AKENTEN-APPIAH MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

MAMPONG- ASHANTI

INFLUENCE OF EXOGENOUS ENZYME (ROVABIO™) ON THE GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, BONE HEALTH AND PROFIT MARGINS IN BROILERS FED DIETARY HIGH LEVELS OF MAIZE BRAN

MUSAH ISSAK

MASTER OF PHILOSOPHY

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BY

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A thesis in the Department of Animal Science Education, Faculty of Agriculture Education, submitted to the school of Graduate Studies in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (Non-Ruminant Nutrition) In the Akenten-Appiah Menka University of Skills Training and Entrepreneurial Development

DECEMBER, 2024

DECLARATION

STUDENT'S DECLARATION

I, Musah Issak, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

Musah Issak (Student)

Signature……

Date: ……/……/…….

SUPERVISOR'S DECLARATION

I hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis as laid down by the Akenten-Appiah Menka University of Skills Training and Entrepreneurial Development.

DR. Holy Kwabla Zanu (Supervisor)

Signature……

Date: ……/……/…….

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DEDICATION

I dedicate this thesis to my parents, Mr. Abdulai Musah and Mrs. Fatimatu Musah, and my beloved wife Rahima Nuhu.

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DEFINITIONS OF ABBREVIATION

ABBREVIATION	Definition
Ca	Calcium
MB	Maize bran
Enz	Enzyme
NSP	non-starch polysaccharide
NSPase	non-starch polysaccharide degrading enzyme
MEC	Multi enzyme complex
AME	Apparent metabolizable energy
AX	Arabinoxylans
P	Phosphorus
FI	Feed intake
WG	Weight Gain
BWG	Body weight Gain
FCR	Feed conversion ratio
SBM	Soyabean meal
IDF	Insoluble dietary fibre
ANF	Anti-nutritional factors
FTU	Phytase unit
DCP	Dicalcium Phosphate
PEI	Production Economic Index
NRC	National Research Council
AOAC	Association of Official Analytical Chemists.

ABSTRACT

Maize bran (MB) is a potential feed ingredient that can be used to reduce the quantity of maize added to Ghanaian poultry diets. However, MB contains a high level of anti-nutritional factors (ANFs) such as phytic acid and non-starch polysaccharides (NSPs) that negatively affect the utilization of nutrients and allow nutrients to escape enzymatic digestion in the gastrointestinal tract (GIT). Exogenous enzymes have been used over the years to degrade and improve the digestion of nutrients in feedstuffs containing these ANFs. Thus, this study was designed to investigate the hypothesis that exogenous enzyme (Rovabio^{TM}) in the presence of high levels of MB could improve the general performance of broilers and increase the profit margins. Three hundred and thirty-six (336) Cobb-500 broiler chicks were allotted to four dietary treatments in a 2 x 2 factorial arrangement in a completely randomized design. The factors were, Enzyme (No vrs yes) and Maize bran MB (No vrs yes) in the starter (d 0 to d 28), grower (d 28 to 42), and finisher diets (d 42 to 56). Weekly intake, body weight (BW), gain, feed conversion ratio, and livability were calculated. The data collected at d 28 and d 56 were nutrient digestibility (crude protein, crude fat, crude fibre and ash), gut pH, carcass traits and bone health (femur and tibial BS), and An Enzyme x MB interaction was detected for FCR ($P < 0.05$) on d 14 indicating that only in birds fed MB did the enzyme improve feed efficiency. On d 28, No MB as a main effect increased both BW ($P < 0.05$) and BW gain ($P < 0.05$) compared to Yes MB diet. The inclusion of enzyme diet increased the gizzard pH of the birds ($P < 0.05$) at d 28. Maize bran increased gizzard weight and reduced breast weight, % bodyweight ($P < 0.05$). There was no consistency in the effect of enzyme or maize bran on bone traits. However, the general outcome suggests that the inclusion of enzymes increased feed cost but also increased profitability. In conclusion, the inclusion of the enzyme in the MB-based diet improves broiler performance.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Food safety, environmental impact, and high production costs are just a few of the difficulties the poultry industry faces as it attempts to feed a growing population with high-quality products at reasonably low cost (Pirgozliev *et al*., 2019). Enhancing the nutritional content of feedstuffs is one strategy to somewhat mitigate the growing cost of feed. Feed additives are chemical and biological supplements, including enzymes, that are used for an added advantage of the feed (Rios *et al.,* 2017). Recent studies have focused on the effects of exogenous enzyme supplementation on nutrient digestibility and performance of chickens. According to Yacoubi *et al*. (2016), exogenous enzymes are safe to use and enhance feed conversion ratio (FCR) and broiler body weight gain.

Globally, maize and soybean meal are primarily used as the main ingredients in chicken feed. Nevertheless, several anti-nutritional factors (ANFs) such as phytic acid, non-starch polysaccharides (NSPs), and anti-trypsin negatively impact their nutritional value (Amerah, 2015; Jlali *et al*., 2020). Phytate and non-starch polysaccharides (NSP) are examples of these ANFs, which allow essential nutrients to escape digestion in the gastrointestinal tract (GIT) (Rios *et al*., 2017; Sun *et al*., 2019). High NSP levels cause digesta viscosity to increase, leading to reduced absorption of important nutrients like proteins, lipids, and starch and also lowering feed efficiency and performance (Amerah, 2015; Jlali *et al*., 2020; Musigwa *et al*., 2020). The apparent metabolizable energy (AME) of feedstuffs varies depending on the composition and structure of the NSP (Yacoubi *et al*., 2017). To improve digestion and optimize energy utilization, non-starch polysaccharide degrading enzymes (NSPase) are

added to feedstuffs. This reduces intestinal viscosity and improves performance (Yacoubi *et al*., 2017; Musigwa *et al*., 2020). The appropriate addition of NSPase, according to Maharjan *et al*. (2019), results in an improvement in the metabolizable energy (ME) of the feed ingredients because the breakdown of NSP releases extra energy. NSPase can be used to boost the nutritional value of low-quality maize (Rios *et al*., 2017).

In addition to NSP, phytate also has an adverse effect on feedstuffs' nutritional value. More than 60 % of the total phosphorous (P) in feedstuffs can be bound by phytate, making P inaccessible for absorption (Lawlor *et al*., 2019). This frequently causes animal feed costs to rise and environmental contamination to increase as the dietary requirements for P inclusion levels are exceeded. To improve growth performance and carcass characteristics of broilers, Jlali *et al*. (2020) reported that phytase inclusion in broiler diets breaks down phytate and releases P along with other minerals and nutrients that are trapped.

1.2 Problem Statement

Maize makes up the majority of the diet of chickens in many African countries whereas, it is used to also prepare the majority of the staple foods for humans, making it a costly commodity. However, Ajila *et al*. (2012) have identified several locally available agricultural and agro-industrial by-products, including rice bran, wheat bran, and maize bran, as suitable alternatives to maize. Maize bran is a potential feed ingredient that can be used to reduce the quantity of maize grain added to the Ghanaian poultry diet. Moreover, maize bran contains high levels of anti-nutrients such as phytic acid and non-starch polysaccharides (NSPs) that negatively affect the utilization of nutrients and allow nutrients to escape digestion in the gastrointestinal tract (GIT) of monogastric animals (Amoah *et al.,*

2018). Several studies have shown that the antinutritional factors (ANFs) in non-conversional feed resources such as rice bran, maize bran and wheat bran could be broken by the activities of exogenous enzymes in the diet. Exogenous enzymes have been used over the years to degrade and improve the digestion of nutrients in feedstuffs containing these ANFs (Alagawany *et al.,* 2018). However, there is a paucity of information on the effect of increasing the inclusion rate of maize bran in broiler diets containing novel exogenous feed enzymes (Rovabio^{TM}). The enzyme product is made up of xylanase, phytase, amylase, cellulase, and proteases which helps to improve the utilization of diets suspected to contain high levels of NSPs and phytic acids. Therefore, it was hypothesized that the inclusion of exogenous enzymes would improve the utilization of maize bran or allow their higher inclusion rate in Ghanaian broiler diets while giving better nutrient digestibility, growth performance, bone health, and improving profit margins.

1.3 Main Objective

The main objective of this study was to evaluate the influence of exogenous enzyme on nutrient digestibility, growth performance, gut pH, bone health and profit margins in broilers fed diets containing high levels of maize bran**.**

1.4 Specific Objective

Specifically, the study was conducted to evaluate;

1. the influence of exogenous enzyme on broilers fed diets containing high levels of maize bran on the growth performance of broiler chickens
2. the influence of exogenous enzyme on broilers fed diets containing high levels of maize bran on gut pH of broiler chickens

3. the influence of exogenous enzyme on broilers fed diets containing high levels of maize bran on carcass characteristics of broiler chickens
4. the influence of exogenous enzyme on broilers fed diets containing high levels of maize bran on bone health of broiler chickens
5. the influence of exogenous enzyme on broilers fed diets containing high levels of maize bran on nutrient digestibility (crude protein, ash, crude fat, and crude fibre)
6. the cost and benefits of feeding broiler chickens with diets containing high levels of maize bran and enzyme supplementation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non-Conventional Feed Resources

The rising cost of conventional feed ingredients, such as maize and soybeans, has made poultry production more expensive, particularly for small and medium-scale farmers. As a result, there is growing interest in utilizing non-conventional feed ingredients that are locally available and often more affordable. Non-conventional feeds refer to alternative ingredients such as agro-industrial by-products, leaves, and other plant materials not traditionally used in poultry diets. These feeds have the potential to reduce feed costs, enhance sustainability, and support the growth of poultry in resource-limited settings (Amata, 2014). However, the use of non-conventional feeds also poses certain challenges regarding nutrient availability, palatability, and potential anti-nutritional factors (ANFs).

Studies have shown that non-conventional feed ingredients can have varying effects on the growth performance of poultry, depending on their nutrient composition and inclusion levels. For example, cassava peels, which are high in fibre but low in protein, may reduce growth rates if included at high levels without proper supplementation (Adeyemo *et al*., 2016). However, when supplemented with protein-rich ingredients such as moringa or soybean meal, cassava peels can be effectively utilized in poultry diets without negatively affecting performance (Ogbuewu & Mbajiorgu, 2023).

Rice bran and maize bran, both commonly used in poultry diets, contain relatively high levels of ANFs such as phytates and non-starch polysaccharides (NSPs), which can reduce nutrient digestibility (Ravindran, 2013). The inclusion of enzymes such as phytase or

xylanase can improve nutrient availability and enhance the overall performance of birds fed diets containing rice bran or maize bran (Selle *et al*., 2009).

One of the main challenges associated with non-conventional feeds is the presence of ANFs, which can inhibit nutrient absorption and reduce the overall performance of poultry. For example, cassava peels contain cyanogenic glycosides, which can be toxic if not properly processed (Devi & Diarra, 2021). Similarly, Leucaena leaves contain mimosine, an alkaloid that can cause toxicity in poultry if consumed in large quantities (Norfadhilah, 2019). Proper processing methods such as drying, boiling, or fermentation can help reduce the levels of ANFs in non-conventional feed ingredients, making them safer for poultry consumption.

Enzyme supplementation has also been widely studied as a strategy to mitigate the negative effects of ANFs in non-conventional feeds. For example, the addition of phytase to diets containing rice bran or maize bran has been shown to increase phosphorus availability and improve growth performance (Selle *et al*., 2009). Similarly, the use of xylanase or other NSP-degrading enzymes can enhance the digestibility of fibre-rich ingredients, leading to better feed efficiency (Ravindran, 2013).

2.1.1 The need for non-conventional feed resources

There are serious shortages in some animal feeds of the conventional type. The grains are required almost exclusively for human consumption. With increasing demand for livestock products as a result of rapid growth in the world population and shrinking land area, future hopes of feeding the animals and safeguarding their food security will depend on the better utilization of non-conventional feed resources that do not compete with human food. The availability of feed resources and their rational utilization for livestock represents possibly

the most compelling task facing planners and animal scientists in the world. The situation is acute in numerous developing countries where chronic annual feed deficits and increasing animal populations are common, thus making the problem a continuing saga (Norfadhilah, 2019).

Thus, non-conventional feeds could partly fill the gap in the feed supply, decrease competition for food between humans and animals, reduce feed cost, and contribute to self-sufficiency in nutrients from locally available feed sources (Rashid, 2020; Beriso, 2022; Kolawole & Mustapha, 2023). It is therefore imperative to examine for cheaper non-conventional feed resources that can improve intake and digestibility of low-quality forages. Feedstuffs such as fish offal, cassava peel, parm kernel cake, sugarcane bagasse, rice bran, maize bran, cocoa bean waste, coconut meal, corn cob, moringa leaf, leucaena leaf, local brewery and distillery by-products, sisal waste, and coffee pulp are commonly used in Ghana and could be invaluable feed resources for small and medium size holders of livestock (Nortey *et al*., 2015; Amoah *et al*., 2017).

2.1.2 Agro-industrial by-products for livestock

Appropriate use of relatively inexpensive agricultural and industrial by-products is of paramount importance for profitable livestock production. However, the high cost and low availability of conventional livestock feedstuffs frequently demand consideration of by-products even if the efficiency of utilization is low (Kolawole & Mustapha, 2023). Efficient use of by-products relies on their chemical and physical properties, which influence production system outputs. In developing countries like Ghana, grains, which form the bulk of concentrate feeds for poultry, is both in short supply and expensive due to direct

competition with human food uses (Kusi *et al*., 2015). The increasing human demands for several foods (i.e. olive oil, vegetables, wine, fruit juices, etc.) led to a considerable increase of lands occupied by crops producing these feeds. Consequently, huge amounts of agro-industrial by-products are available in numerous developing countries (e.g. maize bran, rice bran, wheat bran, copra cake etc.), which are still not fully utilized in poultry feeding. Most of these agro-industrial by-products are low in major nutrients. Moreover, the difficulty of the use of these feed sources as fresh material for extended periods and the lack of efficient ways for their integration in feeding calendars may account for their under-utilization (Onte *et al*., 2019).

Table 2.1: By-Product Feeds from Trees and Crops for Livestocks

Type	**Crop**	**BY-PRODUCT FEEDS**
Tree crop	cocoa	Cocoa bean waste and cocoa pod husks
	Coconut	Coconut meal
	Oil palm	Oil palm sludge (dry), palm press fibre and palm kernel meal
	Rubber	Rubber seed meal
Field crop	Castor	Castor meal
	cotton	Cotton seed meal
	Maize	Maize bran and maize germ meal
	Rice	Broken rice, rice bran, rice husk and rice straw
	wheat	Wheat bran and wheat straw
	Cassava	Tapioca waste
	Surgarcane	Baggase, green tops, and molasses

Source:(Onte *et al*., 2019).

2.1.3 Advantages of non-conventional feed resources (NCFR)

The use of NCFR offers several advantages, such as reducing feed costs, minimizing waste, and enhancing sustainability in animal production. Below are some of the advantages of NCFR;

a) Concerning the feeds of crop origin, the majority are bulky poor-quality cellulosic roughages with high crude fibre and low nitrogen contents, suitable for feeding to ruminants and poultry (Amoah *et al*., 2017).

b) They are mainly organic and can be in a solid, slurry, or liquid form. Their economic value is often very low (Nortey *et al*., 2015).

c) These are end products of production and consumption that have not been used (Beriso, 2022).

d) The feed crops that generate valuable NCFR are excellent sources of fermentable carbohydrates eg. cassava and sweet potato. This is an advantage to ruminants because of their ability to utilize inorganic nitrogen (Amata, 2014).

e) Fruit wastes such as banana rejects and pineapple pulp by comparison have sugars that are energetically very beneficial (Amata, 2014; Nortey *et al*., 2015).

f) They have considerable potential as feed materials and their value can be increased if they are converted into some usable products (Ravindra, 2013).

2.2 Maize Bran

Maize bran is a by-product of dry milling maize, which consists of the bran coating and the maize germ. It is palatable to all classes of farm animals and approaches maize grain in feeding value though it contains more fibre because of the hulls, which are included.

Maize bran consists of the outer coating of the kernels, including the hull and tip cap, with little or none of the starchy part of the germ (Saeed *et al*., 2021). Hussain *et al*. (2024) defined maize bran as a by-product obtained from the milling of maize, which is the removal of the hull. They added that the hull contains about 15 % crude fibre. Kantanka (2013) reported that maize bran is very much sought after by small and medium scale pig and poultry farmers. It is a very good partial replacement for maize for these species partly because milling machines used in the milling process are not very efficient and the by-product contains most of the germ, bran and some proportions of the endosperm. It is therefore a high-energy source, but unfortunately during the manufacturing process, water is added to the maize and thus the maize bran may be wet. If not dried immediately, it can easily become moldy and may also become rancid. Wherever there are large concentrations of poultry and pigs, the demand is high, and therefore it can be scarce leading to high costs. It is highly fibrous and this limits its utilization because its high fibre cannot be digested by the endogenous enzymes of poultry and can have anti-nutritive effects (Saeed *et al*., 2021). Maize bran causes an increase in viscosity of intestinal content and entrap large amounts of well digestible nutrients like starch and proteins. This leads to an impaired digestion and digestive problems (Hussain *et al*., 2024). Table 2.4 shows the proximate composition of maize bran.

Table 2.2: Proximate composition of maize bran

Composition	%
Crude protein	9.85
Crude fat	11.66
Moisture content	16.01
Ash content	4.37
Crude fibre	13.29
Total reducing sugar	19.39
Total carbohydrate	58.12
ME (kJ/100g)	1586.83

Source:(Asuk *et al*., 2016).

Plate 1: Photograph of maize bran. Source:(www.alamy.com)

2.3 Feed Additives in Poultry Production

Feed additives make up a small percentage of animal feed, yet they can have a significant impact by enhancing feed utilization, increasing growth efficiency, and reducing diseases (Cherian, 2020). The commonly used feed additives in poultry production are pro- and

prebiotics, antioxidants, enzymes, and antibiotic growth promoters and each has a unique function. The feed enzyme market has expanded significantly over the last five years, mostly as a result of rising raw material costs (Ravindran, 2013). Even with increased acceptability, there are still many unanswered concerns about how to employ enzymes to provide consistent effects and reactions to enzyme supplementation. However, these inconsistent answers highlight both the existing constraints and the possible ways to improve the advantages of using enzymes. Three essential elements are inescapably linked to limitations in enzyme responses: the substrate, the bird, and the enzyme.

Enzymes decrease nutrient loss and lower environmental pollution by enhancing digestion, which increases nutrient availability (Cherian, 2020). According to Pirgozliev (2019), enzymes are proteins that catalyze particular chemical processes and are specific to a particular substrate. The use of exogenous feed enzymes is one of the ways for nutritionists to create diets more economically while enhancing the efficiency of feed and still giving consumers the most economical source of protein because feed costs make up the greater percentage of all input costs (Davids & Meyer, 2017; Boyd *et al*., 2018).

2.4 Exogenous Enzyme Activities in Broiler Diets

According to Zakaria *et al*. (2010), using exogenous feed enzymes in monogastric diets is a useful strategy for allowing for flexibility in diet composition, as well as for reducing feed costs, improving feed digestibility, and minimizing environmental pollution. According to Doskovic *et al*. (2013), for exogenous enzymes to be as efficient as possible, they must balance out the function of the animal's endogenous substances. Plant-based raw materials have anti-nutritional factors that limit the availability of nutrients by preventing endogenous

enzymes from accessing them, which prevents digestion (Costa *et al*., 2013). Rios *et al*. (2017) reported that the digesta transit rate in modern broilers is too quick for optimum digestion, allowing important nutrients to escape digestion in the GIT. In addition to breaking down bound nutrients, exogenous enzymes reduce the cost of producing chicken diets (Boyd *et al*., 2018).

According to Saleh *et al*. (2019), animal rations contain trace amounts of exogenous enzymes such as phytase, protease, and xylanase which are produced from microbial sources. It has been demonstrated that the use of exogenous enzymes in animal feed improves the body's ability to absorb nutrients that would not otherwise be available (Classen, 1996). The addition of enzymes reduces the adverse effects of ANFs and boosts profitability in poultry production (Costa *et al*., 2013; Sun *et al*., 2019). Approximately 1.67-1.88 MJ of energy per kilogram of feed is not being digested in a conventional corn-soybean diet without enzyme supplementation (Govil *et al*., 2017). Enzymes can be used to increase protein, fat, and carbohydrate availability as well as to increase more energy being made available for utilization.

In poultry feed, enzymes can be added either separately or in the form of multi-enzyme complexes (MEC) (Jlali *et al*., 2020). Positive outcomes have been reported for both MEC and single. There has been contradictory research on the effectiveness of supplementing with non-starch polysaccharide degrading enzymes in addition to phytase. While NSPase and protease possess distinct target substrates, their actions complement one another because NSPase releases many nutrients and reduces mucus production in the gastrointestinal tract (Rahimi *et al*., 2020). According to Jlali *et al*. (2020), the bird's response to MEC use is influenced by its genetic ancestry, age, nutrition, and MEC dose.

Adding enzymes to poultry feed, either separately or in combination, has several advantages:

i. The release of encapsulated starch in the cell wall minimizes the variation in apparent metabolizable energy (AME) and performance (Amerah, 2015).
ii. Less digesta viscosity lowers the amount of wet litter and sticky droppings, which lowers the risk of dermatitis (Wang *et al*., 1998; Amerah *et al*., 2017).
iii. Due to the immature GITs, young chicks are particularly vulnerable to the negative impact of NSP. Enzymes called carbohydrases help to keep the gut healthy so that an inflammatory gut does not impair performance (Yacoubi *et al*., 2017).
iv. By reducing the digesta viscosity and changing gut microbes by promoting the growth of beneficial microbes, BW, gain, and FCR are improved (Saleh *et al*., 2019).
v. Short-chain fatty acids (SCFA) such as butyrate and acetate are produced by multi-enzyme complexes. Butyrate serves as an energy source for the intestinal epithelial cells in the stomach, promoting both their proliferation and differentiation to improve digestive health (Yacoubi *et al*., 2016).
vi. Phytase inclusion enhances growth performance and carcass characteristics of broilers by releasing trapped P and other nutrients (Jlali *et al*., 2020).
vii. Because nutrients are being utilized, feed costs are decreased (Lawlor *et al*., 2019).
viii. The amount of undigested nutrients excreted into the environment is decreased, which reduces its contribution to environmental contamination (Lawlor *et al*., 2019).

2.4.1 Enzymes that break down non-starch polysaccharides (NSPase)

Enzyme use for commercial applications is a relatively new development, beginning about three decades ago with a focus on addressing the anti-nutritional impact of non-starch polysaccharides (NSP) in cereal-based diets for broiler chickens. NSP, characterized by its

high molecular weight, contributes to increased digesta viscosity by forming complex polymers that resist digestion in poultry (Wu *et al*., 2004). Assessing NSP content in raw materials is crucial for determining the appropriate enzyme levels needed to facilitate energy and nutrient release, thus enhancing diet quality.

The specific substrates released by NSPase that contribute to favorable production of short-chain fatty acids (SCFA) in the ceca remain incompletely understood. However, increased fermentation of oligosaccharides and subsequent SCFA production likely play a role in improving apparent metabolizable energy (AME) and influencing gut hormone secretion, which aids in gastric retention and overall gut health. Enhanced growth performance results from improved AME, dry matter retention, and ileal digestible energy. Furthermore, by improving the performance and digestion of nutrients, sticky droppings are reduced in frequency. Furthermore, the proliferation of butyrogenic bacteria in the ceca, facilitated by NSP-degrading enzymes, protects against pathogenic bacteria (Lee *et al*., 2010).

Carbohydrate-degrading enzymes are introduced to high-NSP monogastric feed to break complex carbs down into smaller polymers (Cherian, 2020). Endogenous enzymes are essential for aiding in the breakdown of β 1-3, β 1-4, and β 1-6 links that are present in NSP, but they are absent in monogastric animals. According to Tejede & Kim (2021), the degree of NSP molecule branching affects its solubility, which in turn affects the enzyme's effectiveness (Cherian, 2020). Wu *et al*. (2004) showed that adding xylanase and phytase to the broiler chicks' diet reduced the digesta viscosity from their duodenum ($p < 0.05$). Furthermore, Lee *et al*. (2010) reported that adding phytase and NSPase to the diet decreased the digesta viscosity by 16.8 % and 12.4 %, respectively. These findings imply that NSP

degrading enzymes that are capable of dissolving the matrix of the cell wall may make it simpler for phytase to access nutrients that are encapsulated in cell walls by reducing the viscosity of the intestinal contents.

2.4.2 The enzyme profile of Rovabio

Rovabio is a commercial multi-enzyme blend manufactured by fermenting the fungus *Talaromyces versatilis*, which breaks down arabinoxylans (AX) (Bichot *et al*., 2022). This particular blend consists of 19 enzymatic activities, the most common of which are endo-xylanase and arabinofuranosidase, with a ratio of 3:7, respectively (Table 2.3) (Cozannet *et al*., 2017; Cozannet *et al*., 2019; Bichot *et al*., 2022).

Rovabio was evaluated in broilers and was found to improve gut health and growth performance by improving the utilization of energy, fat, fibre, and protein (Cozannet *et al*., 2019; Saleh *et al*., 2019). This is because endo-xylanases and arabinofuranosidase work together to increase nutrient digestibility.

Table 2.3 Enzyme composition of Rovabio

Enzyme	Composition
Xylanase	β-xylosidase and Endo-1,4 β-xylanase
β-glucanases	Endo-1,3 1,4 β-glucanase, Laminarinase
Proteases	Metallo protease and Aspartic protease
Debranching enzymes	α-glucuronidase Ferulic acid esterase, and α-arabinofuranosidase,
Cellulases	Endo-1,4 β-glucanase, Cellobiohydrolase and β-glucosidase
Pectinases	Endo-1,5 α-arabinanase, Pectin esterase, Polygalacturonase, α-galactosidase and Rhamnogalacturonase
Others	Endo-1,4 β-mannanase, β-mannosidase

Source: (Plouhinec *et al*., 2023).

Chickens raised on diets high in NSP experience adverse consequences, which can be reduced by adding xylanase as an enzyme supplement (Arczewska-Wlosek *et al*., 2019). Endo-xylanase facilitates the hydrolysis of the xylan backbone, which releases encapsulated starch and other nutrients and reduces the digesta viscosity caused by soluble non-starch polysaccharides (sNSP). Arabinoxylan is the primary NSP that accounts for at least 50 % of the total carbohydrate fraction (Amerah, 2015; Ward, 2021).

It has been documented that certain enzyme, such as β-glucanase, improve the nutritional content of cereal by-products in monogastric animals (Cherian, 2020). According to Jlali *et al*. (2020), the activity of xylanase causes the release of oligosaccharides, which in turn alters the hindgut microbial population. This improves intestinal health and enhances the capacity for digestion and absorption, ultimately leading to better growth performance. Reducing intestinal viscosity and enhancing the nutritional content of cereal-based diets is achieved

by supplementing diets with glucanase and xylanase alone, in combination, or as part of a multi-enzyme complex (Yacoubi *et al*., 2016).

The first (1,3-1,4)-β-glucanase enzyme was isolated from a strain of *Bacillus subtilis,* which is now known as *Bacillus amyloliquefaciens.* When introduced to a barley-based diet, broilers responded favourably (Von Wettstein *et al*., 2000). According to studies conducted by Esteve-Garcia *et al*. (1997) and Von Wettstein *et al*. (2000), adding β-glucanase as a supplement to broiler diets has been demonstrated to decrease intestinal viscosity, vent pasting, the frequency of sticky droppings, and increase weight gain. Maize dry matter digestibility has long been enhanced by the use of endo-β-1,4-xylanase and arabinofuranosidase enzyme combinations (Saleh *et al*., 2019).

The avian digestive tract largely passes cellulose and arabinoxylans undigested because no animal enzyme is able to break them down (Cherian, 2020; Ward, 2021). In order to break down the cellulose, which is a contributing factor to the undigested elements in the terminal ileum, microbial cellulase supplements should be given (Khalil *et al*., 2022).

According to Silva *et al*. (2012) and Zyla *et al*. (2012), pectinase is an enzyme that hydrolyzes cell walls. When pectin is hydrolyzed, it changes lipid metabolism, the caeca's ability to function, and the GIT's inflammatory response.

2.4.3 Phytase enzyme

It is now standard procedure to add phytase to poultry feeds to promote sustainable chicken meat production because phytic acid is considered an anti-nutritional factor (Lui *et al*., 2014). The first study to add phytase derived from *Aspergillus ficuum* to a liquid soybean

diet was Nelson *et al*. (2018). The results showed a significant increase in the percentage of bone ash when compared to the control group which did not receive any inorganic P. Ever since, phytase has been a reasonably priced source of inorganic P replacement. According to Outchkourov & Petkov (2019). fungi, bacteria, yeast, and higher plants with different origins can be used to produce phytase at optimal pH and temperature.

The phytase enzyme, also known as Myo-inositol hexakisphosphate phosphohydrolase, primarily functions in the upper portion of the gastrointestinal tract (GIT). Its function is to facilitate the stepwise hydrolysis of penta-to monophosphates, which breaks down phytic acid into lower phytate esters and inositol (Feil, 2008; Amerah, 2015; Rahimi *et al*., 2020; Walk & Roa 2020). It has been reported that inositol release increases broiler growth performance (Bedford & Rousseau, 2017). The animal may now use the phosphorus that was previously bound, and this process also improves the animal's ability to digest and use calcium, amino acids, and energy (Walk & Roa, 2020). According to Rahimi *et al*. (2020), this minimizes the cost of including inorganic phosphorus and restricts the amount of phosphorus excreted into the environment by enabling a reduced inclusion of the aforementioned nutrients without adversely affecting the animal. There is further evidence that supplementing with phytase improves pre-caecal amino acid digestibility (Siegert *et al*., 2019). Phytase effectiveness varies depending on the feedstuff and can be attributed to several characteristics of the enzyme, such as ideal pH or temperature, or the feed source (Cherian, 2020; Siegert *et al*., 2019).

2.5 Exogenous Enzymes' Mode of Action in Broiler Feed

Cowieson *et al*. (2010) reported that the mode of action of exogenous enzymes to increase the income of poultry production is improving the apparent digestibility of dietary nutrients and reducing the animal's nutrient requirements. For exogenous enzymes to be applied to dry diets successfully, several requirements are necessary to be met in order for the animal's digestive tract to be active. It must remain active in the physiological conditions of the animal's digestive tract, it must be resistant to proteolysis by the animal's endogenous proteases (Thorpe & Beal, 2001; Cherian, 2020). An enzyme's capacity to break down different substrates is determined by the solubility of non-starch polysaccharides and the intricate structure of the carbohydrate; on the other hand, an enzyme's mode of action is dependent on its efficacy (Cherian, 2020). This means that it should not conflict with the animal's natural digestive enzymes. Variations in the physiology and morphology of the digestive system between different species are likely to alter exogenous enzyme function in this regard.

Partridge (1993) and Dierick & Decuypere (2002) demonstrated some of the species' differences in the utilization of enzymes between poultry and pigs as follows:

- Bacterial activity: Compared to pigs, chickens' gut microbiota is far less significant.
- Digestive ability: Compared to pigs, poultry has shorter small intestines, which means that there is less chance of enzyme inactivation by microflora. Poultry also have a shorter mean retention time in the small intestine (1 to 2 hours) compared to pigs (4 to 5 hours), and their upper gastrointestinal tracts contain less water.
- Fermentation of fibre: Because chickens have significantly smaller hind guts than pigs, birds ferment fibre less than pigs do.

- Anatomical: In pigs, feed enters the stomach's acid environment right away after consumption, whereas in poultry, feed enters the crop, where enzymes can function for several hours at a pH of about 6.0 before entering the gizzard.

Several commercial enzyme products have been introduced to the feed industry as a result of the efficacy of exogenous feed enzymes in boosting animal performance and raw ingredient utilization, as shown in Table 2.4.

Table 2.4: Target Substrates, Production Organisms, and Types of Exogenous Enzymes

Enzyme name	Classification	Production organism	Targeted function
α – Amylase	Carbohydrase	*Aspergillus ssp, Bacillus spp, Rhizopus*	Starch hydrolysis
β – Amylase	Carbohydrase	*Barley malt*	Starch hydrolysis and production of Maltose
Cellulase	Carbohydrase	*Aspergillus niger*	Cellulose breakdown
α–Galactosidase	Carbohydrase	*Aspergillus niger, Morteirella vinaceae var, Saccharomyces spp*	Oligosaccharides hydrolysis
β – Glucanase	Carbohydrase	*Aspergillus spp, Bacillus spp,*	β-glucans hydrolysis
β – Glucosidase	Carbohydrase	*Aspergillus niger*	Hydrolyses cellulose degradation products to glucose
Hemicellulase	Carbohydrase	*Aspergillus spp, Bacillus spp, Humicola spp, Trichoerma spp*	Break down hemicellulose
Invertase	Carbohydrase	*Aspergillus niger, Sacchatomyces spp*	Hydrolyse sucrose to glucose and Fructose
Lactase	Carbohydrase	*Aspergillus niger, Aspergillus oryzae,*	Hydrolyse lactose to glucose and galactose
β – Mannanase	Carbohydrase	*Aspergillus niger, Bacillus lentus, Trichoderma spp. Trichoderma reeseic*	Beta-mannans hydrolysis
Pectinase	Carbohydrase	*Aspergillus niger, Rhizopus oryzae, Aspergillus aculeatus*	Pectin hydrolysis
Xylanase	Carbohydrase	*spergillus spp, Bacillus spp, Humicola spp., Penicilin spp., Trichoderma spp.*	Xylan hydrolysis
Lipase	Lipase	*Aspergillus niger, Candida spp, Rhizomucor spp, Rhizopus spp.*	Hydrolyses triglycerides, diglycerides and glycerol monoesters
Pepsin	Protease	*Animal stomach*	Protease hydrolysis
Protease	Protease	*Aspergillus niger, Aspergillus spp, Bacillus spp*	Protease hydrolysis
Trypsin	Protease	*Animal pancreas*	Protease hydrolysis
Phytase	Phytase	*Aspergillus niger*	Phytate hydrolysis

Source: (Munir & Maqsood, 2013)

2.6 Factors Influencing Enzymes Effectiveness

According to Cowieson *et al*. (2006), one of the main problems with dietary enzyme product is that, adding enzymes may not necessarily result in improved nutrient digestibility or growth performance, and there are a variety of reasons for this. According to Gracia *et al*. (2003), variations in the types and activities of the microorganisms being used to manufacture the enzyme products as well as their sorts can affect the variation in the results. Additional factors include the degree of inclusion thus single versus mixture (Cowieson & Adeola, 2005; Cowieson *et al*., 2006). According to Cowieson, (2010), dietary nutritional quality is the most significant factor influencing responses to enzyme products; higher responses are anticipated in diets of lower quality. Ravindran (2013) reported that dietary nutrient density, the type of dietary ingredients, and the age of the birds are some of the major factors contributing to variation in the responses of birds to enzyme inclusion.

Ravindran (2013) outlined the key factors necessary for the effective functioning of exogenous enzymes. These include the source of the enzyme, its specific catalytic activity, and its resistance to degradation by pepsin. Additionally, the concentration and accessibility of the substrate, along with the physiological conditions of the digestive tract such as pH, temperature, moisture content, and the duration of digesta in the gut, particularly during the gastric phase where enzyme action is most critical, are essential for optimal enzyme activity.

2.6.1 Impact of dietary nutrient density on enzyme effectiveness

According to Moraes *et al*. (2015), enzyme impacts on performance metrics are typically not noticed when standard diets consisting of nutrients that are highly digested and balanced are fed.

Sorbara *et al*. (2009) reported that adding an enzyme to a broiler's diet that is theoretically perfect is unlikely to yield significant improvement because the birds are already performing to the best of their genetic potential, leaving little possibility for improvement. When improved nutrient utilization is not followed by better growth performance, it is possible that the control diets were not sufficiently limiting in nutrients to reduce growth. A study comparing the effects of xylanases and ß-glucanase with α-galactosidase and ß-mannanase at varying metabolizable energy concentrations discovered that adding these enzymes to the broiler diet increased the digestibility and utilization of energy, which in turn increased the broilers' feed conversion ratio (FCR). Additionally, it was observed that ß-glucanase and xylanases added to a low-energy diet increased feed efficiency (Alqhtani *et al*., 2014). A study by Gitoee *et al*. (2015) assessed the efficacy of feeding xylanase, α-amylase, and protease at three distinct metabolizable energy levels. According to the findings, adding enzymes to broiler diets allowed for a reduction in energy content without impairing the performance of the broiler chickens. In an investigation into the impact of several enzyme combinations on apparent metabolizable energy, it was discovered that not a single combination was able to improve the performance of the standard diet. On the other hand, pectinase, protease, and α-amylase greatly enhanced the ME when added to a lower-calorie diet (Kocher *et al*., 2003).

2.6.2 Effect of dietary ingredients on enzyme efficacy

An experiment was conducted by Bhuiyan *et al*. (2013) to demonstrate the impact of enzyme inclusion on varying diet levels of maize. Enzymes such as xylanase, α-amylase, protease, and phytase were employed in this study. There were three different levels of maize used: 250 g/kg, 500 g/kg, and 750 g/kg. The findings showed that while the FCR remained

unchanged, adding the enzymes to the various levels of maize significantly increased the FI and BW. Meng & Slominski (2005) used a multi-carbohydrase cocktail in several diets, the enzyme consists of xylanase, ß-glucanase, pectinase, cellulase, ß-mannanase, and galactanase. The study used four different diets: one that was semi-purified maize and the other three that had 30 % soybean meal, canola meal, or peas in addition to maize. Only when the enzymes were added to the maize and soybean meal diet was an improvement in BWG and FCR seen. According to Walters (2019), there was no discernible difference in broiler BW or FCR when the effects of drought-affected maize and a carbohydrase enzyme mixture including ß-glucanase, cellulase, and xylanase inclusion were assessed on broiler performance and nutrient digestibility. In an experiment, the reaction of broiler chicks to two concentrations of xylanase and ß-glucanase cocktail with one of three digestible lysine levels in the feed was assessed. Between d 1 and 42, the enzyme supplementation reduced the FI by 4.67 % and increased the FCR by 5.53 %, all without affecting the BWG. The inclusion of enzymes made up for a decrease in breast weights at day 42 caused by 300 g of sunflower meal or 8.0 g of digestible lysine/kg of diet. As a result, the relationship between enzyme and sunflower meal was substantial (Woest, 2019).

Cowieson & Ravindran (2008b) conducted a study to evaluate the reaction of broilers in the starter phase to three different dosages of an enzyme cocktail that included protease, α-amylase, and xylanase. The outcomes showed that adding the enzyme mixture to the control diet improved performance in a dose-dependent way. The quality of the ingredients, the enzyme combinations in the cocktail, and the concentration of substrates in the diet may all have an impact on the dosage sensitivity. The highest BWG was obtained from the birds

with higher doses of the enzyme; however, this may not always be the most cost-effective option.

2.6.3 Influence of the birds' age on the enzyme effectiveness

Enzyme inclusion can be beneficial for both young and adult chickens. However, young broiler chickens are generally expected to benefit more from enzyme supplementation because their digestive tracts have limited endogenous enzyme activities, potentially resulting in less efficient feed digestion (Olukosi *et al*., 2018; Bedford & Apajalahti, 2022). Younger broilers typically have less developed digestive enzyme secretion capacity compared to adult chickens, making the addition of feed enzymes more likely to enhance digestion (Ravindran, 2013). However, the age-dependent effect should be less significant when the supplemented enzyme activities are not naturally present in the chicken's digestive system and are intended to complement the endogenous digestive enzymes (Aftab *et al.,* 2014). The impact of added enzymes may vary with the bird's age as caecal populations grow in size and variability, leading to more pronounced fermentation responses to cell wall fragments in older birds (Bedford & Cowieson, 2012). As broiler chickens get older, their capacity for digestion and microbiota increases. Feed enzymes may affect broiler performance by interacting with microbial populations, which proliferate with broiler chicken age (Bedford & Apajalahti, 2022).

The combined impact of xylanase and arabinofuranosidase debranching enzymes on broiler performance, maize glucuronoarabinoxylan breakdown, and caecal microbial fermentation was recently studied by Ravn *et al*. (2018). Significant improvements in BW and FCR were seen with the addition of the enzymes; these effects were seen throughout the trial but were

especially noticeable on days 21 and 29. The observed improvements in gut morphology and broiler performance were most likely caused by the significantly increased caecal butyrate production.

According to a study conducted by Tahir *et al*. (2012), diets containing phytase along with xylanase or a combination of xylanase, protease, and α-amylase showed significant improvement in the BWG and FCR in broilers at 35 days, but only a partial improvement at 49 days. At seven days of age, Radhi *et al*. (2023) discovered no discernible variations in the impact of different enzymatic supplements. Nevertheless, regardless of the enzyme utilized, the addition of enzymatic complexes improved the performance of broilers at 21 and 35 days in comparison to the control. Two-enzyme supplementation produced comparable performance to the positive control from days 1–21 in a study where broilers were fed diets with lower levels of energy and minerals, but only modest improvements were seen from days 22–42 (Nunes *et al*., 2015).

2.7 The Role of Enzymes in Breaking Down Minerals in Feed Ingredients

Rahimi *et al*. (2020) reported that phosphorus is one of the most expensive nutrients in poultry diets, but it is an essential nutrient with multiple important functions in the animal body. According to Xu *et al*. (2021), insufficient amounts of calcium (Ca) and phosphorus (P) can hinder the growth, mineralization, and strength of bones, respectively. As phytate contains bound P, which accounts for 55 – 85 % of total P in the diet, monogastric animals cannot easily access it (Trayhurn, 2005; Jlali *et al*., 2020). Because of the way it binds to P and prevents it from being absorbed, this is known as the "phytate effect" (Amerah, 2015; Lawlor *et al*., 2019). Trayhurn (2005) reported that this binding effect raises the cost of

production to supply additional phosphorus that is available to the animal and this contributes to environmental pollution as excess P is excreted into the environment.

According to Segobola (2016), the two most prevalent minerals in bone are the macro-minerals, calcium and phosphorus, which account for roughly 37 % and 17 % of bone ash, respectively. To prevent imbalances that might lead to a deficiency of either or both, the animal's intake of Ca and P must be carefully balanced. The primary result of inadequate intake of these minerals is rickets, which can be brought on by either a P or Ca deficiency. This can happen when one nutrient is consumed more than the other or when the dietary intake of one nutrient is excessively high, leading to a deficiency of the other. The availability of P varies greatly depending on the source, but calcium is one of the minerals that is both abundant and highly available from most sources (Whitehead *et al*., 2004). Because of these differences in nutrient availability and the need to maintain a balanced ratio while avoiding excessive use of P to minimize pollution, dietary levels frequently fall short of requirements. The binding of the nutrient in phytate molecules further complicates the availability of dietary P in cereal grains.

Nevertheless, according to Selle & Ravindran (2008), the most widely used standard practice for dephosphorylating phytate and liberating the inherent P component in the diet is the inclusion of exogenous phytases in the diets of pigs and poultry. Naves *et al*. (2016) found that by supplementing broiler feed with 1500 active units of phytase, it is possible to reduce the level of available phosphorus in broiler feed to 1.0 g/kg. Additionally, the calcium level could be fixed at 6.5 g/kg to maintain performance and optimize the bone mineralization of the birds as well as to improve the retention coefficients of calcium, phytate phosphorus,

total phosphorus, and nitrogen, while also decreasing the phosphorus excretion into the environment.

2.8 Classification of Non-Starch Polysaccharides

Plant cell walls contain non-starch polysaccharides, which can differ in composition, size, and structure (Maharjan *et al*., 2019). According to their solubility, they are separated into two factions: soluble NSP (sNSP) and insoluble NSP (iNSP). The NSP classification is shown in Figure 2.1.

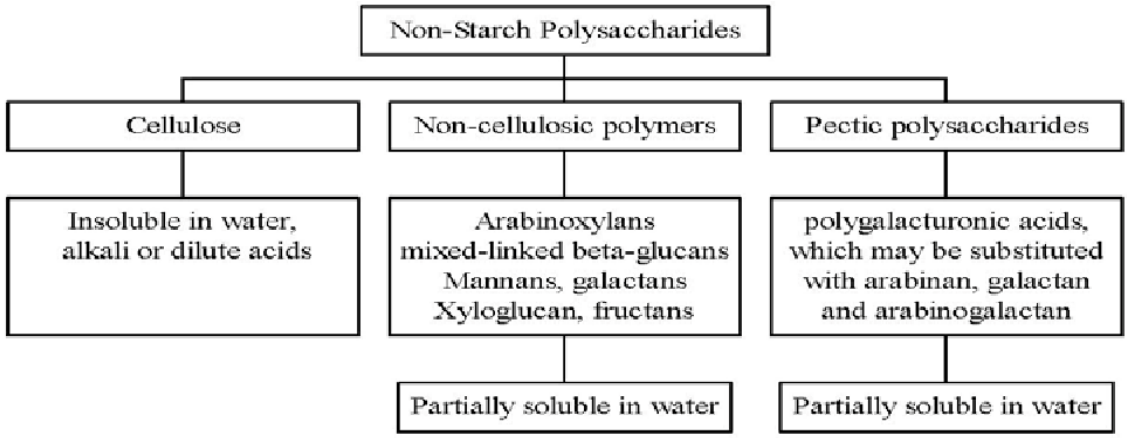

Figure 2.1: Classification of non-starch polysaccharides (Choct *et al*., 2010).

Since soluble NSP (sNSP) has anti-nutritional effects, its inclusion in broiler diet formulation is limited; as a result, the proportion of water-soluble NSP to total NSP in feed is low (Maharjan *et al*., 2019; Tejede & Kim, 2021). Yacoubi *et al*. (2016) reported that the anti-nutritional effects are attributed to arabinoxylans and β-glucans with β-1,4 glycosidic linkage backbones and β-1,3 linkage found in the sNSP fraction. According to Maharaj *et al*. (2019), it results in a sizable amount of water in the digesta binding, which increases the digesta's viscosity as it passes through the small intestine from the proximal to the distal end. Increased intestinal inflammation, poorer nutrient digestion and absorption, and a decrease

in feed AME are all results of this increase in digesta viscosity (Amerah, 2015; Yacoubi *et al*., 2017; Musigwa *et al*., 2020). According to Maharjan *et al*. (2019), valuable nutrients pass through the GIT undigested, resulting in poor feed utilization and potentially poor growth and performance if requirements are not met. The decrease in nutrient digestion is caused by the increase in viscosity, which results in reduced interaction between the intestinal brush border and the digesta, hindering the action of intestinal enzymes. Maharjan *et al*. (2019) reported that an increase in sticky droppings and consequently wet litter is observed due to the increased water-holding capacity. Tejede & Kim (2021) noted that a key factor in the occurrence of foot pad dermatitis is wet litter, as a result of the increased digesta viscosity and decreased pace of digesta transit, the digestive tract may become hypoxic, which might foster the growth of harmful bacteria.

According to Maharjan *et al*. (2019), the insoluble NSP (iNSP) component is thought to be inert and makes up a higher fraction of the total NSP in broiler diets. According to Musingwa *et al*. (2020), insoluble NSP has no appreciable impact on digesta viscosity and consequently no negative impact on nutrient digestibility. This component of NSP causes a physical barrier against enzymes which is referred to as a 'cage effect' (Rios *et al*., 2017; Musigwa *et al*., 2020). According to Rios *et al*. (2017), nutrients are encapsulated, which may affect energy and nutrient digestibility when iNSP is used in diet formulation. Insoluble NSP has laxative qualities, reduces the bacterial load in the hindgut, and in certain situations, may be beneficial in broiler diets. Since enzymes that can break down the β1-4, β1-3, and β1-6 connections are lacking, insoluble NSP has been utilized as a nutritional diluent (Tejede & Kim, 2021). Due to the slowing down and dilution of nutrient intake, too high inclusions reduce performance (Tejede & Kim, 2021).

2.8.1 Effect of non-starch polysaccharides on nutrient uptake in broilers

The costliest raw materials are cereal grains and their by-products, which make up the majority of broiler diets (Cerrate *et al*., 2019). These cereal grains coupled with protein crops contain the anti-nutritional component NSP that causes variance in the ME of broiler diets (Yacoubi *et al*., 2016; Musigwa *et al*., 2020). The NSP concentration in cereal grains varies between 83 and 98 g/kg, according to Sun *et al*. (2019). Maharjan *et al*. (2019) found that the presence of NSP, a collection of molecules with varying sizes, structures, and water solubilities, is negatively correlated with the digestion of carbohydrates. According to Rios *et al*. (2017), this decrease in digestion is caused by increased viscosity of the digesta and intestinal inflammation, which allow important nutrients to escape digestion in the GIT and result in losses of important nutrients. Increased digesta viscosity causes a bacterial overgrowth and delays transit through the gastrointestinal tract (Cherian, 2020; Tejeda & Kim, 2021). Due to the digesta's high viscosity, the intestinal brush border and digesta do not interact well, resulting in limited contact between the digesta and substrates. This prevents breakdown products from being absorbed (Maharjan *et al*., 2019). The author also emphasizes that other important nutrients are included in addition to the carbohydrate portion. In line with these claims, Rios *et al*. (2017) describe the "cage" effect of encapsulation, which lowers digestibility and, as a result, the absorption of nutrients like lipids and amino acids because it creates a physical barrier that inhibits enzyme activity. Non-starch polysaccharides make up a large portion of fibre because fibre is the sum of lignin and NSP, and monogastric animals do not secrete the enzymes required to break down NSP (Cherian, 2020).

According to Rios *et al*. (2017), corn and soybean diets are easier to digest than diets formulated with other cereals, such as wheat and barley, which are known to have higher amounts of NSP. According to Changa *et al*. (2020), broilers obtain their energy and protein from highly digestible raw materials. In a typical broiler corn and soybean diet, maize supplies around 65 % of the total apparent metabolizable energy while soybean meal offers 80 % of the total crude protein (CP) (Rios *et al*., 2017). Because soybean meal is abundant in protein and satisfies poultry's need for certain amino acids, it offers the highest feeding value among all plant-based protein sources (Frempong *et al*., 2019).

A feedstuff's energy value is determined by how well the starch is absorbed, which is rarely an issue in maize-based diet because broilers completely digest the starch component of maize (Zaefarian *et al*., 2015). On the other hand, soybean meal has additional ANFs like phytic acid and trypsin inhibitors in addition to NSP (Frempong *et al*., 2019). The NSP levels in soybean meal present a digestibility issue even though they are lower than those of other vegetable ingredients like wheat and barley (Jamroz *et al*., 2002; Musigwa *et al*., 2020). According to Nguyen *et al*. (2022), diets containing maize and soybeans typically have a total NSP of 10 – 12 % DM, with a water-soluble portion of 1-2.5 % DM. According to Rios *et al*. (2017) and Saleh *et al*. (2019), the contents of NSP in maize and soybean meal are around 9 % (97 g/kg) and 29 % (217 g/kg), respectively. However, according to Zaefarian *et al*. (2015), genetics and environment determine how much NSP is in maize and soybean meal. Frempong *et al*. (2019) claim that appropriate thermal processing of raw materials can reduce the issues caused by ANFs to some degree. Table 2.5 shows how the NSP contents of several cereal grains differ from one another.

Table 2.5: Content of dry matter (g/100 g, as is basis) and non-starch polysaccharides of the plant-based ingredients

Ingredients	DM [1]	sNSP [2]	iNSP [3]	tNSP [4]
Wheat	90.53	14.23	83.08	97.31
Corn	88.97	2.86	64.56	67.42
Barley	90.96	42.36	137.4	179.7
Sorghum	88.45	1.65	53.54	55.2
Soybean meal	90.05	11.22	132.2	143.4
Canola meal	92.16	15.35	146.8	162.1
Wheat bran	91.21	23.16	385.1	408.2
Oat bran	92.40	52.24	65.02	117.3
Soy protein concentrate	93.05	14.69	157.4	172.1

Source: (Nguyen *et al.,* 2022). *DM- [1] Dry matter; sNSP- [2] Soluble non-starch polysaccharides; iNSP- [3] Insoluble non-starch polysaccharides; tNSP- [4] Total non-starch polysaccharides*

2.9 Effect of Phytic Acid on Growth Performance of Broilers

The phosphorylated cyclic sugar alcohol is known as phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate). Phytate, the anion form of phytic acid, is found in all plants. Phytin is the chelated form of phytate that is often found in plants when it is combined with cations, proteins, and/or starches (Wang & Guo, 2021). Angel *et al*. (2015) and Ravindran *et al*. (2000) reported that in addition to limiting the availability of P, phytate also functions as an anti-nutrient in the diet, affecting the metabolizable energy (ME) and overall digestibility of dietary cations and amino acids. Additionally, it was noted that increased losses of endogenous amino acids were correlated with the amount of phytate included in

the diet and had a negative impact on amino acid utilization (Ravindran *et al*., 1999; Cowieson *et al*., 2004a; Cowieson & Ravindran, 2007). It has been suggested that the low levels of endogenous phytase are the reason why over two-thirds of the P in plant-based feedstuffs is not easily accessible in poultry because it is bonded to phytic acid (PA) (Bedford, 2000; Woyengo & Nyachoti, 2011). According to recent research, this is untrue because chickens' intestinal mucosa has enough phytase activity. Poor substrate solubility in the small intestine as a result of cation interactions with Ca is the fundamental problem with phytate digestion in poultry (Maenz & Classen, 1998; Cowieson *et al*. 2011). Phytate phosphorus (PP) and calcium ions combine to create insoluble complexes that inhibit phytase action (Angel *et al*., 2015). Consequently, reducing the amount of calcium in the diet can enhance the impact of exogenous phytase on the breakdown of PP. Nowadays, it's standard practice to provide exogenous phytase to help birds break down PP. Previous research has demonstrated that the performance of young birds was unaffected by the addition of phytase (500 FTU/kg) and the simultaneous reduction of Ca in starter diets from 1.0 % to 0.67 % in conjunction with decreased non-PP (nPP) levels (Létourneau-Montminy *et al*., 2010; Powell *et al*., 2011). According to Lui *et al*. (2014), there are variations in the concentrations of phytate within various raw materials, and it may be found in the aleurone layers of sorghum and wheat while in maize the germ cell. The charge on the molecule makes it a great chelator. At low pH levels, below the isoelectric point of proteins, proteins have a positive charge, which causes insoluble complexes to form with the negatively charged phytic acid (Feil, 2008). Phytic acid directly affects starch digestion and suppresses amylase activity in broilers due to its capacity to form complexes with other nutrients, including calcium, iron (Fe), zinc (Zn), and manganese (Mn) (Lui *et al*., 2014).

According to Bedford & Rousseau (2017), phytic acid also decreases the rate at which pepsinogen is converted to pepsin and raises endogenous losses linked to the breakdown of gastric mucus. These actions effectively lower the quantity of pepsin available in the stomach. Phytic acid is an ANF that has been shown to affect the effectiveness of digestion and, in turn, the performance of chickens (Bedford & Rousseau, 2017). Because chickens cannot obtain P from their diets, particularly when fed maize and soybeans, costly inorganic sources of P must be added to the diets. This frequently results in dietary P levels exceeding the minimum requirements, which increases feed costs and pollution to the environment (Feil, 2008; Lawlor *et al*., 2019; Poernama *et al*., 2021).

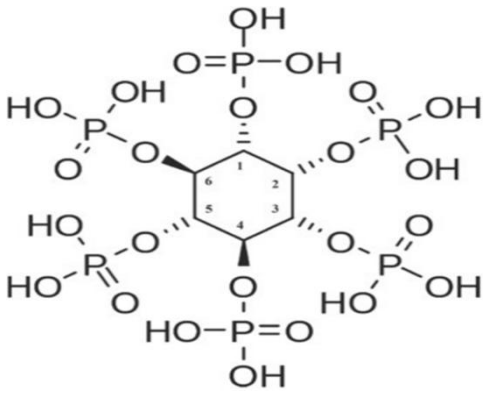

Figure 2.2: The molecular structure of phytic acid (Cherian, 2020)

2.10 Influence of Exogenous Enzyme on Growth Performance of Broilers

The poultry industry has witnessed significant advancements in feed additives aimed at improving the growth performance and overall health of broilers. One such additive gaining attention is the exogenous enzyme which is designed to enhance nutrient utilization in poultry diets, thereby positively influencing broiler growth and performance. Studies by Choct *et al*. (2010) have shown that the inclusion of exogenous enzymes in broiler diets leads to increased enzymatic activity, particularly in the hydrolysis of NSPs and proteins. A

study by Bedford and Cowieson (2012) demonstrated that the addition of exogenous enzyme to broiler diets resulted in improved feed conversion ratios (FCR), indicating enhanced utilization of nutrients for growth. The increased efficiency in converting feed into body mass suggests the economic feasibility of incorporating exogenous enzymes in broiler production systems.

When a multi-enzyme complex was introduced to the diets of broiler chickens fed maize and soybean meal, Rios *et al*. (2017) assessed its effects on the growth performance, energy, and amino acid consumption of the birds. According to the authors, feed conversion ratio, digestible energy, and digestible amino acid levels all improved with enzyme addition. Nadeem *et al*. (2005) found that adding feed enzyme to a diet containing 50 Kcal/kg less ME than the control diet at a rate of 0.05 g/kg did not significantly affect weight gain. However, it did significantly increase feed intake and decrease feed conversion rate (FCR) during the initial (1-28 days) and overall (1-42 days) growing periods (Table 2.6). However, during the finisher phase (29–42 days), these authors did not find any appreciable variations in these parameters. The research conducted by Khan *et al*. (2006) also demonstrated that adding an exogenous enzyme to chicken feed (0.05 g/kg) increased weight gain and feed conversion ratio (FCR) by 8 % when the diet was corn-based and sunflower meal; feed intake was unaffected. Nutrient digestibility was improved in the diet that included enzyme supplements. These results support the hypothesis that, in comparison to high-digestible feedstuffs, the positive effects of NSP-degrading enzymes may be slightly greater with low-digestible feedstuffs such as sunflower meal (14–18 % crude fibre). A study by Chalghoumi *et al*. (2020) reported that supplementing Rovabio Excel at 0.05 g/kg improved final live BW, daily BWG, daily FI, FCR, and production index for broilers from (day 7-21) and (day

22-37) but was statistically similar to the standard control diet. Broilers fed maize and SBM diets containing NSPase showed improvements in BWG and FCR of 3.9 % and 3.2 %, respectively, according to Slominski (2011).

In contrast to the aforementioned findings, research by West *et al*. (2007) shows that the addition of Rovabio Excel (0.022 %) was without impact on growth, feed conversion, and carcass characteristics but decreased (P = 0.06) mortality at days 1-14 and 1-42 respectively.

Table 2.6: Growth performance of broiler chicks fed diets supplemented with or without NSPDE

Parameter	Diet			
	A	B	C	D
Starter phase (0-28 days)				
Average initial body weight (g/bird)	44.00 ± 0.03	45 ± 0.08	43 ± 0.07	44 ± 0.06
Average body weight (g/bird)	1002 ± 12	1069 ± 11	1063 ± 14	1060 ± 16
Average feed intake (g/bird	2200^{b} ± 5	2457^{a} ± 7	2336^{b} ± 5	2414^{a} ± 4
Feed efficiency	2.20^{b} ± 0.04	2.30^{a} ± 0.02	2.20^{b} ± 0.05	2.28^{a} ± 0.03
Finisher phase (29-42 days)				
Average body weight (g/bird)	969 ± 21	969 ± 22	966 ± 19	982 ± 15
Average feed intake (g/bird	2355 ± 6	2443 ± 4	2403 ± 8	2491± 9
Feed efficiency	2.43 ± 0.08	2.52 ± 0.07	2.49 ± 0.03	2.54 ± 0.02
Overall (0-42 days)				
Average body weight (g/bird)	1971 ± 16	2038 ± 9	2029 ± 15	2042 ± 16
Average feed intake (g/bird	4555^{b} ± 5	4900^{a} ± 3	4739^{b} ± 9	4905^{a} ± 8
Feed efficiency	2.31^{b} ± 0.06	2.40^{a} ± 0.00	2.33^{b} ± 0.04	2.40^{a} ± 0.03

Source: (Nadeem *et al*., 2005).

Value (means ± SEM) in rows with different superscripts differ significantly ($p<0.05$).

** Diet A: commercial broiler diet without NSPDE; Diet B: commercial broiler diet without NSPDE; Diet C: commercial broiler diet having 50 Kcal/Kg less ME and without NSPDE and Diet D: commercial broiler diet having 50 Kcal/Kg less ME and with 0.05 g/Kg NSPDE*

2.11 Influence of Exogenous Enzyme on Nutrient Digestibility of Broilers

The carbohydrases such as xylanases and cellulases, target non-starch polysaccharides (NSPs) present in feed ingredients. Through their enzymatic action, they break down complex carbohydrates into simpler, more digestible forms, potentially improving nutrient availability for absorption in the gastrointestinal tract (Bedford, 2018).

The impact of exogenous enzyme on the nutrient digestibility of broilers has been the subject of several investigations. Choct *et al*. (2017) reported increased ileal digestibility of nutrients, including proteins and amino acids, when broilers were fed diets supplemented with feed enzyme. The enhanced digestibility of nutrients suggests that feed enzyme may play a crucial role in breaking down dietary components, making them more accessible for absorption in the small intestine. One aspect of nutrient digestibility influenced by feed enzymes is the degradation of dietary fibres. Carbohydrases target fibrous components, such as arabinoxylans and cellulose, leading to their breakdown. Bedford (2018) highlighted the importance of this enzymatic action in reducing the anti-nutritional effects associated with fibre, ultimately contributing to improved nutrient utilization by broilers.

A study by Choct *et al*. (2017), demonstrated that an exogenous enzyme (0.05 %) contributes to the breakdown of complex carbohydrates into fermentable substrates, potentially increasing the production of short-chain fatty acids in the caeca. This fermentation process can enhance energy availability for the bird, influencing overall nutrient digestibility and utilization. Bedford (2018) proposed that the enzymatic breakdown of NSPs by exogenous enzyme reduces the viscosity of the digesta, promoting nutrient absorption. Additionally, the liberation of oligosaccharides during NSP degradation may serve as prebiotics, fostering a healthier gut environment for improved nutrient assimilation (Choct *et al*., 2017). It has also been demonstrated by Wiseman *et al*. (2000) that there is a strong correlation between the AME values of several wheat varieties and the rate of starch digestion in vitro. The findings indicate that the degree of amylolytic enzyme accessibility to starch granules varies among wheat varieties, with the cell wall architecture of the grain potentially serving as a major determining factor. In the small intestine of chickens, Bedford (2002) reported that a

significant quantity of nutrients, including starch, are retained inside the cell walls and are eliminated when xylanase is added. According to D'Alfonso & McCracken (2003), there is a notable fluctuation in the nutritional content of maize (93 samples analyzed) for hens, with starch digestibility ranging from 84 % to 90 % and ileal digestible energy value varying by 2.04 MJ/kg DM. This variance was decreased using an enzyme product including xylanase, protease, and amylase. According to Cowieson (2005), rather than viscosity reduction, as is frequently the case for viscous grains, the capacity of enzymes, in particular, glycanases, to boost the nutritional content of corn-soy diets is likely mediated by changes in cell wall architecture of the gain.

2.12 Impact of Exogenous Enzyme on Gut pH Of Broilers

Research has shown that exogenous enzyme supplementation influences nutrient utilization in broilers by breaking down NSPs and other complex substrates. Exogenous enzyme facilitates the release of fermentable substrates in the gastrointestinal tract. Choct *et al*. (2017) reported that this enzymatic action may contribute to an increase in short-chain fatty acid production in the ceca, potentially influencing gut pH through the fermentation process.

Digesta viscosity is a critical factor affecting nutrient absorption in the gastrointestinal tract. Bedford (2018) proposed that the enzymatic degradation of NSPs by exogenous enzymes reduce the viscosity of the digesta, creating an environment conducive to nutrient absorption. This reduction in viscosity may contribute to changes in gut pH, as the availability and absorption of nutrients are closely linked to the physical properties of the digesta. The gut microbiota plays a crucial role in maintaining gut health and influencing pH. The breakdown of NSPs by exogenous enzymes generates oligosaccharides, which may act as prebiotics,

promoting the growth of beneficial microorganisms. The modulation of the microbial population in the gut can have downstream effects on fermentation processes and, consequently, on gut pH regulation (Choct *et al*., 2017; Bedford, 2018).

Morgan *et al*. (2022) observed a trend towards a more acidic pH in the caeca of broilers receiving exogenous enzyme supplementation. This observation aligns with the notion that enhanced microbial fermentation, facilitated by exogenous enzyme, can lead to the production of organic acids, influencing gut pH.

2.13 Influence of Exogenous Enzyme on Carcass Traits of Broilers

The composition of broiler carcasses, including the distribution of muscle and fat, is critical for meat quality. Bedford (2018) suggested that the enzymatic action of exogenous enzyme on lipids might impact fat deposition and, subsequently, carcass composition. Additionally, changes in nutrient availability and utilization may influence muscle development, potentially influencing the yield and quality of broiler meat.

Morgan *et al*. (2022) reported improved carcass yield and breast meat percentage in broilers receiving exogenous enzyme supplementation. The authors attributed these effects to enhanced nutrient utilization and the promotion of a healthier gut environment. While Zanella *et al*. (1999) demonstrated that the inclusion of exogenous enzyme had no significant influence on the relative weight of leg, breast muscle, and wings, Selle *et al*. (2003a) found that supplementing wheat-based diets with xylanase plus phytase increased breast weight by 5.8 %. Wu *et al*. (2004) reported that the addition of phytase and xylanase separately resulted in a significant decrease in the small intestine's relative length and weight ($p<0.05$). In contrast, Brenes *et al*. (1993) showed that adding xylanase to wheat-based diets had no effect

on the relative weights of the pancreas, liver, proventriculus, or small intestine in broiler chicks. Similarly, Lee *et al*. (2010) found no significant differences in the relative weights of the liver, abdominal fat, right leg, or right breast muscle between treatment groups when using Rovabio® Max (0.02%). Chicks fed diets containing Rovabio® Max had slightly larger relative weights of the right leg and right breast muscle than chicks in the negative control groups lacking Rovabio® Max, but these changes were not statistically significant.

2.14 Influence of Exogenous Enzyme on Bone Health

Bone mineralization is a key determinant of bone strength and overall skeletal integrity. Research by Morgan *et al*. (2022) demonstrated that broilers receiving exogenous enzyme supplementation exhibited improved bone mineralization. The authors attributed this effect to the enhanced digestion of phytate-bound minerals, releasing phosphorus for better utilization in bone formation. Improved bone mineralization is indicative of enhanced bone strength, which is essential for the overall well-being of broilers. When comparing the tibia-breaking strength and ash of chicks fed diets containing exogenous enzyme to those of the negative control groups that did not contain exogenous enzyme, Lee *et al*. (2010) found no significant differences in the relative weight and length of the tibia among the treatments. Chicks fed diets with exogenous enzyme had tibia-breaking strength and ash considerably higher than those of the negative control groups that did not receive enzyme ($p < 0.05$). The phytase action may have increased the retention of calcium and phosphorus from the phytate-mineral complex, which would have improved the ash percentage. These findings are thought to be a strong indicator of the correlation between enhanced tibia mineralization and the addition of phytase to the diet, as several publications have found comparable results (Martínez-Vallespín *et al*., 2022).

The breakdown of NSPs and phytate by exogenous enzyme may reduce the anti-nutritional effects associated with these compounds, leading to increased mineral availability for bone development (Bedford, 2018). Additionally, the potential prebiotic effects of exogenous enzyme on the gut microbiota may indirectly influence mineral absorption and utilization, contributing to improved bone health in broilers.

In an experiment to determine the effects of multiple enzymes composed of phytase plus carbohydrolases found that the supplementation of the multiple enzyme improved the growth performance and bone mineralization in broiler chicks (Alagawany *et al*., 2018). Another study by Javaid *et al*. (2022) evaluated the impact of an indigenously produced multi-enzyme complex on broiler growth and found that the supplementation led to significantly higher tibia ash values and phosphorus content in the bones of the birds.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Location and Duration

The research was carried out within the poultry unit of the Department of Animal Science at Akenten-Appiah Menka University of Skills Training and Entrepreneurial Development, situated on the Mampong- Ashanti campus. Mampong is positioned in the intermediate region linking the Guinea savannah to the north and the tropical rainforest to the south of Ghana, along the Kumasi-Ejura Road. The research spanned from February 2024 to June 2024.

3.2 Dietary Treatment and Experimental Design

The proximate composition of the primary ingredients (maize, maize bran, soybean meal, and fishmeal) was Analyzed and used to formulate four experimental diets (Table 3). These diets were structured based on a 2 × 2 factorial design, with treatments randomized in a completely randomized design (CRD). The factors include Enzyme (Enz) levels (No vrs Yes) and Maize Bran levels (No vrs Yes) across starter, grower, and finisher diets. The Concept 4 feed formulation program from Creative Formulation Concepts, LLC, Annapolis, MD, was employed for diet formulation. The MB-based diets were made to be a bit deficient in crude protein, energy, lysine and methionine. The addition of enzyme on top of the diets, without using the manufacturer's matrix values as is the practice in Ghana was expected to improve nutrient retention and compensate for the deficiency of the dietary nutrient. Subsequently, the diets were subjected to proximate and chemical analyses following the procedures of AOAC (1990). Throughout the starter (d 0 to 28) and grower-finisher (d 28 to 56) phases, the diets were provided *ad libitum* in mash form.

Table 3.1: Composition and Calculated analysis of experimental diets, %

	Starter				Grower /Finisher			
Ingredients	**T1**	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
Maize grain	63	63	50	50	67	67	56	56
Soybean meal	19.5	19.5	15.5	15.5	16.25	16.25	17	17
Maize bran	0	0	19.76	19.76	0	0	15.2	15.2
Fishmeal	15.18	15.18	12	12	15	15	10	10
Dicalcium Phosphate	0.5	0.5	0.99	0.99	0.5	0.5	0.4	0.4
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Oyster shell	0.32	0.32	0.25	0.25	0.15	0.15	0.2	0.2
Mineral premix	0.5	0.5	0.5	0.5	0.4	0.4	0.5	0.5
TiO_2	0.5	0.5	0.5	0.5	-	-	-	-
DL-Methionine	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3
Enzyme (Rovabio^{TM})	0	0.03	0	0.03	0	0.03	0	0.03
Total	100	100	100	100	100	100	100	100
Calculated Nutrient								
Crude Protein	22.43	22.43	20.15	20.15	21.33	21.33	19.30	19.30
Fibre	2.84	2.84	4.68	4.68	2.81	2.81	4.48	4.48
Calcium	0.9	0.9	0.94	0.94	0.82	0.82	0.76	0.76
Avail Phosphorus	0.34	0.34	0.45	0.45	0.32	0.32	0.32	0.32
M.E. (Kcal/Kg)	2998	2998	2795	2795	3058	3058	2889	2889
Methionine, total	0.59	0.59	0.53	0.53	0.77	0.77	0.7	0.7
Lysine, total	1.43	1.43	1.22	1.22	1.34	1.34	1.12	1.12
Sodium	0.29	0.29	0.28	0.28	0.29	0.29	0.27	0.27
Chloride	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24

Vitamin A, 8,000,000 IU; Vitamin B1, 1300 mg, Vitamin B2, 2500 mg, Vitamin D3, 3000 IU; Vitamin E, 10,000 IU; Vitamin K3, 1,500 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mg, Nicotinic Acid, 5,000 mg,Pantothenic Acid, 4000 mg; Choline Chloride, 8000 mg; Copper, 2,500 mg; Cobalt, 700 mg; Iron, 4,500 mg; Zinc, 55, 000 mg; Methionine, 50,000 mg; Lysine, 200,000 mg; Selenium (1%), 1,300 mg; Iodine, 2,000 mg; Manganese, 60, 000 mg; Antioxidant, 625 mg.

3.3 Experimental Units and Management

Three hundred and thirty-six (336) Cobb 500 chicks (both sexes) in a ratio of 7:7 (7 males:7 females), were raised in an open-sided deep litter housing system. The housing system was bedded with fresh softwood shavings (5 cm deep), and the chicks had unlimited access to starter, grower, and finisher diets as well as water throughout the 56-day experimental period. Upon arrival from the hatchery, chicks of comparable sizes were balanced by weight and randomly distributed among 24-floor pens (each measuring 3.6 m^2), with 6 replicate pens per treatment, accommodating 14 birds each. Each pen was equipped with plastic feeding and watering troughs. Birds were introduced to 24 hours light during the brooding stage through to the Sixth week and then reduced to 12 hours light and 12 hours darkness till the eighth week of the study.

3.4 Parameters Measured

3.4.1 Growth Performance

Weekly calculations were conducted for feed consumption, body weight, gain, feed conversion ratio (FCR), and livability. Feed consumption was determined by subtracting the amount of feed left over from the amount fed. Body weight was calculated by dividing the cumulative weight of birds in the pen by the number of birds. The weight gain was calculated as the difference between the current body weight of birds and their initial body weight. FCR was computed by dividing the difference between the pen weight and the sum of the initial bird weight and dead bird weight by the feed consumption for the same period. Livability was determined by dividing the number of surviving birds by the initial total number of birds and multiplying by 100. Mortality in the pens was monitored twice daily, and post-mortem

examinations were conducted on dead birds throughout the 56-day study period at AAMUSTED-M farm. Feed intake and feed conversion ratio were adjusted for mortality.

3.4.2 Gastrointestinal pH

The gastrointestinal pH was taken following the method of Zanu *et al.* (2023), At day 28 and 56 of the study, two birds per replicate pen were sampled and through cervical head dislocation the sampled birds were killed. The birds were then dissected, and the pH of the crop, proventriculus, gizzard, duodenum, jejunum, ileum, and caeca were measured using a pH tester (Hanna Instruments, UK) by directly inserting the pointed tip into the digesta in the lumen of the proximal end of each segment of the sampled bird, making sure the pH electrode did not touch the walls. The probe was cleaned with distilled water once all of the readings for each bird had been obtained. The next step was calculating the mean of the two readings for each tract section of the two sampled birds.

3.4.3 Bone traits

On days 28 and 56 post-hatch, the femur and tibiae bones were removed from the left leg of the sampled birds and used for gut pH and carcass traits analysis to assess bone weight, dimensions, and bone-breaking strength. The dimensions of the femur and tibia were measured, including length (mm) from the proximal end to the distal end and width (mm) at the medial region, using a digital caliper, and the femur and tibia bones of the two sampled birds were weighed (grams) after air drying for one week using an electronic scale (Mettler Toledo) and the result was express as a percentage of the live body weight. To determine breaking strength (BS), the flesh was removed manually from the femur and tibiae using a scalpel. Subsequently, the femur and tibiae underwent testing on a universal texture analyzer

(Inspekt table50-1, Hegewald & Peschke, Meß- Germany) equipped with a 50 KN load cell and 3-point fixture bed, operating at a test speed of 10 points of data per second. The equipment was controlled by a BenQ computer (24 Inch IPS monitor) using Blue Hill 3 software.

3.4.4 Relative organ weight

The heart, liver, empty gizzard, breast meat, and thigh were taken from the sampled birds used for the determination of gut pH and weighed. Each part was expressed as a percentage of live BW.

3.4.5 Protein determination in diets and digesta

The protein content of the diets and digesta was determined using the AOAC (1990) procedure with a little modification. Protein determination was done in three stages thus digestion, distillation and titration.

Digestion stage

Two (2.0) grams of diet and digesta samples were put into digestion flasks and a half tablet of Selenium-based catalyst was added. 25ml of concentrated Sulphuric acid was added to each of the flasks and shaken thoroughly to ensure complete soaking of the samples. The flasks were then placed on a digestion burner and heated slowly with intermittent shaking until a clear greenish solution was obtained (usually 1-hour period). The digested samples (digests) were allowed to cool and transferred into a 100ml volumetric flask and made up to the mark with distilled water.

Distillation

Kjeldahl nitrogen distillation apparatus (TecatorTM Kjeltec System) was flushed out using distilled water for about 5 minutes. 25 ml of 4 % boric acid was measured into 250 ml conical flasks and two drops of mixed indicator were added. Each of the 100ml diluted digests was transferred into a Kjeldahl distillation tube and then 50ml of 40 % NaOH was added. The apparatus was switched into operation whereby steam was generated and the mixture was heated in a tube. This process liberated gaseous ammonia which was collected into the conical flask until 150ml of the distillate was obtained. The colour change observed in the conical flask was pink to bluish-green. A blank test was conducted without the test samples.

Titration

The distillates were titrated with 0.1N HCl solution. The end-point was noted during the titration which was from the bluish-green colour to colourless. The volume of HCl used (titre values) was then recorded after colour changes to pink by a drop.

Calculation;

Nitrogen (N) =

$$\frac{(1.4007 \times 0.1 \times Titre\ value)}{2} \times 10$$

Crude protein = N × 6.25

3.4.6 Ash determination in diets and digesta

2 g of both diet and digesta samples were weighed into already cleaned and weighed crucibles in duplicate. The crucibles were placed in a furnace (Vecstar) preheated at 600℃ for 2 hours. The crucibles were removed, cooled, and reweighed. The masses of the crucibles and their contents were

found by subtraction. The ash content was calculated by difference and expressed as a percentage of the initial weight of the sample.

3.4.7 Crude fat determination in diets and digesta

2 g of both diet and digesta samples were transferred into a paper thimble plugged at the opening with glass wool and placed into a thimble holder. Petroleum ether of volume 150 ml was measured into a previously dried and weighed 250 ml round-bottom flask and this was assembled with the thimble holder and its contents. The Quick fit condenser was connected to the Soxhlet Extractor and refluxed for sixteen 16 hours on low heat on a heating mantle. After extraction the flask containing the fat was dried at 105°C in an oven for 30 minutes, cooled in a desiccator and the weight of the fat collected was determined and expressed as a percentage of crude fat, (AOAC, 1990).

3.4.8 Crude fibre determination in diets and digesta

The samples after the determination of the crude fat were weighed and transferred into a 500 ml Erlenmeyer flask and few chips were added and 200 ml of 1.25 % sulphuric acid was added to each of the samples. The flask was then set on a hot plate and connected to a condenser. The content was timed at the onset of boiling. At the end of the thirty minutes, the flask was removed and the contents filtered through a linen cloth in a funnel. Boiling water was used to wash the content till the acid was removed. The distillate was discarded and the residue was placed back into the Erlenmeyer flask containing the chips using a spatula 200 ml of 1.25 % NaOH was added and flask reconnected to the condenser. The content was left to boil for thirty minutes after which the flask was disconnected and the content filtered using linen cloth. The content was washed with boiling water till the base was completely removed and then finally washed with ethanol to break any lumps. The residue was transferred into a porcelain crucible and dried in an oven for 2 hours at 130°C.

The weight of the dried residue now composed of the crude fibre and minerals was recorded and then placed in the furnace for 30 minutes to burn off the organic material (crude fibre) leaving the inorganic (minerals). The content after ashing was allowed to cool after which the weight was taken. The crude fibre was obtained by a subtraction of the weight of the ash from the weight of the dried residue before ashing.

3.4.9 Titanium dioxide analysis

The method for spectrophotometric analysis, as outlined by Short *et al*. (1996), was employed with slight modifications to measure the concentration of TiO_2 in both the diet and ileal digesta samples. Titanium dioxide concentrations were assessed in duplicate for both diets and digesta samples. Approximately 0.1 g of oven-dried digesta and 0.2 g of diet samples were weighed into porcelain crucibles and then ashed at 580°C for 13 hours. After cooling, 5 mL of 7.4 M H_2SO_4 was added to the samples and boiled on a hotplate at 300°C for 2 hours to ensure complete dissolution. Following cooling to room temperature, 5 mL of Milli-Q H_2O was added before filtration (using Whatman 541 filters, hardened, ashless, 90 mm, Whatman International Ltd Maidstone, UK) into 50 mL volumetric flasks. Subsequently, 10 mL of H_2O_2 (30 % v/v) was added to each flask, and the mixture was adjusted to 50 mL with Milli-Q H_2O and thoroughly mixed. The resulting solution is stored overnight before measuring the absorbance values. The absorbance of the solutions and prepared standards was measured at 410 nm using a Cecil CE 7400 UV-visible spectrophotometer (Cecil Instruments Ltd., Peterborough, United Kingdom). The TiO_2 content was then calculated using a standard curve.

3.4.10 Digestibility calculation

The apparent ileal digestibility of crude protein, ash, fat, and fibre was calculated using the indigestible marker using the following formula:

Apparent ileal digestibility (%) = (1 − [TiO_2diet (%)/ TiO_2digesta (%)] × [digesta nutrient (%)/ diet nutrient (%)] × 100.

3.5 Statistical analysis of data

The data were analyzed using a 2 × 2 factorial design with the General Linear Models (GLM) procedure in Minitab 20.3 statistical software. This analysis aimed to evaluate the main effects of Enzyme (Enz) at No or Yes levels and Maize bran (MB) levels (No or Yes), as well as the 2-way interaction between Enz and MB (Enz * MB). Pairwise comparisons between treatment means were conducted using the Tukey LSD means separation test ($P < 0.05$).

The data were treated as a fixed effect model, with the effects outlined in the statistical model as follow;

$Yij = \mu + ENZi + MBj + (ENZ*MB)\ ij + eij$

Where Yij = Response expected independent variable

μ = Overall mean

ENZi = Main effect of enzyme (i = No or Yes)

MBj = Main effect of maize bran (j = No or Yes)

(ENZ * MB) ij = Interaction between enzyme and maize bran

eij = Random residual error.

CHAPTER FOUR

4.0 RESULTS

4.1 Analyzed Nutrient Composition of Maize Bran and Experimental Diets

The proximate analysis of maize bran, as presented in Table 4.1, highlights its nutritional profile and potential as a feed ingredient. The moisture content (8.79%) indicates good storage stability, as lower moisture levels reduce the risk of microbial contamination. The ash content (2.96%) reflects the mineral composition of the bran, suggesting moderate mineral availability. Crude protein (8.76%) is relatively low, making maize bran more suitable as an energy source rather than a primary protein contributor. The crude fat (3.54%) content provides an additional energy source, while the crude fiber (7.30%) may support gut health but could limit digestibility if used in high proportions. The high nitrogen-free extract (68.65%) indicates a significant carbohydrate contribution, making maize bran a valuable energy source in feed formulations. Additionally, the metabolizable energy value of 3016.44 kcal/kg underscores its potential to meet energy requirements in livestock diets when used appropriately.

The proximate compositions of the experimental ingredients are shown in Table 4.2 indicating that though the analyzed crude protein and crude fibre were slightly lower than the formulated levels, the diets were similar and standard diets for broilers.

Table 4.1: Analyzed nutrient composition of maize bran

Analyzed Nutrient Composition (%)	Maize Bran
Moisture content	8.79
Ash content	2.96
Crude protein	8.76
Crude fat	3.54
Crude fibre	7.30
Nitrogen free extract	68.65
Metabolizable energy kcal/kg	3016.44

Table 4.2: Analyzed nutrient composition of experimental diets

Analyzed Nutrient Composition (%)	Starter Diets				Grower/Finisher Diets			
	T1	**T2**	**T3**	**T4**	**T1**	**T2**	**T3**	**T4**
Moisture content	10.41	10.94	13.29	13.14	9.53	9.43	11.09	10.60
Ash content	5.17	5.09	6.81	6.42	5.52	4.46	7.16	8.11
Crude protein	21.57	21.45	19.40	19.56	20.96	21.10	18.68	18.82
Crude fat	4.96	4.42	3.77	3.85	3.87	4.54	3.48	3.34
Crude fibre	4.52	4.96	6.85	7.34	2.45	2.99	7.02	6.51
Nitrogen free extract	53.37	53.14	49.88	49.69	57.67	57.48	52.57	52.62
Metabolizable energy kcal/kg	3072	3015	2772	2778	3111	3164	2816	2811

T1= (No MB + No enzyme), T2= (No MB + Enzyme), T3= (MB +No Enzyme), T4= (MB +Enzyme), and MB= Maize Bran.

4.2 Influence of Exogenous Enzyme and Maize Bran on the Performance of Broilers from d 0 to 14

The result from Table 4.2 shows that enzyme as a main effect was not significant for intake ($P > 0.05$), FCRc ($P > 0.05$), BW ($P > 0.05$), BW gain ($P > 0.05$), and livability ($P > 0.05$) at d 14, but maize bran as main effect had a significant effect on FCRc ($P < 0.05$), BW ($P < 0.05$), and BW gain ($P < 0.05$). Birds fed the diet with No MB as a main effect had a higher body weight (272.9 g), gain (236.0 g), and a better FCRc (1.537) as compared to those fed Yes MB diet. Similarly, maize bran as a main effect was not significant for intake ($P > 0.05$), and livability ($P > 0.05$) at d14 but birds on (No MB) had higher feed intake (362.2 g) than those fed Yes MB diet (359.6 g). There was an Ezn × MB interaction ($P < 0.05$) observed for FCRc and feed intake. Birds fed a diet containing No enzyme and No maize bran (No × No) had a better FCRc (1.515) as compared to birds fed enzyme and maize bran (Yes × Yes) diet (1.587) as well as those on No enzyme and maize bran inclusion (No × Yes) diet (1.673). Interaction detected in feed intake indicates that birds fed diet containing enzyme and no maize bran (Yes × No) had a higher feed intake (376.2 g) compared to birds fed the diet containing no enzyme and no maize bran (No × No) (348.2 g) as well as those feed with enzyme and maize bran (Yes × Yes) diet (351.2 g).

Table 4.3: Influence of exogenous enzyme and maize bran on the performance of broilers at d 0 to 14

Effects			Intake, g	BW, g	Gain, g	FCRc	Livability, %
	Enzyme	**Maize bran**					
	No		358.1	262.6	225.2	1.594	99.40
	Yes		363.7	269.1	231.6	1.573	100.0
SEM			5.040	4.120	4.100	0.016	0.421
		No	362.2	272.9^{a}	236.0^{a}	1.537^{b}	100.0
		Yes	359.6	258.7^{b}	220.8^{b}	1.630^{a}	99.41
SEM			5.040	4.120	4.100	0.016	0.421
Interaction							
No × No			348.24^{b}	266.7	230.0	1.515^{c}	100.0
No × Yes			367.9^{ab}	258.5	220.2	1.673^{a}	98.81
Yes × No			376.2^{a}	279.2	241.8	1.558^{bc}	100.0
Yes ×Yes			351.2^{b}	258.9	221.4	1.587^{b}	100.0
P-value							
Enzyme			0.440	0.283	0.279	0.378	0.329
Maize bran			0.715	0.024	0.017	0.001	0.329
Enz × MB			0.005	0.312	0.374	0.011	0.329
SEM			7.130	5.830	5.790	0.023	0.560

^{a,b,c} means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
FCRc, Corrected feed conversion ratio

4.3 Influence of Exogenous Enzyme and Maize Bran on the Performance of Broilers at d 28

On d 28, no Enz × MB interaction was detected ($P > 0.05$) for all performance indices (Table 4.4). Enzyme as a main effect did not affect performance but No MB inclusion as a main effect increased both BW ($P= 0.000$) and gain ($P= 0.000$) compared to Yes MB diet. FCR ($P < 0.05$) was better for No MB diet (1.823) compared to Yes MB diet (1.979) and also maize bran as a main effect was not significant for intake ($P > 0.05$) and livability measured at d 28.

Table 4.4: Influence of exogenous enzyme and maize bran on the performance of broilers at d 28

Effects			Intake, g	BW, g	Gain, g	FCRc	Livability, %
	Enzyme	**Maize bran**					
	No		1418	786.0	748.6	1.899	98.81
	Yes		1445	797.0	759.6	1.906	96.43
SEM			22.40	11.30	11.10	0.016	0.960
		No	1443	827.8[a]	790.8[a]	1.826[b]	97.02
		Yes	1420	755.3[b]	717.4[b]	1.979[a]	98.21
SEM			22.40	11.30	11.10	0.016	0.960
Interaction							
No × No			1429	820.3	783.7	1.823	98.81
No × Yes			1408	751.8	713.5	1.974	98.81
Yes × No			1458	835.3	797.9	1.828	95.24
Yes ×Yes			1432	758.7	721.2	1.985	97.62
P-value							
Enzyme			0.410	0.498	0.493	0.736	0.095
Maize bran			0.472	0.000	0.000	0.000	0.391
Enz × MB			0.940	0.803	0.838	0.901	0.391
SEM			31.70	15.90	15.70	0.023	1.36

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
FCRc, Corrected feed conversion ratio.

4.4 Influence of Exogenous Enzyme and Maize Bran on the Performance of Broilers from d 0 to 42

Results on the performance of birds on d 42 revealed that No MB as a main effect improved BW ($P < 0.05$), gain ($P < 0.05$), FCRc ($P < 0.05$), and livability of birds ($P < 0.05$) and tended to increased feed intake ($P = 0.076$) compared to Yes MB. The main effect of enzyme did not affect ($P > 0.05$) any of the performance indices measured. The interaction of Enz × MB however, did not affect BW ($P > 0.05$), gain ($P > 0.05$), FCR ($P > 0.05$), intake ($P > 0.05$), and livability ($P > 0.05$) measured on d 42 (Table 4.5).

Table 4.5: Influence of Exogenous Enzyme and Maize Bran on the Performance of Broilers at d 42

Effects			Intake, g	BW, g	Gain, g	FCRc	Livability, %
	Enzyme	**Maize bran**					
	No		3181	1575	1538	2.075	75.00
	Yes		3194	1563	1526	2.099	73.21
SEM			44.80	24.90	24.90	0.020	1.370
		No	3247	1654[a]	1617[a]	2.010[b]	72.02[b]
		Yes	3128	1484[b]	1446[b]	2.164[a]	76.19[a]
SEM			44.80	24.90	24.90	0.020	1.370
Interaction							
No × No			3233	1648	1612	2.009	73.81
No × Yes			3130	1502	1464	2.147	76.19
Yes × No			3261	1660	1623	2.011	70.24
Yes ×Yes			3126	1466	1429	2.188	76.19
P-value							
Enzyme			0.848	0.736	0.735	0.398	0.368
Maize bran			0.076	0.000	0.000	0.000	0.044
Enz × MB			0.801	0.505	0.518	0.440	0.368
SEM			63.40	35.20	35.10	0.0287	1.940

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
FCRc, Corrected feed conversion ratio.

4.5 Influence of Exogenous Enzyme and Maize Bran on the Performance of Broilers from d 0 to 56

On d 56, no Enz × MB interaction was observed ($P > 0.05$) for all performance variables measured except feed intake ($P = 0.083$) indicating that Yes MB tended to increase feed intake in birds fed No enzyme diet. Similarly, enzyme as a main effect did not influence ($P > 0.05$) any of the performance variables measured. However, no MB as a main effect improved the FCRc ($P < 0.05$) of birds and tended to increase BW ($P = 0.099$), gain ($P = 0.096$), and livability ($P = 0.068$) compared to Yes MB (Table 4.6).

Table 4.6: Influence of exogenous enzyme and maize bran on the performance of broilers at d 56

Effects			Intake, g	BW, g	Gain, g	FCRc	Livability, %
	Enzyme	**Maize bran**					
	No		5312	2279	2242	2.373	72.62
	Yes		5244	2237	2200	2.388	67.86
SEM			83.4	43.60	43.60	0.028	2.18
		No	5309	2312	2275	2.336[b]	67.26
		Yes	5246	2205	2167	2.425[a]	73.21
SEM			83.40	43.60	43.60	0.028	2.18
Interaction							
No × No			5236	2309	2273	2.308	70.24
No × Yes			5388	2249	2211	2.439	75.00
Yes × No			5383	2314	2276	2.365	64.29
Yes ×Yes			5104	2161	2123	2.411	71.43
P-value							
Enzyme			0.569	0.503	0.503	0.714	0.138
Maize bran			0.596	0.099	0.096	0.035	0.068
Enz × MB			0.083	0.460	0.467	0.286	0.703
SEM			118.0	61.60	61.60	0.039	3.08

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
FCRc, Corrected feed conversion ratio.

4.6 Influence of Exogenous Enzyme and Maize Bran on the Gastrointestinal pH of Broilers, d 28

As shown in Table 4.7, No main effects or interactions ($P > 0.05$) were observed for the pH of the crop, proventriculus, duodenum, jejunum, and caeca at d 28. enzyme as a main effect increased the gizzard pH (1.990) compared to No enzyme (1.752). Also, enzyme as a main effect tended to ($P = 0.069$) decrease the ileal pH compared to No enzyme inclusion.

Table 4.7: Influence of exogenous enzyme and maize bran on the gastrointestinal pH of broilers, d 28

Effects			Crop	Gizzard	Proventricul	Duodenum	Jejunum	Ileum	Caeca
	Enzyme	**Maize bran**							
	No		5.062	1.752[b]	1.300	5.155	5.579	6.586	6.536
	Yes		4.972	1.990[a]	1.290	5.119	5.524	6.370	6.428
SEM			0.087	0.079	0.054	0.135	0.085	0.079	0.196
		No	5.089	1.952	1.336	5.103	5.522	6.492	6.596
		Yes	4.945	1.790	1.255	5.170	5.582	6.464	6.365
SEM			0.087	0.079	0.054	0.135	0.085	0.079	0.196
Interaction									
No × No			5.184	1.878	1.398	5.136	5.601	6.567	6.568
No × Yes			4.935	1.626	1.203	5.173	5.557	6.605	6.504
Yes × No			4.959	2.055	1.275	5.071	5.442	6.417	6.624
Yes ×Yes			4.955	1.955	1.306	5.168	5.605	6.323	6.233
P-value									
Enzyme			0.473	0.045	0.893	0.854	0.647	0.069	0.703
Maize bran			0.255	0.162	0.300	0.728	0.623	0.803	0.422
Enz × MB			0.382	0.421	0.158	0.878	0.397	0.559	0.561
SEM			0.123	0.111	0.077	0.191	0.120	0.112	0.277

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
Enz, Enzyme
MB, Maize bran.

4.7 Influence of Exogenous Enzyme and Maize Bran on the Gastrointestinal pH of Broilers at d 56

On d 56, there was an Enz × MB interaction observed for jejunal pH ($P < 0.05$) indicating that in the group fed Yes enzyme, No MB increased the jejunal pH while in those fed No enzyme, Yes MB increased jejunal pH. Similarly, an Enz × MB interaction was observed for crop pH ($P = 0.076$) indicating that in birds fed No enzyme, Yes MB tended to increase the crop pH. Also, No MB as a main effect increased gizzard pH ($P = 0.056$) and proventriculus pH ($P < 0.05$) compared to Yes MB at d 56 (Table 4.8).

Table 4.8 Influence of exogenous enzyme and maize bran on the gastrointestinal pH of broilers, d 56

Effects			Crop	Gizzard	Proventriculus	Duodenum	Jejunum	Ileum	Caeca
	Enzyme	**Maize bran**							
	No		4.832	2.063	1.364	5.321	5.789	5.988	6.933
	Yes		4.694	2.175	1.365	5.232	5.798	5.868	6.933
SEM			0.101	0.105	0.058	0.150	0.062	0.110	0.062
		No	4.786	2.267[a]	1.450[a]	5.338	5.797	5.952	6.974
		Yes	4.740	1.970[b]	1.279[b]	5.215	5.790	5.905	6.893
SEM			0.101	0.105	0.058	0.150	0.062	0.110	0.062
Interaction									
No × No			4.722	2.236	1.404	5.428	5.699[b]	5.944	6.999
No × Yes			4.942	1.889	1.324	5.213	5.878[a]	6.033	6.868
Yes × No			4.850	2.299	1.496	5.248	5.894[a]	5.959	6.948
Yes ×Yes			4.538	2.051	1.234	5.216	5.702[b]	5.777	6.918
P-value									
Enzyme			0.344	0.458	0.992	0.681	0.918	0.450	1.000
Maize bran			0.753	0.059	0.050	0.567	0.940	0.766	0.369
Enz × MB			0.076	0.744	0.279	0.672	0.047	0.396	0.569
SEM			0.142	0.149	0.082	0.212	0.088	0.156	0.088

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
Enz, Enzyme
MB, Maize bran.

4.8 Influence of Exogenous Enzyme and Maize Bran on the Carcass Traits (% BW) of Broilers from d 0 to 28

At day 28 (Table 4.9), Yes MB as a main effect increased empty gizzard weight, % bodyweight ($P < 0.05$) but reduced breast weight, % bodyweight ($P < 0.05$) compared to No MB diet. Enzyme as a main effect had no significant ($P > 0.05$) effect on all the carcass traits measured on day 28 of the study and also, no interaction was detected.

Table 4.9: Influence of exogenous enzyme and maize bran on the carcass traits (% BW) of broilers from d 0 to 28

Effects			Heart	Empty Gizzard	Liver	Thigh	Breast
	Enzyme	**Maize bran**					
	No		0.763	3.230	3.243	8.658	3.230
	Yes		0.823	3.248	3.357	8.643	3.455
SEM			0.030	0.126	0.118	0.099	0.118
		No	0.809	3.049^{b}	3.402	8.701	3.520^{a}
		Yes	0.777	3.429^{a}	3.197	8.500	3.165^{b}
SEM			0.030	0.126	0.118	0.099	0.118
Interaction							
No × No			0.768	3.105	3.297	8.580	3.364
No × Yes			0.757	3.356	3.188	8.736	3.096
Yes × No			0.849	2.994	3.507	8.822	3.677
Yes ×Yes			0.797	3.502	3.206	8.463	3.233
P-value							
Enzyme			0.163	0.922	0.505	0.915	0.191
Maize bran			0.462	0.047	0.235	0.479	0.045
Enz× MB			0.634	0.480	0.572	0.081	0.601
SEM			0.042	0.179	0.168	0.140	0.166

^{a,b,c} means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
Enz, Enzyme
MB, Maize bran.

4.9 Influence of Exogenous Enzyme and Maize Bran on the Carcass Traits (% BW) of Broilers from d 0 to 56

According to Table 4.10, enzyme as a main effect increased the fat pad, % bodyweight ($P < 0.05$) compared to the No enzyme. Enzyme as a main effect had no significant influence on the heart weight, % bodyweight ($P > 0.05$), liver weight, % bodyweight ($P > 0.05$), empty gizzard weight, % bodyweight ($P > 0.05$), thigh weight, % bodyweight ($P > 0.05$) and breast weight ($P > 0.05$) at d 56. Also, Yes MB as a main effect increased empty gizzard weight, % bodyweight ($P < 0.05$) but reduced breast weight, % bodyweight ($P < 0.05$). No MB as a main effect increased breast weight, % bodyweight ($P < 0.05$) compared to Yes MB. There was no interaction detected.

Table 4.10: Influence of exogenous enzyme and maize bran on the carcass traits (% BW) of broilers at d 56

Effects			Heart	Empty Gizzard	Liver	Thigh	Breast	Fatpad
	Enzyme	**Maize bran**						
	No		0.479	2.079	2.009	10.06	5.036	0.917[b]
	Yes		0.503	1.999	2.032	10.00	5.030	1.206[a]
SEM			0.018	0.059	0.073	0.115	0.172	0.085
		No	0.497	1.845[b]	1.996	10.15	5.372[a]	0.975
		Yes	0.485	2.233[a]	2.049	9.919	4.694[b]	1.147
SEM			0.018	0.058	0.073	0.115	0.172	0.085
Interaction								
No × No			0.489	1.836	1.960	10.24	5.249	0.854
No × Yes			0.470	2.322	2.057	9.887	4.823	0.980
Yes × No			0.505	1.854	2.023	10.06	5.495	1.097
Yes ×Yes			0.501	2.143	2.040	9.951	4.565	1.315
P-value								
Enzyme			0.381	0.343	0.828	0.715	0.982	0.025
Maize bran			0.662	0.000	0.587	0.176	0.012	0.166
Enz× MB			0.769	0.246	0.703	0.454	0.314	0.707
SEM			0.026	0.083	0.103	0.163	0.244	0.120

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
Enz, Enzyme
MB, Maize bran.

4.10 Influence of Exogenous Enzyme and Maize Bran on the Femur and Tibial Weight (% BW) of Broilers, d 28 and d 56

No enzyme as a main effect increased tibial weight, % bodyweight ($P < 0.05$) compared to the Yes enzyme on d 28. No main effects or interactions were detected for femur weight, % bodyweight at d 28 (Table 4.11**)**. At d 56, no Enz × MB interaction was detected for tibial and Femur weight, % bodyweight ($P > 0.05$). Also, No MB as a main effect tended to increase femur weight, % bodyweight ($P = 0.086$) compared to Yes MB.

Table 4.11: Influence of exogenous enzyme and maize bran on the femur and tibial weight (% BW) of broilers, d 28 and d 56

Effects			day 28		day 56	
			Tibial WT	**Femur WT**	**Tibial WT**	**Femur WT**
	Enzyme	**Maize bran**				
	No		0.304[a]	0.228	0.340	0.261
	Yes		0.286[b]	0.225	0.331	0.256
SEM			0.005	0.006	0.008	0.007
		No	0.292	0.222	0.328	0.250
		Yes	0.297	0.231	0.343	0.267
SEM			0.005	0.006	0.008	0.007
Interaction						
No ×No			0.300	0.227	0.333	0.254
No × Yes			0.307	0.229	0.347	0.269
Yes × No			0.284	0.217	0.323	0.246
Yes × Yes			0.288	0.233	0.339	0.266
P-value						
Enzyme			0.031	0.670	0.449	0.573
Maize bran			0.493	0.278	0.221	0.086
Enz × MB			0.794	0.382	0.939	0.790
SEM			0.008	0.008	0.012	0.010

[a,b,c] *means in the same column with different superscripts are significantly different (P < 0.05)*
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran and WT, Weight

4.11 Influence of Exogenous Enzyme and Maize Bran on the Tibial and Femur Breaking Strength (N) of Broilers, d 28 and d56

As shown in Table 4.12, no main effects or interaction ($P > 0.05$) were detected for the femur and tibial BS on d 28. However, on d 56, No MB as a main effect increased tibial BS (293.9 N) and femur BS (292.67 N) compared to Yes MB. No Ezn × MB interaction ($P > 0.05$) was detected for tibial and femur BS at d 56.

Table 4.12: Influence of exogenous enzyme and maize bran on the tibial and femur breaking strength (N) of broilers, d 28 and 56

Effects			day 28		day 56	
			Tibial BS	Femur BS	Tibial BS	Femur BS
	Enzyme	**Maize bran**				
	No		125.7	146.2	266.4	256.8
	Yes		125.1	163.4	259.7	274.2
SEM			7.090	12.70	12.10	9.790
		No	130.7	171.7	293.9[a]	292.7[a]
		Yes	120.0	137.9	232.2[b]	238.3[b]
SEM			7.090	12.70	12.10	9.790
Interaction						
No ×No			131.6	156.5	294.3	292.0
No × Yes			119.8	135.8	238.5	221.6
Yes × No			129.8	186.8	293.5	293.3
Yes × Yes			120.3	139.9	225.8	255.0
P-value						
Enzyme			0.954	0.350	0.699	0.224
Maize bran			0.300	0.075	0.002	0.001
Enz × MB			0.908	0.474	0.731	0.260
SEM			10.00	18.00	17.10	13.80

[a,b,c] *means in the same column with different superscripts are significantly different (P < 0.05)*
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran and BS, Breaking Strength.

4.12 Influence of Exogenous Enzyme and Maize Bran on Apparent Ileal Digestibility of Protein, Ash, Fat and Fibre at d 28

According to Table 4.13, at d 28 of the study, Ezn × MB interaction affected the apparent ileal digestibility of fat ($P < 0.05$) indicating that in birds fed No enzyme, No MB increased fat digestibility while in those fed No enzyme, Yes MB reduced fat digestibility. Also, No MB as a main effect increased apparent ileal digestibility of fat ($P < 0.05$) compared to Yes MB at d 28. Enzyme as a main effect had no significant effect ($P > 0.05$) on crude protein, ash, fat and fibre digestibility at d 28 of the study.

Table 4.13: Influence of exogenous enzyme and maize bran on apparent ileal digestibility of protein, ash, fat and fibre at d 28

Effects			CP digestibility	Ash, digestibility	Fat digestibility	Fibre digestibility
	Enzyme	**Maize bran**				
	No		0.829	0.526	0.849	0.697
	Yes		0.837	0.635	0.876	0.736
SEM			0.009	0.041	0.014	0.032
		No	0.856[a]	0.589	0.885[a]	0.696
		Yes	0.810[b]	0.573	0.840[b]	0.736
SEM			0.009	0.041	0.014	0.032
Interaction						
No × No			0.853	0.526	0.892[a]	0.668
No × Yes			0.806	0.527	0.805[b]	0.726
Yes × No			0.860	0.652	0.879[ab]	0.724
Yes ×Yes			0.814	0.619	0.874[ab]	0.747
P-value						
Enzyme			0.550	0.076	0.162	0.408
Maize bran			0.002	0.782	0.027	0.389
Enz × MB			0.975	0.775	0.042	0.703
SEM			0.013	0.058	0.019	0.045

[a,b,c] means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
CP, Crude protein.

4.13 Influence of Exogenous Enzyme and Maize Bran on Production Economics, d 56

At d 56, there was an Enz × MB interaction effect on the price per bird ($P < 0.05$) indicating that in the group fed Yes enzyme, No MB increased the price per bird while in those fed No enzyme, Yes MB increased the price per bird. Similarly, an Enz × MB interaction was observed for profit per bird ($P < 0.05$) indicating that in birds fed Yes enzyme, No MB increased the profit per bird while in those fed No enzyme, Yes MB increased the profit per bird. Also, there was an Enz × MB interaction observed for the production economic index (PEI) ($P < 0.05$) indicating that in the group fed Yes enzyme, No MB increased the production economic index while in those fed No enzyme, Yes MB increased the production economic index. Production economics analysis at d 56 revealed that feeding a No MB-based diet increased ($P = 0.007$) the price per bird, profit per bird ($P = 0.000$), feed cost: profit ($P = 0.037$), price per Wt: feed cost ($P = 0.037$), and the production economic index (PEI) ($P = 0.042$) compared to Yes MB (Table 4.14)

Table 4.14: Influence of exogenous enzyme and maize bran on production economics, d 56

Effects			Total Feed Cost	Price per bird	Profit per bird	Feed cost: Profit	Price per Wt: Feed Cost	PEI
	Enzyme	**Maize bran**						
	No		31.76	67.96	36.20	1.143	2.143	156.5
	Yes		31.21	67.54	36.33	1.166	2.166	155.1
SEM			0.667	1.020	0.550	0.023	0.023	5.050
		No	32.00	69.94^{a}	37.94^{a}	1.191^{a}	2.191^{a}	163.6^{a}
		Yes	31.00	65.56^{b}	34.59^{b}	1.117^{b}	2.117^{b}	148.0^{b}
SEM			0.667	1.020	0.550	0.023	0.023	5.050
Interaction								
No × No			31.83	67.85^{ab}	36.02^{b}	1.137^{ab}	2.137^{ab}	154.7^{ab}
No × Yes			31.69	68.07^{ab}	36.38^{b}	1.150^{ab}	2.150^{ab}	158.2^{ab}
Yes × No			32.16	72.02^{a}	39.86^{a}	1.246^{a}	2.246^{a}	172.4^{a}
Yes ×Yes			30.25	63.05^{b}	32.80^{c}	1.085^{b}	2.085^{b}	137.8^{b}
P-value								
Enzyme			0.564	0.772	0.869	0.508	0.508	0.848
Maize bran			0.290	0.007	0.000	0.037	0.037	0.042
Enz × MB			0.359	0.005	0.000	0.017	0.017	0.015
SEM			0.943	1.440	0.778	0.033	0.033	7.150

^{a,b,c} means in the same column with different superscripts are significantly different (P < 0.05)
2-way interaction separated by least-square difference
BW, Bodyweight
Enz, Enzyme
MB, Maize bran
PEI= Production economic index,
SEM= Standard error mean, and Wt= Weight.

CHAPTER FIVE

5.0 DISCUSSION

The present study was designed to test the hypothesis that the application of feed enzyme in diets containing MB improves nutrient digestibility, growth performance, gut pH, bone health, and profit margins in broilers. In this study, four diets (starter and grower-finisher) were formulated in a 2 × 2 factorial arrangement in which the factors were MB (no or yes) and enzyme (no or yes). Two of the diets (one with enzyme and the other without) were formulated to be nutrient-adequate as expected in a Ghanaian poultry diet. The other two diets (one with enzyme and the other without), containing MB, were made deficient in Crude protein, energy, lysine, and methionine with the hypothesis that the addition of the enzyme to one of the diets would liberate nutrients during digestion and make up for the nutrient deficiency. The enzyme was added on top rather than formulated into the diet without using the manufacturer's matrix value as is mostly practiced in Ghana.

The proximate compositions of the experimental diet indicated that though the analyzed crude protein and crude fibre were slightly lower than the formulated levels, the diets were similar and standard diets for broilers as recommended by the NRC, (1998).

5.1 Analyzed Nutrient Composition of Maize Bran and Experimental Diet

The proximate analysis of maize bran indicates that it is a nutrient-dense by-product with notable levels of nitrogen-free extract (68.65 %) and metabolizable energy (3016.44 kcal/kg). These findings align with those reported in other studies on maize bran. For instance, Jaworski *et al*. (2015) reported maize bran's nitrogen-free extract at approximately 66 %, with metabolizable energy ranging from 2950 to 3100 kcal/kg, emphasizing its value as an energy source in animal feed. The crude protein content of maize bran in this study

(8.76 %) is within the range of 8–10% typically reported in the literature (Donkoh and Attoh-Kotoku, 2009; Ajila *et al*., 2012). While this level makes maize bran a moderate protein source, its protein quality may need supplementation with higher-protein ingredients, especially in poultry and livestock diets. This finding is corroborated by Ayeni and Adeleke (2019), who suggested combining maize bran with protein-rich ingredients to meet the dietary requirements of monogastric animals.

The crude fibre content of 7.30 % in maize bran observed here is similar to values reported by Hussain *et al*. (2024), who noted that the fibre content ranged between 6.8 % and 7.5 %. This moderate fibre level makes maize bran a good feed ingredient for improving gut motility without overburdening the digestive system, especially in poultry. However, high fibre levels could potentially limit nutrient digestibility and energy utilization if not properly balanced in feed formulations (Yacoubi *et al*. 2018; Musigwa *et al*. 2020). Crude fat content in maize bran (3.54 %) is consistent with previous studies, which report a range of 3 – 4 % (Amoah *et al.* 2018). This moderate fat content contributes to energy density and feed palatability. However, the ash content (2.96 %) indicates that maize bran has a lower mineral content compared to other cereal by-products. The moisture content of 8.79 % is relatively low, favouring storage stability and minimizing the risk of microbial spoilage. This result is comparable to the findings of Ibrahim and Anyaehie (2019), who reported moisture levels in maize bran ranging from 8 – 10 %, depending on processing and storage conditions.

5.2 Influence of Exogenous Enzyme and Maize Bran on Growth Performance of Broiler Chickens

The study's main finding is that as the birds get older, those fed with enzymes plus no maize bran (Yes ×No) are unable to outperform those fed only the basal diets (No ×No). This observation was contrary to the hypothesis of this study and much of the literature as it was expected that exogenous enzyme would improve the performance of broilers. The reasons for the current findings are discussed as follows. Firstly, it may well be that the attention of this work should not be on the enzyme effect alone but rather on the nutrient density and the substrates available in the diet as this potentially influences the efficacy of the enzyme. It may be that the enzyme diet was not sufficiently limiting in nutrients to reduce growth so adding enzymes to the diet will not have any significant impact on the birds. It is well recognized that when broilers are fed an ideal diet, there is minimal chance that adding an enzyme on top of the diet would result in any improvement because the birds are already performing to the best of their abilities. This result is consistent with the findings of Moraes *et al*. (2015), who reported that when normal diets consisting of nutrients that are highly digestible and balanced are fed, enzyme impacts on performance indicators are typically not evident. Secondly, the substrate available in the diet also plays a major role in the efficacy of the enzyme. It may be possible that the substrate concentration in the diet may be low which limits the number of available active sites for the enzyme to act on thereby reducing the impact of the enzyme on the growth performance of the birds. Also, the dosage of the enzyme used can influence the effectiveness of the enzyme on the birds as it is evident that a high dose (0.05%) of exogenous enzyme improved the growth performance of broilers fed a standard base diet. This result validates the findings of Cowieson & Ravindran (2008b),

who reported dose-dependent increases in performance when the control diet was supplemented with the enzyme cocktail.

The effect of enzyme on the maize bran diets was observed during the early stage of the bird's growth (d 0 - 28). The reason for this finding is discussed as follows. Firstly, during the early stages of the bird's growth, there is limited endogenous enzyme activity in the digestive tract which could lead to less efficient feed digestion. Because younger broilers typically lack the developed capacity to secrete digestive enzymes like mature broilers do, the benefits of adding feed enzymes to their diet have a greater potential to aid digestion. A literature search on the effect of exogenous enzyme on growth traits in poultry indicates a consistent outcome among studies. For example, Recent research by Ravn *et al*. (2018) examined the combined effects of arabinofuranosidase and xylanase debranching enzymes on caecal microbial fermentation, maize glucuronoarabinoxylan breakdown, and broiler performance. Significant improvements in BW and FCR were seen with enzyme addition; these improvements were shown throughout the trial, although in this instance, they were more noticeable on days 21 and 29. In addition, Tahir *et al*. (2012) found that diets containing phytase together with xylanase or a mix of xylanase, protease, and α-amylase significantly improved the broiler's BWG and FCR at d 35, but only partial improvement was noticed at d 49. Similar research was conducted by (Gitoee *et al*., 2015; Nunes *et al*., 2015; Ravindran, 2013) in which broilers were fed feeds with lower energy and mineral levels. From days 1–21, the enzyme supplementation promoted performance similar to the positive control, but during days 22–42, only partial improvements were observed.

In the first stages (d 0-28) of the investigation, sudden-death syndrome (SDS) and coccidiosis accounted for the bulk of deaths reported. Runts were also eliminated and counted against the total number of mortalities. Despite being unclear, the pathophysiology of this condition has been observed to be widespread in fast growing broilers (Zanu *et al*., 2023).

Moreover, higher mortality was observed in the group fed enzyme in the last few weeks of the study. Birds on the maize bran diets without enzyme might have been protected from SDS by the health benefits of feeding insoluble NSP as reported by Musigwa *et al*. (2020). The beneficial effect of insoluble NSP reduces the hindgut bacterial load and in some cases may be beneficial in broiler diets. Moreover, although the body weight observed in this trial did not match the breeder standard of 3950g and 3876 g for both male and female broilers at d 56, this performance level is not unusual in Ghanaian commercial poultry production. This might be for a number of reasons.

Firstly, the formulation of diets based on crude protein rather than amino acids in the Ghanaian poultry industry could contribute to reduced growth performance (Zanu *et al*., 2023; Kidd *et al*., 2021). Secondly, the open curtain housing technique used to raise chickens in Ghana (as it was in the current study) probably did not offer the optimal circumstances advised by the breeder, which decreased the broiler's potential for growth. Lower body weights like those seen in this study were noted in several Ghanaian broiler studies (Dei *et al*., 2011a; Dei & Bumbie, 2011b; Atuahene, Akowuah, & Adjei, 2013; Affedzie-Obresi *et al*., 2022; Agyekum *et al*., 2022; Zanu *et al*., 2023).

5.3 Influence of Exogenous Enzyme and Maize Bran on the Gastrointestinal pH of Broiler Chickens

In the current study, at d 28 enzyme and maize bran had no significant effect on the gastrointestinal pH of the broiler chickens. The birds fed the enzyme diet had increased gizzard pH and reduced ileal pH. The gizzard pH was significantly reduced in birds fed with enzyme-supplemented diets, particularly when maize bran was included. This finding is supported by Singh *et al*. (2016), who found that the addition of fibre-degrading enzymes, such as those in Rovabio, can lower the pH of the gizzard by enhancing the breakdown of fibrous components in the diet. The reduction in gizzard pH may improve the efficiency of mechanical digestion, as a more acidic environment can activate pepsinogen to pepsin, thus improving protein digestion. At d 56 the pH of the proventriculus did not significantly differ between the enzyme-treated and non-enzyme-treated groups. This is consistent with studies such as Gao *et al*. (2017), which reported that the pH of the proventriculus is relatively stable and less susceptible to changes from dietary modifications, including enzyme supplementation. The proventriculus primarily functions as a glandular stomach, and its pH is maintained within a narrow range to facilitate enzymatic digestion of proteins, which might explain the lack of significant change. In the duodenum, enzyme supplementation slightly increased the pH, though this change was not statistically significant. Similar findings were reported by Olukosi *et al*. (2018), who noted that while enzyme supplementation can alter nutrient digestibility in the duodenum, the pH might not change drastically due to the buffering action of bile and pancreatic secretions. The duodenum's role in neutralizing acidic chyme from the stomach could also explain the stability in pH. The jejunum pH showed minimal variation between treatments, with no significant effect of

enzyme supplementation. Adeola & Cowieson (2020), observed similar results, indicating that the pH in the jejunum is typically well-regulated by the absorption of nutrients and the presence of bicarbonate secretions. The slight reduction in pH with enzyme supplementation might suggest enhanced nutrient absorption, but it does not appear to be substantial enough to reflect in the pH values. The pH of the ileum was also unaffected by enzyme supplementation, with a significant interaction effect between enzyme and maize bran. This finding aligns with Ravindran *et al*. (2019), who reported that enzyme supplementation in high-fibre diets could improve nutrient digestibility in the ileum without significantly altering pH. The interaction effect suggests that maize bran's fibre content, when combined with enzyme supplementation, might influence fermentation processes in the ileum, potentially altering pH levels slightly. The caeca pH remained stable across treatments, with no significant differences observed. The stability in caeca pH might be due to the caeca's role in microbial fermentation, which is more affected by the type of fibre present rather than enzyme supplementation. Bedford & Partridge (2021) suggested that while enzymes can enhance overall digestion, the caeca's pH is largely driven by microbial fermentation of undigested residues, and thus, enzyme supplementation may have minimal impact.

5.4 Influence of Exogenous Enzyme and Maize Bran on the Carcass Traits (% BW) of Broiler Chickens

The effects of the enzyme and maize bran was not seen in any of the carcass traits (% BW) until day 56 where there was a significant influence on empty gizzard weight, % body weight, and breast weight, % bodyweight. The current finding conforms to that reported by Selle *et al*. (2003a) that supplementation of wheat-based diets with xylanase plus phytase

increased breast weight by 5.8 %, but Zanella *et al*. (1999) reported that enzyme addition did not affect the relative weight of the leg, breast muscle, and wings of the broilers.

The birds fed the maize bran diet without enzyme had increased empty gizzard weight, % bodyweight. Due to the inability of the endogenous enzyme to digest NSPs in the gizzard, the mechanical activities of the gizzard increase thereby making the gizzard to increase in size. Also, the reduced gizzard weight (% bodyweight) observed in the birds fed the enzyme diet may be attributed to the fact that the enzyme was able to digest the NSPs present in the diet which might have reduced the mechanical activities of the gizzard on the high fibre thereby reducing muscle development in the gizzard. The birds fed enzyme plus no maize bran (Yes ×No) diet had higher breast weight and % bodyweight. The highest breast meat observed in the birds fed the enzyme plus no maize bran (Yes ×No) may be due to higher body weight recorded as the enzyme was able to improve nutrient availability and hence the growth performance of the birds. This result contradicts Café *et al*. (2002) who reported no significant effect on breast, thigh, and wing components when a corn-soy-based diet was supplemented with 1000mg multi-enzyme (Avizyme 1500®) per kg of the diet.

5.5 Influence of Exogenous Enzyme and Maize Bran on the Bone Traits of Broiler Chickens

The effects of enzyme and maize bran were not seen in any of the bone traits until day 56 perhaps suggesting that ranges in enzyme and maize bran employed in this study had little influence on the early mineralization of bone. This may not be surprising as it is consistent with the findings of Estefania *et al*. (2019) who reported that the degree of mineralization of bones is low in the earlier ages up to 3 to 7 d of broilers. Furthermore, in the present work,

the dietary contents of Ca and P were the same in all diets, it was just the presentation of the enzyme and the level of maize bran that differed. According to Estefania *et al*. (2019), at later ages (up to 37 d), bone mineralization increases to a maximum of 65 % total ash and perhaps it is only later on that the subtle differences between diets in this study would become evident.

The reduced tibial and femur weight observed in the birds fed the Yes enzyme and Yes MB was at variance with the hypothesis of the present study. Yes, enzyme in the presence of Yes MB was expected to result in rapid degradation of nutrients in the GIT resulting in the release of minerals such as Ca and P which are responsible for bone development. Bone weight, however, is not necessarily reflective of mineralization and may be more related to amino acid availability for matrix formation.

The reduced breaking strength observed in birds fed diets with no enzyme supplementation in the presence of maize bran aligns with the study's hypothesis that the absence of enzymes in maize bran-based diets negatively impacts bone mineralization and breaking strength (BS). This can be attributed to the fact that in the absence of enzyme supplementation, many nutrients, particularly minerals, escape digestion. Enzymes, such as phytase, are known to improve the availability of phosphorus and calcium by breaking down phytate complexes in grains, including maize bran. Without enzymes, the release and subsequent absorption of these critical minerals are reduced, leading to impaired bone mineralization and lower breaking strength in birds. This finding is consistent with previous studies that have demonstrated the importance of enzyme supplementation in poultry diets containing high levels of maize bran. For example, Selle *et al*. (2009) have shown that enzyme

supplementation in poultry diets improves phosphorus availability, which directly impacts bone mineralization.

5.6 Influence of Exogenous Enzyme and Maize Bran on Apparent Ileal Digestibility of Protein, Ash, Fat, and Fibre at d 28

The findings indicate that crude protein digestibility is significantly influenced by the inclusion of enzyme in the diet, with the birds' fed diets without maize bran showing higher CP digestibility. This result aligns with studies by Cowieson *et al*. (2006) & Ravindran *et al*. (2019), who reported that maize bran, due to its high fibre content and anti-nutritional factors, can impede protein digestion. The addition of enzymes slightly improved CP digestibility, although the difference was not statistically significant. This suggests that while enzymes may aid in protein breakdown, the challenge posed by maize bran's fibre content still limits CP digestibility.

Ash digestibility, which reflects the availability of minerals in the diet, showed an improvement with enzyme supplementation, although the results were not statistically significant. Previous studies, such as those by Selle *et al*. (2009) and Ravindran *et al*. (1999**)**, have demonstrated that enzyme supplementation enhances mineral absorption by reducing the anti-nutritional effects of dietary fibres like those found in maize bran. However, the lack of significance in this study might be attributed to variations in experimental conditions or differences in enzyme formulations used.

Fat digestibility was significantly affected by the interaction between enzyme supplementation and maize bran inclusion. Birds fed diets with no maize bran and enzyme supplementation showed the highest fat digestibility, whereas birds fed no enzyme and maize

bran had the lowest fat digestibility. This finding is consistent with Siyal *et al*. (2017), who noted that enzyme supplementation helps in breaking down complex structures in fibrous ingredients like maize bran, enhancing fat absorption. The improvement in fat digestibility with enzyme addition underscores the importance of enzymes in mitigating the negative effects of high-fibre diets.

Fibre digestibility improved with enzyme supplementation, although the results were not statistically significant. The inclusion of maize bran increased the challenge of fibre digestion, as shown by lower digestibility in birds fed no enzyme and maize bran diets. The slight improvement with enzyme supplementation suggests that enzymes help in breaking down the fibre in the diet.

5.7 Influence of Exogenous Enzyme and Maize Bran on the Production Economics of Broiler Chickens

The current study discovered that enzyme plus no maize bran (Yes ×No) increased feed cost by 10 %, price per bird, profit per bird and production economic index when compared to the basal diet (No × No). Despite the higher cost of feeding bird's enzyme plus no maize bran diet, it was discovered that livability was lower in birds on that diet, and those birds also weighed more and attracted higher prices during sales. The increased growth performance of birds fed the enzyme plus no maize bran (Yes ×No) diet, as well as the high prices they attracted during sales, compensated for the high feed costs incurred in raising the birds thereby increasing the profit by 30 %. This finding contradicts with the findings of Ravindran (2013) who reported that, enzyme supplementation in broiler chick diets reduced feed costs per kg weight gain compared to non-enzyme supplemented diets. In a recent study,

Sens *et al*. (2021) reported that increasing the amount of phytase in the diet of broiler chickens reduced the cost of nutrition and increases revenue. The findings of these studies are in line with the observations made in the present study because the cost of feed was high in diets containing enzymes but this was compensated by high sales of birds and profit gain per birds. Moreover, feeding birds with maize bran without enzyme reduced feed cost and increase revenue compared to the birds on the maize bran with enzyme diet. This observation contradicts the findings of Ravindran (2013) and Sens *et al*. (2021) who reported that the inclusion of enzymes in the diets of broilers increases the profit margins.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings of the 8-week experimental period, the following conclusions were drawn;

1. The addition of the enzyme (Rovabio) to the maize bran-based diet resulted in better growth performance of the birds at the early stages (d 0 - d 28) of the production cycle.
2. The inclusion of enzymes and No MB diet increased the proventricular and gizzard pH of the birds.
3. The inclusion of maize bran and No enzyme diet increased empty gizzard weight, % bodyweight but reduced breast weight, and % bodyweight of the birds.
4. Neither enzyme nor the maize bran level in the diet had an effect on the bone weight of the broiler chickens. However, feeding birds with maize bran with enzyme increased BS of the tibia and femur of the birds.
5. Fat digestibility was significantly affected by both enzyme and maize bran inclusion. Birds fed diets with no maize bran and enzyme supplementation showed the highest fat digestibility, whereas birds fed no enzyme and maize bran had the lowest fat digestibility.
6. Enzyme inclusion increased feed cost but it increased revenue hence increasing the productive economic index.

6.2 Recommendations

It is recommended that;

1. Maize bran as the test ingredient in this study is recommended at an inclusion level not exceeding 19 % in the diet for Ghanaian poultry farmers and reduce the quantity of maize grain used in feed preparation with or without feed enzyme.
2. Further research should be done on the effect of high levels of maize bran and exogenous enzyme supplementation on the growth performance of broiler chickens.

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APPENDICES

Week 2

Appendix A1: Calculation of Livability d 0-14

$$count\ d14 \div count\ d0 \times 100$$

Appendix A2: Calculation of Body weight d 0-14

$$Pen\ weight\ d14 \div count\ d14$$

Appendix A3: Calculation of Gain d 0-14

$$(Pen\ weight\ d14 \div count\ d14) - (Initial\ weight\ d0 \div count\ d0)$$

Appendix A4: Calculation of Consumption d 0-14

$$Feed\ in\ d0 - Feed\ out\ d9 + Feed\ in\ d9 - Feed\ out\ d13 + Feed\ in\ d\ 13 - Feed\ out\ d14$$

Appendix A5: Calculation of FCR d 0-14

$$Consumption\ d0 - 14 \div (Pen\ weight\ d14 - initial\ weight\ d0 + dead\ bodyweight\ d0 - 14)$$

Appendix A6: Calculation of Feed intake d 0-14

$$Gain\ d14 \times FCR\ d14$$

Week 4

Appendix B1: Calculation of Livability d 0- 28

$$count\ d28 \div count\ d0 \times 100$$

Appendix B2: Calculation of Body weight d 0- 28

$$Pen\ weight\ d28 \div count\ d28$$

Appendix B3: Calculation of Gain d 0- 28

$(Pen\ weight\ d28 \div count\ d28) - (Initial\ weight\ d0 \div count\ d0)$

Appendix B4: Calculation of Consumption d 14- 28

$$Feed\ in\ d14 - Feed\ out\ d18 + Feed\ in\ d18 - Feed\ out\ d21 + Feed\ in\ d21 - Feed\ out\ d24 + Feed\ in\ d24 - Feed\ out\ d26 + Feed\ in\ d26 - Feed\ out\ d28$$

Appendix B5: Calculation of FCR d 0-28

$$(Consumption\ d0 - 14 + Consumption\ d14 - 28) \div (Pen\ weight\ d28 - initial\ weight\ d0 + dead\ bodyweight\ d0 - 14 + dead\ bodyweight\ d14 - 28)$$

Appendix B6: Calculation of Feed intake d 0-28

$Gain\ d28 \times FCR\ d28$

Week 6

Appendix C1: Calculation of Livability d 0-42

$count\ d42 \div count\ d0 \times 100$

Appendix C2: Calculation of Body weight d 0-42

$Pen\ weight\ d42 \div Count\ d42$

Appendix C3: Calculation of Gain d 0-42

$(Pen\ weight\ d42 \div Count\ d42) - (Initial\ weight\ d0 \div Count\ d0)$

Appendix C4: Calculation of Consumption d 28-42

$$Feed\ in\ d28 - Feed\ out\ 31 + Feed\ in\ d31 - Feed\ out\ d33 + Feed\ in\ d33$$
$$- Feed\ out\ d36 + Feed\ in\ d36 - Feed\ out\ d39 + Feed\ in\ d\ 39$$
$$- Feed\ out\ d42$$

Appendix C5: Calculation of FCR d 0-42

$$(Consumption\ d0 - 14 + Consumption\ d14 - 28 + Consumption\ d28 - 42$$
$$\div (Pen\ weight\ d42 - initial\ weight\ d0 + dead\ bodyweight\ d0 - 14$$
$$+ dead\ bodyweight\ d14 - 28 + dead\ body\ weight\ d28 - 42)$$

Appendix C6: Calculation of Feed intake d 0-42

$$Gain\ d42 \times FCR\ d42$$

Week 8

Appendix D1: Calculation of Livability d 0- 56

$$count\ d56 \div count\ d0 \times 100$$

Appendix D2: Calculation of Body weight d 0- 56

$$Pen\ weight\ d56 \div Count\ d56$$

Appendix D3: Calculation of Gain d 0- 56

$$(Pen\ weight\ d56 \div Count\ d56) - (Initial\ weight\ d0 \div Count\ d0)$$

Appendix D4: Calculation of Consumption d 42- 56

$$Feed\ in\ d42 - Feed\ out\ d45 + Feed\ in\ d45 - Feed\ out\ d49 + Feed\ in\ d49$$
$$- Feed\ out\ d52 + Feed\ in\ d52 - Feed\ out\ d56$$

Appendix D5: Calculation of FCR d 0- 56

$$(Consumption\ d0-14 + Consumption\ d14-28 + Consumption\ d28-42 + Consumption\ d42-56 \div (Pen\ weight\ d56 - initial\ weight\ d0 + dead\ bodyweight\ d0-14 + dead\ bodyweight\ d14-28 + dead\ body\ weight\ d28-42 + sample\ bird\ weight + dead\ body\ weight\ d42-56$$

Appendix D6: Calculation of Feed intake d 42- 56

$Gain\ d56 \times FCR$ d56

Digestibility calculation

Apparent ileal digestibility (%) = (1 − [TiO_2diet (%)/ TiO_2digesta (%)] × [digesta nutrient (%)/ diet nutrient (%)] × 100.