Improving broiler performance with dietary supplementation of a multi-enzyme complex at three different inclusion levels of phytase

by

Elsabé Schutte
17057176

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Supervisor: Dr C Jansen van Rensburg

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Declaration

I, Elsabe Schutte, declare that this dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by myself or another individual for a degree at this or any other institution.

Schutte

Pretoria

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Abstract

In South Africa, maize and soybean form the main component of broiler diets. The anti-nutritional properties in these ingredients negatively influence the performance of broilers. Non-starch polysaccharides and phytate are some of the main anti-nutritional factors that nutritionists aim to reduce. Through the use of exogenous feed enzymes these anti-nutritional factors can be mitigated to improve digestibility of the feed and thus the nutritional value of the feed. This study was conducted to evaluate the efficacy of the enzyme complex Rovabio Advance T-Flex® that is produced by *Talaromyces versatilis*, in releasing digestible amino acids and energy in a maize and soybean-based diet. The performance and carcass traits of the broilers that received different inclusion levels of phytase were also evaluated. The treatments consisted of two basal diets. Basal diet one was the standard commercial diet fulfilling the requirements of the bird while basal diet two had a 3% reduction in metabolisable energy and digestible amino acids. The enzyme complex Rovabio Advance T-Flex® were added to treatments 2, 4, 6 and 8 with no enzyme inclusion in the other treatments. Phytase was supplemented at three different levels. Treatments 1, 2, 7 and 8 received 1000 FTU; treatments 3 and 4 received 1500 FTU; and treatments 5 and 6 received 2000 FTU. The production parameters of the standard nutrient diets showed no improvement with the addition of the enzyme complex. An improvement in body weight gain, feed intake and feed conversion ratio were observed in the reduced nutrient treatments that was supplemented with the enzyme complex, compared to those without any enzyme inclusion. There was no observed difference in production parameters or carcass traits between the treatments receiving different inclusion levels of phytase, with and without the enzyme complex supplementation. From the present study it can be concluded that production parameters can be improved with the addition of the enzyme complex Rovabio Advance T-Flex® in maize and soybean meal diets when diets are fed containing reduced levels of metabolisable energy and digestible amino acids. No benefit was observed when increasing the dose of phytase above the level of 1000 FTU.

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List of abbreviations

ADF	Acid detergent fibre
AGP	Antibiotic growth promoters
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy, nitrogen corrected
ANF	Anti-nutritional factors
ATP	Adenosine triphosphate
AX	Arabinoxylans
BWG	Body weight gain
Ca	Calcium
CP	Crude protein
DCP	Dicalcium phosphate
DM	Dry matter
FCR	Feed conversion ratio
Fe	Iron
FI	Feed intake
FTU	Phytase unit
G	Gram
GIT	Gastrointestinal tract
GPV	Gross protein value
LSD	Least significant difference
K	Potassium
Kg	Kilogram
iNSP	Insoluble non-starch polysaccharides
IP6	Phytate
MCP	Monocalcium phosphate
ME	Metabolisable energy
MEC	Multi-enzyme complexes
Mn	Manganese

N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharides
NSPase	Non-starch polysaccharide degrading enzymes
P	Phosphorous
PEF	Performance efficiency factor
RFI	Residual feed intake
SAS	Statistical analysis system
SCFA	Short chain fatty acids
SEM	Standard error of mean
sNSP	Soluble non-starch polysaccharides
T	Tonne
Zn	Zinc

Chapter 1

General introduction

In South Africa the poultry industry dominates all agriculture and animal production systems with 20% and 41.3%, respectively (SAPA, 2019). High production costs lower the local competitiveness of the broiler industry, being especially influenced by high feed costs which can account for 65-75% of total production costs (Nkukwana, 2018). The broiler industry is the major consumer of commercial feed in South Africa (SAPA, 2019) with the most common raw materials being used in the feed being maize and soybean meal (Jlali *et al*., 2020). Any increase in raw material prices, especially that of maize, will decrease the profit of producers.

Energy in diets is expressed as metabolisable energy (ME) and encompasses 65-70% of the total costs of broiler diets (Saleh *et al*., 2019). Energy is derived primarily from cereal grains and their co-products (Musigwa *et al*., 2020). In maize-soybean diets, maize provides up to 65% of the total apparent metabolisable energy (AME) (Rios *et al*., 2017). Improvements in broiler performance is most often associated with improvement in the bird’s capacity to utilise energy (Rios *et al*., 2017; Sun *et al*., 2019). The efficiency for energy utilisation for fat or lean deposition is dependent on the capacity of the bird to control its feed intake (FI), and thus energy intake (Musigwa *et al*., 2020).

The poultry industry faces a lot of challenges including food safety, environmental impact, and high production costs, all while trying to feed a growing population with high quality products at relatively affordable prices (Pirgozliev *et al*., 2017). One way to somewhat overcome the rising cost of feed, is to increase the availability of nutrients in feedstuffs. Feed additives, such as enzymes, are chemical and biological supplements that have been used to combat high feed prices. In recent years, the focus of research has been on the effect of exogenous carbohydrase supplementation on the growth performance and nutrient digestibility of broilers (Rios *et al*., 2017). It has been shown that carbohydrase is safe to use and have led to improvements in broilers such as body weight gain (BWG) and feed conversion ratio (FCR (Yacoubi *et al*., 2016).

Maize and soybean meal are used globally as feed ingredients in poultry feedstuffs, but the presence of various anti-nutritional factors (ANF) negatively impacts its nutritional value (Jlali *et al*., 2020). These ANF include non-starch polysaccharides (NSP) (Sun *et al*., 2019), and phytate, which causes valuable nutrients to pass intact through the gastro-intestinal tract (GIT) (Rios *et al*., 2017). The presence of high NSP levels causes the viscosity of the digesta to increase (Amerah, 2015) leading to a reduction in the efficiency of and absorption of valuable nutrients such as starch, lipids, and proteins (Musigwa *et al*., 2020) as well as decreased performance (Amerah, 2015) and decreased feed efficiency (Jlali *et al*., 2020). The structure and content of the NSP causes variability in the AME of the feedstuffs (Yacoubi *et al*., 2016; Yacoubi *et al*., 2017). Non-starch polysaccharide degrading enzymes (NSPase) are added to diets to maximise energy utilisation (Musigwa *et al*., 2020) by reducing the intestinal viscosity resulting in improved performance through enhanced digestion (Yacoubi *et al*., 2017). Maharjan *et al*. (2019) state that the appropriate inclusion of enzymes that degrade NSP will result in an overall enhancement of the ME of the feedstuffs due to the degradation of dietary NSP releasing additional energy. According to Rios *et al*. (2017) the value of low-quality maize can be increased with the use of NSPase. Along with NSP, phytate also plays an antagonistic role in influencing the nutritional value of feedstuffs. Lawlor *et al*. (2019) state that more than 60% of the total phosphorous (P) in feedstuffs can be bound by phytate, resulting in P being unavailable for digestion. This often leads to the dietary recommendations of P inclusion levels being exceeded which results in increases in feed costs and environmental pollution. According to Jlali *et al*. (2020), phytase is added to broiler diets to break down phytate and release P along with other trapped minerals and nutrients leading to improved carcass traits and growth performance in broilers. The concept of ‘superdosing’ of phytase has become more prevalent in recent years, where phytase is added to diets at equal to or more than 1000 FTU/kg above the standard dose of 500 FTU/kg. Jlali *et al*. (2020) state that the higher dose of phytase leads to further improvement of BWG and feed efficiency of broilers.

The presence of both phytate and NSP significantly reduced the availability of nutrients and has thus led the production of multi-enzyme complexes (MEC) which contain phytase and/or NSPase that can increase the availability of these otherwise unavailable nutrients (Lawlor *et al*., 2019). In a study done by Dos Santos *et al*. (2017) positive effects were observed in performance traits and carcass yield when supplementing an MEC to a broiler diet. Rovabio Advance T-Flex® (Adisseo, France) is a commercially available enzyme cocktail consisting of 19 enzymatic activities with improvements mainly being attributed to the improved digestibility of endo-xylanases and arabinofuranosidase (Saleh *et al*., 2019).

Aim and objective of study

The aim of this trial was to evaluate the efficacy of the MEC, Rovabio Advance T-Flex®, to improve nutrient availability of broiler diets at three concentrations of phytase. To achieve this, the first objective of the trial was to determine whether the performance of broilers that received a diet with 3% less energy and digestible amino acid concentrations would be improved when supplemented with the multi-enzyme complex, Rovabio Advance T-Flex®. The second objective of this trial was to determine if increasing the concentration of phytase above the standard industry inclusion level of 1000 FTU, would improve broiler performance. The last objective of this trial was to determine if there exists an interaction effect between the level of phytase inclusion and the addition of the multi-enzyme complex, Rovabio Advance T-Flex®. Body weight, feed intake, feed conversion ratio, and mortalities were measured as indicators of broiler performance. Additionally, bone ash was determined by incinerating the left tibia at two different stages of growth.

Hypotheses of the study

The first null hypothesis (H_0) was that the birds receiving a diet containing a reduced concentration of AME and digestible amino acids with added multi-enzyme complex, Rovabio Advance T-Flex®, will yield a decreased performance when compared to the standard commercial diet in broilers.

The first alternative hypothesis (H_A) of this study was that the birds receiving diets with reduced nutrient concentrations with the added multi-enzyme complex, Rovabio Advance T-Flex®, will yield the same level of performance as the standard commercial diet in broilers.

The second null hypothesis (H_0) was that increasing the phytase level from 1000 FTU to 1500 FTU and 2000 FTU in the broiler diet will not improve performance.

The second alternative hypothesis (H_A) was that increasing the phytase level from 1000 FTU to 1500 FTU and 2000 FTU in the broiler diet will improve performance.

The third null hypothesis (H_0) was that there will be no interaction effect between phytase level and the efficacy of the added multi-enzyme complex, Rovabio Advance T-Flex®.

The third alternative hypothesis (H_A) was that there will be an interaction between phytase level and the efficacy of the added multi-enzyme complex, Rovabio Advance T-Flex®.

Chapter 2

Literature review

2.1 Introduction

The South African poultry industry is the largest agriculture sector providing the main protein source for the consumer at affordable prices. The poultry sector is divided into broilers for meat production and layers for egg production with each contributing 74% and 26% to the industry, respectively (SAPA, 2020). The struggle of the South African poultry industry to remain competitive in the international market stems from the unfavourable macroclimate (Nkukwana, 2018; SAPA, 2018). High production costs, especially high feed costs that can account for 65-75% of production costs, put a hamper on profit (SAPA, 2019). The main sources of energy and protein in the diet of broilers are maize and soybean meal. These feed ingredients are expensive, and the ever-changing prices has a significant impact on profit. In order to help combat the unstable prices of raw materials, nutritionists are tasked to formulate diets that are more bioavailable and economical. The introduction of additives into the market has led to the reformulation of diets to account for their beneficial response on the digestibility of all nutrients (Sun *et al*., 2019). The focus of this literature review is to discuss broiler production and feed utilisation and the subsequent role of feed additives in broiler diets, with emphasis on NSP degrading enzymes and phytase.

2.2 Feed utilisation in broilers and feed additives

According to the Bureau of Food and Agricultural Policy (BFAP, 2021) the poultry industry is the largest contributor to gross protein value (GPV). Broiler meat and eggs continue the be the most affordable sources of animal protein on a rand to kilogram basis (SAPA, 2020) and the consumption of poultry products continue to exceed the consumption of any other animal protein source (Nkukwana, 2018). The gross value of agriculture is dominated by the poultry sector, with 33.8% of all animal products. The poultry sector consumes the largest amount of feed in the country, with broiler feed accounting for about 40% and layer feed about 15% of feed produced by the formal feed industry (SAPA, 2020). Feed cost contributes at least 70% of total input cost of an intensive system such as poultry operations, which significantly influences profitability (BFAP, 2021; Nkukwana, 2018). Maize is the main source of energy (Nkukwana, 2018) and accounts for 65-70% of the total broiler diet cost (Saleh *et al*., 2019). South Africa is normally a net exporter of maize and a net importer of soybeans and the erratic exchange rates as well as the trade policies of South America, such as export tax, increase the price of soybeans (David & Meyer, 2017). Any surge in feed costs will lead to higher prices for day-old chicks (SAPA, 2019). Feed costs and day-old chicks form the bulk of production costs as seen in table 2.1. The growing unrest between Russia and Ukraine has put tremendous strain on the supply of raw materials used in animal feed as well as the availability of crude oil resulting in increases in input prices and further increases are expected (BFAP, 2022). The occurrence of loadshedding in South Africa hampers the efficiency of production and decreases profitability in intensive production systems (SAPA, 2019). The technical efficiency of poultry production systems depends on the mortality rates, FCR, and performance efficiency factor (PEF) while the economic efficiency is driven by feed costs (David & Meyer, 2017). Quality feed formulation is a driving factor in the efficiency of the poultry industry (Nkukwana, 2018). Good quality feed formulation and increased growth performance are key drivers in the efficiency of the poultry sector.

Table 2.1 Variable production cost breakdown of South African broiler production (David & Meyer, 2017)

Variable cost component	Average share of variable production cost
Feed	71.3%
Day old chicks	20.0%
Labour	1.3%
Heating and electricity	3.3%
Bedding, waste removal and cleaning	1.7%
Vitamins and vaccinations	0.6%
Maintenance	0.7%
Catching	0.4%
Other	0.7%

The combination of maize and soybean meal, which make up the largest proportion of South African broiler feed, contain large quantities of NSP and phytate, which negatively affect the feed's nutritional value (Jlali *et* al., 2020). Loar and Corza (2011) state that the pellet quality should also be considered in addition to the nutritional composition since increased physical pellet quality increases bird performance. The particle size also plays an important role as it influences the voluntary FI as well as the development of the GIT (Chang'a *et al*., 2020). According to Amerah (2015), the raw materials, physical form of the diet, and processing method changes the efficacy of exogenous enzymes when added to the feed. Exogenous enzymes are supplemented to aid in the digestibility of nutrients and improve the energetic efficiency (Cerrate *et al*., 2019). However, to realise its true value, feed must be formulated specifically to account for the possible improved digestibility when adding exogenous enzymes (Sun *et al*., 2019).

Feed additives form a small portion of animal feed but may play a big role, such as improving feed utilisation, efficiency of growth, and preventing diseases (Pirgozliev, 2017; Cherian, 2020). The most common feed additives include enzymes, antioxidants, antibiotic growth promoters (AGP), and pro- and prebiotics, each with its own unique function. Enzymes improve the availability of nutrients by improving digestion and therefore limits the loss of nutrients and reducing environmental pollution (Cherian, 2020). Enzymes are defined as proteins that promote specific chemical reactions and are specific to a substrate (Pirgozliev, 2017). Since feed cost accounts for the highest proportion of all input costs, exogenous enzymes are a means for nutritionists to formulate diets in a more economical manner (Boyd *et al*., 2018) while improving feed efficiency and still provide the most affordable source of protein to the consumer (Davids & Meyer, 2017).

2.3 Carbohydrates in broiler feed

The largest portion of plant tissue are carbohydrates, mainly starch, oligosaccharides and NSP (Tejede & Kim, 2021) which makes up 60-90% of dry matter (DM) (Cherian, 2020). Carbohydrates are present as sugar or starch or is associated with the cell wall structure such as cellulose (Cherian, 2020). The most abundant carbohydrate in nature is cellulose, which is also highly stable (McDonald *et al*., 2011). Sugars are identified by the suffix “ose” of the biochemical name (Cherian, 2020) and is divided into monosaccharides and oligosaccharides depending on the amount of carbon atoms that are present in the molecule (McDonald *et al*., 2011; Cherian, 2020). Starch is divided into two groups of polysaccharides which are polymers of monosaccharide units and complex carbohydrates that are not well defined as they contain not only carbohydrates, but also non-carbohydrate molecules (McDonald *et al*., 2011). The classification of carbohydrates is illustrated in figure 2.1.

The largest component of poultry diets is plant-based starch (Tejeda & Kim, 2021), which is a glucan and the principal form of carbohydrates in cereal grains. Starch serves as the primary energy source in the diet of a monogastric animal (Cherian, 2020). The natural form of starch is a granule and the shape and size differ between plant sources (McDonald *et al*., 2011). All animals need energy to perform the various bodily processes such as movement, digestion, and reproduction (Cherian, 2020). Metabolic processes in animal cells make the energy from carbohydrates available to the animal in order to carry out bodily processes (Cherian, 2020). Energy is obtained in the diet from chiefly carbohydrates and are stored for later use due to there not being a constant supply of nutrients (Reece *et al*., 2015). Starches differ in their chemical composition and are most often the combination of the two polysaccharides, amylose and amylopectin (McDonald *et al*., 2011) as illustrated in figures 2.2 and 2.3. Amylose is a water-soluble linear structure with glucose molecules joined in a α-1,4 linkage and constitute 15-30% of the total plant starch, whereas amylopectin is not water soluble and has a bush-like structure with α-1,4 linkages as well as several α-1,6 linkage and constitutes 70-85% of the total plant starch (Cherian, 2020; McDonald *et al*., 2011).

According to Classen (1996), the digestion of starch is dependent on many factors such as different grain species and variation within species. The digestion of carbohydrates primarily occurs in the small intestine where the starch granules amylose and amylopectin are broken down by the enzyme amylase that is secreted by the pancreas (Cherian, 2020). In monogastric animals the end-product of carbohydrate digestion is mainly glucose that diffuses into the brush border to allow the final digestive processes to occur (Cherian, 2020). This capacity to absorb the end-products is highly influenced by the development and maturation of the small villi in the intestines (Yacoubi *et al*., 2017).

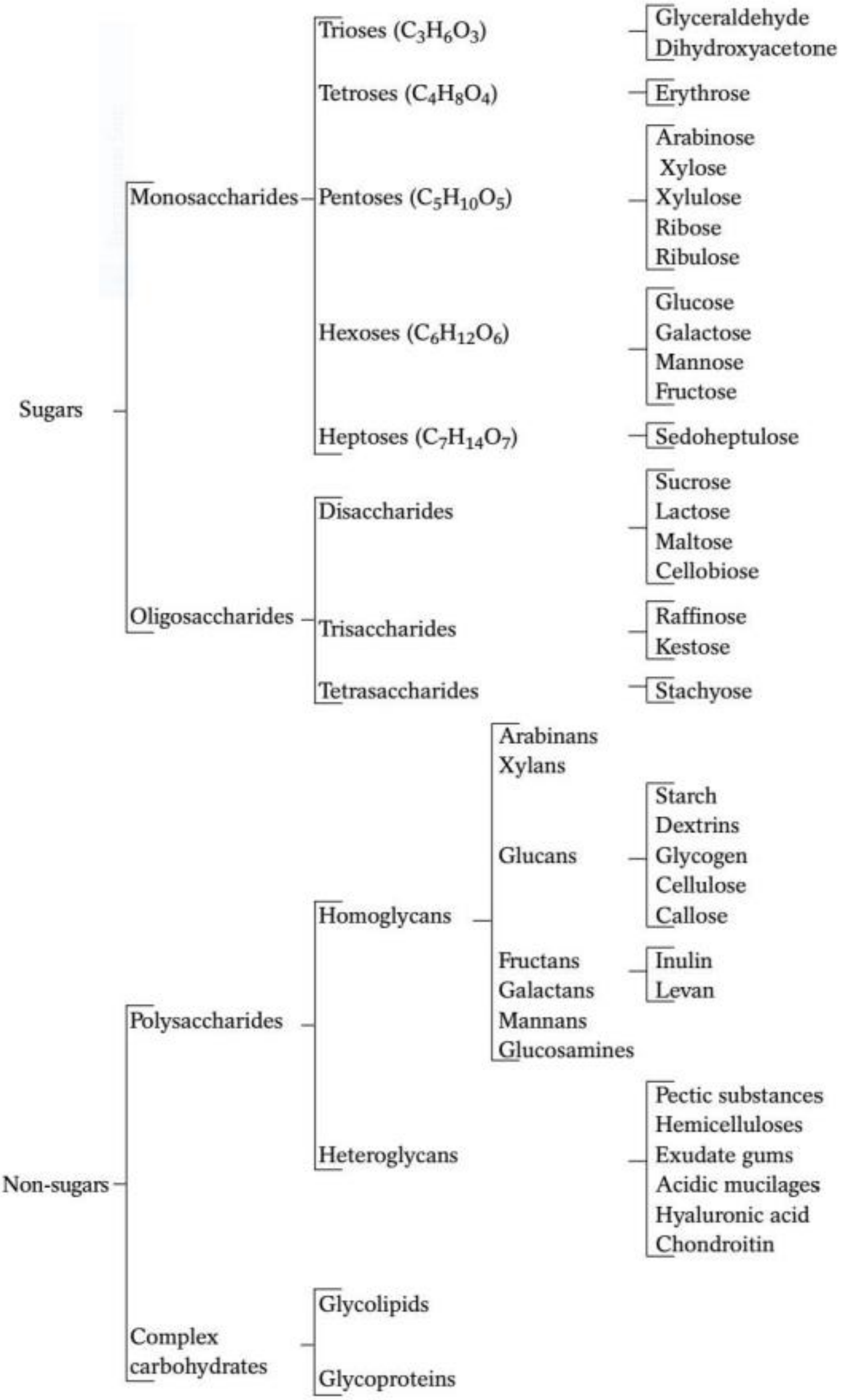

Figure 2.1 Classification of carbohydrates (McDonald *et al*., 2011)

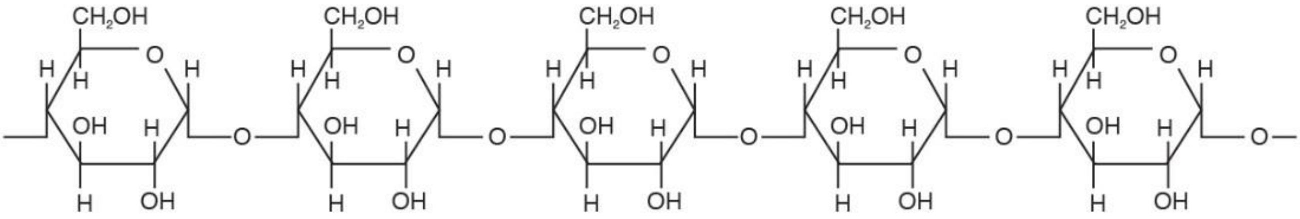

Figure 2.2 Amylose structure (Cherian, 2020)

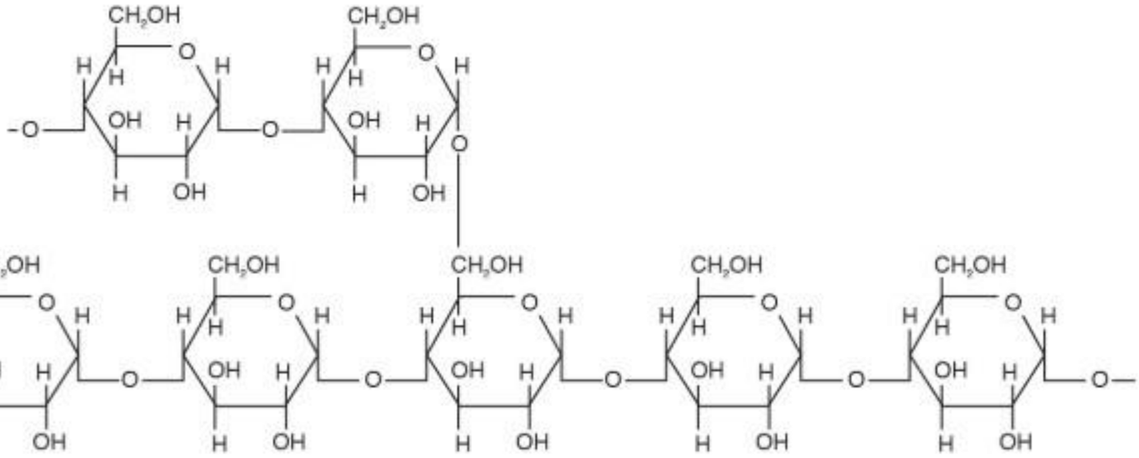

Figure 2.3 Amylopectin structure (Cherian, 2020)

2.3.1 Classification of non-starch polysaccharides

Non-starch polysaccharides are found in plant cell walls and can vary in content, size and structure (Maharjan *et al*., 2019). They are divided into two factions that are based on their solubility, namely soluble NSP (sNSP) and insoluble NSP (iNSP) (Musigwa *et al*., 2020). The classification of NSP is illustrated in figure 2.4.

The inclusion of sNSP is limited when formulating broiler diets due to its anti-nutritional effects (Tejede & Kim, 2021), thus the water-soluble fraction to the total NSP in feed is low (Maharjan *et al*., 2019). The sNSP fraction includes arabinoxylans (Yacoubi *et al*., 2017) and β-glucans with β-1,4 glycosidic linkage backbones and β-1,3 linkages (Tejede & Kim, 2021) that exert the anti-nutritional effects (Yacoubi *et al*., 2016). It causes the binding of a significant volume of water in the digesta leading to increased viscosity of the digesta as it moves in the small intestine from the proximal to distal end (Maharjan *et al*., 2019). This increase in digesta viscosity causes increased intestinal inflammation (Yacoubi *et al*., 2017) and decreased digestion and absorption of nutrients (Amerah, 2015) which ultimately decreases the feed AME (Musigwa *et al*., 2020). The decrease in nutrient digestion is due to the increase in viscosity that causes diminished interaction between the intestinal brush border and the digesta which hinders the action of intestinal enzymes and is not limited to only the carbohydrate fraction (Maharjan *et al*., 2019). Valuable nutrients pass undigested through the GIT (Rios *et al*., 2017) leading to poor feed utilisation and a possible decrease in growth and performance if requirements are not met (Saleh *et al*., 2019; Cherian, 2020). The increased digesta viscosity also decreases the rate of digesta passage, which can create hypoxic conditions in the intestinal tract that can create favourable conditions for pathogenic bacterial growth (Tejede & Kim, 2021). An increase in sticky droppings and thus wet litter is observed due to the increased water holding capacity (Maharjan *et al*., 2019). According to Wang *et al*. (1998) a key factor in the occurrence of foot pad dermatitis is wet litter.

The iNSP portion is the larger contributor to the total NSP in broiler diets and is considered inert (Maharjan *et* al., 2019). Insoluble NSP has no significant effect on the viscosity of digesta and thus no detrimental effect on nutrient digestibility (Musigwa *et al*., 2020). This portion of NSP creates a physical hindrance against enzymes (Musigwa *et* al., 2020) which is references to as a ‘cage effect’ (Rios *et al*., 2017). Nutrients are encapsulated which may impact the energy and nutrient digestibility (Rios *et al*., 2017). Insoluble NSP has laxative properties and reduces the hindgut bacterial load and in some cases may be beneficial in broiler diets (Musigwa *et al*., 2020). Insoluble NSP has been used as a diluent for nutrients due to the lack of enzymes that can digest the β1-4, β1-3, and β1-6 linkages (Tejede & Kim, 2021). Too high inclusions impair performance due to the slowing down and dilution of nutrient intake (Tejede & Kim, 2021).

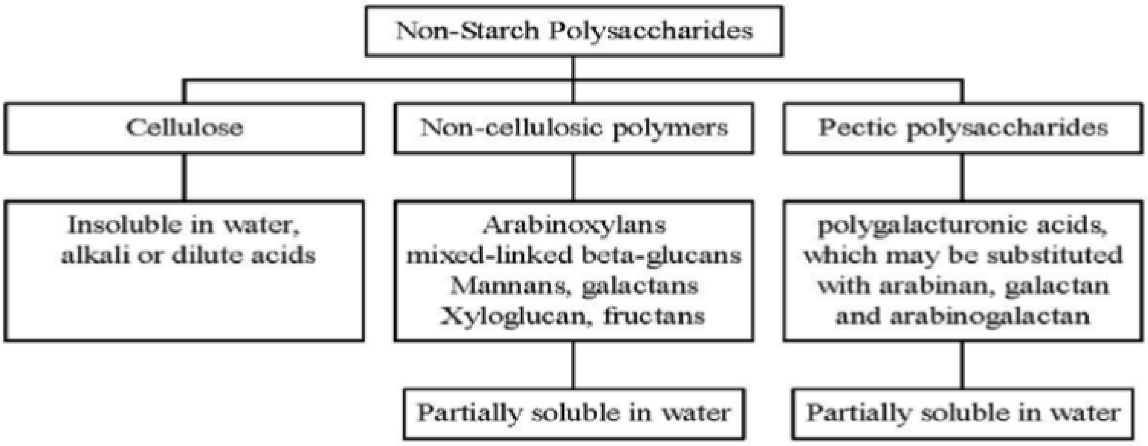

Figure 2.4 Classification of non-starch polysaccharides (Choct *et al*., 2010)

2.3.2 The effect of non-starch polysaccharides on digestion

Cereal grains and their co-products constitute the largest portion of broiler feeds (Musigwa *et al*., 2020) and are the most expensive raw materials (Cerrate *et al*., 2019). These cereal grains along with protein crops contain the anti-nutritional component NSP (Musigwa *et al*., 2020) that causes variation in the ME of broiler diets (Yacoubi *et al*., 2016). According to Sun *et al*. (2019) the NSP content in cereal grains are present in varying content of 83-98 g/kg. Maharjan *et al.* (2019) state that a negative correlation exists between the digestion of carbohydrates and the presence of NSP, which are a group of molecules that differ in water solubility, size, and structure. This reduction in digestion is related to the increased digesta viscosity and intestinal inflammation that causes valuable nutrients to pass through the GIT undigested (Rios *et al*., 2017), leading to losses of valuable nutrients. The increase in digesta viscosity delays transit through the GIT and creates an opportunity for pathogenic bacterial overgrowth (Cherian, 2020; Tejeda & Kim, 2021). The high viscosity of the digesta causes poor interaction between the digesta and intestinal brush border leading to limited contact between the digesta and substrates which hinders breakdown products from being digested and absorbed (Maharjan *et al*., 2019). This author also reiterates that this is not limited to only the carbohydrate fraction, but other valuable nutrients as well. Concurring with these statements, Rios *et al*. (2017) describes an encapsulation ‘cage’ effect which is exerted, and it reduces the digestibility and consequently the absorption of nutrients such as amino acids and lipids due to the physical barrier it poses against enzymes. Musigwa *et al.* (2020) describes a dietary energy dilution and gut filling effect that can have laxative properties and result in a reduction in the hindgut bacterial load. Non-starch polysaccharides are a major part of fibre, as fibre is the sum of lignin and NSP, and monogastric animals do not secrete the necessary enzymes to break down NSP (Cherian, 2020).

The predominant polymers of NSP includes arabinoxylans (xylose and arabinose) (Musigwa *et al*., 2020) that make up to 70% of the cell walls in the starchy endosperm (Yacoubi *et al*., 2017). The physiological and biological properties of NSP correspond to dietary fibre (Agiriga & Siwela, 2017) which is the sum of sNSP and iNSP and lignin (Tejeda & Kim, 2021). Cell walls in the endosperm are not digested by monogastric animals as they do not secrete the necessary enzymes (Cherian, 2020), resulting in nutrients such as starch and protein being encapsulated and not released for digestion (Amerah, 2015). The NSP in the cell walls of cereals and legumes are comprised of cellulose (linear α-glucan chains), non-cellulosic polysaccharides (mixed-linked α-glucans, arabinoxylans, xyloglucan, galactans, mannans) and pectic polysaccharides (polygalacturonic acids, which may be exchanged with galactan, arabinan and arabinogalactan) (Varley & Wiseman, 2001; Cherian, 2020). Soluble NSP increases digesta viscosity (Amerah, 2015) due to its capacity to hold water resulting in an increase in water consumption and excreted moisture having a poor litter quality effect, as well as increased endogenous secretions and nutritional losses (Nguyen *et al*., 2022). Body weight gain may be suppressed due to changes in the water-balance of the body and the gut microflora (Jamroz *et al*., 2002). The increase in viscosity in the pre-caecal part of the GIT disturbs the secretion of endogenous enzymes and bile acids leading to intestinal changes resulting in reduced nutrient digestibility (Jamroz *et al*., 2002). The effects of high concentrations of NSP in young poultry are more severe as they are especially sensitive to the anti-nutritional effects of NSP (Jamroz *et al*., 2002) due to the undeveloped digestive tract and unstable microbiota environment (Yacoubi *et al*., 2017). The transit of the digesta is delayed with high NSP concentrations and creates the ideal hypoxic environment that favours pathogenic bacterial growth (Cherian, 2020; Tejeda & Kim, 2021). In contrast to this, sNSP can also aid in the production of short chain fatty acids (SCFA) by providing the beneficial bacteria with a source of fermentable substrates to perform selective fermentation (Nguyen *et al*., 2022). The SCFA provide a source of energy (Nguyen *et al*., 2022) and is absorbed and utilised in the body (Jamroz *et al*., 2002). In addition, SCFA acts as a stimulator of GIT growth, immune system modulation, and secretion of some gut hormones that are linked to the satiety of the animal (Lee *et al*., 2017).

2.3.3 Non-starch polysaccharides in maize-soybean diets

Broilers rely on highly digestible raw materials as sources of energy and protein (Chang'a *et al*., 2020). Maize-soybean diets are more digestible than other cereal-based diets such as wheat and barley, which are known to have higher levels of NSP (Rios *et al*., 2017). Maize provides about 65% of the total AME and soybean meal provides 80% of the total crude protein (CP) in a typical maize-soya based broiler diet (Rios *et al*., 2017). According to Frempong *et al*. (2019) soybean meal has the highest feeding value compared to all other plant-based protein sources due to it being high in protein and meeting the amino acid demand of poultry.

The energy value of a feedstuff is influenced by the digestion of starch which is rarely a problem in maize-based diets as the starch component in maize is essentially fully digested by broilers (Zaefarian *et al*., 2015). Soybean meal, however, contain not only NSP but also other ANF such as trypsin inhibitors and phytic acid (Frempong *et al*., 2019). Albeit the NSP levels are lower than other vegetable ingredients such as wheat and barley, it still poses a digestibility problem (Jamroz *et al*., 2002; Musigwa *et al*., 2020). Maize-soybean diets contain on average total NSP of 10-12% DM with the water-soluble portion being 1-2.5% of DM (Nguyen *et al*., 2022). The concentrations of NSP in maize and soybean meal is around 9% (97 g/kg) and 29% (217 g/kg), respectively (Rios *et al*., 2017; Saleh *et al*., 2019). However, the NSP content of maize and soybean meal depends on genetics and environment (Zaefarian *et al*., 2015). The difference in NSP content between different cereal grains are illustrated in table 2.2. According to Frempong *et al.* (2019) the problems posed by ANF can be minimised to an extent by using proper thermal processing of the raw materials.

Table 2.2 Carbohydrate and lignin (gram/kilogram dry matter) in whole grain cereals (adapted from Knudsen, 1997)

NSP type	Maize		Wheat		Barley			
					Hulled		Hulless	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
β-glucan	1	1	8	1	42	5	42	6
S-NCP	9	7	25	4	56	10	50	10
Rhamnose	0	0	0	0	0	0	0	0
Arabinose	3	2	7	2	6	1	3	1
Xylose	2	2	9	4	6	3	4	1
Mannose	2	1	2	1	2	1	1	< 1
Galactose	1	1	2	1	1	1	1	< 1
Glucose	1	1	4	3	39	7	41	8
Uronic acids	1	1	1	1	2	1	1	1
I-NCP	66	11	74	6	88	10	64	11
Rhamnose	0	0	0	0	0	0	0	0
Arabinose	19	2	22	1	22	1	17	1
Xylose	28	3	38	3	50	4	24	4
Mannose	1	1	1	1	2	1	3	0
Galactose	4	1	2	1	2	1	2	0
Glucose	9	4	7	3	8	6	17	6
Uronic acids	6	1	4	1	4	0	1	0
Cellulose	22	3	20	4	43	5	10	3
Total NSP	97	2	119	11	186	11	124	10

I-NCP: Insoluble non-cellulosic polysaccharides
NSP: Non-starch polysaccharide
SD: Standard deviation
S-NCP: Soluble non-cellulosic polysaccharides

2.4 Phosphorous in broiler feed

Phosphorous is a crucial nutrient that serves numerous important functions in the animal body and is one of the costliest nutrients in the diets of poultry (Rahimi *et al*., 2020). Calcium (Ca) and P are needed to ensure bone strength, bone mineralisation, and growth, with deficiencies in either affecting the growth of the bird (Xu *et al*., 2021). The largest portion of P in the diet is provided by bound P in phytate (55-85% of the total P) (Jlali *et al*., 2020), but it is not readily accessible to monogastric animals (Trayhurn, 2005). This is referred to as the 'phytate effect' (Amerah, 2015) due to its binding effect on P, making it unavailable for digestion (Lawlor *et al*., 2019). According to Trayhurn (2005) this binding effect contributes to environmental pollution as the P is excreted into the environment and it increases the production cost to supply additional P that is available to the animal.

Phosphorous and Ca are two of the most abundant macro-minerals found in animal tissue (Jlali *et al*., 2020). It is estimated that deposition of P is around 80% in the skeletal system of and 20% being distributed in bodily tissues and fluid (Jlali *et al*., 2020). Numerous chemical reactions and metabolic processes in the skeletal system is dependent on P to ensure adequate growth, development and maintenance (Fernandes *et al*., 2019). In addition, it also controls cell metabolism, forms part of adenosine triphosphate (ATP) to transfer energy and activates numerous enzymatic processes (Jlali *et al*., 2020). It is also said FI may be controlled partly by P thus affecting broiler performance (Jlali *et al*., 2020). In theory, requirements of the broiler should be met by the P that originates from plant materials (Trayhurn, 2005). Phosphorous is in majority present in the form of phytic acid, an myo-inositol hexakisphosphate molecule, which is not digested by monogastric animals since they do not produce the enzymes that are necessary in their GIT to free the trapped P (Rahimi *et al*., 2020). The phytic acid structure and the existence of twelve replaceable protons suggest a tremendous potential for chelation (Feil, 2008). Phytic acid not only binds P, but it also has a strong affinity to bind to nutrients such as amino acids, soluble proteins at low pH, starch, and minerals (Fernandes *et al*., 2019). The binding of these nutrients makes them unavailable for absorption. The resultant impaired bone growth causes economic losses due to bone fragility, the incidence of black bone disease and consumer rejection (Fernandes *et al*., 2019). The bone is a complex structure composed of an inorganic phase mineralising an organic matrix and water, with its development and properties being influenced by multiple factors such as genetics, nutrition, sex, and the environment (Sanchez-Rodriguez *et al*., 2019). According to Sanchez-Rodrigues *et al*. (2019), several studies have been done with the aim to explore the changes in bone morphology throughout the bird’s lifespan, however, the is no detailed work indicating how the structural organization and chemistry of the bone changes during the growth of the broiler. The inclusion of other sources of P such as dicalcium phosphate (DCP) and monocalcium phosphate (MCP) is warranted with P in plant sources being mostly unavailable, but this increases feed cost and contributes to environmental pollution through the excretion of undigested P (Rahimi *et al*., 2020).

2.5 Exogenous enzymes in broiler diets

According to Costa *et al*. (2013) raw materials that are of plant origin contain ANF that reduce the availability of nutrients by creating a barrier that inhibits access of endogenous enzymes and thus prohibiting digestion. In the modern-day broiler, the passage rate of digesta is too accelerated for optimal digestion, and valuable nutrients pass undigested through the GIT (Rios *et* al., 2017). Through the use of exogenous enzymes cost of diets can be reduced in addition to degrading bound nutrients (Boyd *et al*., 2018). A challenge for all sectors in the agriculture industry, including the poultry sector, is to produce enough food for a growing population while reducing the environmental impact (Pirgozliev *et al*., 2017).

Exogenous enzymes such as xylanase, phytase and protease are produced using microbial sources (Saleh *et al*., 2019) and form a minor component of animal rations (Cherian, 2020). Exogenous enzymes used in animal feed has been proven to have beneficial effects on the utilisation of otherwise unavailable nutrients (Classen, 1996). The negative effects of ANF are alleviated by the addition of enzymes (Sun *et al*., 2019) while also increasing the profit in a poultry production system (Costa *et al*., 2013).

In a typical maize-soybean diet without enzymatic supplementation around 1.67-1.88 MJ of energy per kilogram of feed is not being digested (Govil *et al*., 2017). Through the usage of enzymes, the availability of fat, protein, and starch will increase in addition to more energy being made available for utilisation (Govil *et al*., 2017). Enzymes can be supplemented alone or in combination as an MEC in a poultry diet (Jlali *et al*., 2020). Positive results for both single and MEC has been illustrated (Yacoubi *et al*., 2016). Results regarding the efficacy of NSPase supplementation along with phytase is inconsistent (Rahimi *et al*., 2020). Phytase and NSPase have different target substrates, but NSPase have indirect benefits by releasing numerous nutrients and decreasing the production of mucus in the GIT, thus their effects complement each other (Rahimi *et al*., 2020). The response of using MEC depends on the diet, dose of the MEC and age and genetic background of the bird (Jlali *et al*., 2020).

There are numerous benefits of using enzymes in broiler feed, either alone or in combination:

a. The variation in AME and performance is minimised by releasing encapsulated starch in the cell wall (Amerah, 2015).
b. Reduced digesta viscosity decreases the incidence of sticky droppings (Amerah *et* al., 2015) as well as wet litter resulting in a reduced occurrence of dermatitis (Wang *et al*., 1998).
c. Young birds are extremely sensitive to the harmful effects of NSP as they do not have a fully developed GIT. Carbohydrase enzymes aid in maintaining gut health so as performance is not hampered by an inflamed gut (Yacoubi *et al*., 2017).
d. Body weight gain and FCR is improved through the decrease in the viscosity of digesta and the alterations of gut microbes by enhancing proliferation of microbes that are beneficial (Saleh *et al*., 2019).
e. Multi-enzyme complexes promote the production of SCFA (butyrate and acetate), and butyrate is an energy source for the gastro-intestinal epithelial cells that enhances their proliferation as well as differentiation to increase digestive health (Yacoubi *et al*., 2016).
f. Phytase releases trapped P and other nutrients which improve growth performance and carcass traits (Jlali *et al*., 2020).

g. Endogenous protein losses are decreased with an increase in body protein accretion which may be the result of a decrease in endogenous enzyme secretion and an increase in amino acid utilisation (Saleh *et al*., 2019).
h. Feed costs are reduced due to nutrients being utilised (Lawlor *et al*., 2019).
i. There is a reduction in the excretion of undigested nutrients into the environment and thus the contributing to environmental pollution is reduced (Lawlor *et al*., 2019).

2.5.1 Non-starch polysaccharide degrading enzymes

The commercial use of enzymes is a relatively new concept as it came into working only 30 years ago with the first phase focussing on the removal of the anti-nutritional effects of NSP in diets that are cereal-based for broilers (Choct, 2006). The high molecular weight of NSP causes the increase in digesta viscosity through coalescing to form complex polymers that are not digested by poultry (Collet, 2012). Analysis of the NSP in raw materials is important in order to choose the appropriate level of enzyme inclusion (Maharjan *et al*., 2019) to aid in the release of energy and nutrients to enhance the quality of diets (Rios *et al*., 2017). According to Yacoubi *et al*. (2016) it is not fully understood or explained what substrates released by NSPase aid in favourable production of SCFA in the caeca (Yacoubi *et al*., 2016). The increased fermentation of oligosaccharides and resulting release of SCFA may be a contributing factor in increasing the AME of diets treated with carbohydrase (Maharjan *et al*., 2019) while also influencing the release of gut hormones that contributes towards gastric retention and gut health (Jlali *et al*., 2020). The improvement in growth performance is a result of the increased AME, DM retention, and ileal digestible energy (Klein *et al*., 2015). Through the improvement of nutrient digestibility and consequently performance, the occurrence of sticky droppings is reduced (Amerah *et al*., 2015). Protection against pathogenic bacteria is achieved by the proliferation of butyrogenic bacteria in the caeca (Yacoubi *et al*., 2016). Figure 2.5 illustrates a summary of the possible effects of adding NSP degrading enzymes to a cereal-based diet is illustrated in figure 2.5.

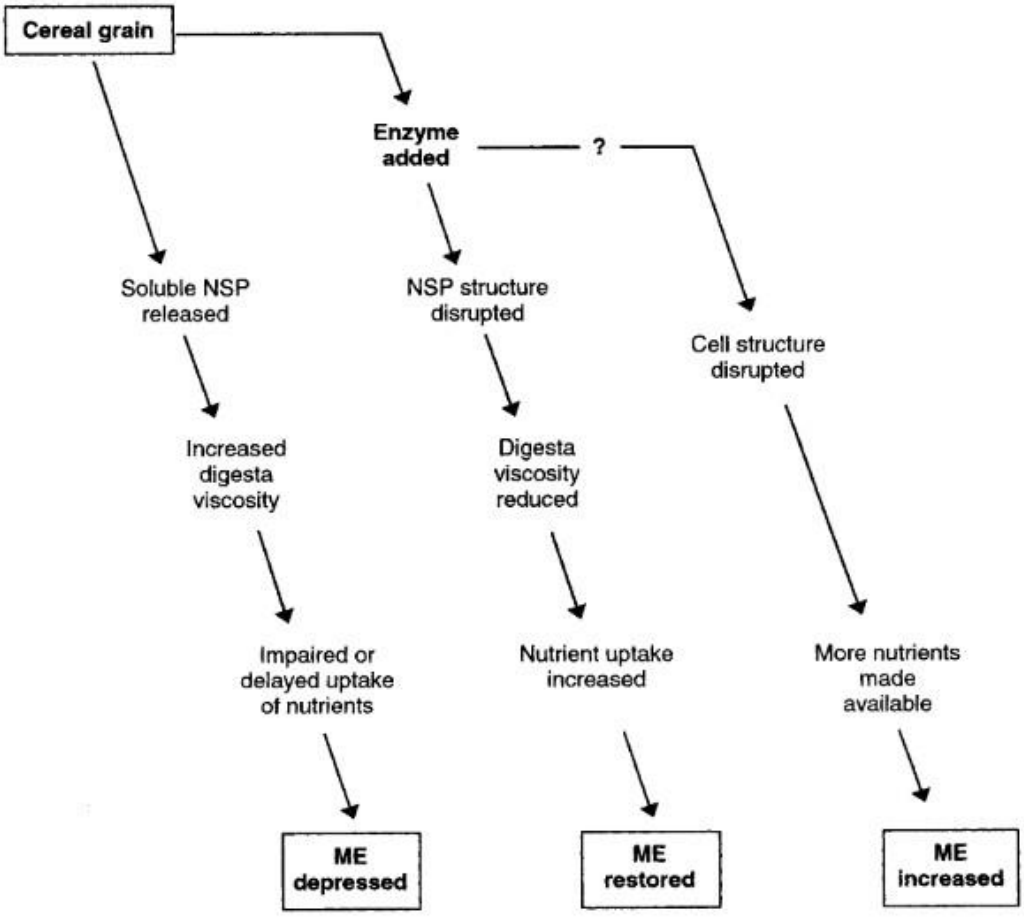

Figure 2.5 Summary of the possible effects of the addition of non-starch polysaccharide degrading enzymes to cereal-based diets for poultry (adapted from Chesson, 2001)

Carbohydrate degrading enzymes are added to monogastric diets that contain high quantities of NSP to break down complex carbohydrates into smaller polymers (Cherian, 2020). Monogastric animals lack the endogenous enzymes that are crucial aid in the digestion of the β 1-4, β 1-3, and β 1-6 linkages that occur in NSP (Tejede & Kim, 2021). Tejede & Kim (2021) state that the degree of branching of the NSP molecule determines the degree of solubility thus influencing the efficacy of the enzyme (Cherian, 2020). Birds do not have the capacity to digest the complex carbohydrates such as NSP (Saleh *et al*., 2019) and warrant the use of exogenous enzymes. Young animals have smaller endogenous enzyme reserves and warrant the use of supplemental amylase to aid in starch digestion (Classen, 2020).

2.5.2 The multi-enzyme approach for maize-soya diets

The efficacy of an enzyme to degrade different substrates depends on the solubility of the NSP and the complex nature of the carbohydrate (Cherian, 2020) while the mode of action of the enzyme depends on the efficacy of the enzyme (Rios *et al*., 2017). The commercial enzyme cocktail Rovabio Advance T-Flex® (Bichot *et al*., 2022) is produced by the fermentation of the fungus *Talaromyces versatilis* (IMI378536 and DSM26702; Adisseo France S.A.S. proprietary strains) (Cozannet *et al*., 2017) and overexpressing XlnR, a transcript factor that is involved in enzymes that degrade arabinoxylans (AX) (Cozannet *et al*., 2019). It is a unique combination of 19 enzymatic activities listed in table 2.3. The main enzyme activity in Rovabio Advance T-Flex® comes from endo-xylanase and arabinofuranosidase with a ratio of arabinofuronidase:endo-xylanase 3.7 (Cozannet *et al*., 2019). When tested in broilers, Rovabio Advance T-Flex® enhanced the utilisation of energy, fat, fibre and protein that resulted in an improvement in growth performance and gut health (Saleh *et al*., 2019). Improvements can be attributed to the enhanced nutrient digestibility instigated by endo-xylanases and arabinofuranosidase (Saleh *et al*., 2019).

Table 2.3 The enzymatic profile of Rovabio Advance T-Flex® (Adisseo, France)

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

Supplementation of South African maize-soybean diets with only endo-β-1,4-xylanase and/or β-glucanase or in combination of protease, α-amylase, and endo-β-1,4-xylanase has proven marginally effective at most (Ward, 2021), indicating that a multi-enzyme approach might be more advantageous. Diets with high concentrations of NSP have negative effects on poultry and these negative effects can be ameliorated by supplementing the enzyme xylanase (Arczewska-Wlosek *et al*., 2019). Endo-xylanase aids in the degradation of AX, the main NSP making up at least 50% of the total carbohydrate fraction (Ward, 2021), through the hydrolysation of the xylan backbone (Saleh *et al*., 2019) resulting in the release of encapsulated starch and other nutrients while also reducing the digesta viscosity caused by sNSP (Amerah, 2015). The release of oligosaccharides caused by xylanase action modulates the hindgut microbial population that will improve intestinal health and benefit the digestion and absorption capabilities resulting in improved growth performance (Jlali *et al*., 2020). Supplementing diets with glucanase and xylanase separately, in combination or as part of an MEC reduce the intestinal viscosity and improve the nutritional value of cereal-based diets (Yacoubi *et al*., 2016). Various enzymes, including β-glucanase, have been reported to enhance the nutritional value of cereal by-products in monogastric animals (Cherian, 2020). Birds lack the enzyme needed to depolymerise (1,3-1,4)-β-glucan which is a component of the endosperm cell wall (Von Wettstein *et al*., 2000). The first (1,3-1,4)-β-glucanase enzyme was purified from a strain of *Bacillus subtilis*, now named *Bacillis amyloliquefaciens*, and indicated a positive response in broilers when it was added to a barley-based diet (Von Wettstein *et al*., 2000). Supplementation of β-glucanase in broiler diets has shown to reduce intestinal viscosity, vent pasting (Esteve-Garcia *et al*., 1997), the incidence of sticky droppings, as well as an increase in weight gain (Von Wettstein *et al*., 2000). Enzyme mixtures of endo-β-1,4-xylanase and arabinofuranosidase have long been used to improve the DM digestibility of maize (Saleh *et al*., 2019).

Research suggest that using debranching enzymes such as arabinofuranosidase enhances the efficiency of endo-β-1,4-xylanase in poultry diets (Poernama *et al*., 2021). Arabinofuranosidase hydrolyses the release of a-Larabinofuranosyl residues that are attached to backbone of xylan or arabinan in pectin and lignocellulosic constituents of cell walls in a plant while the glucuronidase liberates methylglucuronic acid and glucuronic residues (Ward, 2021). This action of arabinofuranosidase of splitting the xylose backbone in arabinose gives access to endo-β-1,4-xylanase activity (Saleh *et al*., 2019). Supplementation of arabinofuranosidase in sequence with endo-β-1,4-xylanase can increase the solubilisation of maize arabinoxylans more than two-fold (Ward, 2021).

Cellulose along with arabinoxylans pass through the avian digestive system virtually undigested (Ward, 2021) as no animal enzyme can digest it (Cherian, 2020). The presence of cellulose contributes to the undigested components in the terminal ileum (Khalil *et al*., 2022) and thus microbial cellulase should be supplemented to degrade the cellulose (Cherian, 2020).

Pectinase is a cell wall hydrolysing enzyme (Zyla *et al*., 2012) that hydrolyses the pectin which alters lipid metabolism, the functioning of the caeca, and causes an inflammatory response of the GIT (Silva *et al*., 2012).

Soybean meal not only has a high protein content, but is also highly digestible, however the protein is not always available to the bird due to the ANF such as NSP, lectin, and trypsin inhibitors (Doskovic *et al*., 2013). The supplementation of protease has been primarily together with other enzymes to improve the broiler's performance parameters (Barekatain *et al*., 2013). Supplementing protease in the diets of broilers causes the hydrolysation of the protein matrix in the endosperm thereby improving the accessibility of starch granules for the amylolytic enzymes (Amerah, 2015). Supplementing protease is more beneficial in young birds to overcome the deficiency in enzymes as they do not yet have developed digestive systems that can secrete sufficient amount of digestive secretions (Doskovic *et al*., 2013). Through the supplementation of exogenous protease together with the endogenous peptidase it reduces the necessity to include extra amino acids and energy in the diet (Doskovic *et al*., 2013; De Keyser *et al*., 2016).

Numerous pathogens have mannans on the surface and cause an innate immune response thus diverting energy towards an immune response instead of growth (Klein *et al*., 2015). By including β-mannanase these negative effects are alleviated and there is an increase in nitrogen corrected AME (AMEn), BWG, and FCR (Klein *et al*., 2015).

2.5.3 Phytase

The most abundant mineral in animal tissue is Ca and is followed by P (Jlali *et al*., 2020) and both are essential in the survival of an animal (Guo *et al*., 2009). The largest P reserve in plant feedstuffs is phytic acid (IP6) (Poernama *et al*., 2021), which bounds up to two thirds of the total P (Guo *et al*., 2009). The location of phytate caries between different raw materials, for example, it is located in the aleurone layers of wheat and sorghum but in the germ of maize; while also exhibiting different concentrations within raw materials (Lui *et al*., 2014). The scientific nomenclature for the phytic acid molecule is myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (Feil, 2008) with recognised anti-nutritional properties in monogastric nutrition (Lui *et al*., 2014). The phytic acid molecule is highly charged with six groups of phosphate extending from the central inositol structure (Feil, 2008) as seen in figure 2.6. The molecule is an excellent chelator due to its charge and at a low pH, below the isoelectric point of proteins, the charge of proteins are positive and thus insoluble complexes are formed with the negatively charged phytic acid (Feil, 2008). The ability of phytic acid to form complexes with other nutrients such as manganese (Mn), zinc (Zn), iron (Fe), Ca, and starch causes it to directly influences the digestion of starch and inhibits amylase activity (Lui *et al*., 2014). Phytic acid also increases endogenous losses associated with gastric mucin and reduces the rate of conversion of pepsinogen to pepsin, thus effectively reducing the amount of pepsin in the stomach (Bedford & Rousseau, 2017). It has now been well established that phytic acid is an ANF that influences the efficiency of digestion and ultimately the growth performance of poultry (Bedford & Rousseau, 2017). Due to the P being unavailable to poultry, especially in maize-soybean diets (Feil, 2008), it is necessary to include costly inorganic sources of P in the diets (Poernama *et al*., 2021) which often result in the dietary P exceeding the minimum requirements that leads to environmental pollution and increased feed cost (Lawlor *et al*., 2019).

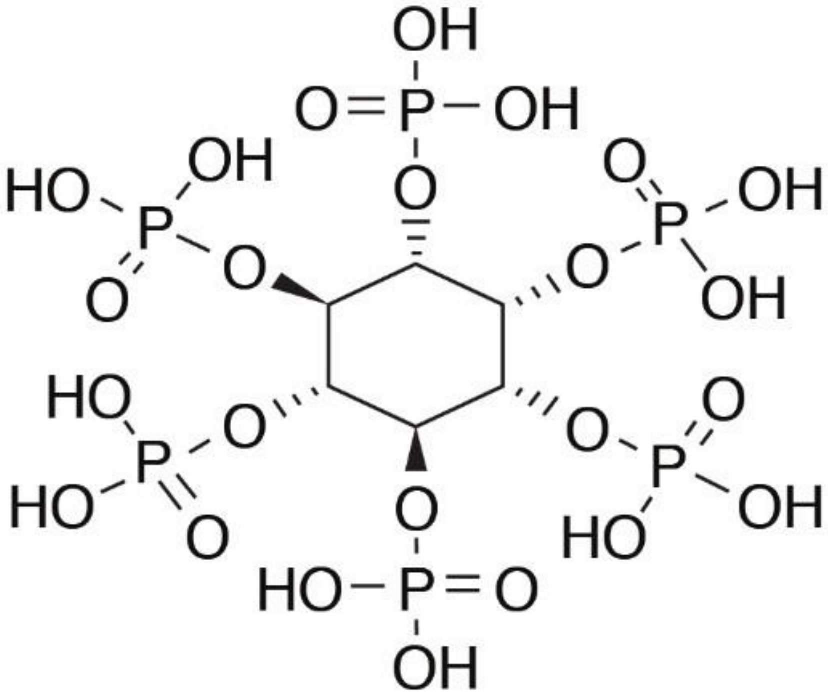

Figure 2.6 Phytic acid molecule (Cherian, 2020)

Due to the anti-nutritional nature of phytic acid it has become routine practice to include phytase in poultry diets to facilitate sustainable chicken meat production (Lui *et al*., 2014). Nelson *et al*. (1968) was the first to supplement phytase produced from *Aspergillus ficuum* to a liquid soybean diet and the result indicated a significant enhancement in bone ash percentage when it was compared to the control group that received no inorganic P. Since then, phytase has been a cost-effective replacement source for inorganic P. Phytase can be produced from fungi, bacteria, yeast, and higher plants with different origins having different pH and temperature optimal conditions (Feil, 2008). Phytase (myo-inositol hexakisphosphate phosphohydrolase) (Cherian, 2020) functions mainly in the upper part of the GIT (Rahimi *et al*., 2020) and breaks down phytic acid into lower phytate esters and inositol (Walk & Roa,

2020) by facilitating the stepwise hydrolysis via penta-to monophosphates (Feil, 2008; Amerah, 2015). The release of inositol has been shown to increase growth rate in broilers (Bedford & Rousseau, 2017). This process allows the P that was previously bound to become available for use to the animal while simultaneously enhancing the digestibility and utilisation of Ca, amino acids, and energy (Walk & Roa, 2020). This allows for a reduced inclusion of above-mentioned nutrients without negatively influencing the animal (Walk & Roa, 2020) thus lowering the cost of supplementing inorganic P and limits the excretion of P into the environment (Rahimi *et al*., 2020). Supplementing phytase has additionally been proven to enhance pre-caecal amino acid digestibility (Siegert *et al*., 2019). The efficacy of phytase varies among feedstuffs (Cherian, 2020) and can be explained by distinct features of the phytase itself such as optimal temperature or pH (Siegert *et al*., 2019) or by the feed source.

A more recent practice is superdosing phytase (Lee *et al*., 2018) where high phytase levels are added to diets to limit the anti-nutritional effects instigated by phytate, instead of just focussing on P release (Dos Santos *et al*., 2017). This requires supplying levels of phytase above the current industry recommendation of 500-1000 FTU/kg (Woyengo & Wilson, 2019). Higher levels of P and myo-inositol is available to the animal and reduces the negative impact on the performance, leg and breast weight, as well as skeletal development (Jlali *et* al., 2020). It is shown in some research that superdosing phytase improves animal performance through the enhanced digestibility of energy, amino acids, Ca, and trace minerals (Fernandes *et al*., 2019). There is however limited research available of superdosing phytase on nutrient digestibility of maize for poultry (Woyengo & Wilson, 2019). The optimal dosage of phytase is unknown because P equivalency of phytase is affected by numerous factors such as the dietary concentrations of phytate-P, non-phytate-P, Ca, and inclusion levels of phytase, the source of exogenous phytase and the level of endogenous phytase in the ingredients (Guo *et al*., 2009). Jlali et al. (2020) states the following benefits when supedosing phytase:

a. Decrease in undesirable microbiota activity that may be the result of changes in the pH of the digesta brought on by the higher levels of available Ca and P.
b. Improvements in BWG and feed efficiency above standard dose.
c. The chelation of phytate is reduced with other nutrients which allows the animal to preserve and maintain its performance, carcass composition and the mineral status of its skeletal system.
d. More rapid breakdown of phytate leading to improved bioavailability of, amino acids, energy, fatty acids, and numerous minerals.

2.6 Conclusion

Maize and soybean meal are often the primary components of broiler chicken diets around the world, including South Africa, with maize being the primary source of energy and soybean meal the most important source of dietary protein. However, these raw materials contain NSP and phytate, both considered as ANF. Non-starch polysaccharides are divided into soluble and insoluble NSP. The sNSP forms the bigger portion and exerts its anti-nutritional effect by increasing the digesta viscosity in the GIT by binding large quantities of water. The increase in digesta viscosity decreases the digestion and absorption of valuable nutrients leading to a decrease in the AME of the feedstuff. Nutrients are being wasted as it passes through the GIT without being digested, affecting the growth and health performance of the bird. The increased digesta viscosity causes slow movement of the digesta through the GIT thus creating hypoxic conditions that favour pathogenic bacterial growth. The iNSP portion exerts a ‘cage effect’ that inhibits the access of enzymes to the substrates affecting nutrient and energy digestibility. Through the inclusion of NSP degrading enzymes these anti-nutritional effects can be alleviated. It also promotes fermentation that result in the release of SCFA that may contribute towards gut health. The digesta viscosity is reduced allowing enzymes access to substrates that will be digested and utilised. Rovabio Advance T-Flex® is an MEC that contain 19 enzymatic activities to aid in the alleviation of the anti-nutritional effects exerted by NSP. Along with NSP, feedstuffs also contain the ANF phytate that not only binds two thirds of P, but also numerous other nutrients. These bound nutrients are unavailable for the animal to utilise. The enzyme phytase catalyses the stepwise removal of P from phytate and releases other bound nutrients. There is a debate regarding the most optimal inclusion level of phytase. Currently, the standard recommended level is 500-1000 FTUs, but superdosing levels as high as 2500 FTUs are becoming more popular. Benefits of higher inclusion levels have been investigated, but variation exists due to numerous factors such as feed source, phytase source, and the bird itself. Therefore, the purpose of this trial was to investigate whether the multi-enzyme product, Rovabio Advance T-Flex®, could increase the release of energy and amino acids from a maize-soybean diet. In addition, superdosing of phytase was evaluated against current standard inclusion levels, and possible interaction effects with the MEC were investigated.

Chapter 3

Materials and Methods

The trial was conducted on the Innovation Africa @UP Research Farm (University of Pretoria, Hillcrest, Pretoria). A large broiler house containing 96 pens was used, equipped with environmental control units (Skov system). All animal procedures were reviewed and approved by the Animal Ethics of the University of Pretoria (approval number NAS256/2021).

3.1 Birds and Housing

Prior to the arrival of the chicks, the broiler facility was cleaned and disinfected, and all water lines were flushed. On the day of hatching, two thousand five hundred (2500) chicks were selected at Eagle's Pride Hatchery, choosing males by the feather sexing technique. Chicks with visible signs of weakness or deformity were discarded. One hundred (100) extra chicks were ordered to replace mortalities, weak chicks or sexing errors (females) at placement. The following morning the selected chicks were delivered to the experimental farm where they were sexed again to ensure only males were placed. After sexing, 25 birds were randomly selected, weighed, and allocated to a pen with the dimensions of 1.5 m x 1.5 m (2.25 m^2). Each pen was labelled with the pen number and treatment.

The birds were exposed to the standard environmental conditions recommended by Aviagen Broiler Management Handbook Aviagen (2018). Prior to the arrival of the chicks the facility was pre-heated to 35ºC (32ºC floor temperature). Electrical heaters were used to increase house temperature as required. The environment was controlled by a combination of electrical heaters, automated electric exhaust and stirring fans and mist sprayers. Minimum ventilation was always maintained to ensure clean air inside the house and to also prevent accumulation of toxic gases such as ammonia. The conditions were monitored twice daily by checking bird behaviour and sensor readings, and adjustments were made if needed.

The following heating programme was implemented:

During Days 0 and 1, an air temperature of 33ºC was maintained. The temperature was then decreased to 31ºC for Days 2 and 3. From Day 4 onwards the temperature was decreased with an additional 2ºC every day until 18ºC was reached. The temperature was then maintained until termination of the trial on Day 35.

The following lighting programme was implemented:

Day 0 to 6, the broilers were exposed to 1 hour dark and 23 hours light; Day 7 to 13, they were exposed to 3 hours dark and 21 hours light, whereafter they were exposed to 6 hours dark and 18 hours light until the termination of the trial on Day 35.

A standard vaccination programme was followed. The birds received the sprayed live vaccines for Newcastle Disease and Gumboro (Infectious Bronchitis) immediately post hatch at the hatchery. Booster vaccines were administered on Day 12 and 15 for Newcastle Disease and Gumboro, respectively. The water lines were lifted for approximately two hours to deprive the birds of water and induce thirst and ensure adequate water intake when the water fountains were provided. Bird behaviour was used as an indicator of discomfort due to thirst. The vaccines were mixed with water according to the guidelines from the manufacturer and then supplied by means of water fountains for approximately an hour to ensure all birds had adequate water intake and access to the vaccine. After vaccine administration the water lines were lowered to appropriate height to supply water *ad libitum.*

At placement on Day 0, each pen was equipped with a water fountain to supplement the nipple drinkers to ensure adequate water intake. The water fountains were replenished every morning to ensure clean cool water in the pens. After seven days the water fountains were removed as the chicks were accustomed to the nipple drinkers as their primary water source. There were five nipples per pen fixed on water lines that were connected to the municipal water source. The water lines were adjusted frequently to be at eye level with the birds to ensure easy access to the water. During the brooding phase, chick paper and a feeder tray were placed to supplement the tube feeder. On Day 4, the chick paper was removed and on Day 7 the feeder trays were removed. The height of the tube feeders was frequently adjusted to allow easy access to the feed for adequate feed intake, and to limit feed wastage. Feed was available *ad libitum* for the duration of the trial. One-day prior to arrival of chicks, the starter feed was weighed out into feeder bins to allow the feed to warm to room temperature before being consumed by the chicks.

3.2 Experimental design and treatments

A randomised block design with eight dietary treatments were followed. Each treatment was replicated 12 times where one pen, containing 25 birds, were considered an experimental unit (pen replicate).

Trial feed for Treatments 1 to 6 was formulated based on Ross 308 broiler nutrient specifications to meet or exceed daily nutrient requirements of the chicks. Trial feed for Treatments 7 and 8 was formulated to be 3% deficient in metabolisable energy and digestible amino acids compared to Treatments 1 to 6. All feed was mixed at the SimpleGrow Feedmill (South Africa). A three-phase feeding program was followed, whereby the starter feed was fed from Day 0 to day 14 in crumbled form; the grower feed was fed from Day 14 to Day 28 in pelleted form; and the finisher, also in pelleted form, was fed from Day 28 to Day 35.

Two basal diets were formulated for each of the three phases. Basal diet 1 was the standard commercial diet with and without Rovabio Advance T-Flex® supplementation and different inclusion levels of phytase (Treatments 1-6). Basal diet 2 had a 3% reduction in metabolisable energy and digestible amino acids with the standard level phytase (1000 FTU) with and without Rovabio Advance T-Flex® (Treatments 7 and 8). The reduced energy and digestible amino acids were achieved by decreasing the quantity of maize, and soybean meal and increasing the wheat bran and full fat soya quantities. A summary of the dietary treatments used is displayed in Table 3.1 while raw material composition of the treatment diets is shown in Tables 3.2, 3.3 and 3.4 for starter, grower and finisher, respectively.

Rovabio Advance T-Flex® (Adisseo, France) is a commercially available enzyme that degrades NSP. The main ingredients are endo-1,4-beta-xylanase and endo-1,3(4)-beta-glucanase that degrades the xylanase and glucanase components of NSP, respectively. Rovabio Advance T-Flex® was added to Treatments 2, 4, 6 and 8 at the inclusion level of 50 g/t, according to the manufacturer's recommendations.

Phytase is included in all commercial broiler diets as a standard ingredient. Different levels of phytase were included in the diets, i.e. standard inclusion levels and increased levels which is referred to as superdosing. Dietary Treatments 1, 2, 6 and 8 had the standard level of 1000 FTU, Treatments 2 and 3 contained 1500 FTU, and Treatments 4 and 5 contained 2000 FTU.

The additives were supplied and added to the treatments in powder form. During manufacturing of the diets, samples of approximately 500 g were collected from all the dietary treatments. Only the basal diets (Treatment 1 and 7) for the three feeding phases were chemically analysed for crude protein, crude fibre, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), starch, sugar and the minerals calcium, phosphorous, sodium, magnesium and potassium. The calculated nutrient composition and analysed nutrient composition of the dietary treatments are listed in Tables 3.5 to 3.10. Procedures used for the chemical analyses of the feed are described in Section 3.3.

Table 3.1 Description of the treatment groups and experimental diets

Treatment	**Rovabio AdvanceT-Flex®**	**Phytase**
1. Standard Diet + 1000 FTU	-	1000 FTU
2. Standard Diet + 1000 FTU + NSPase[1]	50 g/t	1000 FTU
3. Standard Diet + 1500 FTU	-	1500 FTU
4. Standard Diet + 1500 FTU + NSPase	50 g/t	1500 FTU
5. Standard Diet + 2000 FTU	-	2000 FTU
6. Standard Diet + 2000 FTU + NSPase	50 g/t	2000 FTU
7. Low Concentration Diet[2] + 1000 FTU	-	1000 FTU
8. Low Concentration Diet + 1000 FTU + NSPase	50 g/t	1000 FTU

[1] NSPase: non-starch polysaccharide degrading enzymes

[2] Low concentration diet contained 3% less metabolisable energy and digestible amino acids than the standard diet

Table 3.2 Raw material composition (%) of the starter diets (on an as ‘fed basis’)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Yellow maize	47.6	47.6	47.6	47.6	47.6	47.6	41.7	41.7
Soya oilcake	22.9	22.9	22.9	22.9	22.9	22.9	19.6	19.6
Wheat bran	18.1	18.1	18.1	18.1	18.1	18.1	24.8	24.8
Full fat soya	5.56	5.56	5.56	5.56	5.56	5.56	8.00	8.00
Sunflower oilcake	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Soya oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium phosphate	1.22	1.22	1.22	1.22	1.22	1.22	1.13	1.13
Limestone	0.84	0.84	0.84	0.84	0.84	0.84	0.90	0.90
Salt	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium bicarbonate	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.10
DL-Methionine	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
L-Lysine	0.14	0.14	0.14	0.14	0.14	0.14	0.12	0.12
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05
Broiler premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Unike plus	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc bacitracin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Axtra PHY	0.01	0.01	0.015	0.015	0.02	0.02	0.01	0.01
Rovabio Advance T-Flex®	-	0.005	-	0.005	-	0.005	-	0.005

CP: crude protein
Unike plus: mycotoxin binder (Adisseo, France)

Table 3.3 Raw material composition (%) of the grower diets (on an as 'fed basis')

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Yellow maize	50.03	50.03	50.03	50.03	50.03	50.03	49.9	49.9
Soya oilcake	13.5	13.5	13.5	13.5	13.5	13.5	18.6	18.6
Wheat bran	17.6	17.6	17.6	17.6	17.6	17.6	20.04	20.04
Full fat soya	12.0	12.0	12.0	12.0	12.0	12.0	4.70	4.70
Sunflower oilcake	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soya oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium phosphate	0.86	0.86	0.86	0.86	0.86	0.86	0.84	0.84
Limestone	0.57	0.57	0.57	0.57	0.57	0.57	0.59	0.59
Salt	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium bicarbonate	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
DL-Methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.06	0.06
L-Lysine	0.14	0.14	0.14	0.14	0.14	0.14	0.07	0.07
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Pellibond	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Unike plus	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc bacitracin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Axtra PHY	0.01	0.01	0.015	0.015	0.02	0.02	0.01	0.01
Rovabio Advance T-Flex®	-	0.005	-	0.005	-	0.005	-	0.005

CP: crude protein
Unike plus: mycotoxin binder (Adisseo, France)
Pellibond: pellet binder (Simple Grow, South Africa)

Table 3.4 Raw material composition (%) of the finisher diets (on an as 'fed basis')

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Yellow maize	52.7	52.7	52.7	52.7	52.7	52.7	47.8	47.8
Soya oilcake	4.91	4.91	4.91	4.91	4.91	4.91	3.50	3.50
Wheat bran	20.5	20.5	20.5	20.5	20.5	20.5	26.2	26.2
Full fat soya	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sunflower oilcake	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soya oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium phosphate	0.62	0.62	0.62	0.62	0.62	0.62	0.54	0.54
Limestone	0.63	0.63	0.63	0.63	0.63	0.63	0.67	0.67
Salt	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Sodium bicarbonate	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03
DL-Methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07
L-Lysine	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18
L-Threonine	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Pellibond	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Unike plus	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Zinc bacitracin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Axtra PHY	0.01	0.01	0.015	0.015	0.02	0.02	0.01	0.01
Rovabio Advance T-Flex®	-	0.005	-	0.005	-	0.005	-	0.005

CP: crude protein
Unike plus: mycotoxin binder (Adisseo, France)
Pellibond: pellet binder (Simple Grow, South Africa)

Table 3.5 Calculated nutrient concentrations (%) in the starter diets (on an ‘as is’ basis)

Calculated	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry matter	88.9	88.9	88.9	88.9	88.9	88.9	89.2	89.2
AMEn Poultry (MJ/kg)	11.5	11.5	11.5	11.5	11.5	11.5	11.2	11.2
Crude protein	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
Crude fibre	4.50	4.50	4.50	4.50	4.50	4.50	5.02	5.02
Crude fat	4.35	4.35	4.35	4.35	4.35	4.35	4.86	4.86
Ash	5.78	5.78	5.78	5.78	5.78	5.78	5.92	5.92
Linoleic acid	2.04	2.04	2.04	2.04	2.04	2.04	2.26	2.26
Starch	36.3	36.3	36.3	36.3	36.3	36.3	33.8	33.8
Total Ca	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total P	0.69	0.69	0.69	0.69	0.69	0.69	0.72	0.72
Phytic P	0.38	0.38	0.38	0.38	0.38	0.38	0.41	0.41
Available P Poultry	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Na	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Cl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
K	0.96	0.96	0.96	0.96	0.96	0.96	1.00	1.00
Ca/P	1.45	1.45	1.45	1.45	1.45	1.45	1.39	1.39
Total Lysine	1.11	1.11	1.11	1.11	1.11	1.11	1.09	1.09
Total Methionine	0.41	0.41	0.41	0.41	0.41	0.41	0.42	0.42
Dig. Lysine	1.05	1.05	1.05	1.05	1.05	1.05	1.02	1.02
Dig. Methionine	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Dig. Cysteine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Dig. Methionine+Cysteine	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Dig. Threonine	0.71	0.71	0.71	0.71	0.71	0.71	0.69	0.69
Dig. Tryptophan	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Dig. Arginine	1.25	1.25	1.25	1.25	1.25	1.25	1.24	1.24
Dig. Isoleucine	0.75	0.75	0.75	0.75	0.75	0.75	0.73	0.73
Dig. Leucine	1.54	1.54	1.54	1.54	1.54	1.54	1.50	1.50
Dig. Valine	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Dig. Histidine	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Dig. Phenylalanine	0.88	0.88	0.88	0.88	0.88	0.88	0.85	0.85
Dig. Glycine	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Dig. Serine	0.82	0.82	0.82	0.82	0.82	0.82	0.81	0.81

AMEn: Apparent metabolisable energy (nitrogen corrected)
Dig.: Digestible (poultry)

Table 3.6 Calculated nutrient concentrations (%) in the grower diets (on an 'as is' basis)

Calculated	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry matter	88.9	88.9	88.9	88.9	88.9	88.9	88.8	88.8
AMEn Poultry (MJ/kg)	12.0	12.0	12.0	12.0	12.0	12.0	11.6	11.6
Crude protein	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Crude fibre	4.74	4.74	4.74	4.74	4.74	4.74	4.72	4.72
Crude fat	5.51	5.51	5.51	5.51	5.51	5.51	4.33	4.33
Ash	4.90	4.90	4.90	4.90	4.90	4.90	5.00	5.00
Linoleic acid	2.66	2.66	2.66	2.66	2.66	2.66	2.02	2.02
Starch	38.2	38.2	38.2	38.2	38.2	38.2	38.5	38.5
Total Ca	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Total P	0.62	0.62	0.62	0.62	0.62	0.62	0.63	0.63
Phytic P	0.36	0.36	0.36	0.36	0.36	0.36	0.38	0.38
Available P Poultry	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Na	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Cl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
K	0.89	0.89	0.89	0.89	0.89	0.89	0.90	0.90
Ca/P	1.30	1.30	1.30	1.30	1.30	1.30	1.27	1.27
Total Lysine	1.02	1.02	1.02	1.02	1.02	1.02	0.95	0.95
Total Methionine	0.37	0.37	0.37	0.37	0.37	0.37	0.35	0.35
Dig. Lysine	0.95	0.95	0.95	0.95	0.95	0.95	0.89	0.89
Dig. Methionine	0.36	0.36	0.36	0.36	0.36	0.36	0.34	0.34
Dig. Cysteine	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Dig. Methionine+Cysteine	0.65	0.65	0.65	0.65	0.65	0.65	0.63	0.63
Dig. Threonine	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Dig. Tryptophan	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Dig. Arginine	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
Dig. Isoleucine	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Dig. Leucine	1.46	1.46	1.46	1.46	1.46	1.46	1.45	1.45
Dig. Valine	0.78	0.78	0.78	0.78	0.78	0.78	0.77	0.77
Dig. Histidine	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Dig. Phenylalanine	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Dig. Glycine	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Dig. Serine	0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76

AMEn: Apparent metabolisable energy (nitrogen corrected)
Dig.: Digestible (poultry)

Table 3.7 Calculated nutrient concentrations (%) in the finisher diets (on an ‘as is’ basis)

Calculated	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry matter	89.1	89.1	89.1	89.1	89.1	89.1	89.3	89.3
AMEn Poultry	12.2	12.2	12.2	12.2	12.2	12.2	11.8	11.8
Crude protein	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0
Crude fibre	4.90	4.90	4.90	4.90	4.90	4.90	5.33	5.33
Crude fat	6.14	6.14	6.14	6.14	6.14	6.14	6.24	6.24
Ash	4.52	4.52	4.52	4.52	4.52	4.52	4.67	4.67
Linoleic acid	2.98	2.98	2.98	2.98	2.98	2.98	2.98	2.98
Starch	40.2	40.2	40.2	40.2	40.2	40.2	38.3	38.3
Total Ca	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Total P	0.58	0.58	0.58	0.58	0.58	0.58	0.61	0.61
Phytic P	0.36	0.36	0.36	0.36	0.36	0.36	0.39	0.39
Available P Poultry	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Na	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Cl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
K	0.80	0.80	0.80	0.80	0.80	0.80	0.83	0.83
Ca/P	1.30	1.30	1.30	1.30	1.30	1.30	1.24	1.24
Total Lysine	0.92	0.92	0.92	0.92	0.92	0.92	0.89	0.89
Total Methionine	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.33
Dig. Lysine	0.85	0.85	0.85	0.85	0.85	0.85	0.82	0.82
Dig. Methionine	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.32
Dig. Cysteine	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Dig. Methionine+Cysteine	0.59	0.59	0.59	0.59	0.59	0.59	0.58	0.58
Dig. Threonine	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Dig. Tryptophan	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Dig. Arginine	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.97
Dig. Isoleucine	0.57	0.57	0.57	0.57	0.57	0.57	0.55	0.55
Dig. Leucine	1.31	1.31	1.31	1.31	1.31	1.31	1.27	1.27
Dig. Valine	0.70	0.70	0.70	0.70	0.70	0.70	0.69	0.69
Dig. Histidine	0.37	0.37	0.37	0.37	0.37	0.37	0.36	0.36
Dig. Phenylalanine	0.69	0.69	0.69	0.69	0.69	0.69	0.67	0.67
Dig. Glycine	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Dig. Serine	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67

AMEn: Apparent metabolisable energy (nitrogen corrected)
Dig.: Digestible (poultry)

Table 3.8 Analysed nutrient concentrations (%) in the starter diets (on an ‘as is’ basis)

Analysed	**Basal A** **Treatment 1-6**	**Basal B** **Treatment 7-8**
Dry matter	89.6	89.4
Crude protein	20.0	19.4
Crude fibre	3.41	4.36
Crude fat (EE)	4.25	4.52
Ash	5.22	4.99
Neutral detergent fibre	12.5	13.8
Acid detergent fibre	6.57	7.13
Starch	40.3	36.3
Sugar (WSE)	5.62	6.53
Ca	1.55	1.26
P	0.68	0.73
Na	0.03	0.05
K	0.91	1.04
Ca/P	2.28	1.73

EE: ether extract

WSE: water soluble extract

Table 3.9 Analysed nutrient concentrations (%) in the grower diets (on an ‘as is’ basis)

Analysed	**Basal A** **Treatment 1-6**	**Basal B** **Treatment 7-8**
Dry matter	89.3	89.4
Crude protein	18.4	18.7
Crude fibre	5.29	5.04
Crude fat (EE)	5.22	4.07
Ash	4.28	4.69
Neutral detergent fibre	13.8	15.1
Acid detergent fibre	6.40	6.99
Starch	40.5	42.2
Sugar (WSE)	6.21	5.69
Ca	0.74	0.67
P	0.70	0.77
Na	0.05	0.05
K	0.98	1.01
Ca/P	1.06	0.87

EE: ether extract

WSE: water soluble extract

Table 3.10 Analysed nutrient concentrations (%) in the finisher diets (on an 'as is' basis)

Analysed	**Basal A** **Treatment 1-6**	**Basal B** **Treatment 7-8**
Dry matter	89.5	89.7
Crude protein	16.4	16.7
Crude fibre	5.71	6.38
Crude fat (EE)	6.09	6.37
Ash	4.15	3.95
Neutral detergent fibre	15.1	17.1
Acid detergent fibre	7.66	7.77
Starch	42.7	40.2
Sugar (WSE)	5.73	6.21
Ca	1.16	0.75
P	0.63	0.73
Na	0.03	0.05
K	0.80	0.91
Ca/P	1.84	1.03

EE: ether extract
WSE: water soluble extract

3.3 Analysis of experimental diets

Representative samples of the basal diets of from each phase were collected after the feed was produced as well as during the trial. Each sample was analysed to determine the nutritional content as well as accuracy of formulation and feed production. The analysis of DM, ash, crude protein, crude fat and crude fibre was done at NutriLab at the University of Pretoria (Pretoria, South Africa). Whereas the analysis of NDF, ADF, starch and sugar were done through Adisseo (France) at GMP Laboratories (South Africa) using a near infrared (NIR) spectrometer. GMP Laboratories (South Africa) also analysed the minerals calcium, phosphorous, sodium and potassium.

Dry matter, ash, crude protein, crude fat, crude fibre and the minerals were analysed following the Official Method of Analysis from the AOAC (AOAC, 2000). For DM, Method 942.05 was used while Method 988.05 was used for crude protein. Crude fibre was analysed using Method 962.09, while crude fat was determined using Method 920.09. The minerals were analysed using Method 935.13 for Ca, Na and K while Method 965.17 was used to analyse for P.

3.4 Measurement of performance parameters

3.4.1 Body weight and body weight gain

Body weight was measured by weighing birds from the same pen together. On the day of placement, the initial body weight of each pen was measured. Thereafter, the birds were weighed on Day 7, 14, 21, 28 and 35. The average body weight (g / bird), weekly body weight gain (g / bird / day) and body weight gain (g / bird) for the entire period was calculated per pen.

3.4.2 Feed intake

Feed was weighed into a bin allocated to each pen, prior to placement and at the start of each 7-day period thereafter. The amount of feed consumed per pen was measured on Days 7, 14, 21, 28 and 35 by subtracting feed left in the bin as well as feed orts in the feeders, from the total feed weighed into the bin at the start of the period. The average feed intake was calculated for each pen on a weekly basis. Cumulative feed intake over the entire period was also calculated.

3.4.3 Feed conversion ratio, corrected for mortalities

Mortalities and culls were recorded twice daily. The dead birds were weighed, and autopsies were done to determine the probable cause of death. The body weight gained, and feed consumed were used to calculate the feed conversion ratio (unit of feed consumed per unit of live mass gained) per pen replicate. The mortalities were used to correct the feed conversion ratio by adding the total weight of the mortalities in a pen during a period to the live weights of that pen at the end of the corresponding period.

3.5 Measurement of tibia ash

On Day 21 and 35, all the birds in each pen were weighed. One bird per pen, close to the average weight of the pen, was selected for slaughter and tagged using a cable tie on the left leg. On Day 22 and 36, the selected birds were euthanised at the abattoir on the Innovation Africa @UP Research Farm (University of Pretoria, Hillcrest, Pretoria). Both legs were dislocated to separate the femur from the hip and the legs were removed with the aid of a scalpel. The removed legs were placed into pre-labelled zip lock bags. The legs were left to rot for three days in a closed-off room so that the meat could be easily removed from the bone to expose the tibia. The bones were separated at the tibio-tarsal junction and the tibio-femoral junction, and the cartilaginous caps were removed. The bones were patted dry using paper towels and the weight recorded as initial weight. The bones were placed in an oven for approximately 12 hours at 70ºC after which they were weighed again as dry final weight to determine moisture loss (Equation 1).

Equation 1: Moisture loss = Initial Weight - Dry Final Weight

The bones were subjected to defatting using analytical grade petroleum for 48 hours, followed by drying in an oven at 70ºC for 8-12 hours. Crucibles were weighed to determine dry crucible weight and the crucible was weighed with the bone (Dry Crucible + Dry Bone Weight). The weight of the dry defatted bones was calculated using Equation 2. The samples were placed in a muffle furnace for ashing at 600ºC for at least 12 hours. After ashing the weight of the crucible with the bone ash was weighed as Dry Crucible + Dry Bone Ash Weight. Tibia ash was then determined using Equation 3 and 4.

Equation 2: Weight of Dry Defatted Bone = (Dry Crucible + Dry Bone Weight) – Dry Crucible Weight

Equation 3: Weight of Dry Tibia Ash = (Dry Crucible + Dry Bone Ash Weight) – Dry Crucible Weight

Equation 4: % Defatted Bone Ash = $\frac{Dry\ Tibia\ Ash\ Weight}{Dry\ Defatted\ Bone\ Weight} x\ 100$

3.6 Statistical analysis

A two-way ANOVA was used to analyse all the data by using the General Linear Model procedure of SAS (SAS Institute, 2004) over repeated measures of variance analysis. Pen means were used to analyse the data with the procedures being appropriate for the complete randomized block design. A significant level of α = 0.05 was set as the data was presented as mean values with pooled standard error of mean (SEM) estimates. The least significant procedure was used to evaluate the difference in means between the treatments (Carmer and Walker, 1985), and overall, treatment effects with a probability of $P < 0.05$ were assumed to be statistically significant. Dietary supplemental phytase and Rovabio Advance T-Flex® concentrations were independent variables in this model, whereas FCR, FI and BWG, over a period body weight and bone ash were regarded as dependent variables.

Chapter 4

Results

Following are the production parameters and tibia ash results recorded from this trial.

4.1 Performance parameters

4.1.1 Body weight

The weekly body weight of the broilers is shown in Tables 4.1 to 4.12.

Phytase inclusion levels

No significant difference ($P > 0.05$) was observed between the body weights on the day of arrival until Day 7 as well as from Day 21 until the conclusion of the trial. At Day 14, the body weight of the birds receiving the treatment with 1000 FTU with no added Rovabio Advance T-Flex®, was significantly higher ($P < 0.05$) than the body weights of the birds in the other treatments with no Rovabio Advance T-Flex® inclusion. However, there was no significant difference ($P > 0.05$) at Day 21 between the body weights of the treatments with no Rovabio Advance T-Flex® and the treatments with Rovabio Advance T-Flex®.

Table 4.1 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers on placement day (0-days-of age) (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	41.93 (± 0.31)	41.93 (± 0.31)	41.93 (± 0.22)
1500	42.60 (± 0.31)	42.13 (± 0.31)	42.37 (± 0.22)
2000	42.27 (± 0.31)	42.20 (± 0.31)	42.37 (± 0.22)
Mean	42.27 (± 0.18)	42.09 (± 0.18)	

Table 4.2 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 7-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**	**Mean**

	None	**Included**	
1000	158.04 (± 2.20)	154.28 (± 2.20)	156.16 (± 1.56)
1500	156.34 (± 2.20)	152.93 (± 2.20)	154.64 (± 1.56)
2000	157.52 (± 2.20)	154.05 (± 2.20)	155.78 (± 1.56)
Mean	157.30 (± 1.27)	153.75 (± 1.27)	

Table 4.3 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 14-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	384.33^{a} (± 7.99)	365.88 (± 7.99)	375.10^{a} (± 5.65)
1500	356.04^{b} (± 7.99)	359.93 (± 7.99)	357.99^{b} (± 5.65)
2000	365.77^{b} (± 7.99)	356.93 (± 7.99)	361.35^{ab} (± 5.65)
Mean	368.71 (± 4.61)	360.91 (± 4.61)	

$^{a,\ b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.4 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	694.53 (± 12.60)	684.82 (± 12.60)	689.67 (± 8.91)
1500	667.68 (± 12.60)	674.39 (± 12.60)	671.03 (± 8.91)
2000	692.06 (± 12.60)	679.71 (± 12.60)	685.88 (± 8.91)
Mean	684.75 (± 7.27)	679.64 (± 7.27)	

Table 4.5 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 28-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1145.17 (± 20.38)	1120.48 (± 20.38)	1132.82 (± 14.41)
1500	1096.58 (± 20.38)	1116.37 (± 20.38)	1106.47 (± 14.41)
2000	1117.51 (± 20.38)	1125.62 (± 20.38)	1121.56 (± 14.41)
Mean	1119.75 (± 11.77)	1120.82 (± 11.77)	

Table 4.6 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance Inclusion**		**Mean**
	None	**Included**	
1000	1740.05 (± 27.27)	1704.64 (± 27.27)	1722.34 (± 19.28)
1500	1683.57 (± 27.27)	1701.32 (± 27.27)	1692.45 (± 19.28)
2000	1701.75 (± 27.27)	1734.80 (± 27.27)	1718.27 (± 19.28)
Mean	1708.46 (± 15.74)	1713.59 (± 15.74)	

Nutrient concentration

There was no significant difference ($P > 0.05$) observed between the body weights of within the standard diet treatments. From Day 14 until the end of the trial the birds on the low concentration diet with added Rovabio Advance T-Flex® were significantly heavier ($P < 0.05$) than the low concentration diets without Rovabio Advance T-Flex®. At Day 7, 21, 28 and 35 the birds receiving the standard diet with no added Rovabio Advance T-Flex® had significantly higher ($P < 0.05$) body weights than the birds receiving the low concentration diets with no added Rovabio Advance T-Flex®. At Day 14 and 35 the birds on the standard diet treatments receiving Rovabio Advance T-Flex® had significantly higher ($P < 0.05$) body weights than the birds on the low concentration diets that received Rovabio Advance T-Flex®.

Table 4.7 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers on placement day (0-days-of age) (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	41.93 (± 0.29)	41.93 (± 0.29)	41.93 (± 0.21)
Low concentration diet#	41.93 (± 0.29)	41.87 (± 0.29)	41.90 (± 0.21)
Mean	41.93 (± 0.21)	41.90 (± 0.21)	

Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.8 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 7-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	158.04^{a} (± 2.14)	154.28 (± 2.14)	156.16 (± 1.51)
Low concentration diet$^{\#}$	151.14^{b} (± 2.14)	155.65 (± 2.14)	153.40 (± 1.51)
Mean	154.59 (± 1.51)	154.96 (± 1.51)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.9 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 14-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	384.33 (± 9.20)	365.88^{b} (± 9.20)	375.10 (± 6.51)
Low concentration diet$^{\#}$	361.40^{B} (± 9.20)	394.18^{Aa} (± 9.20)	377.79 (± 6.51)
Mean	372.86 (± 6.51)	380.03 (± 6.51)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.10 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	694.53^{a} (± 15.71)	684.82 (± 15.71)	689.67 (± 11.11)
Low concentration diet$^{\#}$	644.78^{bB} (± 15.71)	712.45^{A} (± 15.71)	678.61 (± 11.11)
Mean	669.66 (± 11.11)	698.63 (± 11.11)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.11 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 28-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1145.17^{a} (± 25.88)	1120.48 (± 25.88)	1132.82^{a} (± 18.30)
Low concentration diet$^{\#}$	972.25^{Bb} (± 25.88)	1082.63^{A} (± 25.88)	1027.44^{b} (± 18.30)
Mean	1058.71 (± 18.30)	1101.55 (± 18.30)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.12 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average body weight (g) of broilers at 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1740.05^{a} (± 38.86)	1704.64^{a} (± 38.86)	1722.34^{a} (± 27.48)
Low concentration diet$^{\#}$	1390.05^{Bb} (± 38.86)	1576.68^{Ab} (± 38.86)	1483.36^{b} (± 27.48)
Mean	1565.05 (± 27.48)	1640.66 (± 27.48)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

4.1.2 Feed intake

Feed intake, measured on Day 7, 14, 21, 28 and 35, is shown in Tables 4.13 to 4.30. Cumulative FI for the periods 0 to 7, 14, 28 and 35 days, respectively, is shown in Tables 4.31 to 4.38.

Phytase inclusion levels: Weekly feed intake

At Day 14 and 35 there was no significant difference ($P > 0.05$) in weekly FI between any of the treatments. There was no significant difference ($P > 0.05$) in weekly FI between the birds within the treatments receiving Rovabio Advance T-Flex® throughout the whole trial. On Day 21 and 28 the weekly FI of the birds in the 1000 FTU groups with no Rovabio Advance T-Flex® was significant higher ($P < 0.05$) than the group with Rovabio Advance T-Flex®.

Table 4.13 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 7-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	149.24^{a} (± 3.83)	144.66 (± 3.83)	146.95^{a} (± 2.71)
1500	133.92 (± 3.83)	142.11 (± 3.83)	138.01^{b} (± 2.71)
2000	142.46^{a} (± 3.83)	147.10 (± 3.83)	144.78^{ab} (± 2.71)
Mean	141.87 (± 2.21)	144.62 (± 2.21)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)

Table 4.14 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 14-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	321.21 (± 6.82)	302.04 (± 6.82)	156.16 (± 4.82)
1500	302.88 (± 6.82)	303.69 (± 6.82)	154.64 (± 4.82)
2000	313.82 (± 6.82)	310.80 (± 6.82)	155.78 (± 4.82)
Mean	312.65 (± 3.94)	305.51 (± 3.94)	

Table 4.15 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	587.78^{Aa} (± 22.99)	497.67^{B} (± 22.99)	542.73 (± 16.26)
1500	500.08^{b} (± 22.99)	516.73 (± 22.99)	508.41 (± 16.62)
2000	529.52^{ab} (± 22.99)	479.96 (± 22.99)	504.74 (± 16.62)
Mean	539.13^{A} (± 13.37)	498.12^{B} (± 13.37)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)
$^{A, B}$ Means within a row without common superscripts differ significantly (P < 0.05)

Table 4.16 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 28-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	800.28^{Aa} (± 24.07)	719.96^{B} (± 24.07)	760.12 (± 17.02)
1500	699.37^{b} (± 24.07)	752.35 (± 24.07)	725.86 (± 17.02)
2000	758.92^{a} (± 24.07)	728.59 (± 24.07)	743.75 (± 17.02)
Mean	752.86 (± 13.90)	733.63 (± 13.90)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)
$^{A, B}$ Means within a row without common superscripts differ significantly (P < 0.05)

Table 4.17 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1047.56 (± 33.35)	1018.39 (± 33.35)	1032.98 (± 23.58)
1500	985.60 (± 33.35)	1041.86 (± 33.35)	1013.73 (± 23.58)
2000	1049.33 (± 33.35)	1056.91 (± 33.35)	1053.12 (± 23.58)
Mean	1027.50 (± 19.25)	1039.05 (± 19.25)	

Phytase inclusion levels: Cumulative feed intake

There was no significant difference (P > 0.05) in the cumulative FI of the birds between all the treatments receiving Rovabio Advance T-Flex®. Throughout the whole trial the the cumulative FI of the birds receiving 1000 FTU within the groups receiving no Rovabio Advance T-Flex® was significantly higher (P < 0.05) than the birds receiving 1500 FTU. No significant difference (P > 0.05) was observed between the birds receiving 1000 FTU and 2000 FTU as well as 1500 FTU and 2000 FTU within the group with no addition of Rovabio Advance T-Flex®. From Day 21 onwards the cumulative FI of the birds in the 1000 FTU group with no Rovabio Advance T-Flex® was significantly higher (P < 0.05) than that of the groups receiving Rovabio Advance T-Flex®.

Table 4.18 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 7-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	Rovabio Advance T-Flex® Inclusion		Mean
	None	Included	
1000	149.24^{a} (± 3.83)	144.66 (± 3.83)	146.95^{a} (± 2.71)
1500	133.92^{b} (± 3.83)	142.11 (± 3.83)	138.01^{b} (± 2.71)
2000	142.46^{ab} (± 3.83)	147.10 (± 3.83)	144.78^{ab} (± 2.71)
Mean	141.87 (± 2.21)	144.62 (± 2.21)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.19 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 14-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	Rovabio Advance T-Flex® Inclusion		Mean
	None	Included	
1000	470.45^{a} (± 9.17)	446.70 (± 9.17)	458.57 (± 6.49)
1500	436.79^{b} (± 9.17)	445.80 (± 9.17)	441.30 (± 6.49)
2000	456.31^{ab} (± 9.17)	457.89 (± 9.17)	457.10 (± 6.49)
Mean	454.52 (± 5.30)	450.13 (± 5.30)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.20 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	Rovabio Advance T-Flex® Inclusion		Mean
	None	Included	
1000	1058.24^{Aa} (± 28.96)	944.37^{B} (± 28.96)	1001.30 (± 20.48)
1500	936.87^{b} (± 28.96)	962.53 (± 28.96)	949.70 (± 20.48)
2000	985.83^{ab} (± 28.96)	937.85 (± 28.96)	961.84 (± 20.48)
Mean	993.64 (± 16.72)	948.25 (± 16.72)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.21 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 28-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1858.15^{Aa} (± 47.77)	1664.32^{B} (± 47.77)	1761.42 (± 33.78)
1500	1636.24^{b} (± 47.77)	1714.88 (± 47.77)	1675.56 (± 33.78)
2000	1744.75^{ab} (± 47.77)	1666.44 (± 47.77)	1705.59 (± 33.78)
Mean	1746.50 (± 27.58)	1681.88 (± 27.58)	

$^{a,\,b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A,\,B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.22 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	2906.08^{Aa} (± 71.80)	2682.72^{B} (± 71.80)	2794.40 (± 50.77)
1500	2621.84^{b} (± 71.80)	2756.74 (± 71.80)	2689.29 (± 50.77)
2000	2794.08^{ab} (± 71.80)	2723.35 (± 71.80)	2758.72 (± 50.77)
Mean	2774.00 (± 41.46)	2720.94 (± 41.46)	

$^{a,\,b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A,\,B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Phytase inclusion levels: Feed intake over the phases

There was no significant difference ($P > 0.05$) between the weekly FI of the birds in all the phases in the treatment groups receiving Rovabio Advance T-Flex®. In the grower phase the weekly FI of the birds in the treatment 1000 FTU with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the group receiving Rovabio Advance T-Flex®. During the starter and grower phase in the groups receiving no Rovabio Advance T-Flex® the weekly FI of the birds receiving 1000 FTU was significantly higher ($P < 0.05$) than that of the birds receiving 1500 FTU, but no significant difference ($P > 0.05$) between the birds receiving 1000 FTU and 2000 FTU along with the 1500 FTU and 2000 FTU groups were observed. There was no significant difference ($P > 0.05$) between any of the birds during the finisher phase.

Table 4.23 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the starter phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	470.45^{a} (± 9.17)	446.70 (± 9.17)	458.57 (± 6.49)
1500	436.79^{b} (± 9.17)	445.80 (± 9.17)	441.30 (± 6.49)
2000	456.31^{ab} (± 9.17)	457.89 (± 9.17)	457.10 (± 6.49)
Mean	454.52 (± 5.30)	450.13 (± 5.30)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.24 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the grower phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1388.06^{Aa} (± 42.47)	1217.63^{B} (± 42.47)	1302.84 (± 30.03)
1500	1199.45^{b} (± 42.47)	1269.08 (± 42.47)	1234.27 (± 30.03)
2000	1288.44^{ab} (± 42.47)	1208.54 (± 42.47)	1248.49 (± 30.03)
Mean	1291.98 (± 24.52)	1231.75 (± 24.52)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.25 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the finisher phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1047.56 (± 33.35)	1018.39 (± 33.35)	1032.98 (± 23.58)
1500	985.60 (± 33.35)	1041.86 (± 33.35)	1013.73 (± 23.58)
2000	1049.33 (± 33.35)	1056.91 (± 33.35)	1053.12 (± 23.58)
Mean	1027.50 (± 19.25)	1039.05 (± 19.25)	

Nutrient concentration: Weekly feed intake

At Day 14 and 35 no significant difference ($P > 0.05$) was observed in the weekly FI between any of the birds. At Day 7, 21 and 28 the weekly FI of the birds receiving the standard diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the low concentration diet. On Day 21 and 28 the weekly FI of the birds receiving the standard diet with no added Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the standard diet with Rovabio Advance T-Flex®. At Day 28, the weekly FI of the birds receiving the low concentration diet with added Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the low concentration diet with no added Rovabio Advance T-Flex®.

Table 4.26 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 7-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	149.24^{a} (± 3.49)	144.66 (± 3.49)	146.95^{a} (± 2.46)
Low concentration diet$^{\#}$	137.84^{b} (± 3.49)	140.02 (± 3.49)	138.93^{b} (± 2.46)
Mean	143.54 (± 2.46)	142.34 (± 2.46)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.27 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 14-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	321.21^{a} (± 7.20)	302.04 (± 7.20)	311.63 (± 5.09)
Low concentration diet$^{\#}$	306.16^{b} (± 7.20)	304.20 (± 7.20)	305.18 (± 5.09)
Mean	313.68 (± 5.09)	303.12 (± 5.09)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.28 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	587.78^{Aa} (± 26.37)	497.67^{B} (± 26.37)	542.73 (± 18.64)
Low concentration diet$^{\#}$	498.39^{b} (± 26.37)	513.06 (± 26.37)	505.36 (± 18.64)
Mean	543.09 (± 18.64)	505.36 (± 18.64)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.29 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 28-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
Standard diet	800.28^{Aa} (± 20.26)	719.96^{B} (± 20.26)	760.12^{a} (± 14.32)
Low concentration diet[#]	678.15^{Bb} (± 20.26)	747.81^{A} (± 20.26)	712.98^{b} (± 14.32)
Mean	739.21 (± 14.32)	733.89 (± 14.32)	

[a, b] Means within a column without common superscripts differ significantly ($P < 0.05$)
[A, B] Means within a row without common superscripts differ significantly ($P < 0.05$)
[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.30 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average weekly feed intake (g) of broilers at 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
Standard diet	1047.56 (± 30.50)	1018.39 (± 30.50)	1032.98 (± 21.57)
Low concentration diet[#]	977.15 (± 30.50)	1049.64 (± 30.50)	1013.39 (± 21.57)
Mean	1012.36 (± 21.57)	1034.02 (± 21.57)	

[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Nutrient concentration: Cumulative feed intake

Throughout the entire trial the cumulative FI of the birds receiving the standard diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the low concentration diet with no Rovabio Advance T-Flex®. From Day 21 onwards the cumulative FI of the birds receiving the standard diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the standard diet with Rovabio Advance T-Flex®.

Table 4.31 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 7-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	149.24^{a} (± 3.49)	144.66 (± 3.49)	146.95^{a} (± 2.46)
Low concentration diet$^{\#}$	137.84^{b} (± 3.49)	140.02 (± 3.49)	138.93^{b} (± 2.46)
Mean	143.54 (± 2.46)	142.34 (± 2.46)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.32 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 14-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	470.45^{a} (± 9.16)	446.70 (± 9.16)	458.57 (± 6.48)
Low Concentration Diet	443.99^{b} (± 9.16)	444.23 (± 9.16)	444.11 (± 6.48)
Mean	457.22 (± 6.48)	445.46 (± 6.48)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.33 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1058.24^{Aa} (± 34.00)	944.37^{B} (± 34.00)	1001.30 (± 24.09)
Low concentration diet$^{\#}$	942.38^{b} (± 34.00)	957.28 (± 34.00)	949.83 (± 24.09)
Mean	1000.31 (± 24.09)	950.82 (± 24.09)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.34 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 28-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1858.51^{Aa} (± 50.02)	1664.32^{B} (± 50.02)	1761.42 (± 35.37)
Low concentration diet#	1620.54^{b} (± 50.02)	1705.10 (± 50.02)	1662.82 (± 35.37)
Mean	1739.52 (± 35.37)	1684.71 (± 35.37)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
Low concentration had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.35 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on average cumulative feed intake (g) of broilers from day 0 to 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	2906.08^{Aa} (± 67.19)	2682.72^{B} (± 67.19)	2794.40 (± 47.51)
Low concentration diet#	2597.68^{b} (± 67.19)	2754.74 (± 67.19)	2676.21 (± 47.51)
Mean	2751.88 (± 47.51)	2718.73 (± 47.51)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Nutrient concentration: Feed intake over the phases

There was no significant difference ($P > 0.05$) between the FI of the birds during the finisher phase. During the starter and grower phase the FI for the birds receiving the standard diet with no added Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the low concentration diet with no added Rovabio Advance T-Flex®. During the grower phase the FI for the birds receiving the standard diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than the birds receiving the standard diet with Rovabio Advance T-Flex®.

Table 4.36 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the starter phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	470.45^{a} (± 9.16)	446.70 (± 9.16)	458.57 (± 6.48)
Low concentration diet$^{\#}$	443.99^{b} (± 9.16)	444.23 (± 9.16)	444.11 (± 6.48)
Mean	457.22 (± 6.48)	445.46 (± 6.48)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.37 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the grower phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1388.06^{Aa} (± 42.85)	1217.63^{B} (± 42.85)	1302.84 (± 30.30)
Low concentration diet$^{\#}$	1176.54^{b} (± 42.85)	1260.87 (± 42.85)	1218.71 (± 30.30)
Mean	1282.30 (± 30.30)	1239.25 (± 30.30)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.38 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed intake (g) of broilers during the finisher phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1047.56 (± 30.50)	1018.39 (± 30.50)	1032.98 (± 21.57)
Low concentration diet$^{\#}$	977.15 (± 30.50)	1049.64 (± 30.50)	1013.39 (± 21.57)
Mean	1012.36 (± 21.57)	1034.02 (± 21.57)	

$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

4.1.3 Feed conversion ratio

The FCR are shown in Tables 4.39 to 4.64. The treatments are divided into two groups with subdivisions to illustrate cumulative FCR and FCR within phases.

Phytase inclusion levels: Weekly feed conversion ratio

At Day 21 and 28 there was no significant difference ($P > 0.05$) in the FCR between any of the birds. At Day 7, the FCR of the birds in the 2000 FTU group with Rovabio Advance T-Flex® had a significant higher ($P < 0.05$) FCR than the other birds receiving Rovabio Advance T-Flex®, which did not differ significantly ($P > 0.05$) from each other. At Day 7, the FCR for the birds receiving 2000 FTU with Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than the birds receiving no Rovabio Advance T-Flex®. At Day 14, the FCR for the birds receiving 1500 FTU without Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than the birds receiving 1000 FTU. There was no significant difference ($P > 0.05$) between the birds in the treatments 1500 FTU and 2000 FTU along with 1000 FTU with 2000 FTU. At Day 35, the FCR for the birds receiving 2000 FTU without Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds in the 1000 FTU treatments. However, there was no significant difference ($P > 0.05$) between the birds receiving 1500 FTU and 2000 FTU along with 1000 FTU with 1500 FTU.

Table 4.39 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 7-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.22 (± 0.03)	1.23^{b} (± 0.03)	1.22 (± 0.02)
1500	1.25 (± 0.03)	1.22^{b} (± 0.03)	1.23 (± 0.02)
2000	1.21^{B} (± 0.03)	1.30^{Aa} (± 0.03)	1.25 (± 0.02)
Mean	1.23 (± 0.02)	1.25 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.40 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 14-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1.41^{b} (± 0.03)	1.45 (± 0.03)	1.43^{b} (± 0.02)
1500	1.50^{a} (± 0.03)	1.47 (± 0.03)	1.48^{ab} (± 0.02)
2000	1.46^{ab} (± 0.03)	1.51 (± 0.03)	1.49^{a} (± 0.02)
Mean	1.46 (± 0.02)	1.48 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.41 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1.61 (± 0.04)	1.56 (± 0.04)	1.59 (± 0.03)
1500	1.61 (± 0.04)	1.58 (± 0.04)	1.59 (± 0.03)
2000	1.61 (± 0.04)	1.62 (± 0.04)	1.62 (± 0.03)
Mean	1.61 (± 0.02)	1.59 (± 0.02)	

Table 4.42 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 28-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion** **None**	 **Included**	**Mean**
1000	1.79 (± 0.05)	1.78 (± 0.05)	1.79 (± 0.04)
1500	1.89 (± 0.05)	1.82 (± 0.05)	1.86 (± 0.04)
2000	1.79 (± 0.05)	1.82 (± 0.05)	1.81 (± 0.04)
Mean	1.82 (± 0.03)	1.80 (± 0.03)	

Table 4.43 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.93^{b} (± 0.03)	1.97 (± 0.03)	1.95^{ab} (± 0.02)
1500	1.96^{ab} (± 0.03)	1.92 (± 0.03)	1.94^{b} (± 0.02)
2000	2.02^{a} (± 0.03)	1.99 (± 0.03)	2.01^{a} (± 0.02)
Mean	1.97 (± 0.02)	1.96 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Phytase inclusion levels: Cumulative feed conversion ratio

At Day 7, 14 and 21 the cumulative FCR of the birds receiving 2000 FTU with Rovabio Advance T-Flex® had a significant higher ($P < 0.05$) FCR than the other birds receiving Rovabio Advance T-Flex®, which did not differ significantly ($P > 0.05$) from each other. At Day 7 and 14 the FCR for the birds receiving 2000 FTU with Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than the birds not receiving Rovabio Advance T-Flex®. At Day 28 and 35 no significant difference ($P > 0.05$) was observed between the cumulative FCR of any of the birds.

Table 4.44 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed conversion ratio of broilers from day 0 to 7-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.22 (± 0.03)	1.23^{b} (± 0.03)	1.22 (± 0.02)
1500	1.25 (± 0.03)	1.22^{b} (± 0.03)	1.23 (± 0.02)
2000	1.21^{B} (± 0.03)	1.30^{Aa} (± 0.03)	1.25 (± 0.02)
	1.23 (± 0.02)	1.25 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.45 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed conversion ratio of broilers from day 0 to 14-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.32 (± 0.02)	1.33^{b} (± 0.02)	1.33 (± 0.02)
1500	1.37 (± 0.02)	1.34^{b} (± 0.02)	1.36 (± 0.02)
2000	1.33^{B} (± 0.02)	1.41^{Aa} (± 0.02)	1.37 (± 0.02)
Mean	1.34 (± 0.01)	1.36 (± 0.01)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.46 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed conversion ratio of broilers from day 0 to 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.41 (± 0.02)	1.41^{b} (± 0.02)	1.41^{b} (± 0.01)
1500	1.45 (± 0.02)	1.42^{b} (± 0.02)	1.44^{ab} (± 0.01)
2000	1.43 (± 0.02)	1.49^{a} (± 0.02)	1.45^{a} (± 0.01)
Mean	1.43 (± 0.01)	1.44 (± 0.01)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Table 4.47 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed conversion ratio of broilers from day 0 to 28-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.51 (± 0.02)	1.50 (± 0.02)	1.51 (± 0.02)
1500	1.56 (± 0.02)	1.52 (± 0.02)	1.54 (± 0.02)
2000	1.52 (± 0.02)	1.56 (± 0.02)	1.54 (± 0.02)
Mean	1.53 (± 0.01)	1.53 (± 0.01)	

Table 4.48 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on average cumulative feed conversion ratio of broilers from day 0 to 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.59 (± 0.02)	1.60(± 0.02)	1.60 (± 0.02)
1500	1.64 (± 0.02)	1.60 (± 0.02)	1.62 (± 0.02)
2000	1.62 (± 0.02)	1.65 (± 0.02)	1.63 (± 0.02)
Mean	1.62 (± 0.01)	1.62 (± 0.01)	

Phytase inclusion levels: Feed conversion ratio for the phases

During the starter phase the FCR of the birds in the treatment group 2000 FTU with Rovabio Advance T-Flex® had a significantly higher ($P < 0.05$) FCR than the other birds receiving Rovabio Advance T-Flex®. There was no significant difference ($P > 0.05$) during the grower phase. During the finisher phase the FCR for the birds receiving 2000 FTU without Rovabio Advance T-Flex® was significantly higher than that of the birds in the 1000 FTU treatments while there was no significant difference ($P > 0.05$) between the birds in the1000 FTU and 1500 FTU treatments as well as the 1500 FTU and 2000 FTU.

Table 4.49 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the starter phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.32 (± 0.02)	1.34^{b} (± 0.02)	1.33 (± 0.02)
1500	1.37 (± 0.02)	1.34^{b} (± 0.02)	1.36 (± 0.02)
2000	1.33^{Ba} (± 0.02)	1.41^{Aa} (± 0.02)	1.37 (± 0.02)
Mean	1.34 (± 0.01)	1.36 (± 0.01)	

$^{a,\ b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A,\ B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.50 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the grower phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	

1000	1.70 (± 0.04)	1.67 (± 0.04)	1.69 (± 0.03)
1500	1.75 (± 0.04)	1.70 (± 0.04)	1.72 (± 0.03)
2000	1.70 (± 0.04)	1.72 (± 0.04)	1.71 (± 0.03)
Mean	1.72 (± 0.02)	1.70 (± 0.02)	

Table 4.51 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the finisher phase (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	1.93^{b} (± 0.03)	1.97 (± 0.03)	1.95^{ab} (± 0.02)
1500	1.96^{ab} (± 0.03)	1.92 (± 0.03)	1.94^{b} (± 0.02)
2000	2.02^{a} (± 0.03)	1.99 (± 0.03)	2.01^{a} (± 0.02)
Mean	1.97 (± 0.02)	1.96 (± 0.02)	

$^{a,\ b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

Nutrient concentration: Weekly feed conversion ratio

Throughout the whole trial the FCR for the birds in the low concentration diet group without Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the standard diet. On all the days except for day 28 the FCR for the birds receiving the low concentration diet without Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving low concentration diet with Rovabio Advance T-Flex®. From day 21 onwards the FCR for the birds receiving the low concentration diet with Rovabio Advance T-Flex® was significantly higher than that of the birds receiving the standard diet.

Table 4.52 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 7-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.22^{b} (± 0.04)	1.23 (± 0.04)	1.22^{b} (± 0.03)

Low concentration diet$^{\#}$	1.38^{Aa} (± 0.04)	1.26^{B} (± 0.04)	1.32^{a} (± 0.03)
Mean	1.30 (± 0.03)	1.24 (± 0.03)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.53 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 14-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.41^{b} (± 0.02)	1.45 (± 0.02)	1.43 (± 0.01)
Low concentration diet$^{\#}$	1.51^{Aa} (± 0.02)	1.40^{B} (± 0.02)	1.47 (± 0.01)
Mean	1.46 (± 0.01)	1.43 (± 0.01)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.54 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.61^{b} (± 0.04)	1.56^{b} (± 0.04)	1.59^{b} (± 0.03)
Low concentration diet$^{\#}$	1.75^{Aa} (± 0.04)	1.88^{aB} (± 0.04)	1.81^{a} (± 0.03)
Mean	1.68 (± 0.03)	1.72 (± 0.03)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.55 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 28-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.79^{b} (± 0.05)	1.78^{b} (± 0.05)	1.79^{b} (± 0.04)

Low concentration diet$^{\#}$	2.22^{a} (± 0.05)	2.07^{a} (± 0.05)	2.15^{a} (± 0.04)
Mean	2.01 (± 0.04)	1.93 (± 0.04)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.56 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the weekly feed conversion ratio of broilers at 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.93^{b} (± 0.04)	1.97^{b} (± 0.04)	1.95^{b} (± 0.02)
Low concentration diet$^{\#}$	2.34^{Aa} (± 0.04)	2.10^{Ba} (± 0.04)	2.21^{a} (± 0.02)
Mean	2.14^{A} (± 0.02)	2.03^{B} (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Nutrient concentration: Cumulative feed conversion ratio

For the entire period the cumulative FCR for the birds receiving the low concentration diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the standard diet. On all days, with the exception of Day 21, the cumulative FCR for the birds receiving the low concentration diet with no Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving Rovabio Advance T-Flex®. From Day 21 onwards the cumulative FCR for the birds receiving the low concentration diet with Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the standard diet.

Table 4.57 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average cumulative feed conversion ratio of broilers from day 0 to 7-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.22^{b} (± 0.04)	1.23 (± 0.04)	1.22^{b} (± 0.03)
Low concentration diet$^{\#}$	1.38^{Aa} (± 0.04)	1.26^{B} (± 0.04)	1.32^{a} (± 0.03)

Mean	1.30 (± 0.03)	1.24 (± 0.03)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)
$^{A, B}$ Means within a row without common superscripts differ significantly (P < 0.05)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.58 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average cumulative feed conversion ratio of broilers from day 0 to 14-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.32^{b} (± 0.03)	1.34 (± 0.03)	1.33^{b} (± 0.02)
Low concentration diet$^{\#}$	1.45^{Aa} (± 0.03)	1.33^{B} (± 0.03)	1.39^{a} (± 0.02)
Mean	1.38 (± 0.02)	1.33 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)
$^{A, B}$ Means within a row without common superscripts differ significantly (P < 0.05)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.59 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average cumulative feed conversion ratio of broilers from day 0 to 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.41^{b} (± 0.02)	1.41^{b} (± 0.02)	1.41^{b} (± 0.01)
Low concentration diet$^{\#}$	1.54^{a} (± 0.02)	1.51^{a} (± 0.02)	1.53^{a} (± 0.01)
Mean	1.48 (± 0.01)	1.46 (± 0.01)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.60 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average cumulative feed conversion ratio of broilers from day 0 to 28-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.51^{b} (± 0.02)	1.50^{b} (± 0.02)	1.51^{b} (± 0.01)
Low concentration diet$^{\#}$	1.71^{Aa} (± 0.02)	1.65^{Ba} (± 0.02)	1.68^{a} (± 0.01)
Mean	1.61 (± 0.01)	1.58 (± 0.01)	

$^{a, b}$ Means within a column without common superscripts differ significantly (P < 0.05)

[A, B] Means within a row without common superscripts differ significantly ($P < 0.05$)
Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.61 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average cumulative feed conversion ratio of broilers from day 0 to 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.59^{b} (± 0.02)	1.60^{b} (± 0.02)	1.60^{b} (± 0.01)
Low concentration diet[#]	1.84^{Aa} (± 0.02)	1.74^{Ba} (± 0.02)	1.79^{a} (± 0.01)
Mean	1.72^{A} (± 0.01)	1.67^{B} (± 0.01)	

[a, b] Means within a column without common superscripts differ significantly ($P < 0.05$)
[A, B] Means within a row without common superscripts differ significantly ($P < 0.05$)
[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Nutrient concentration: Feed conversion ratio over the phases

During the starter phase the FCR for the birds receiving the low concentration diet without Rovabio Advance T-Flex® was significantly higher ($P < 0.05$) than that of the birds receiving the low concentration diet with Rovabio Advance T-Flex® as well as the standard diet without Rovabio Advance T-Flex®. During both the grower and finisher phase the birds receiving the low concentration diets had significant higher FCR ($P < 0.05$) than the birds receiving the standard diets.

Table 4.62 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the starter phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.32^{b} (± 0.03)	1.34 (± 0.03)	1.33^{b} (± 0.02)
Low concentration diet[#]	1.46^{Aa} (± 0.03)	1.33^{B} (± 0.03)	1.39^{a} (± 0.02)
Mean	1.38 (± 0.02)	1.33 (± 0.02)	

[a, b] Means within a column without common superscripts differ significantly ($P < 0.05$)
[A, B] Means within a row without common superscripts differ significantly ($P < 0.05$)
[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.63 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the grower phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.70^{b} (± 0.03)	1.67^{b} (± 0.03)	1.69^{b} (± 0.02)
Low concentration diet$^{\#}$	1.98^{a} (± 0.03)	1.97^{a} (± 0.03)	1.98^{a} (± 0.02)
Mean	1.84 (± 0.02)	1.82 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.64 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the average feed conversion ratio of broilers during the finisher phase (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	1.93^{b} (± 0.04)	1.97^{b} (± 0.04)	1.95^{b} (± 0.02)
Low concentration diet$^{\#}$	2.34^{a} (± 0.04)	2.08^{a} (± 0.04)	2.21^{a} (± 0.02)
Mean	2.14^{A} (± 0.02)	2.03^{B} (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)
$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)
$^{\#}$ Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

4.1.4 Mortality

The total mortality rate of this broiler trial was 6.8%. The mortalities were even distributed throughout both houses and all the treatments. There were no observed differences of the mortalities between the treatments.

4.2 Tibia ash

At Day 21, the birds receiving 2000 FTU without Rovabio Advance T-Flex® had significant heavier ($P < 0.05$) bone ash than the birds receiving Rovabio Advance T-Flex® at 2000 FTU. At day 35, within the treatments without Rovabio Advance T-Flex® supplementation, the birds receiving 1500 FTU had significant heavier ($P < 0.05$) bone ash than the birds in the treatment group 1000 FTU with no significant difference ($P > 0.05$) between the birds in the treatments

1000 FTU and 2000 FTU as well as 1500 FTU and 2000FTU. On Day 35, within the treatments with Rovabio Advance T-Flex® included, the birds receiving 2000 FTU had significant heavier ($P < 0.05$) bone ash than the birds receiving 1500 FTU with no significant difference ($P > 0.05$) between birds in treatments 1000 FTU and 2000 FTU as well as between the birds receiving 1000 FTU and 1500 FTU.

Table 4.65 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the bone ash (g) of broilers at 21-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	45.58 (± 0.60)	45.45 (± 0.60)	45.51 (± 0.42)
1500	45.05 (± 0.60)	44.54 (± 0.60)	45.80 (± 0.42)
2000	46.35^{A} (± 0.60)	44.32^{B} (± 0.60)	45.33 (± 0.42)
Mean	45.66 (± 0.34)	44.77 (± 0.34)	

$^{A, B}$ Means within a row without common superscripts differ significantly ($P < 0.05$)

Table 4.66 Interaction effect of three different levels of phytase and Rovabio Advance T-Flex® inclusion on the bone ash (g) of broilers at 35-days-of-age (± standard error of mean)

Phytase Inclusion (FTU)	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
1000	48.05^{b} (± 0.03)	49.30^{ab} (± 0.03)	48.67^{b} (± 0.02)
1500	50.47^{a} (± 0.03)	48.99^{b} (± 0.03)	49.73^{ab} (± 0.02)
2000	50.27^{ab} (± 0.03)	51.45^{a} (± 0.03)	50.86^{a} (± 0.02)
Mean	49.60 (± 0.02)	49.91 (± 0.02)	

$^{a, b}$ Means within a column without common superscripts differ significantly ($P < 0.05$)

At Day 21, the birds receiving the standard diet with Rovabio Advance T-Flex® had a significant heavier ($P < 0.05$) bone ash than the birds receiving the low concentration diet. No other significant differences were observed.

Table 4.67 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the tibia bone ash (g) of broilers at 21-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	45.58 (± 0.77)	45.45^{a} (± 0.77)	45.51^{a} (± 0.55)
Low concentration diet[#]	44.01 (± 0.77)	43.21^{b} (± 0.77)	43.31^{b} (± 0.55)
Mean	44.79 (± 0.55)	44.33 (± 0.55)	

[a, b] Means within a column without common superscripts differ significantly ($P < 0.05$)

[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Table 4.68 Interaction effect of two different nutrient concentrations and Rovabio Advance T-Flex® inclusion on the tibia bone ash (g) of broilers at 35-days-of-age (± standard error of mean)

Nutrient Concentration	**Rovabio Advance T-Flex® Inclusion**		**Mean**
	None	**Included**	
Standard diet	48.05 (± 0.77)	49.30 (± 0.77)	48.67 (± 0.54)
Low concentration diet[#]	49.31 (± 0.77)	48.73 (± 0.77)	49.02 (± 0.54)
Mean	48.68 (± 0.54)	49.01 (± 0.54)	

[#] Low concentration diet had a lower concentration of apparent metabolisable energy and digestible amino acids compared to the standard diet

Chapter 5

Discussion

The most expensive component of broiler production is feed, which accounts for up to 75% of the total production costs (Nkukwana, 2018). The success of formulated feed is measured by the accuracy with which nutrient requirements of the broilers can be met by nutrients supplied to achieve the target performance (Panda *et al*., 2013). The main source of energy in poultry diets originate from maize (Panda *et* al., 2013) and protein from soybean meal (Frempong *et al*., 2019). These raw materials contain the anti-nutritional factors NSP and phytate, which influence the nutritional value of feedstuffs (Jlali *et al*., 2020). Feed additives such as enzymes are needed to combat the anti-nutritional factors and improve the quality of feed while improving the performance of the animal (Pirgozliev *et al*., 2017).

Rovabio Advance T-Flex®, a multi-enzyme complex with 19 enzymatic activities, along with different inclusion levels of phytase, were evaluated in this trial. It was hypothesised that the enzymatic activities would allow the release of encapsulated nutrients thus increasing the digestion and absorption of nutrients that would otherwise be unavailable. If this was true, it would be possible to reduce the energy and digestible amino acid concentration of broiler feeds when including Rovabio Advance T-Flex® without reducing broiler performance. Lawlor *et al*. (2019) stated that the use of an MEC can allow the formulation of diets that would otherwise be deficient in digestible energy, amino acids, Ca and P which would reduce feed costs and nutrient excretion.

It was also hypothesised that by increasing the inclusion levels of phytase (superdosing) the performance of the broilers would improve compared to the standard industry recommended concentrations. However, the optimal dose of phytase is a heavily debated topic as some researchers are of opinion that superdosing is not economically viable while some researchers are of the opinion that superdosing phytase will impact the nutritional matrix which will lower the cost of feed and increase profitability (Nacimento *et al*., 2021).

Several studies (Jia-Cheng *et al*., 2020; Jlali *et al*., 2020) found a complementary effect between an MEC and phytase on the digestibility of nutrients. Jia-Cheng *et al*. (2020) stated that this effect is most likely due to the fact that the MEC degrades NSPs on the aleurone layer where phytate is bound which allows phytase better access to phytate, resulting in a more pronounced effect of phytase in increasing the availability of P and other nutrients.

The result of this study shows that the production parameters did not significantly improve when the MEC Rovabio Advance T-Flex® was added to diets that meet the dietary requirements of the birds at different inclusion levels of phytase. The production parameters did however improve when a nutrient deficient diet was fed with the addition of an MEC. This is consistent with other experiments done (Lawlor *et al*., 2019; Jlali *et al*., 2020). The production parameters between the different inclusion levels of phytase with and without the MEC showed no significant difference.

Moraes *et al.* (2015) state that performance parameters may be unaffected if enzymes are supplemented in a highly digestible diet. When enzymes are added to diets that meet the requirements of the bird, improvements in performance parameters are not always observed as the diets allow the bird to perform close to its genetic potential and further improvements are not feasible (Sorbara *et al*., 2009). In a trial conducted by West *et al*. (2007), it was found that although the feed digestibility increased, it did not have an influence on production parameters as there was already enough nutrients to sustain the performance. It should also be noted the response of the bird to enzymatic activities is dependent on the NSP content of the diet (Musigwa *et al*., 2020).

In the present study the addition of the MEC to the standard diet as well as to the different inclusion levels of phytase did not improve the body weight (Tables 4.1 to 4.16). This is similar to what was found in an experiment done by Kaczmarek *et al*. (2014) where no improvement was observed when an MEC was added to a positive control treatment. No significant difference was observed between the different phytase inclusion levels, which is consistent with results from Siegert *et al*. (2019) where growth was not further increased with increased phytase concentrations. The results of this trial show that the body weight of the birds receiving the low concentration diet with no added Rovabio Advance T-Flex® was significantly lower than that of the birds receiving the standard diet (Tables 4.7 to 4.12) showing that the basal diet 2 was indeed lower in nutrient concentration than basal diet 1, as intended. This is consistent with results from other trials (Lawlor *et al*., 2019; Saleh *et al*., 2019; Jlali *et al*., 2020) where birds were fed nutrient deficient diets. Jlali *et al*. (2020) found when birds were fed diets deficient in ME, digestible amino acids, avP, and Ca, the body weight of the birds were on average 7.2% lower than those of the birds receiving the positive control diet that meets the nutritional requirements. They found that when supplementing an MEC together with 1000 FTU phytase that the bird performance and feed efficiency were restored to the level of the birds fed the positive control diet. Lawlor *et al*. (2019) found that when supplementing nutrient deficient diets with an MEC, the growth performance did not match that of the adequate diet.

In this study the feed intake of the birds that received the 1000 FTU with no MEC supplementation treatments, were higher than that of the treatments supplemented with the increased phytase concentrations (Tables 4.13 to 4.22). This was reflected in the starter and grower phase, however there was no significant difference within the finisher phase (Tables 4.23 to 25). These results correspond with the results from an experiment done by Siegert *et al*. (2019) where the FI did not change when higher concentrations of phytase were included in the diet. The FI for the standard diet was significantly higher than that of the low concentration diets within the groups that were not supplemented with the MEC (Tables 4.26 to 4.35). These results are the same for other studies (Lawlor *et al*., 2019) where FI was reduced in diets that were nutrient deficient. In a trial conducted by Lawlor *et al*. (2019), the FI was the primary parameter that was influenced when feeding a nutrient deficient diet. The standard diet with no MEC had significant higher FI than that of the standard diet with the MEC which was predominant in the grower phase (Table 4.37). This is in accordance with another study done (Musigwa *et al*., 2020) where the FI lowered when a carbohydrase enzyme was added to the diet. This result shows that broilers control their FI in order to adjust their energy intake. The ability of a bird to utilise energy for lean muscle and fat deposition is highly dependent on the capacity of the bird to control FI (Musigwa *et al*., 2020).

In the present study the FCR for the birds receiving the 2000 FTU diet supplemented with the MEC was significantly higher than that of the other inclusion levels of phytase as well as the 2000 FTU treatment that was not supplemented with MEC. The FCR for the birds receiving the low concentration diets were higher than that of the standard diets which confirms that the diets were indeed deficient in energy and digestible amino acids. These results are reiterated in the results of other trials (Amerah *et al*., 2017; Rios *et al*., 2017; Jlali *et al*., 2020). In a study done by Saleh *et al*. (2019) the growth performance in broilers improved when the MEC was added, and they suggested that the improvement may be due to the enhanced nutrient digestibility instigated by the enzymes, xylanases and arabinofuronidase.

In the present study, birds receiving the 2000 FTU treatment without the MEC supplementation had significantly higher bone ash on Day 21 than the birds receiving the treatments supplemented with the MEC. On Day 35, the birds receiving the phytase concentration of 1500 FTU without the MEC supplementation had significantly higher bone ash than the birds receiving the 1000 FTU concentration. These results are the same as with an experiment done by Dos Santos *et al*. (2017) where no difference in tibia ash was observed between the different treatments. The authors state that the improvement in the growth performance parameters in the super-dosed treatments were not related to the increased absorption of P, but rather a reduction in the anti-nutritional effects of phytate. On the other hand, Fernandes *et al*., (2019) did observe a positive effect on bone integrity and attributed it to the greater availability of P which allowed better bone development.

Chapter 6

Conclusion

In the monogastric feed industry enzyme supplementation is becoming crucial to ensure success in an environment where high feed costs and limited resources impact the survival of the poultry industry. Exogenous enzymes play an important role in reducing the anti-nutritional factors present in feedstuffs, allowing animals to utilise nutrients that would otherwise be unavailable for digestion and utilisation. The supplementation of exogenous enzymes is seen as an aid for animals where there is no endogenous secretion of the necessary enzymes, or the secretion of these enzymes is limited such as in young broilers. The anti-nutritional factors, NSP, may not be as prevalent in the maize-soybean diet that are used in South Africa, but the effects should not be dismissed. Through the supplementation of NSP-degrading enzymes these negative effects can be alleviated with an ultimate cost saving effect. Phytate remains a challenge in all feedstuffs as it not only binds the majority of the P, but also other nutrients such as proteins and numerous minerals. By including the right amount of phytase this anti-nutritional factor can be combatted and the environmental impact can be reduced or alleviated.

According to this trial it is possible to reduce the energy and digestible amino acids while adding the MEC Rovabio Advance T-Flex®. The decrease in energy and digestible amino acids is a cost-saving approach since it decreases the inclusion levels of the costliest raw materials such as maize and soybean and allow the inclusion of raw materials that are mostly seen as fillers and are relatively inexpensive. The supplementation of Rovabio Advance T-Flex® to these nutrient deficient diets facilitates the release of otherwise bound nutrients, thus allowing the digestion and utilisation of more nutrients that become available to the animal for production. The FI of the standard diets that were not supplemented with Rovabio Advance T-Flex® were higher than the standard diets with Rovabio Advance T-Flex®. This suggests that the birds altered their FI to satisfy their dietary requirements. The diets with Rovabio Advance T-Flex® supplementation gave the birds better access to nutrients thus decreasing their FI. These results are reflected in the FCR since the treatments not receiving Rovabio Advance T-Flex® supplementation had significant higher FCR thus more feed is needed and ultimately results in an increase in feed costs. The results for the different inclusion levels of phytase shows that there is no significant difference in performance parameters between the different inclusion levels. The tibia bone results show no significant difference in bone ash.

This study showed that the inclusion of the MEC Rovabio Advance T-Flex® does improve performance parameters and can be used to decrease the nutrient concentration (energy and digestible amino acids) of maize-soya based diets and save feed costs. Due to the fact that there was no significant differences in the performance parameters as well as tibia bone ash, it may suggest that the inclusion levels of phytase is not as important as long as phytase is included in the diet at a concentration of at least 1000 FTU.

Critical Review and Recommendations

Further studies to evaluate the efficacy of the enzyme Rovabio Advance T-Flex® along with the effect of superdosing phytase are recommended. The following factors should be considered when conducting the study as it may have had an influence on the results of the current trial:

1. During this study the temperature control unit struggled to cope with the high ambient temperature resulting in temperature fluctuations during the day leading to heat stress. This impacts the performance of the birds and increases variation within treatments.

2. No enzyme recovery was done to verify the mixing efficiency of the enzyme complex Rovabio Advance T-Flex®. This should be done by Adisseo (France), however due to the location of the laboratory it may result in long turnaround times.

3. During formulation of the dietary treatments, care should be given to ensure that the specifications are limiting enough to allow for significantly lower performance in order to accurately determine the efficacy of the supplemented enzymes.

4. The accuracy of the feed analyses could have been improved by taking more samples per feed and analysing the samples at different laboratories in order to get a more accurate estimation of the nutrient content of the different treatment diets.

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