake.



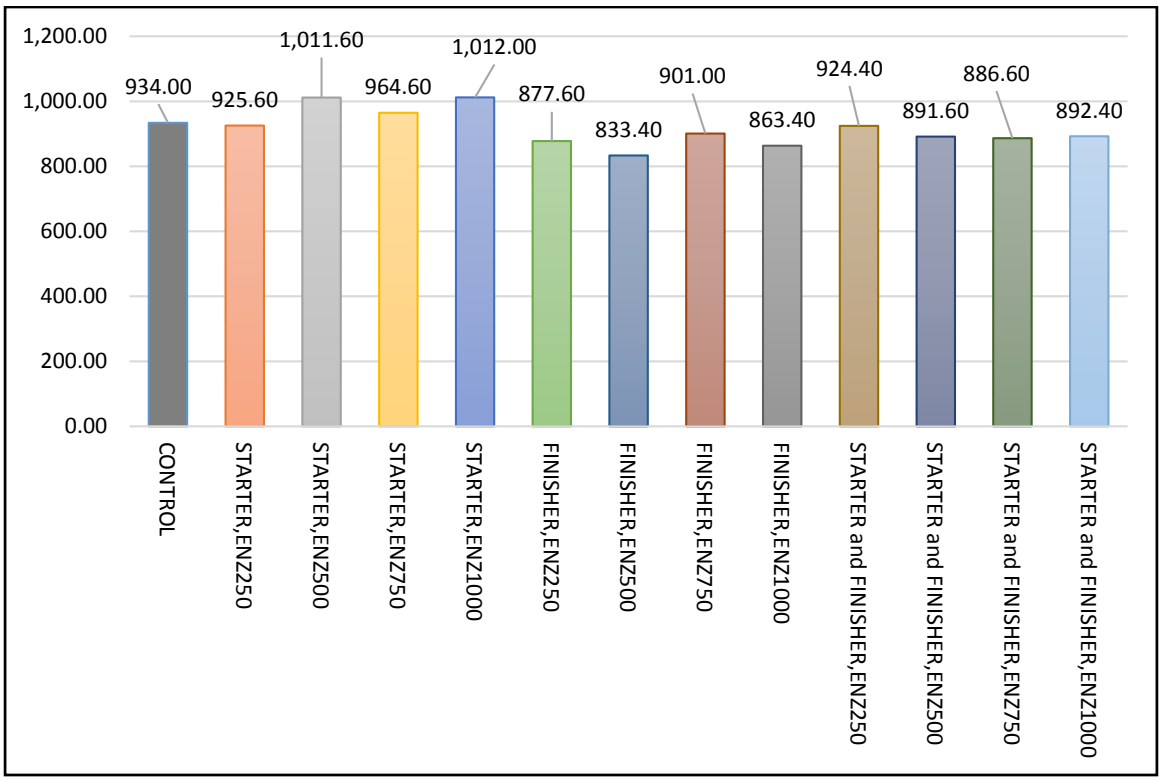
Appendix (26): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 3rd week feed intake.



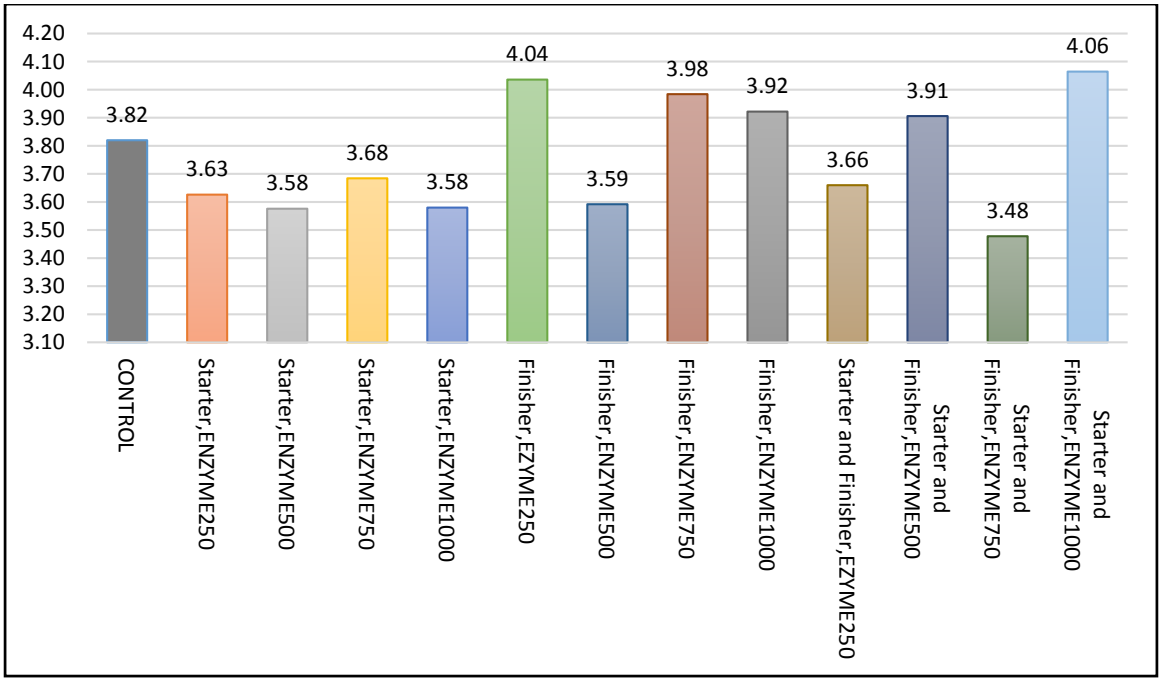
Appendix (27): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 4th week feed intake.



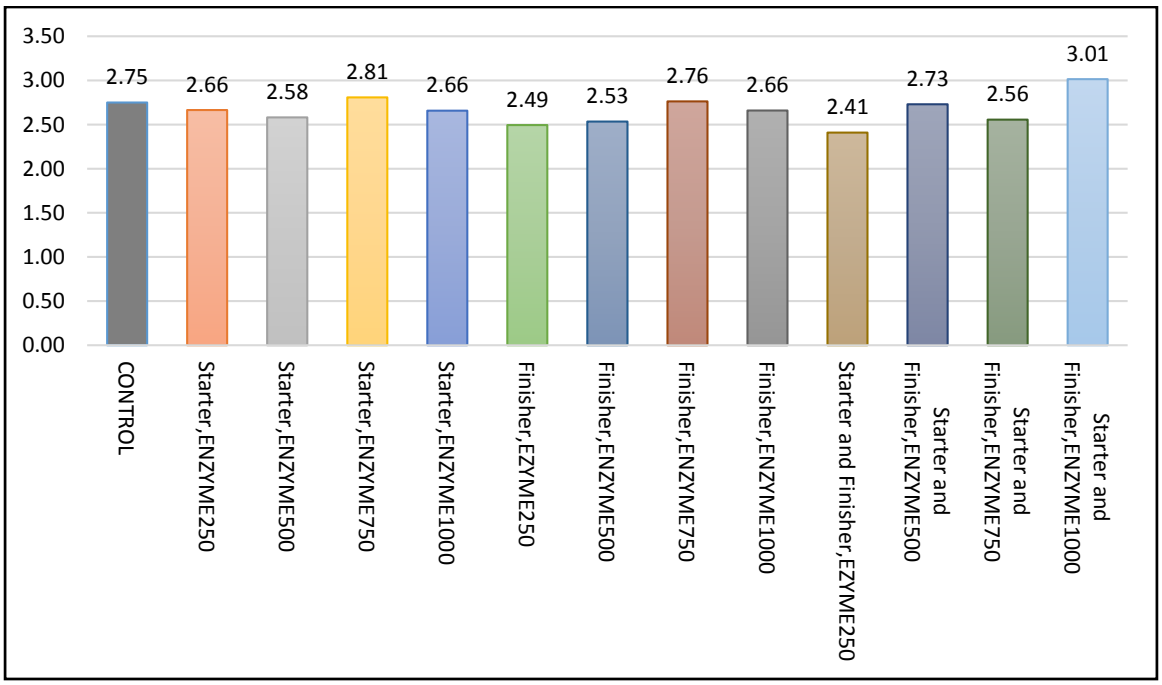
Appendix (28): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on 5th week feed intake.



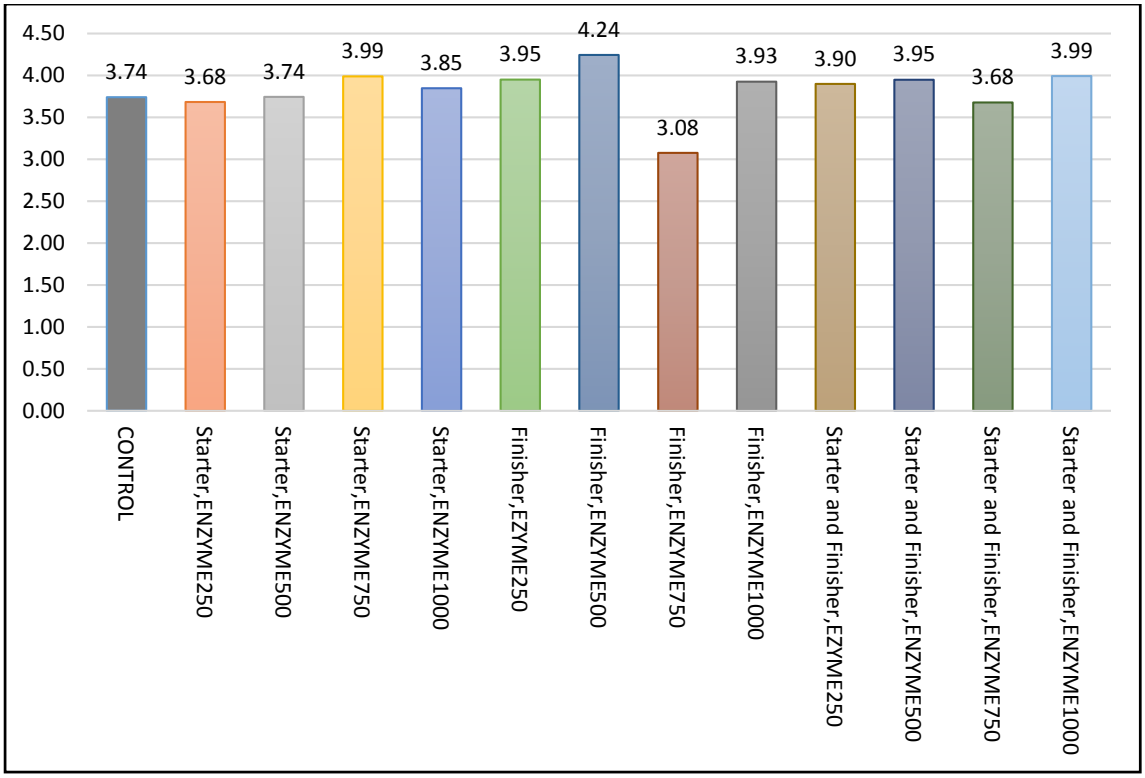
Appendix (29): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on legs relative weight.



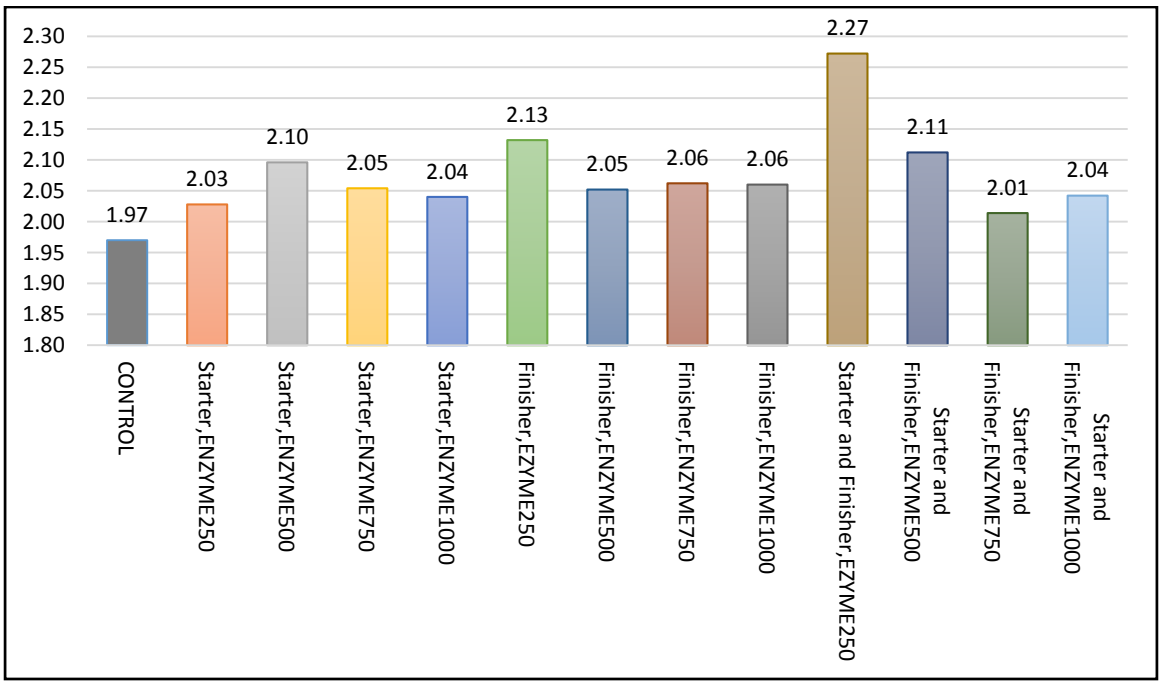
Appendix (30): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on head relative weight.



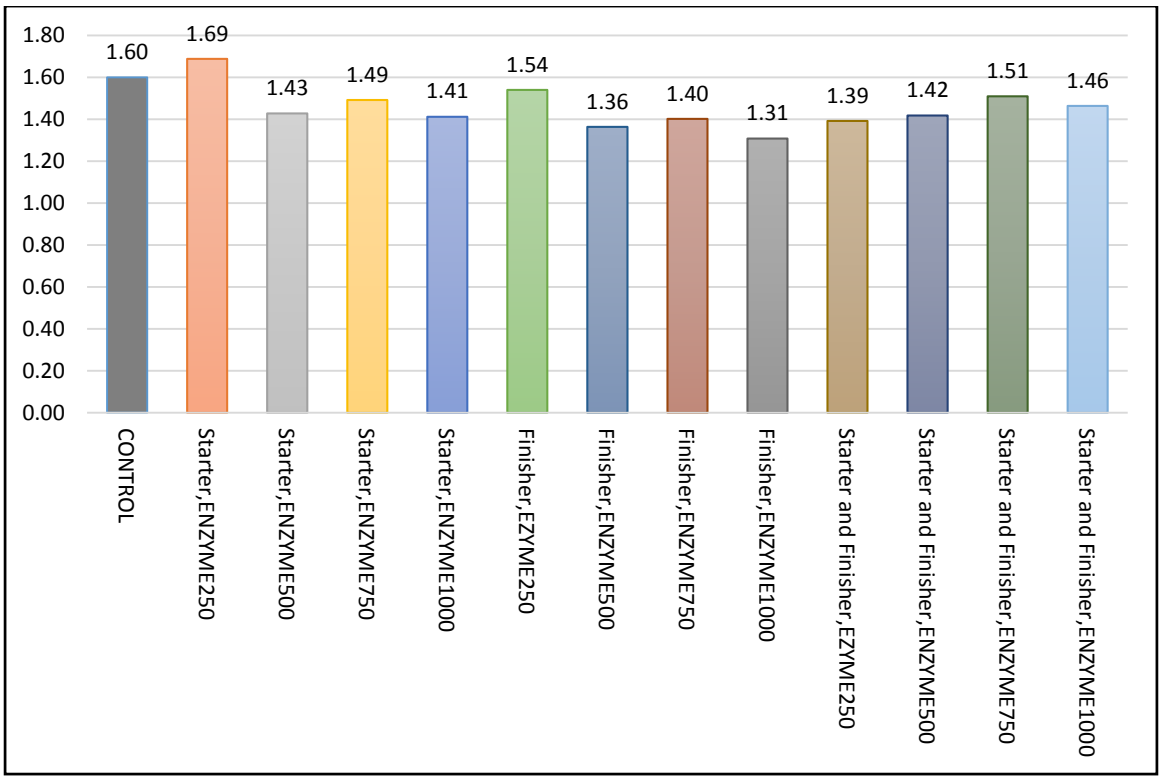
Appendix (31): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on neck relative weight.



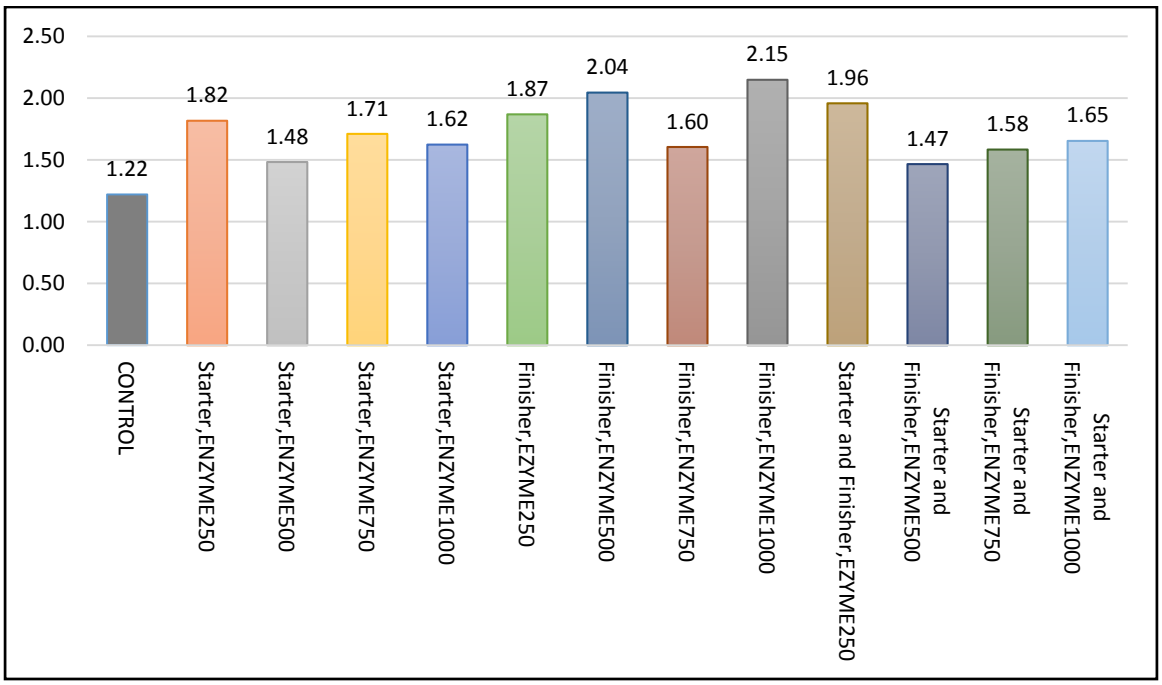
Appendix (32): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on liver relative weight.



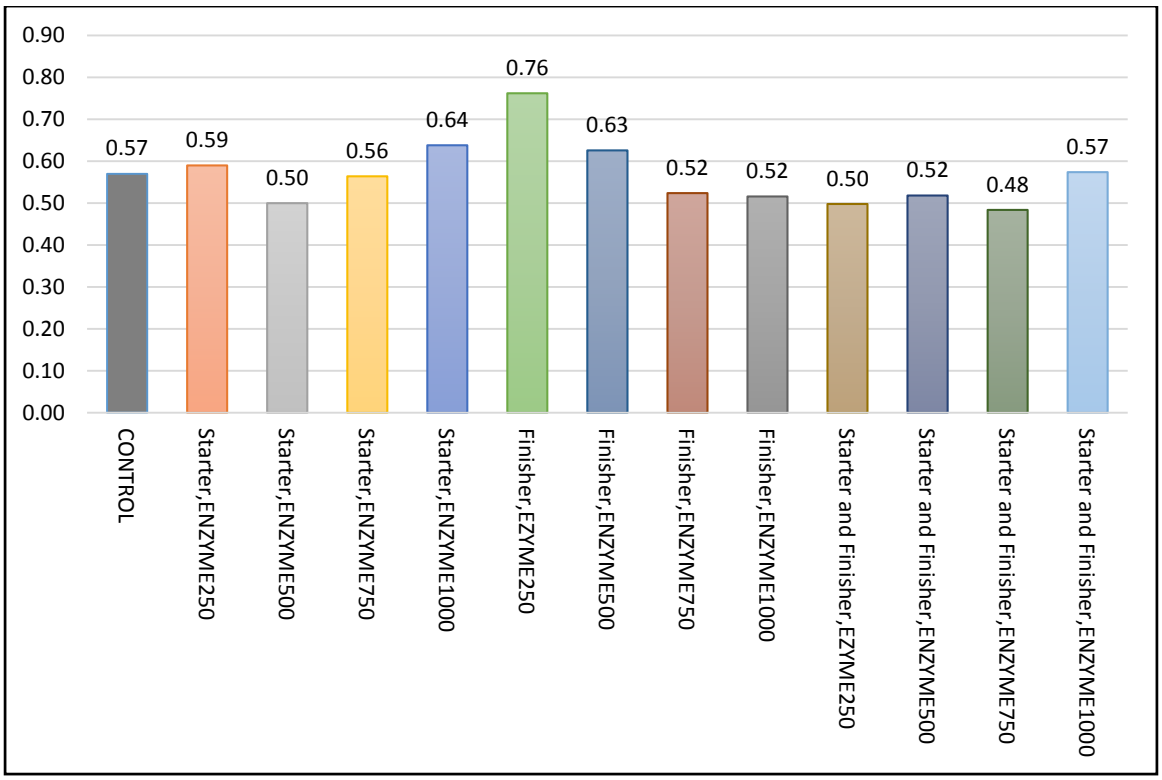
Appendix (33): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on gizzard relative weight.



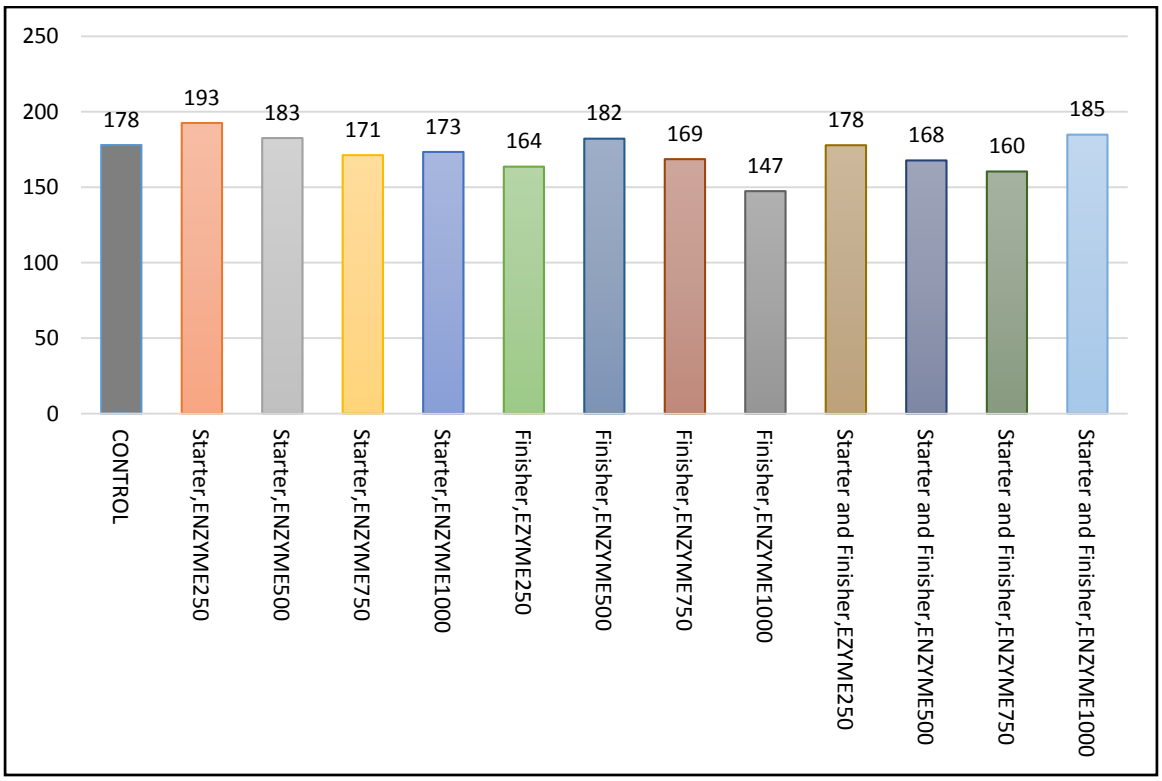
Appendix (34): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on abdominal fat relative weight.



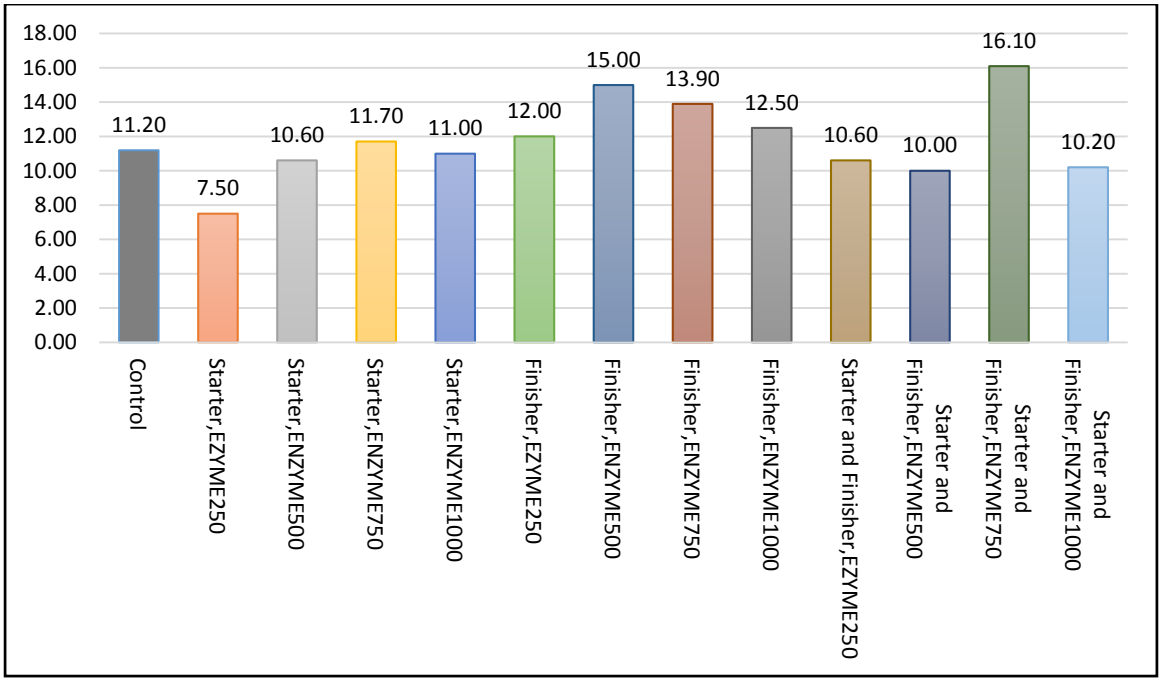
Appendix(35): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on heart relative weight.



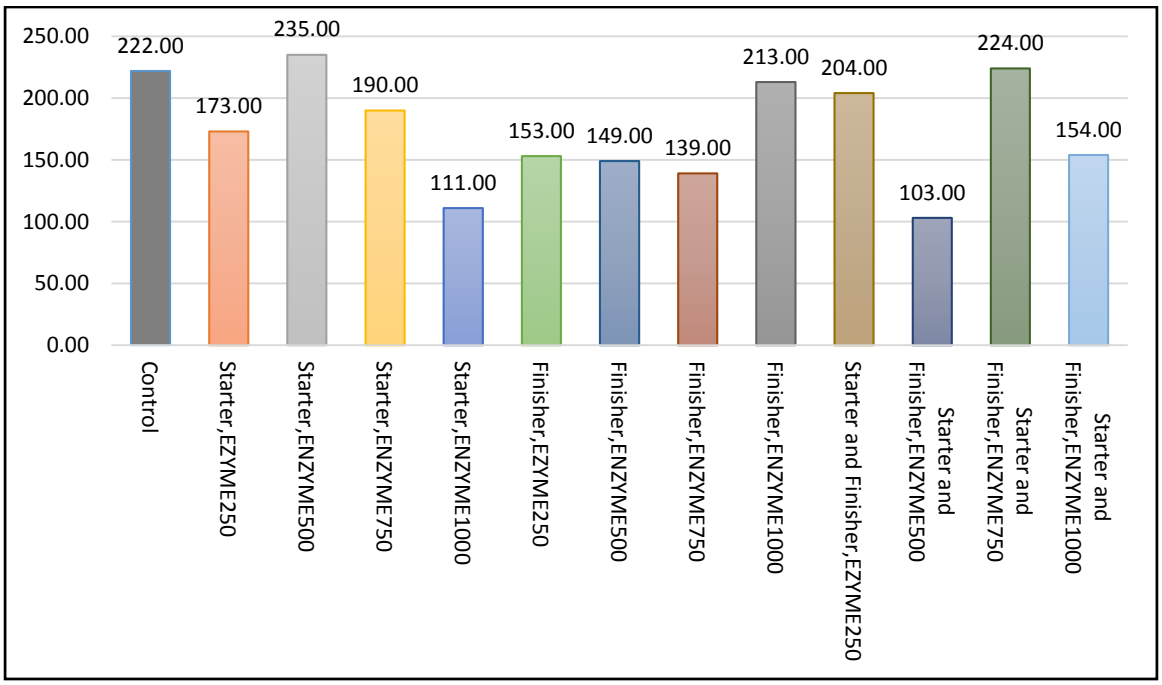
Appendix (36): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on intestinal length.



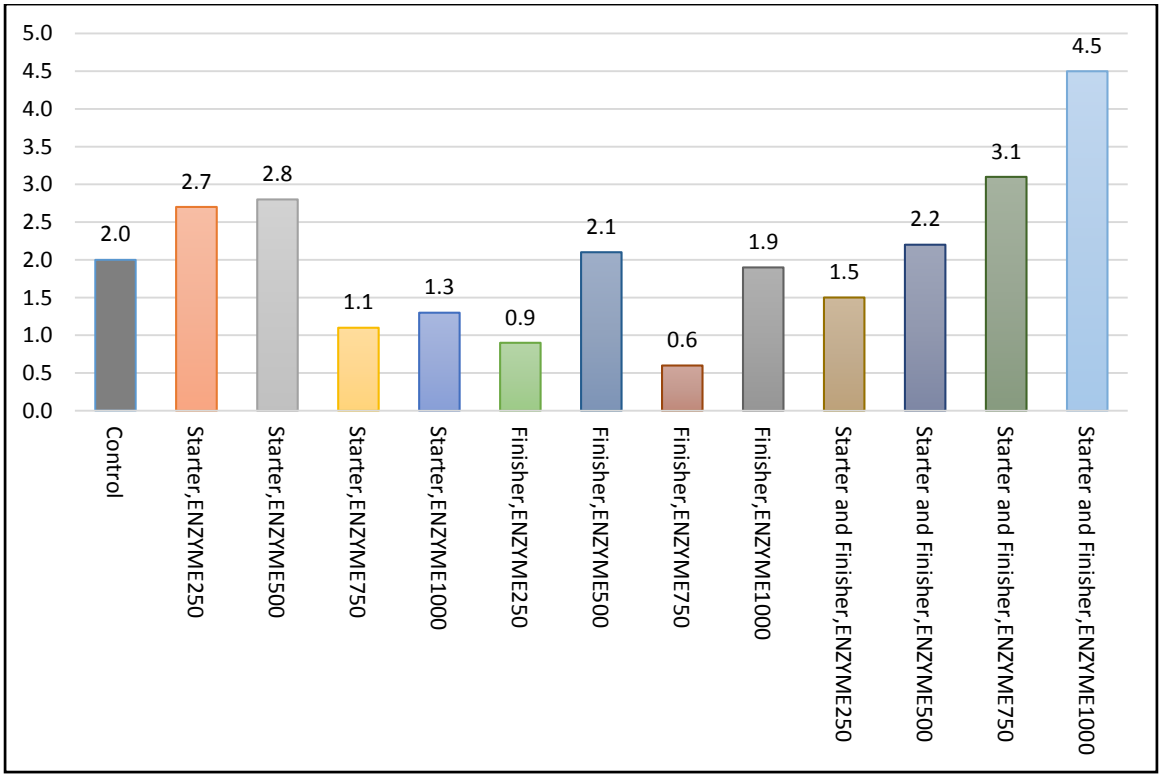
Appendix(37): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on blood hemoglobin.



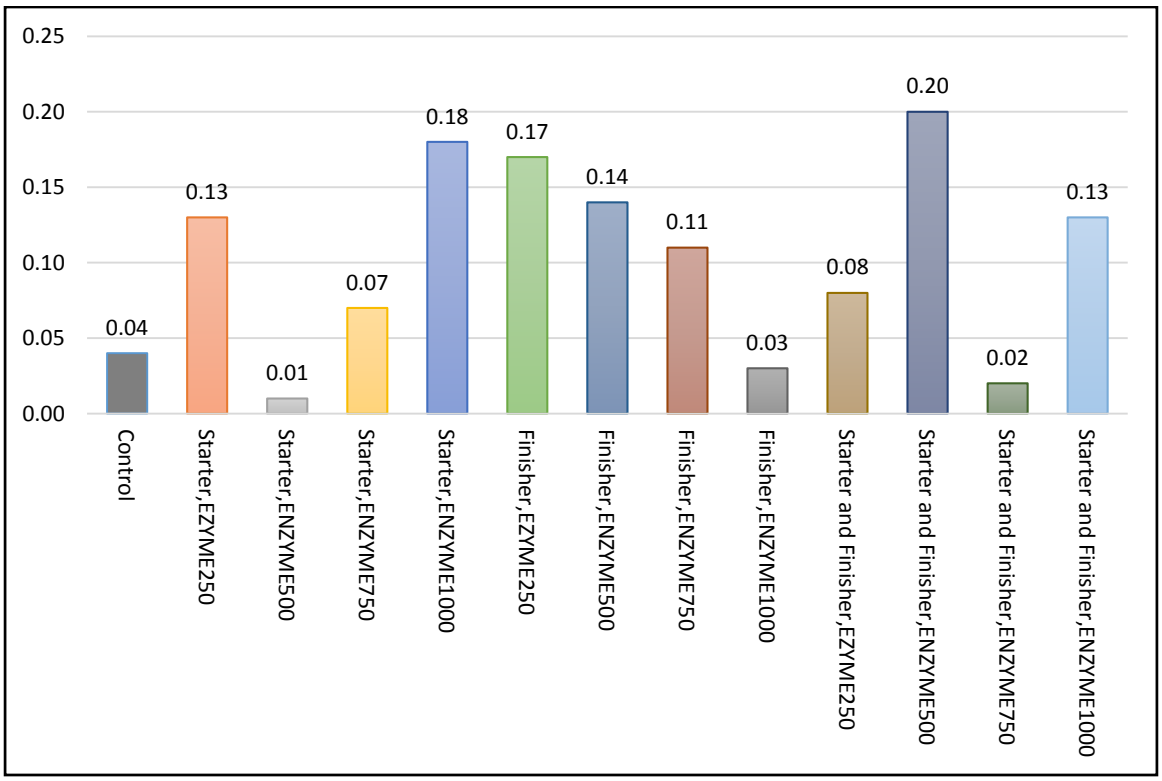
Appendix (38): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on blood glucose.



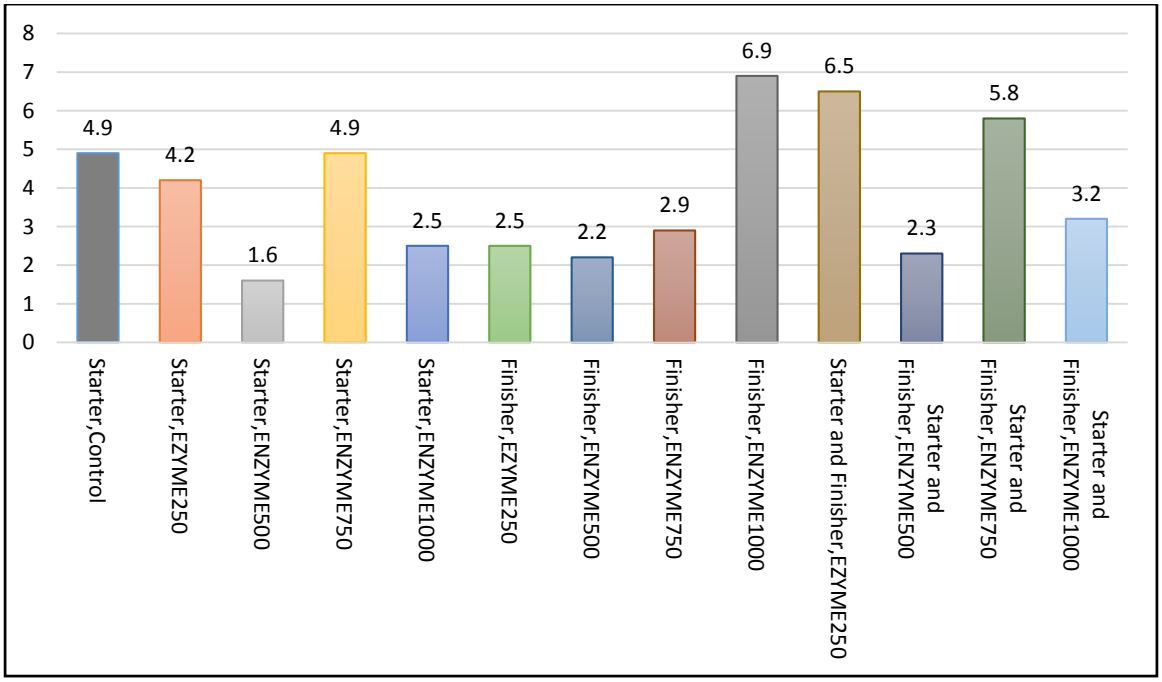
Appendix (39): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum urea.



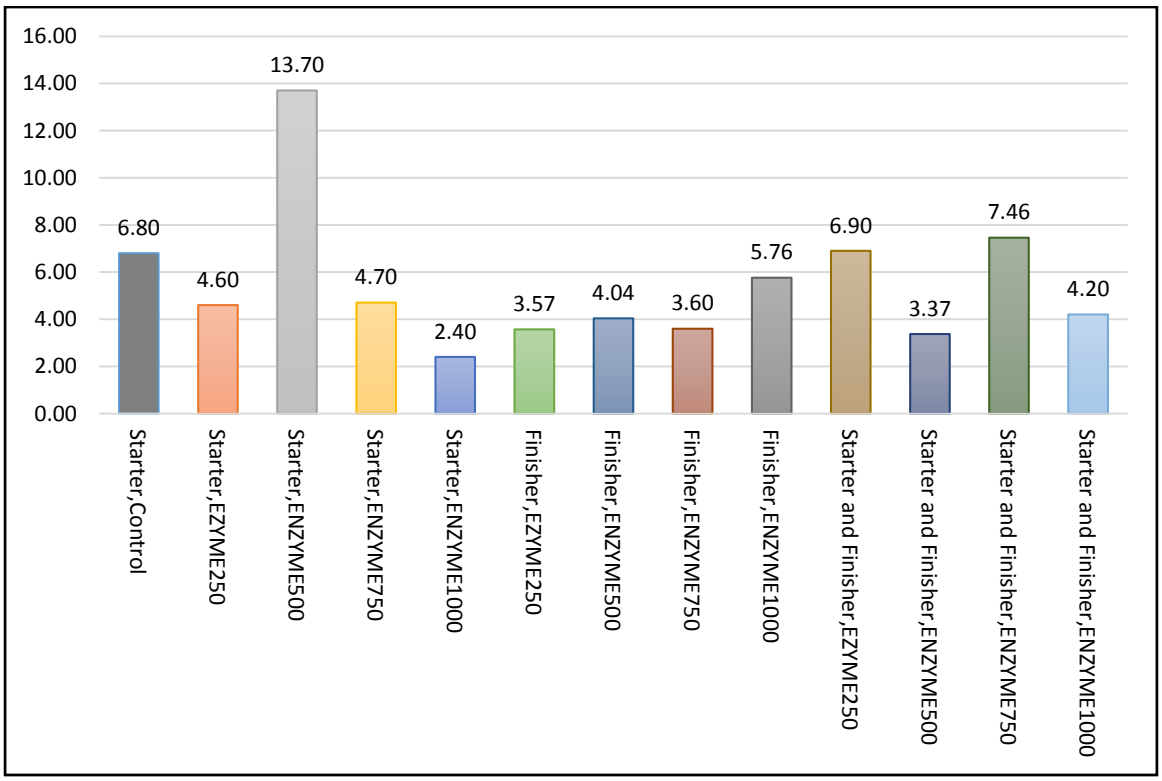
Appendix (40): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum creatinine.



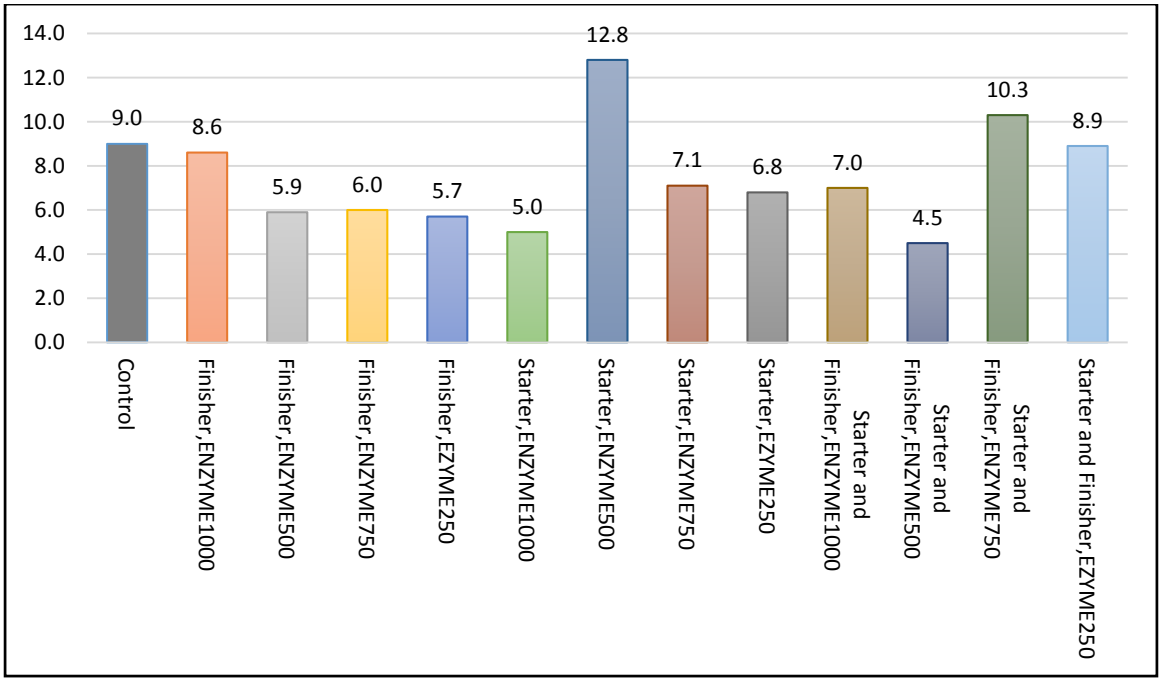
Appendix (41): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum uric acid.



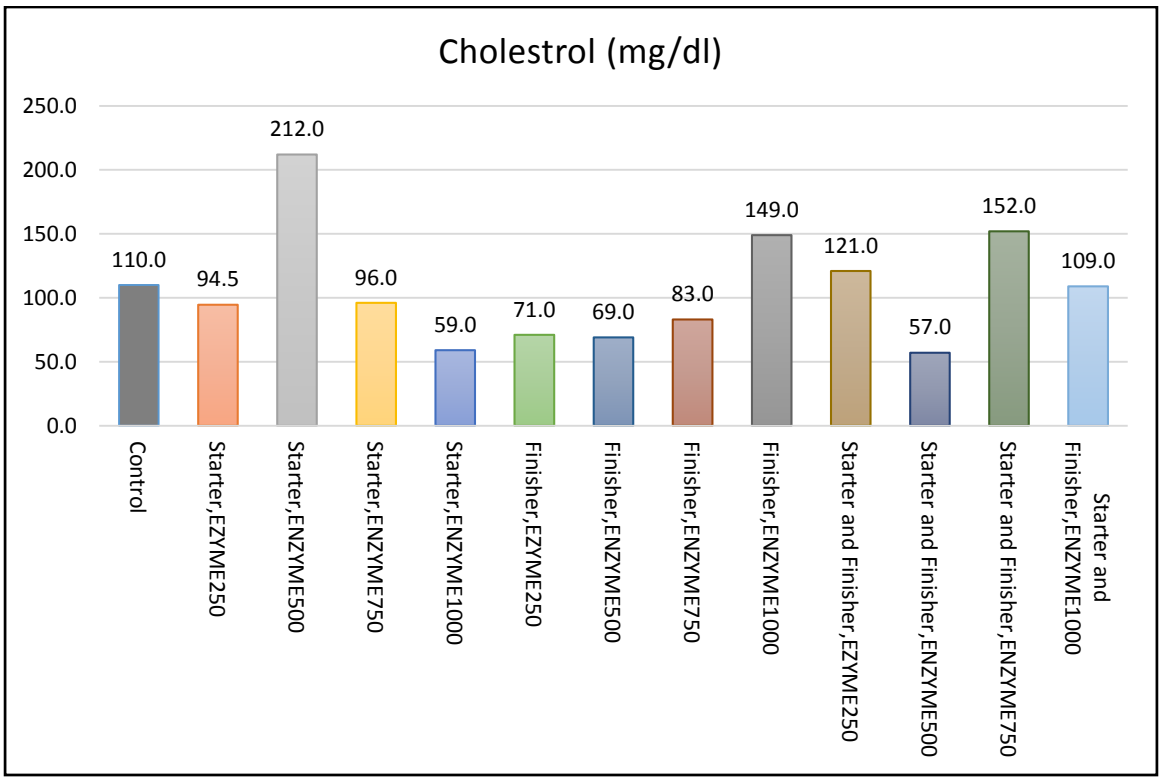
Appendix(42): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on blood phosphorus level.



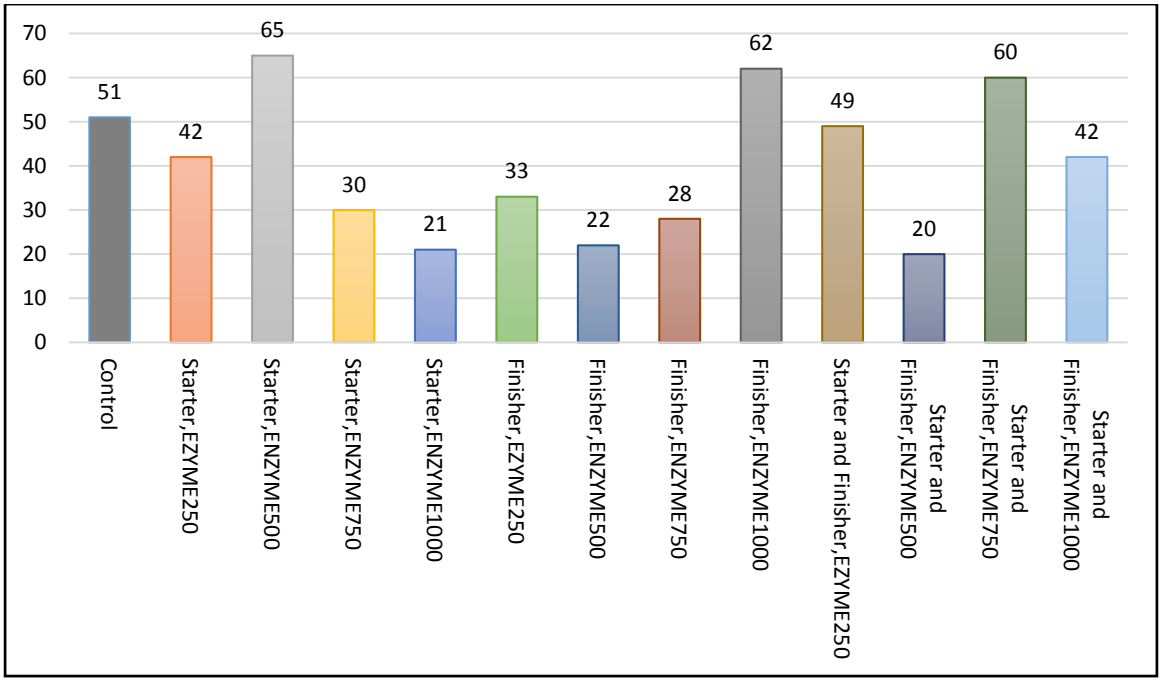
Appendix(43): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on blood calcium level.



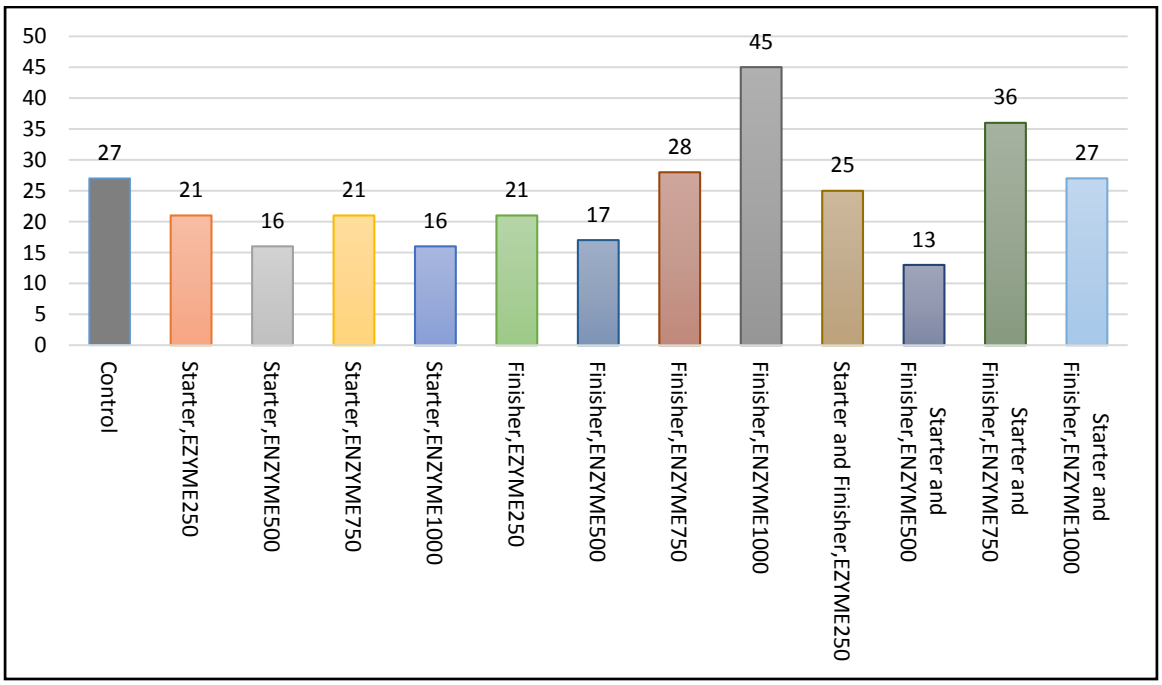
Appendix(44): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum cholesterol.



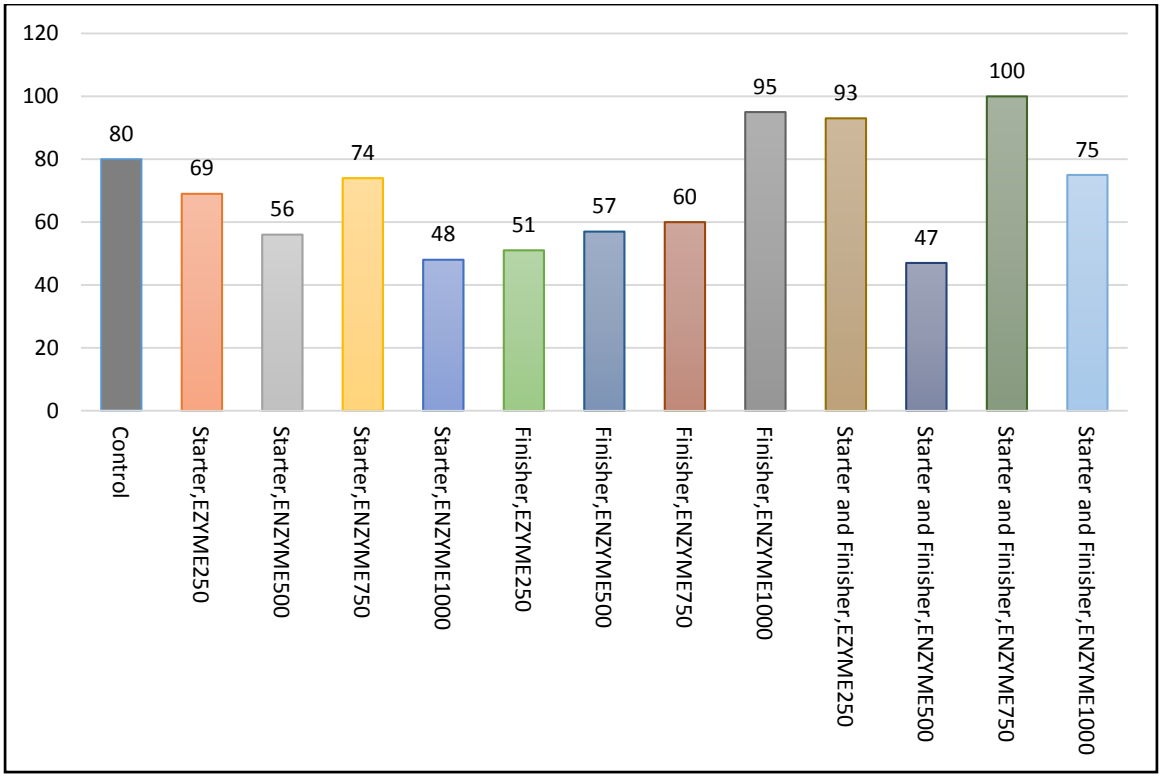
Appendix (45): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum triglycerides.



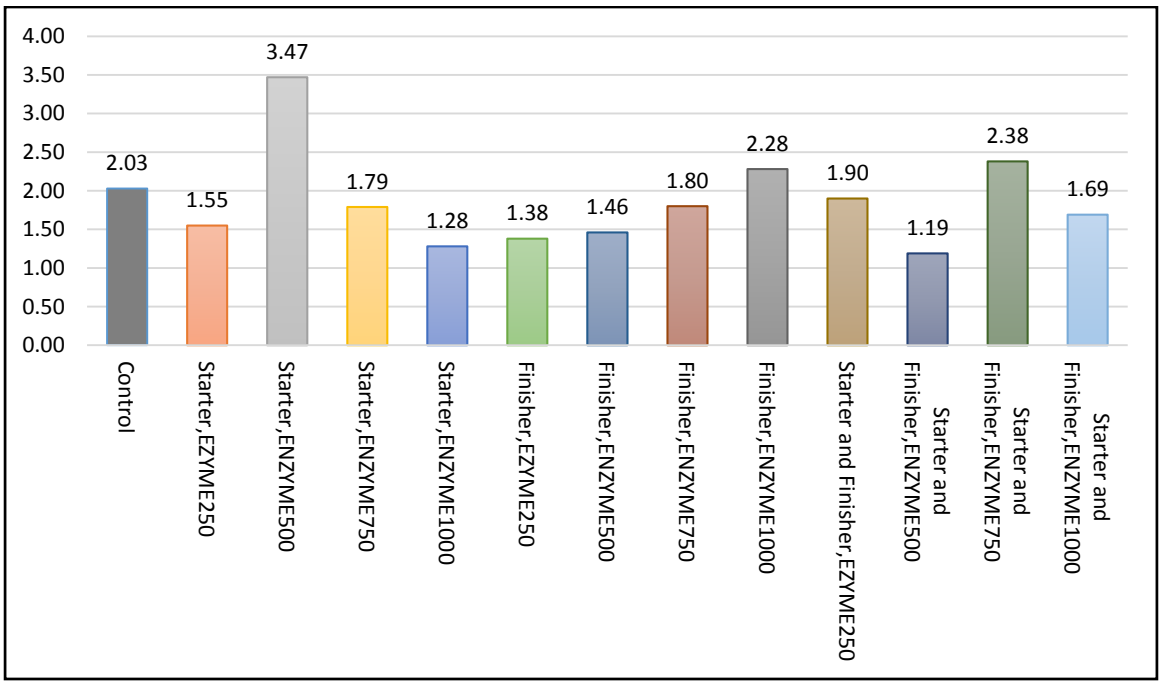
Appendix (46): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum low density lipids.



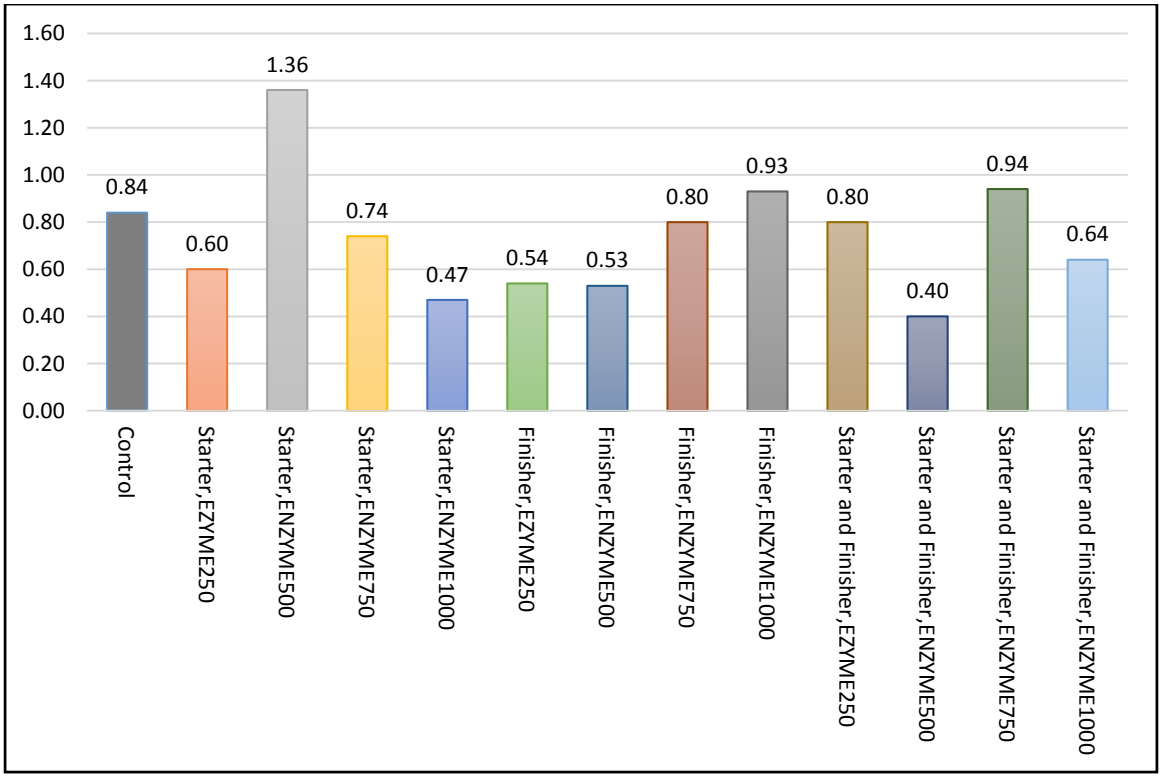
Appendix (47): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum high density lipids.



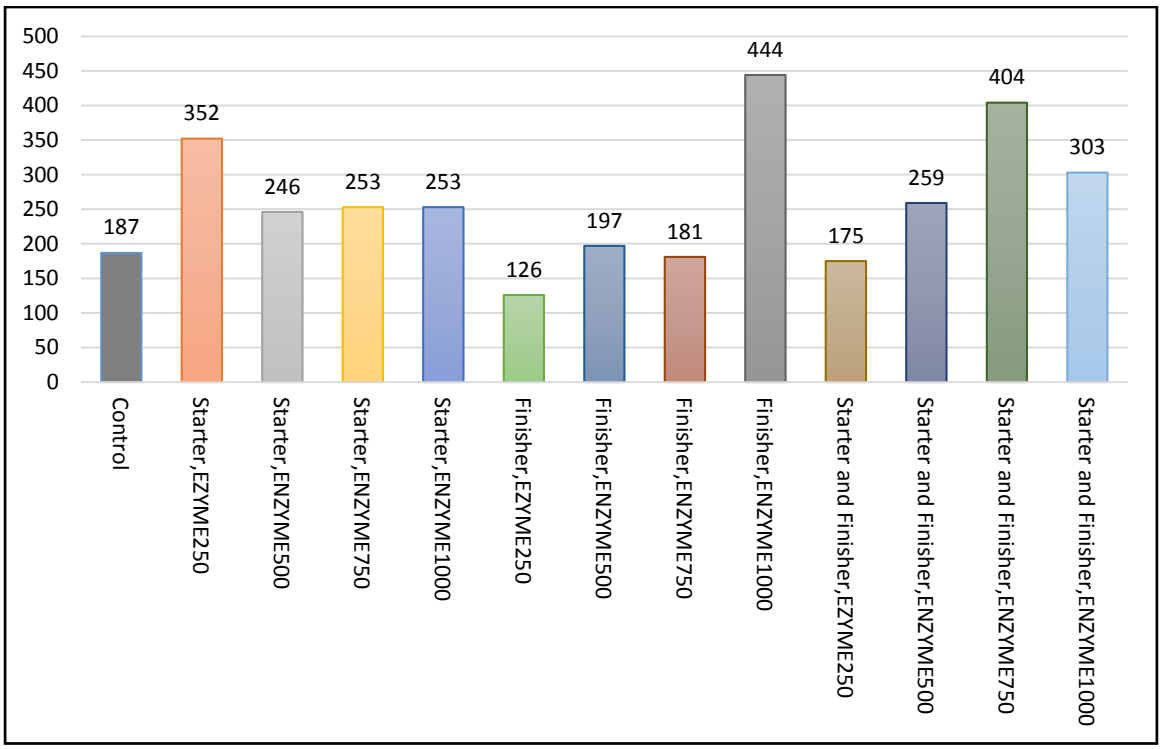
Appendix (48): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum total proteins.



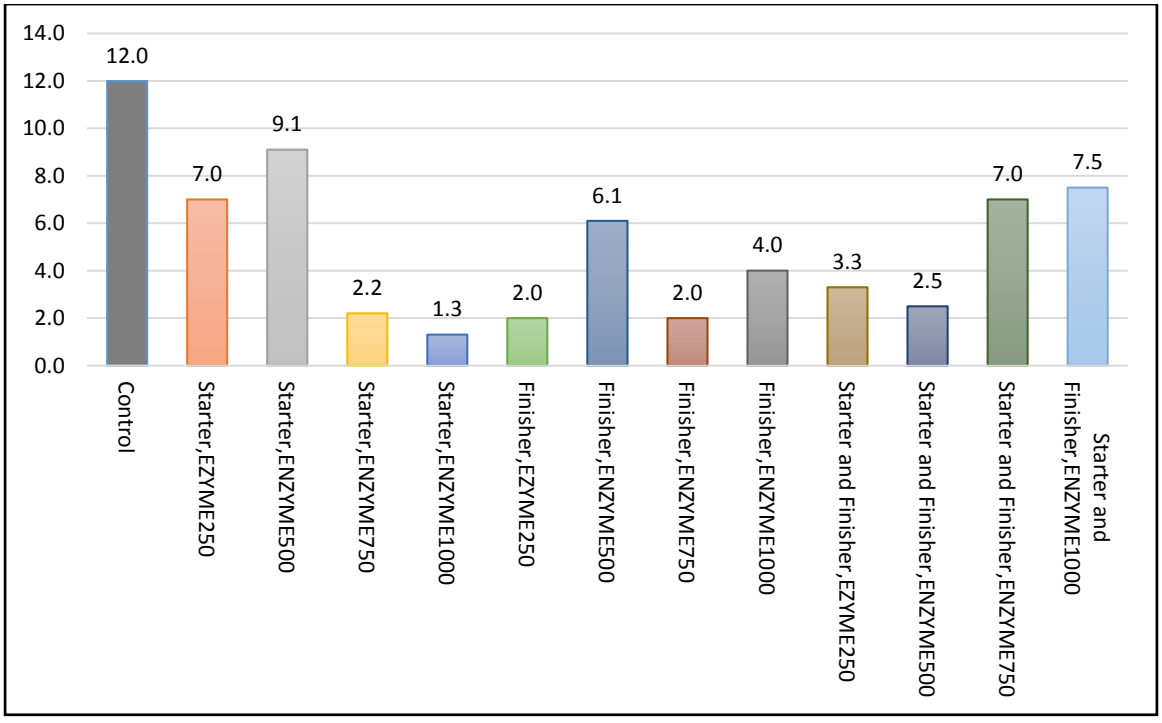
Appendix (49): Effect of feeding durations or the levels of included multi-enzymes and their interactive impact on serum albumin.



Appendix (50): Effect of durations or the levels of included multi- enzymes and their interactive impact on liver enzyme aspartate aminotransferase.



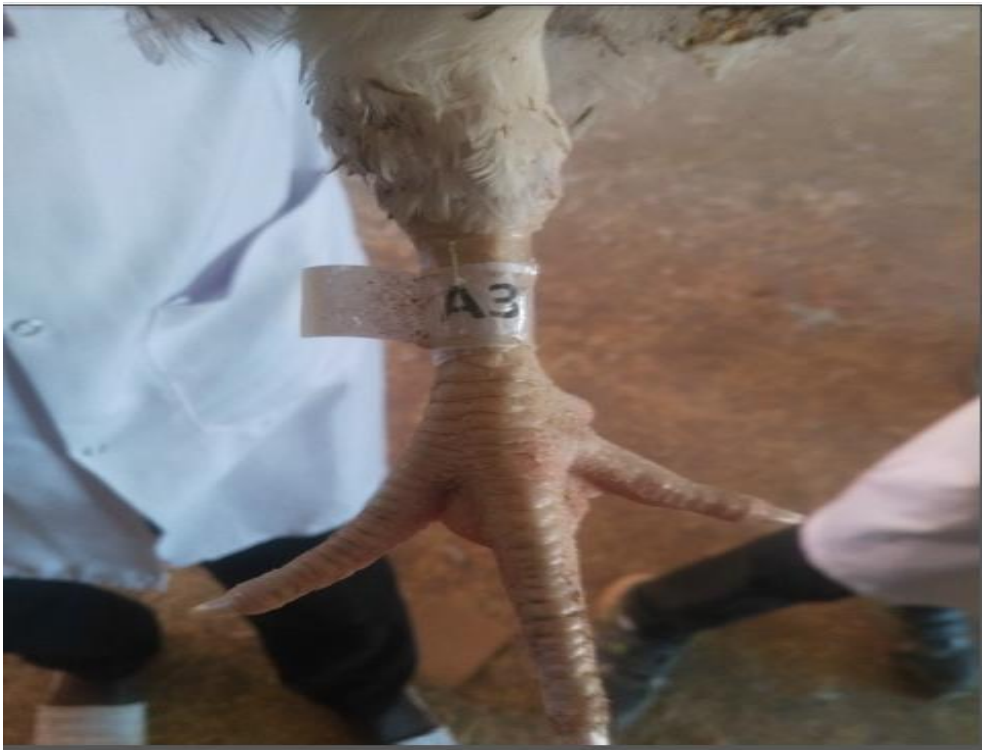
Appendix(51): Effect of feeding durations or levels of multi- enzymes and their interactive impact on liver enzyme aniline aminotransferase.




M2
B4


K1
F2
G3


2545 2 10.00
5345


A3


1720
TOTAL PRICE
1 2 3
0 .


فراش
طيب


SF – 400A
Electronic Compact Scale