Improving ileal amino acid digestibility and broiler performance with dietary protease and xylanase

by

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DECLARATION

I, Claire Norton, hereby declare that this dissertation, submitted for the MSc (Agric) Animal Science: Animal Nutrition degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any tertiary institution.

Signature: 

Date: 08/12/2021

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ABSTRACT

The use of exogenous enzymes in broiler diets is well documented, and in recent years their popularity has increased. With the rise of the global population leading to an increased demand in animal protein, farmers are forced to increase production with limited resources thus making production efficiency vital and the use of exogenous enzymes imperative. The aim of this project was to determine the effects that exogenous protease and xylanase have on broiler chickens. A digestibility trial with broiler feed containing protease, and a broiler performance trial including xylanase and protease individually and in combination in the treatment diets were conducted.

The digestibility trial was done to determine the effect that various levels of protease have on ileal amino acid digestibility of broilers. Six hundred male broilers were reared until day 17 on the same standard maize-soya diet, thereafter 480 male birds closest to the average body weight were transferred to 60 metabolic cages and randomly divided into six treatment groups with 10 replicates per treatment. The first treatment was a negative control (NC) with no added enzymes and formulated to replicate a typical South African maize soy-based broiler diet. Four treatments used the negative control diet as a base with the addition of increasing doses of a protease product not previously used in South Africa, Kemzyme (Kemin). A fifth treatment was regarded as a positive control diet with the addition of a well-known protease product often used in South Africa, Proact (DSM). The supplementation of protease in broiler diets did not result in significantly ($P<0.05$) beneficial results in terms of crude protein (CP) and dry matter (DM) digestibility. The CP digestibility is the amount of dietary crude protein which is absorbed by the broiler and not excreted in the animal's faeces while the DM digestibility is the portion of dry matter which is digested by the broiler for a given amount of feed intake. No dose response was observed as initially predicted. The negative control group either had significantly ($P<0.05$) better performance than those treated with protease, or the exogenous protease resulted in insignificant differences compared to the NC. The majority of the ileal amino acid digestibilities were not significantly affected by the addition of different doses of protease to the diet.

The performance trial was done with the aim to determine the effect of dietary xylanase and protease both alone and in combination on the production performance of broiler chickens. Two thousand male broilers were randomly divided into one of a total of 11 treatments. A typical maize soya diet was fed to simulate commercial conditions. A positive control (PC) diet was formulated using standard commercial energy and amino acid levels.

Three negative control (NC) diets were included either with 0.418 MJ/kg less energy (NC1), 4% lower amino acid levels (NC2) or lower in both energy and amino acid levels (NC3). Three concentrations of xylanase (Xygest HT) were added to NC1, Kemzyme protease was added to NC2 at two concentration levels, as well as Proact protease. Both Kemzyme and Xygest HT were added to NC3 to form the last treatment.

The addition of xylanase showed no clear or predictable benefit on the weekly or cumulative bird performance. During the first three weeks there was little difference between the treatment groups for weekly performance measurements. During the final two weeks of the trial a supplementation of 10 mg/kg of xylanase resulted in a significantly ($P<0.05$) lower feed conversion ratio (FCR). The FCR assists in determining how efficiently the broilers grow. It is the amount of feed that needs to be eaten in order to gain 1 kg of body mass. The cumulative FCR for the various xylanase treatments was either insignificant or not beneficial throughout the trial. Results for protease supplementation showed no difference between treatments for both cumulative and weekly performance parameters for the first two weeks. The 28d body weight of birds supplemented with 300 mg/ton protease was significantly higher than the NC and the same birds had the highest weekly feed intake from day 28 to day 35. The weekly and cumulative FCR was not impacted by the addition of protease. The cumulative feed intake was significantly reduced with the addition of 200 mg/kg ProAct and 150 mg/kg Kemzyme protease compared to the PC. The combination of xylanase and protease did not result in any beneficial results regarding weekly and cumulative performance parameters. The portion yield of the broilers was not enhanced with the supplementation of dietary xylanase, protease or a combination of protease and xylanase. The dressing percentage of broilers fed 30 mg/kg of xylanase was significantly higher compared to the NC.

This trial suggests that exogenous enzymes should be used with caution as they do not always provide benefits. Enzymes may decrease the performance of an animal or lead to no difference at all when not used in the correct manner or under the correct circumstances. Therefore, careful application of dietary enzymes is suggested.

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LIST OF ABBREVIATIONS

ANF	Anti-nutritional factor
AME	Apparent metabolisable energy
CO_2	Carbon dioxide
CP	Crude protein
˚C	Degrees Celsius
d	Days
FCR	Feed conversion ratio
g	Gram
GLM	General linear model
H_0	Null hypothesis
H_1	Alternative hypothesis
kg	Kilogram
LSM	Least square mean
m	Meter
m^2	Square meter
MJ	Mega joules
ME	Metabolisable energy
N_2	Nitrogen
NC	Negative control
O_2	Oxygen
PC	Positive control
SEM	Standard error of the mean

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CHAPTER 1. INTRODUCTION

1.1 Introduction

Feed contributes a major portion to the costs of broiler production. The cost of raw materials is continuing to rise, especially those raw materials which are sources of high-quality protein. The human demand for animal protein is anticipated to rise as the global population grows therefore farmers are faced with the challenge of increasing production with decreasing and limited resources, thus making high efficiency of production crucial (FAO, 2011; Recoules *et al*., 2017). There is an increasing concern about the negative effect which animal production has on the environment. Undigested nutrients, unavailable for absorption, are wasted and will not contribute to the performance of the animal. The aim of these studies was to improve the dietary nutrient utilisation and absorption for broilers through dietary supplementation of xylanase and protease.

Overfeeding protein in a broiler diet will result in economic losses and increased nitrogen excretion (Kamel *et al*., 2015), adding to the pollution of the environment. The excess protein which is excreted may also result in an increased incidence of welfare issues such as hock burn and breast blisters (de Jong *et al*., 2014). However, underfeeding protein, in particular essential amino acids, will result in a reduction in performance of the birds (Bregendahl *et al.,* 2002).

The major ingredients of most broiler feeds are products derived from plants. These plant materials have complex structures containing varying amounts of carbohydrates, protein and oil. The main components of South African broiler feed are maize and soya. Approximately 65% of the apparent metabolisable energy in broiler diets is from the maize portion of the diet (Rios *et al*., 2017). Normal digestion by an animal is brought about by the action of various endogenous enzymes on the feed mixture. Different feed compositions will differ in terms of digestibility. One of the challenges of the nutritionist is to formulate feeds with acceptable low costs while having a high degree of digestibility. This situation gives an opportunity for the application of various supplements in feed formulations as a means of improving overall diet digestibility.

Protease is an enzyme which degrades complex proteins into more digestible amino acids thus improving the digestibility of protein (Xu *et al*., 2017). By making use of the correct dosage of exogenous protease supplementation it will be possible to decrease the dietary CP requirements of broilers (Borda-Molina *et al*., 2019). The CP digestibility is the amount of

dietary crude protein which is absorbed by the broiler and not excreted in the animal's faeces. This will allow for more efficient use of raw materials, thus providing the opportunity to improve the efficiency of use and decrease the cost of the feed whilst decreasing the negative effects which production can have on the environment. Certain welfare issues can be avoided and performance of broilers can be optimised. *In vivo* studies are typically done to assess the digestibility of various nutrients. An *in vivo* trial is the preferred method to evaluate the digestibility of nutrients, as an accurate and reliable laboratory simulation of the digestive processes is difficult to achieve. Collection of ileal digesta samples for CP and amino acid digestibility determination assists in eliminating the lower portion of the gut as a source for errors. Such errors include nitrogen in poultry excreta originating from urine and amino acid in faecal material of microbial origin (Lemme *et al*., 2004).

Diets which are rich in non-starch polysaccharides (NSPs) result in highly viscous feed mixtures which could consequently inhibit nutrient diffusion and transport in the gastrointestinal tract (Rios *et al*., 2017). Non-starch polysaccharides (NSPs) are carbohydrates which are indigestible to the endogenous enzymes of broilers, thereby rendering them anti-nutritional factors (ANFs). An ANF is a portion of the feed, usually a biological component, which reduces the digestibility of the feed by decreasing the availability of one or more components of the feed. Viscosity of the contents of the intestine is an important factor influencing the nutritive value of many cereals. Xylanase is an enzyme which degrades the structure of hemicellulose, a main constituent of plant cell walls. The addition of dietary xylanase has been shown to reduce gut viscosity in broilers (Moss *et al*., 2018). Thus, supplementation of xylanase should allow the bird to digest its feed more efficiently and less wastage will occur (Engberg *et al*., 2004).

An animal's nutritional status influences its ability to reach its genetic potential for growth, reproduction and longevity and to respond to pathogens and other environmental stressors. Ensuring that feed is highly digestible means that the nutrients contained in the feed are easily made available for absorption through the intestinal wall. Improving the efficiency of feed utilisation, including feed digestion and nutrient absorption, is essential for profitable farming.

1.2 Aims and objectives

1.2.1 Protease digestibility trial

The aim of this research was to determine the optimal dose of a coated, compound protease (Kemzyme protease) required in commercial broiler maize-soya diets.

The objective of the protease digestibility trial was to measure ileal digestibility of CP, dry matter (DM), the DM digestibility is the portion of dry matter which is digested by the broiler for a given amount of feed intake, and amino acids when broilers were fed feed supplemented with various levels of Kemzyme protease (Kemin Industries, Des Moines, Iowa, United States) and compared its performance with a competitor protease (Ronozyme ProAct, DSM, Heerlan, Netherlands).

1.2.2 **Protease and xylanase performance trial**

The first aim of the trial was to determine the efficacy of Xygest HT enzyme (Kemin Industries, Des Moines, Iowa, United States) supplemented at three doses on performance of broiler chickens fed typical South African soya-maize based diets, from 0–35 days-of-age. The results will contribute to the determination of the matrix value (dietary specification values) for the Xygest HT enzyme for South African Ross 308 broilers that received feed materials available in South Africa.

The second aim of this trial was to determine and quantify the effects of Kemzyme supplementation on the performance of Ross 308 broiler chickens from day 0-35 days-of-age which were fed soya-maize based diets.

The third and final aim of this broiler performance trial was to determine the efficacy of the Kemzyme on performance of broilers when fed in combination to Xygest HT.

The objective of the broiler performance trial was to measure broiler performance in terms of feed intake, body weight gained, feed conversion ratio (FCR) and portion yield when the birds received feed supplemented with varying doses Kemzyme, Xygest HT and both Kemzyme and Xygest HT in combination. The FCR assists in determining how efficiently the broilers grow. It is the amount of feed that needs to be eaten in order to gain 1 kg of body mass.

1.3 Hypotheses

1.3.1 **Protease digestibility trial**

$H_{0:}$ The ileal digestibility of amino acids will not increase regardless of the level of protease added to the feed.

H_1: The ileal digestibility of amino acids will increase as the level of protease added to the feed increases until an optimal point is reached. Thereafter the digestibility will not continue to increase regardless of the dose of supplemented protease.

1.3.2 **Protease and xylanase performance trial**

1) H_0: Adding protease to broiler diets containing 4% less amino acids than standard commercial diets will not enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher amino acid levels.
   H_1: Adding protease to broiler diets containing 4% less amino acids than standard commercial diets will enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher amino acid levels.

2) H_0: Adding xylanase to broiler diets containing 0.418 MJ/kg less energy than standard commercial diets will not enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher energy levels.
   H_1: Adding xylanase to broiler diets containing 0.418 MJ/kg less energy than standard commercial diets will enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher energy levels.

3) H_0: Adding xylanase and protease to broiler diets containing 0.418 MJ/kg less energy and 4% less amino acids than standard commercial diets will not enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher energy and amino acid levels.
   H_1: Adding xylanase and protease to broiler diets containing 0.418 MJ/kg less energy and 4% less amino acids than standard commercial diets will enhance production performance to the same level than that of broilers receiving the standard commercial diets with higher energy and amino acid levels.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Broilers are incapable of optimally digesting feed using only endogenous enzymes hence the need for dietary supplemented enzymes. Exogenous enzymes are a way to cost effectively enhance production whilst improving efficiency of production. This literature review aims to discuss exogenous enzymes, how they are used in poultry diets, their modes of action and their effects.

2.1.1 The South African poultry industry

South Africa is classified as a developing country with a large portion of the population living below the breadline. An accessible and affordable protein source is crucial for the nutrition of the population. During 2017, the total consumption of poultry meat and eggs was 2.649 million tonnes; 80.9% more than the combined 1.464 million tonnes of beef, pork, mutton and goat consumed over the same period (DAFF, 2017). According to the South African Poultry Association (SAPA), 20% of the total gross value of the agricultural industry stems from the poultry industry. SAPA also stated that more than 110 000 jobs are created both directly and indirectly in this industry. In order for the poultry industry to remain a crucial part of the agricultural industry and to keep supplying an affordable protein source to the population, it is essential that the production of poultry is as efficient as possible, and that the poultry industry evolves to conquer the numerous challenges it faces. One such challenge is the rising cost of raw materials and the inconsistent supply of high-quality feed ingredients.

Feed accounts for 60 to 70% of the total production cost in the production cycle of animal production systems (Abdollahi *et al*., 2013). Thus, the profitability and success of the production cycle can be greatly influenced by the relative feed cost and nutritive value (Khusheeba *&* Sajid, 2013). South Africa is currently the largest producer of animal feeds in Africa. In 2016, the combined production of poultry and swine feed amounted to 70% of the total feed manufactured (FAO, 2011). Apart from the monetary costs of farming, the cost of farming on the environment must also be taken into account as consumers become more conscious.

Given the above information, it is easy to understand the importance and relevance of the poultry industry in feeding the nation and contributing to the economy. Due to the large

role that feed plays in the poultry industry, it is an aspect which cannot be overlooked and should be further investigated.

2.1.2 **Standard broiler diets used in South Africa**

The composition of a broiler diet varies depending on where in the world it is manufactured and the raw materials that are available in that region. A typical South African broiler diet has a maize-soya base with maize meal and soya oil cake often contributing more than 75% of the total volume of ingredients. The majority of the energy derived by the broilers from their diet is from the starch found in cereal grains present in the diet (Stefanello *et al*., 2016), i.e. maize in South Africa. Soybean meal is frequently the main protein source in broiler feed due to its relatively high protein content and a favourable amino acid profile. However, its contribution towards dietary energy in terms of apparent metabolisable energy (AME) is low and inconsistent due to the high levels of NSPs (Vahjen *et al*., 2005). Apparent metabolisable energy is the difference between the gross energy present in feed and the amount of gross energy excreted in faeces. The addition of phytase to commercial broiler diets is standard practice in South Africa. Maize contributes approximately 690 g/kg of energy, which equates to approximately 65% of the total AME of the diet. However, maize also contributes about 68-94 g/kg of NSP with some studies reporting a total NSP value of up to 97 g/kg of maize (Stefanello *et al*., 2016). When these values are compared to soybean meal it is apparent that the values of soybean meal show greater variation and have higher NSP values, with reports of up to 220 g/kg NSPs in soybean meal. This is 55% higher than the NSP contribution from maize (Vahjen *et al*., 2005; Rios *et al*., 2017). It should be noted that a large portion of the variation seen in energy and nutrient utilisation of feed by broilers is due to the soybean meal in the feed (Stefanello *et al*., 2016).

The digestibility of raw feed materials is dependent on various factors such as inherent chemical properties of the raw material and how the ingredients are processed. The digestibility of soybean in particular is dependent on how the ingredient is processed (Mahmood *et al*., 2017). Soybean meal must be cooked before added to a broiler diet to reduce the ANFs, such as trypsin inhibitors. Under-cooking soybean meal results in high levels of ANFs, whilst overcooking soybean meal will lead to a reduced protein digestibility due to the high temperature damaging the protein structure. It has hence been reported that pelleting of a diet may increase the protein digestibility of the feed, however, this is not always the case due to the Maillard reaction occurring during the pelleting process (Roza *et al*., 2018). The Maillard reaction is a chemical process which involves the joining of the azanide group of an amino acid with a reducing sugar in the presence of heat. This renders the amino acid

indigestible thereby reducing the overall digestibility of the raw material and negatively influencing animal performance. Lysine is particularly susceptible to this process as it has an exposed azanide group. Whilst lysine is not the first limiting amino acid in broiler diets, it is the second limiting amino acid. Feeding a pelleted diet to broilers is a standard procedure due to the various benefits such as an enhanced overall performance, reduction in the amount of feed wasted and a homogenous intake of nutrients (Roza *et al*., 2018).

When compared to other cereals sometimes included in broiler diets, such as wheat or barley, a maize-based diet has generally higher digestibility values due to the lower NSPs, which leads to a decreased diet viscosity (Rios *et al*., 2017). This has led to the majority of research aiming to alleviate the effect of NSPs within wheat-based diets, while research on maize-based diets is lacking.

Although the use of unconventional feed ingredients has not been a common practice in South Africa, there is a renewed interest in the field. These feedstuffs are often high in fibre and the bird is unable to adequately digest the fibre with its endogenous enzymes. Therefore, there is a need for exogenous enzymes to improve the digestibility of certain grains due to the enzyme's ability to degrade the NSP and other complex nutrients. The bird's inability to efficiently digest the various components of raw feed ingredients, in particular fibre, frequently presents itself as a challenge when formulating a dietary ration. Due to this inefficiency, there is a gap between the potential nutritive value of feed and the actual nutritive value of the feed derived by the animal (Khusheeba & Sajid, 2013). Given the discussed inefficiencies of broiler digestion, the need for exogenous enzymes becomes clear.

2.2 Exogenous enzymes

Enzymes act as catalysts to increase the rate of a reaction and or improve the efficiency of a process. Majority of enzymes are proteins and function by using the lock and key mechanisms thereby making them substrate specific. All animals produce their own enzymes known as endogenous enzymes, however, these are not always sufficient for the desired level of nutrient utilisation and performance. Therefore, enzymes are being produced and supplemented, which are known as exogenous or dietary enzymes. Ruminant animals possess the ability to utilise alloenzymes, enzymes differing by just one allele, from their microbiota giving them the ability to optimally digest complex feed. In contrast, monogastric animals lack the endogenous enzymes required to achieve optimal utilisation of dietary nutrients (Khusheeba & Sajid, 2013). Thus, the addition of exogenous enzymes for monogastric animals such as broilers is necessary to achieve high production rates. These

additional enzymes frequently increase the digestion of nutrients after consumption within the digestive tract, however they can also degrade certain components of the feed during feed processing (Khusheeba & Sajid, 2013). The role that exogenous enzymes play in increasing the level of available nutrients and decreasing the effects of anti–nutritional factors found in common feed ingredients is crucial and its importance is only expected to rise. It is now common practice to include exogenous enzymes in animal feed and this is frequently done by supplementing multiple enzymes in one diet often referred to as an enzyme cocktail (Mahmood *et al*., 2017).

There are reports dating back to as far as 1926 stating that feed enzymes were used commercially to enhance the level of available nutrients in the diet (Khusheeba & Sajid, 2013). It began with the patenting of the process to produce alpha-amylase from a specific fungus from the genus *Aspergillus* (Pariza *&* Cook, 2010). Interestingly, most commercially used exogenous enzymes are still from this same genus. In the past, the research focused mostly on the use of NSP degrading enzymes, such as xylanase, in conventional broiler diets (Choct, 2006). As most of this research was not conducted in South Africa, the use and acceptance of NSP degrading enzymes occurred first in barley-based diets and then in wheat-based diets (Bedford, 2003). However, information on how NSP degrading enzymes will perform in a maize-based diet is lacking. The original aim with NSP enzyme supplementation and use was to improve litter quality and overall performance of the broilers (Khusheeba & Sajid, 2013). By providing a dietary xylanase, researchers hoped to increase nutrient digestibility and increase gut viscosity which would lead to the aforementioned benefits.

More recently, dietary enzymes have been supplemented to enhance nutrient utilisation, thereby reducing the amount of nutrients excreted into the environment (Khusheeba & Sajid, 2013). This is beneficial in terms of the environmental impact of production being reduced as well as a decrease in the nutrients wasted. Thereby ensuring that diets are formulated in a cost-effective way, as a lower nutrient level can be provided to the bird without a significant decrease in performance (Stefanello *et al*., 2016).

Numerous enzymes have been found to enhance overall bird performance. The main enzymes used on an industrial level include cellulase, xylanase, phytase, protease, lipase and galactosidase as summarised in Table 2.1 (Khusheeba & Sajid, 2013).

Table 2.1. The main enzymes used in the broiler industry as well as their classification, production organisms and their function (Khusheeba & Sajid, 2013)

Enzyme Name	Classification	Production organisms	Function
α-Amylase	Carbohydrase	*Aspergillus spp., Bacillus spp., Rhizopus spp.*	Hydrolyses starch
β -Amylase	Carbohydrase	Barley Malt	Hydrolyses starch with production of maltose
Cellulase	Carbohydrase	*Aspergillus niger.*	Breaks down cellulose
α-Galactosidase	Carbohydrase	*Aspergillus niger, Morteirella vinaceae var Saccharomyces sp.*	Hydrolyses oligosaccharides
β-Glucanase	Carbohydrase	*Aspergillus spp., Bacillus spp.*	Hydrolyses B-glucans
Hemicellulase	Carbohydrase	*Aspergillus spp., Bacillus spp., Humicola sp., Trichoderma sp.*	Breaks down hemicellulose
Lactase	Carbohydrase	*Aspergillus niger, Aspergillus oryzae, Candida pseudotropicalis, Kluyveromyces marxianis.*	Hydrolyses lactose to glucose and galactose
β-Mannanase	Carbohydrase	*Aspergillus niger, Bacillus lentus, Trichoderma reeseic*	Hydrolyses beta-mannans
Pectinase	Carbohydrase	*Aspergillus aculeatus, Aspergillus niger, Rhizopus oryzae*	Breaks down pectin
Xylanase	Carbohydrase	*Aspergillus spp., Bacillus spp., Humicola sp., Penicillum sp., Trichoderma sp.*	Hydrolyses xylans
Lipase	Lipase	*Aspergillus niger, Candida sp., Rhizomucor sp., Rhizopus sp.,*	Hydrolyses triglycerides, diglycerides, and glycerol monoesters

Bromelain	Protease	Pineapple *(Ananas comosus)* stem and fruit	Hydrolyses proteins
Pepsin	Protease	Animal stomachs	Hydrolyses proteins
Protease	Protease	*Aspergillus niger, Aspergillus sp., Bacillus spp.*	Hydrolyses proteins
Trypsin	Protease	Animal pancreas	Hydrolyses proteins
Phytase	Phosphatase	*Aspergillus spp., Penicillium sp., Phytase canola, Pichia pastoris, Aspergillus niger, d-Escherichia coli.*	Hydrolyses phytate

The stability of an exogenous enzyme in feed is an area of concern. Most feed which is fed to poultry is heat treated and fed in a pellet form. It is therefore important that the exogenous enzyme is heat stable and able to withstand the pelleting process or the capability of the enzyme will be severely reduced. A potential solution to overcome this issue is to add enzymes in the liquid form after the feed has been pelleted. Poor homogeneity of the final product as well as a costly process and the need for specialised equipment can be problematic. Due to these problems, it is common practice to include the dietary enzyme prior to pelleting in a powder form as this is more economical and convenient (Marron *et al*., 2001). There are two main ways for nutritionists to include an enzyme to a diet. The first way is to simply add the enzyme "on top" without taking into account the effect that the enzyme will have on the nutrient value of the diet. This method often results in an improved performance, but at a greater dietary cost. The second way that nutritionists incorporate dietary enzymes into feed is to formulate them into the dietary ration using the enzyme's nutrient matrix. The dietary matrix of an enzyme provides the amount of expected nutrient release when that specific enzyme is added to feed. This method allows the nutritionist to decrease the nutrient level of the diet and therefore the cost of the diet whilst still maintaining bird performance. The second approach seems to offer greater benefits; however it is not always feasible due to the enzyme matrix not being known or the feed formulation program not having this feature. When either method is applied numerous benefits can be seen.

2.2.1 **Benefits of supplementing a dietary enzyme**

There are several reasons why a dietary enzyme is supplemented including (Khusheeba & Sajid, 2013; Stefanello *et al.,* 2016):

- To reduce the level of ANFs naturally found in the feed.
- To increase the availability of various feed components such as starch, proteins or minerals, by breaking down cell walls that encapsulate the nutrients.
- To increase the release of nutrients by degrading certain chemical bonds which are resistant to endogenous enzymes and digestive processes.
- To provide younger animals with an additional enzyme supply whilst their digestive tract is not yet fully developed.
- Improve the accuracy of feed formulations leading to less wasted nutrients and thus a reduced feed cost.
- To decrease the amount of natural variation seen within feed ingredients with regard to nutritive value as the dietary enzyme is able to compensate for any nutrient deficiencies in raw materials. This will assist with flock management as it will lead to more uniform birds.

- Enhance the general health of birds by decreasing general digestive upsets caused by improving fibre digestibility.
- Exogenous enzymes may cause reduced water content of excreta thus a reduced incidence of pasty vents and an improved overall bird wellness.
- Improve the overall digestibility of feed, reducing the amount of energy used for digestion. This allows for more energy to be partitioned towards growth and performance.
- The digesta viscosity will be reduced largely due to the enhanced ability to digest fibre. This is important for increasing the availability of fat-soluble fats.
- Improved broiler performance.
- Decrease environmental impact. Globally, the poultry industry produces a total of more than 10000 tons of manure per million birds which is high in nitrogen and can have harmful effects on the environment. With the use of dietary enzymes, less nutrients will be excreted by the birds thereby reducing the impact of poultry manure on the environment.

The supplementation of exogenous enzymes allows for a decrease in feed costs whilst maintaining broiler performance in terms of weight gain, feed efficiency and decreasing the impact of broiler production on the environment by minimising nutrient excretion (Stefanello *et al.,* 2016). However, these benefits cannot all be attained when only one exogenous enzyme is supplemented. More often than not, a greater benefit is seen when numerous enzymes are supplemented in the diet compared to a single dietary enzyme (Rios *et al*., 2017). Many commercially available enzymes are a blend of several different enzymes thus increasing their efficacy.

Enzymes are complicated and there are a plethora of factors that need to be taken into account when feeding them. As a result, not all the potential benefits are always seen when supplementing dietary enzymes.

2.2.2 Factors affecting the efficacy of exogenous enzymes

The list below represents factors which should be considered when supplementing a dietary enzyme:

1. The response to enzyme supplementation depends on the age of the bird. The age of the bird influences its response to feed additives. The younger the age of the bird, the less developed its digestive tract and the less able it is to digest complex feed ingredients such as non-starch polysaccharides (Marquardt *et al*., 1996; Rios *et al*., 2017). Therefore, younger birds are more susceptible to the detrimental effects of non-starch polysaccharides. Due to the bird's reduced digestive capabilities at a young age, exogenous enzymes may prove more beneficial in the diets of young chicks compared to older birds (Mahmood *et al*., 2017).

2. The dietary enzyme must consist of the correct spectrum of enzymes which are capable of targeting specific substrates in the diet which will neutralise the anti-nutritive effects (Khusheeba & Sajid, 2013). Therefore, making use of the correct type and concentration of enzyme is crucial. The appropriate enzyme or enzyme cocktail should be used given the diet being fed.

3. The activity of the exogenous enzyme should not be altered by the feed manufacturing process, the endogenous enzymes of the bird, the digestive processes feed undergoes, or the pH range found in the numerous digestive tract microenvironments. The exogenous enzyme should be stable and resistant enough to reach the target substrate without being altered (Khusheeba & Sajid, 2013; Ravn et al., 2018).

4. The response of an enzyme treatment also depends on the level of ANFs which are susceptible to enzymes (Sheppy, 2003; Stefanello *et al.,* 2016). Even within a given cereal, this level varies and so too will the response to the dietary enzyme. Birds on diets which contain lower levels of ANFs will experience a smaller enzyme response compared to birds on a diet with a higher level of ANFs.

5. The degree to which a response is observed is affected by the feed ingredients used, as individual ingredients respond to different enzymes and enzyme concentrations differently (Khusheeba & Sajid, 2013).

6. The amount of substrate available for the exogenous enzyme to act on influences the efficacy of the dietary enzyme (Selle *et al*., 2009). When a diet is deficient in nutrients a larger response to exogenous enzyme supplementation on production performance is seen. This is because there is a greater room for improvement. Thus, the effect of exogenous enzymes is often more apparent in nutrient deficient diets.

7. Making use of the correct dose of an exogenous enzyme is vital. An oversupply of a dietary enzyme could interfere with the animal's own endogenous enzyme capabilities rendering the exogenous enzyme detrimental to bird performance. On the other end of the spectrum, an undersupply of an exogenous enzyme may not be sufficient to see a result (Khusheeba & Sajid, 2013).

8. The type of animal will impact the effect of the exogenous enzyme. Ruminants are far more capable of digesting complex feed materials and therefore will not experience the same benefits to enzyme supplementation as a monogastric animal like a broiler (Pariza & Cook, 2010; Khusheeba & Sajid, 2013).

9. As mentioned, enzymes are active site specific and thus efficacy of the enzyme depends on how efficiently the enzyme and digestive process is able to degrade different substrates in a feed ingredient (Marquardt *et al*., 1996). The greater the ability of the enzyme to degrade the material, the more active sites will become available. In return for more active sites, greater enzymatic activity can occur.

The use of exogenous enzymes in broiler diets requires further research to fully understand the underlying mechanisms and results that can be achieved. For the development of new feed enzymes, it is suggested that the safety margin of the product should be relatively high. It is also important that the enzyme conforms to the relevant specifications with regards to chemical and microbial contamination (Stefanello *et al.,* 2016). The interaction of numerous enzymes supplemented in one diet should be further studied as well as the ability of enzymes fed in a typical South African maize soy-based diet. Finally, the composition of the microbial population in the chicken gut and how it is affected by enzyme supplementation and enzyme response should also be investigated (Stefanello *et al.,* 2016).

2.3 Non-starch polysaccharides

Non-starch polysaccharide (NSP) is a term used to describe complex carbohydrates which form the major part of dietary fibre and include: cellulose, pectins, glucans, xylans, inulin and chitin. The main NSPs present in the raw feed ingredients found in broiler feed includes: pectins, arabinoxylans and mixed link β-glucans. Arabinoxylans are one of the main NSPs found in all grains. It is well known that NSPs are found in plant cell walls and that these NSPs are considered as ANFs when present in broiler diets (Marron *et al*., 2001). Non-ruminant animals such as broilers are not capable of digesting NSPs by exclusively using endogenous enzymes (Vahjen *et al*., 2005; Lee *et al*., 2017). Common enzymes such as xylanase are often added to a broiler diet to combat the effects of non-starch polysaccharides (Stefanello *et al.,* 2016).

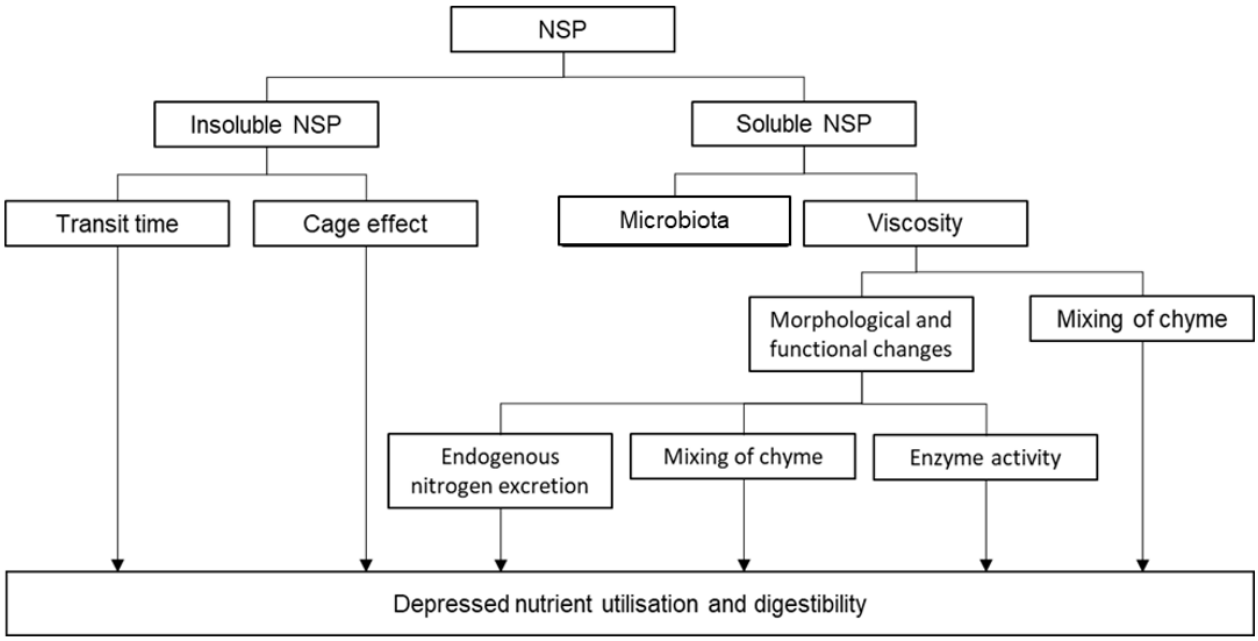

Figure 1. The main two modes of action of non-starch polysaccharides (NSPs) to decrease feed digestibility (adapted from Halas *&* Babinszky, 2014)

The mode of action of NSPs to depress nutrient digestibility and feed utilisation, is not yet fully understood, however, there are two main hypotheses as suggested in Figure 1. The first hypothesis refers to the fact that NSPs are part of the cell wall and therefore are involved in the containment of nutrients within the cell wall. The containment of the nutrients within the cell wall can be referred to as the "cage effect" (Amerah *et al*., 2008; Rios *et al*., 2017). A result of the cage effect is that the amount of nutrients and energy available to the bird is reduced. Thus, providing a dietary enzyme which targets NSPs leads to a greater release of energy and nutrients and has the potential to increase the nutritive value of the diet (Rios *et al*., 2017). This can lead to a reduced FCR due to the increase in availability of free sugars to the bird (Barekatain *et al*., 2013). In a typical maize-soya diet, the supplementation of exogenous

enzymes can increase the amount of energy available to the birds for utilisation by up to 1.88 MJ/kg (Stefanello *et al.,* 2016). The second hypothesis regarding the mode of action of NSPs is related to gut viscosity and gut microbiota. When soluble NSPs are digested, the viscosity in the gut increases (Amerah *et al*., 2008). This results in a decrease in the rate at which both nutrients and endogenous enzymes diffuse through the intestinal wall thereby reducing the effectiveness at which they are able to interact when at the mucosal surface of intestine. Due to the decreased efficacy at which diffusion occurs through the intestinal cell wall, the amount of fermentable material may increase. The fermentable material is digested by anaerobic microbes in the gut of the chicken therefore an increase in fermentable material will lead to a rise in the population of microbes and a change in the composition of the gut microbiome (Marron *et al*., 2001). This change in the microbes influences the morphology of the gut (Amerah *et al*., 2008). Apart from the morphological changes which occur, as the population of microbes increases, the competition for nutrients between the bird and the microbes will increase thereby leaving less nutrients available for the chicken itself to utilise (Marron *et al*., 2001).

Cereal grains consist of many layers. Arabinoxylans form a part of the endosperm's cell wall and surround the granule. Poultry are unable to naturally digest arabinoxylans thereby reducing the nutritive value of the cereal grain. Several methods have been introduced to overcome the effects of NSPs in broiler diets. One such way is the supplementation of vitamin C. Vitamin C can result in a decrease in gut viscosity of up to 20%, although it is instable and expensive (Marron *et al*., 2001). Therefore, supplementing an NSP degrading enzyme is preferable. Such an enzyme will decrease gut viscosity due to enhanced digestion of starch in the diet which has an effect on the microbes in the gut. Exogenous enzymes can be used to access intracellularly stored starch by breaking down oligosaccharides such as arabinoxylans. This allows pancreatic enzymes to act on the nutrients which would be otherwise encapsulated within the plant cell, releasing both oligosaccharides and monosaccharides which can be directly or indirectly used by the bird. These simple sugars can either be directly absorbed and utilised through the gut or indirectly absorbed and utilised by first being degraded to volatile fatty acids by intestinal microbes. The volatile fatty acids stimulate a feedback mechanism which extends digesta retention time thus increasing energy absorption. The overall enhancement of performance of broilers often shares a direct relationship with an increase in the availability of nutrients and energy. Thus, it can be said that NSP degrading enzymes have the potential to improve starch digestion and performance (Marron *et al*., 2001; Stefanello *et al.,* 2016).

Another benefit which comes with the breaking down of NSPs is that the moisture level of the excreta is reduced. When NSPs are partially hydrolysed their ability to absorb water is decreased. This leads to a decrease in the moisture content of excreta which is significant as it reduces paste venting thus lowering the incidence of spoiled eggs in layers and mortalities of young broilers (Stefanello *et al.,* 2016). The decrease in moisture also leads to less wet litter which can reduce the incidence of several welfare related matters such as hock burn and breast blisters.

Many of the oligosaccharides which are present in soybean meal cannot be hydrolysed by endogenous enzymes as they belong to alpha-galactosidase (Vahjen *et al*., 2005). Therefore, the NSPs need to be degraded by alternative methods such as mechanical breakdown in the gizzard, degradation due to microbe actvity in the gut or by exogenous enzyme supplementation (Lee *et al*., 2017).

2.4 Protease

2.4.1 Protein digestion

Digestion of protein in poultry begins in the proventriculus and is strictly regulated by pepsin. The proteins are degraded into peptides which are further degraded by endogenous enzymes such as trypsin in the small intestine of the bird. The activation of trypsinogen is crucial to the release of other endogenous pancreatic enzymes (Walk *et al*., 2019). The digestive tract of poultry encompasses a wide range of pH values and consists of an acid, neutral and alkaline microenvironment. Thus, providing a feed additive which is capable of working in all three environments could be beneficial. It was previously believed that the microbiome of poultry plays an insignificant role in the digestion of feed. However, as more research becomes available its importance is becoming clear. There are several factors which are capable of influencing the microorganisms in the caeca of the bird, including cereal type and dietary enzyme supplementation. These two factors show the potential to decrease the population size of the potentially pathogenic *Enterobacteriaceae* (Stefanello *et al.,* 2016).

All animals make use of endogenous enzymes when digesting feed. These enzymes are either produced by the intestinal microbes of the bird or by the bird itself. Because a large portion of protein is excreted/undigested, it can be said that digestion by endogenous enzymes is not an optimally effective process. Thus, there is room for improvement in terms of protein catabolism, hence the need for exogenous enzymes to achieve optimal digestion (Stefanello *et al.,* 2016). Therefore, exogenous enzymes capable of breaking down CP should result in more optimal protein digestion thus leading to several benefits including: enhanced bird

growth, better growth parameters and less nitrogen excreted into the environment (Mahmood *et al*., 2017).

Protein forms a crucial part of a broiler diet. However, both under and over supplying protein can have detrimental effects. Diets which contain high levels of CP are both financially and metabolically expensive. When excessive levels of CP are fed, the bird must excrete this into the environment in the form of uric acid which also has a negative impact on the environment. Through the use of studies, specifically *in vivo* studies, the amount of dietary CP required for broilers can be calculated accurately to supply the correct amount to the bird and avoid any negative repercussions due to the incorrect nitrogen level being fed. Through determining the correct amount of CP needed, the cost of the feed may decrease. However, broilers require a minimum amount of CP to perform optimally, therefore when insufficient amounts of CP are fed, the performance of a bird is negatively influenced (Mahmood *et al*., 2017).

2.4.2 **Protease mode of action**

Protease is an enzyme which improves the digestibility and nutrient retention of the diet by hydrolysing proteins and peptides found in specific ingredients into more simple and easily digestible peptides and amino acids to be absorbed in the small intestine (Walk *et al*., 2019; Xu *et al.,* 2017). Amino acid utilisation and digestion is improved due to the hydrolyses which occurs (Rios *et al*., 2017). The exact mode of action of protease remains unknown and is an area for further research. It is thought that the supplementation of protease reduces the amount of endogenous enzymes required by the bird thereby reducing the maintenance requirements of the bird. The reduction in maintenance requirements could be the reason for enhanced bird performance (Barekatain *et al*., 2013). Protease supplementation has been known to reduce the incidence of coccidiosis infections by increasing the mucous layer along the lining of the gut. The increased mucous layer aids in gut protection against pathogens. There is concern that this thickened mucosal layer will result in a reduction of bird performance due to a reduction in the absorption of nutrients (Peek *et al*., 2009).

The response to an exogenous protease supplementation is well recorded, however literature offers contradictory results. There is a theory that exogenous enzymes may influence the birds own natural ability to produce endogenous enzymes therefore negatively impacting the bird's growth and performance (Mahmood *et al*., 2017). Yet, numerous studies have shown that supplementation with an exogenous protease results in an enhanced nutrient digestibility and thus performance in poultry (Xu *et al.,* 2017). Walk *et al*. (2019) reported that broiler growth

and nitrogen digestibility was not significantly enhanced due to protease supplementation and reported a decrease in the growth performance of broilers when the broilers were supplemented with a novel protease. It is not yet fully understood how exogenous protease supplementation influences feed intake, digestion and hormones in the digestive tract and this depends on several factors such as the specific substrate available, the type and dose of protease supplemented and finally the level of available nutrients in the diet (Walk *et al*., 2019). It is thought that exogenous protease works in conjunction with the bird's endogenous enzymes to hydrolyse proteins, especially those proteins which would be otherwise unresponsive to the endogenous enzymes (Mahmood *et al*., 2017). Protease is often supplemented with various other exogenous enzymes and forms part of an enzyme cocktail, however the interacting effects are poorly understood (Barekatain *et al*., 2013).

2.4.3 **The impact of adding protease to poultry feed**

Protease has been shown to have larger effects when the feed ingredients are of a poor quality as there is more room for improvement such as in the case of poorly processed soybean (Barekatain *et al*., 2013; Mahmood *et al*., 2017). It has been reported that when birds are fed an amino acid deficient diet, they tend to show an increased feed intake to compensate for the lack of amino acids in the diet (Walk *et al*., 2019). Thus, it is difficult to conclude with certainty that if broilers have an increased feed intake when supplemented with protease, whether a higher feed intake is due to protease supplementation or the amino acid deficient diet. Walk *et al*. (2019) reported an increase in the level of protease activity when a grain protein such as soybean meal was present. This could indicate that, as previously mentioned, the specific type of substrate available influences the efficiency of dietary protease. A higher dose of dietary protease has not proven to improve growth performance of broilers (Walk *et al*., 2019). Xu *et al.* (2017) reported several findings as a result of a multi-component protease being supplemented including: an increased villus height indicating an enhanced absorption ability in the intestine; a reduction in the crypt depth in all three regions of the small intestine; an improved meat quality due to a decreased breast muscle drip loss, as well as a significantly increased breast muscle weight and an increase in the overall slaughter weight of the birds. Barekatain *et al*. (2013) found a significant decrease in feed intake and an increased body weight gained at 21 days-of-age when broilers were supplemented with exogenous protease. The performance of birds is thought to improve when supplemented with protease partially due to the increased ability to digest both proteins and fat, and thus the AME of the feed (Mahmood *et al*., 2017). Mahmood *et al*. (2017) found broilers that were supplemented with 0.02% exogenous protease while being fed a diet containing 20% CP showed significantly enhanced feed intake, an increased body weight gain and percent carcass yield, but the FCR

of the birds were not affected, highlighting the somewhat inconclusive results for protease supplementation in broilers.

It has been frequently reported that an improvement in ileal amino acid digestibility is seen when birds are supplemented with exogenous enzymes. This has several benefits including an enhanced growth performance, reduction in the level of nitrogen excreted thus reducing the environmental impact of broiler production as well as improved gut health (Walk *et al*., 2019). Marron *et al*. (2001) found no significant improvement in feed intake or live weight gain but the FCR was significantly reduced. However, it is also frequently observed that results from digestibility studies indicate an enhanced broiler performance in terms of nutrient digestion and energy utilisation and not in terms of animal performance parameters such as growth and feed efficiency (Stefanello *et al*., 2016). A protease trial done by Angel *et al*. (2011) found improved performance when birds were fed a nutrient deficient diet supplemented with 200 mg/kg of protease. The supplementation of protease allowed the birds on the protein deficient diet to perform to the same standard as those on the positive control diet.

Xu *et al. (*2017) found that uncoated protease had a release rate of below 35%, however, when a coated protease was supplemented, the release rate in the small intestine increased to 85%. It was also reported that the retention rate of the coated protease in the stomach proved to be over 90%. Thus, it can be concluded that when a protease is coated it results in increased retention rates due to a less enzymatic degradation occurring in the stomach which, likely, leads to increased nutrient retention and possible enhanced performance.

2.5 Xylanase

Xylanase has the ability to degrade both soluble and insoluble non-starch polysaccharides (Selle *et al*., 2009; Moss *et al*., 2018). This enzyme has been frequently used for more than 20 years in the broiler industry. The use of xylanase in broiler diets is well reported, often showing beneficial effects on broiler performance (Marron *et al*., 2001; Cowieson *et al*., 2010; Barekatain *et al*., 2013). This is thought to be through the release of nutrients trapped in the cell wall and a reduced gut viscosity allowing endogenous enzymes to reach nutrients more efficiently. The increased gut viscosity associated with high levels of NSPs reduces the transit rate of digesta, interferes with the normal functioning of the endogenous enzymes, reduces nutrient absorption through the gut wall and can increase the amount of bacterial fermentation in the gut (Lee *et al*., 2017). Supplementation of dietary xylanase can have several benefits, but mainly it is believed that hydrolysis of the cell wall leads to enhanced access to intracellular cell contents/portions and therefore improves

nutritional value of the diet (Stefanello *et al.,* 2016). A further benefit includes a reduction of the level of ANFs typically found in a standard South African broiler diet. Rios *et al*. (2017) found that when broilers are supplemented with an exogenous xylanase when being fed a maize-soya diet there was an enhanced ileal digestibility of dry matter and amino acids, an improved FCR and an overall improvement in the total amino acid digestibility. Lee *et al*. (2017) also noted an enhanced starch, fat and protein digestibility due to the reduced gut viscosity. Barekatain *et al*. (2013) also concluded that xylanase supplementation is capable of significantly reducing the FCR of the broilers whilst, reducing the concentration of NSPs found in the ileum due to insoluble arabinoxylans. However, effects are not always noted when exogenous xylanase is supplemented (Amerah *et al*., 2008). Poultry do not possess the necessary endogenous enzymes required to break down arabinoxylans (Marron *et al*., 2001). The extent of the effect of xylanase is dependent upon the concentration of NSP occurring in the broiler diet and the digestibility of the diet (Cowieson *et al*., 2010). The beneficial effects of xylanase in wheat-based diets are well documented, however the effect of xylanase in a maize-soya based diet is not as large when compared to the effect that xylanase has when supplemented in a wheat-based diet (Lee *et al*., 2017). This may be due to the fact that wheat has a higher NSP content than maize. Despite this, broiler producers are still interested in making use of xylanase to maximise their profits, therefore it is necessary to determine the required dose of enzyme in a maize soya-based diet where the concentration of NSPs is low as typically used in South Africa (Cowieson *et al*., 2010).

When supplementing glucanase with xylanase a synergistic response can be expected. The benefits are a reduced FCR as well as an enhanced ileal digestibility (Stefanello *et al.,* 2016). Moss *et al*. (2018) found that when feeding broilers an atypical diet consisting of canola meal, the dietary supplementation of both phytase and xylanase resulted in a two-fold factor increase in phosphorus digestibility. This is known as a synergistic response, when the effect of two enzymes in combination is greater than the sum of the individual effects of the enzymes. This is due to the increased amount of substrate available for the phytase when the plant cell walls are being degraded by xylanase (Selle *et al*., 2009).

More recent studies show a trend of the beneficial effects of xylanase decreasing. This could be due to the birds themselves become more efficient and adapted to a high grain diet (Cowieson *et al*., 2010).

2.6 Interaction of xylanase and protease

Exogenous enzymes are often fed in combination with various different enzymes. Literature offers contradictory results in terms of the effect that protease and xylanase have on nutrient digestibility and broiler performance. When both xylanase and protease are supplemented, the ileal amino acid digestibility is not enhanced (Cowieson *et al*., 2010). This lack of a synergistic effect between xylanase and protease could be due to insufficient substrate for the enzymes to both work efficiently (Cowieson *et al*., 2010). As previously mentioned, the amount of substrate available for an exogenous enzyme impacts its efficacy. Barekatain *et al*. (2013) found that protease has a more beneficial effect on apparent ileal digestibility of amino acids when used alone leading to the conclusion that perhaps there is a negative interaction between protease and xylanase. There are also reports in literature stating beneficial responses to an enzyme cocktail containing both xylanase and protease. Cowieson *et al*. (2006) observed that the supplementation of both xylanase and protease as part of an enzyme cocktail resulted in enhanced broiler performance even when broilers were fed a diet that was sufficient in all nutrients.

This is an area for further research as protease is often supplemented in conjunction with various other exogenous enzymes but the mode of action of protease is not yet clearly defined and therefore the interaction between the various exogenous enzymes is poorly understood.

2.7 Conclusion

Exogenous enzymes have been used for decades in broiler nutrition and have the potential to allow for the formulation of more cost effective, digestible, and environmentally friendly feed (Engberg *et al*., 2004; Lemme *et al*., 2004; Khusheeba & Sajid, 2013). However, there is a gap in research on enzyme efficacy in maize-based diets which differ vasty from the well-researched wheat-based diets. There are also a vast number of factors which can affect the efficacy of the enzyme such as the amount of substrate available for the enzyme, the level of ANFs in feed as well as the specific feed ingredients used which need to be taken into account when supplementing enzymes in broiler feed (Selle *et al*., 2009; Rios *et al*., 2017; Stefanello *et al.,* 2016). Should the feed which is being fed to the broilers already be highly digestible with low level of ANFs the impact of an exogenous enzyme will be severely limited. More recent studies show a trend that the beneficial effects of certain exogenous enzymes are decreasing. This could be due to the birds themselves becoming better adapted to a typical broiler diet which is often high in grain (Cowieson *et al*., 2010). Therefore, the context in which exogenous enzymes are used is crucial to achieve the potential benefits.

CHAPTER 3. MATERIALS AND METHODS

The purpose of these studies was firstly to determine the optimal dose of coated protease and secondly to determine the effects of xylanase and protease supplementation on broiler performance, both alone and in combination. All experimental procedures for both the digestibility trial and the broiler performance trial were approved by the University of Pretoria's Animal Ethic Committee (NAS479/2019) proving both studies to be humane and ethical.

3.1 Protease digestibility trial

3.1.1 *Animals and experimental design*

A total of six hundred day-old Ross 308 chicks which were in good health were sourced from Eagles Pride Hatchery (Gauteng, South Africa). Day-old chicks were individually sexed using the feather sexing method and only male chicks were selected. The average body weight of the day-old chicks was 36.82 g at placement and the parent flock was 33 weeks-of-age.

This trial was conducted at the University of Pretoria's Experimental farm in Hatfield, Pretoria, at the Poultry Unit (Pretoria, South Africa). One broiler house was used throughout the trial and the environmental conditions of the close-sided house were carefully monitored by a SKOV environmental control system. The house was preheated to 35°C prior to the birds' arrival to minimise the stress of placement. Each chick received a neck-tag with a unique number on day 0 before placement for identification. The pre-experimental/rearing period occurred from day 0 until day 18. During this period, birds were placed into mesh wire pens with a surface area of 2.25m^2, concrete flooring and covered with fresh pine shavings. Chicks were randomly allocated to 24 of these pens with 25 chicks in each pen. All pens contained five nipple drinkers and a tube feeder. For the first seven days, an additional square of chick paper, a bell drinker and tray feeder was placed in each pen to ensure that chicks had easy access to feed and water. Water and feed was available *ad libitum* throughout the trial. Birds were vaccinated for Gumboro disease at 13d and for Newcastle disease at 16d. Vaccines were administered through the drinking water by using fountain drinkers. Before the birds were vaccinated, the water lines were lifted for approximately one hour to ensure sufficient intake of the vaccine. A commercial lighting and temperature programme was followed throughout the trial (Aviagen, 2018).

1. Lighting program

1. 23 hours light, 1-hour dark from day 0 until day 7
2. 21 hours light, 3 hours dark from day 8 until day 14
3. 18 hours light, 6 hours dark from day 15 until the end of the trial

2. Heating program
    1. Pre-heated to 35°C, with a floor temperature of 32°C
    2. 33°C on day 1 and 2,
    3. 31°C on day 3 and 4,
    4. then a 3°C decrease every 2 days until 18°C.

The temperature was controlled by automatic heaters to keep the birds in their desired comfort zone as indicated by the Aviagen Ross management guide (Aviagen, 2018).

On day 17, all birds were individually weighed and body weight per bird was recorded. On the following day (day 18), 480 broilers with body weight closest to the population average (686.33 g) were moved to 60 metabolic cages, with eight broilers per cage. These birds were randomly assigned to one of six dietary treatments.

3.1.2 **Dietary treatment and feed analysis**

The Ross 308 broilers were fed a two-phase feeding program: a maize-soya based starter diet from day-old until seven days-of-age and a grower diet from eight days to eighteen days-of-age (Table 3.1). The feed was received as mash and a pellet for the starter and grower diet, respectively. Once birds entered the experimental period at day 18, a typical maize-soya diet was fed to simulate commercial conditions, with the only difference between treatments being the concentration of the protease enzyme. The nutrient composition of the experimental diets were the same as for the grower diet fed during rearing from eight to eighteen days-of-age. All diets contained fine phytase (1000 FTUs) as this is standard practice for commercial broiler producers. All treatment diets contained titanium dioxide at 4 g/kg feed as an indigestible marker. Five treatment diets contained the test protease in increasing concentrations (0, 150, 200, 300, 400 mg/kg) and the sixth treatment contained another commercial protease product, RONOZYME® ProAct, as positive control at manufacturers recommended inclusion level (200 mg/kg). The feed ingredient composition of the experimental diets are shown in Table 3.2. Proximate analysis of feed samples was conducted according to standard methods described by the Association of Official Analytical Chemists (AOAC, 2000). Feed was further analysed for concentrations of titanium dioxide, CP and DM content.

Dry matter was determined by placing 5.0 grams of feed sample into crucibles. The crucibles had been previously oven dried for one hour at 105˚C. The crucibles were then placed into an oven and left to dry overnight (24 hours) at a temperature of 105˚C. After being oven dried, the samples were cooled in a desiccator and weighed. This method is according to AOAC (2000), Official Method of Analysis 934.01 and the DM percentage was calculated using the below formula:

$$\%\, DM = \frac{mass\ of\ crucible\ and\ sample\ after\ drying}{mass\ of\ crucible\ and\ sample\ before\ drying}\ x\ 100$$

The Dumas method was used to determine the CP following the AOAC's Official Method of Analysis (AOAC, 2000, Official Method of Analysis 968.06). The percentage of CP was determined by multiplying the nitrogen content by using a conversion factor of 6.25.

The moisture percentage was determined by subtracting the DM percentage from 100.

The titanium marker's concentration was determined by making use of an inductively coupled plasma optical emission spectrometer (Ravindran *et al*., 2017). This method was followed to determine the concentration of titanium in feed, faecal and ileal samples.

Table 3.1. The feed ingredient composition (%) and calculated nutrient composition (%) of broiler diets fed during the rearing of chicks from 0-18 days-of-age in the protease digestibility trial

Feed ingredient composition (%)	Starter	Grower
Maize	52.30	55.10
Soya oil cake (46.5%)	24.10	17.60
Full fat soya	10.80	14.30
Soya oil (crude)	3.50	3.50
Sunflower oil cake (34.7%)	3.00	3.00
Gluten 60	2.31	2.72
Lysine HCl (78%)	0.34	0.33
Methionine (DL 98%)	0.30	0.27
Threonine (98%)	0.13	0.11
Valine	0.02	0.02
Limestone	1.51	1.43
Dicalcium phosphate	0.67	0.60
Salt	0.25	0.26
Sodium bicarbonate	0.32	0.32
Broiler premix	0.25	0.25
Toxfin dry	0.10	0.10
Salinomycin 12%	0.05	0.05
Zinc bacitracin 15%	0.07	0.07
AxtraPHY 100g (10 000ftu) tpt	0.01	0.01
Nutrient composition (%)	**Starter**	**Grower**
Dry matter	89.90	89.70
AME broiler (MJ/kg)	12.50	12.80
Crude protein	22.20	20.80
Crude fat	7.56	8.29
Crude fibre	3.34	3.32
Ash	5.85	5.51
Calcium	0.88	0.83
Total phosphorus	0.67	0.64
Digestible phosphorus	0.40	0.38
Sodium	0.19	0.19
Chloride	0.25	0.25
Potassium	0.95	0.88
Calcium: available phosphorus	2.20	2.18
Dietary cation anion balance (mEq/kg)	234.77	216.00
Digestible lysine	1.20	1.10
Digestible methionine	0.60	0.56
Digestible cysteine	0.29	0.27
Digestible methionine + cysteine	0.89	0.83
Digestible threonine	0.81	0.74
Digestible tryptophan	0.21	0.19
Digestible isoleucine	0.81	0.75
Digestible arginine	1.28	1.18
Digestible valine	0.90	0.83

Table 3.2. The feed ingredient composition (%) of broiler diets fed during the protease digestibility trial (18-23 days-of-age)

Name	No protease [1]	Kemzyme (150 mg/kg) [2]	Kemzyme (200 mg/kg) [3]	Kemzyme (300 mg/kg) [4]	Kemzyme (400 mg/kg) [5]	Proact (200 mg/kg) [6]
Maize	55.10	55.10	55.10	55.10	55.10	55.10
Gluten 60	2.72	2.72	2.72	2.72	2.72	2.72
Soya oil (crude)	3.50	3.50	3.50	3.50	3.50	3.50
Soya oil cake (46.5%)	17.60	17.60	17.60	17.60	17.60	17.60
Full fat soya	14.30	14.30	14.30	14.30	14.30	14.30
Sunflower oil cake (34.7%)	3.00	3.00	3.00	3.00	3.00	3.00
Lysine HCl(78%)	0.33	0.33	0.33	0.33	0.33	0.33
Methionine (DL 98%)	0.27	0.27	0.27	0.27	0.27	0.27
Threonine (98%)	0.11	0.11	0.11	0.11	0.11	0.11
Valine	0.02	0.02	0.02	0.02	0.02	0.02
Limestone	1.43	1.43	1.43	1.43	1.43	1.43
Dicalcium phosphate	0.60	0.60	0.60	0.60	0.60	0.60
Salt	0.26	0.26	0.26	0.26	0.26	0.26
Sodium bicarbonate	0.32	0.32	0.32	0.32	0.32	0.32
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25
Toxfin dry	0.10	0.10	0.10	0.10	0.10	0.10
Salinomycin 12%	0.05	0.050	0.05	0.05	0.05	0.05
Zinc bacitracin 15%	0.07	0.07	0.07	0.07	0.07	0.07
AxtraPHY 100g (10 000ftu) tpt	0.01	0.010	0.01	0.01	0.01	0.01
Titanium dioxide	0.30	0.30	0.30	0.30	0.30	0.30
Kemzyme protease		0.015	0.020	0.030	0.040	
Proact protease (DSM)						0.02

[1] Negative control (NC) (no protease added)

[2] NC + 150 mg/kg Kemzyme Protease

[3] NC + 200 mg/kg Kemzyme Protease

[4] NC + 300 mg/kg Kemzyme Protease

[5] NC + 400 mg/kg Kemzyme Protease

[6] NC + 200 mg/kg Proact Protease (DSM)

3.1.3 **Sampling and processing**

Representative feed samples were collected daily during the experimental period (day 18-22 of age) by pooling of a grab sample from fresh feed being fed to each cage per treatment. On day 22, grab faecal samples were collected from the trays beneath each cage. The faecal samples were frozen immediately after collection in marked and sealed bags. Frozen faecal samples were freeze-dried, ground using a 1 mm sieve (Retsch, ZM 200) and stored in airtight containers before analysis for DM, the titanium marker, CP and moisture. The methods used for faecal analysis were the same methods followed as the methods described for feed analysis.

At 23 days-of-age all birds were euthanised using a two-step gas method to prevent struggling and flapping of wings. First the birds were exposed to a gas mixture (30% O_2, 35% CO_2, 35% N) for 4 minutes. Once rendered unconscious, the birds were exposed to pure CO_2 for 2 minutes or until confirmed dead. The small intestine was immediately exposed after death had been confirmed. The small intestine was placed on an ice-cold granite slab to cease enzymatic and microbial digestion. Illeal samples were collected from the lower (distal) half of the ileum with the ileum being defined as the portion of the small intestine from Meckel's diverticulum to 40 mm proximal to the ileo-caecal junction. Digesta was gently flushed from the ileum using distilled water. The digesta from all birds per cage was pooled together into one marked container. The container was kept on ice during collection of samples from all eight birds to cease enzymatic and microbial activity, where after the container was placed in a freezer. Frozen digesta samples were freeze-dried, ground using a 1 mm sieve (Retsch, ZM200) and stored in airtight containers before analysis for DM, the titanium marker, CP, amino acids and moisture.

The DM, CP, moisture and titanium levels were determined by making use of the methods previously described for the feed analysis.

The amino acid digestibility was determined by the bellow formula:

$$AA\ ileal\ digestibility\ coefficient = 1 - \frac{\left(\frac{AA}{Ti}\right) i}{\left(\frac{AA}{Ti}\right) d}$$

Where:

(AA/Ti)d = ratio of AA to titanium in diet

(AA/Ti)i = ratio of AA to titanium in ileal digesta

3.1.4 Statistical analysis

The trial was designed as a complete randomised block design with six treatments and 10 cage replicates per treatment. The facility was divided into 10 blocks and each treatment had one replicate per block. Blocking was done to minimise possible environmental effect on variance between treatments.

SAS (Statistical Analysis System) software was used to statistically analyse data such as treatment means. Significant treatment means will be separated using Tukey HSD test at $P<0.05$. Data was analysed by ANOVA using a general linear model (GLM) as described by the following equation:

$$Y = \mu + Ti + Lj + TLij + eij$$

Where:
Y= variable studied during the period
μ=overall mean of the population
Ti = effect of the ith treatment
Li = effect of the jth source
TLij = effect of the kth level
eij = error associated with each Y

Means and standard errors were calculated and the significance of difference ($P < 0.05$) between means determined by Fisher's Test (Samuels, 1989).

3.2 Protease and xylanase performance trial

3.2.1 Animals and experimental design

A total of 2100 day-old male Ross 308 broiler chickens were reared in pens with concrete floors. All birds were sourced from a commercial hatchery in Gauteng (Eagles Pride Hatchery, South Africa) and only birds in good health were used. The trial was conducted at the University of Pretoria at the broiler unit in fully environmental controlled housing. The house was fitted with a SKOV computer system which strictly regulates the environmental conditions in the house by controlling the electrical heaters and fan speed. Housing and care of the birds was done in such a way as to represent commercial conditions. Prior to placing the day-old chicks, the broiler house was washed, disinfected, and pre-heated to the comfort zone of the chicks of 35°C ambient temperature and at least 32°C litter (floor) temperature. The broiler house was divided into eight blocks, with one repetition of each treatment in each

block. Twenty three chickens were placed in each pen. All birds were placed in mesh wire pens with each pen measuring 1.5 m x 1.5 m. As 23 chicks were placed in each pen, the stocking density was 19 birds/m^2.

Clean pine shavings were used on the concrete floor of the pens to absorb waste and to assist in heat insulation. All pens contained five nipple drinkers and a tube feeder. For the first seven days, an additional square of chick paper, a fountain drinker and tray feeder were placed in each pen to ensure that chicks had easy access to feed and water. Water and feed were available *ad libitum* throughout the trial. On day 13, all birds were vaccinated for New Castle disease through the drinking water. On day 19, all birds were vaccinated for Gumboro disease. This vaccination was also administered through the birds' drinking water.

A commercial lighting and temperature programme was followed throughout the trial as recommended in the production manual for Ross 308 broilers (Aviagen, 2018).

Lighting program

1. 23 hours light, 1-hour dark for the first week
2. 21 hours light, 3 hours dark for the second week
3. 18 hours light, 6 hours dark for the remainder

Heating program

1. Pre-heated to 35°C, with a floor temperature of 32°C
2. 33°C on day 1 and 2,
3. 31°C on day 3 and 4,
4. then a 3 °C decrease every 2 days until 18 °C.

3.2.2 **Dietary treatment and feed analysis**

A total of three feed phases were fed throughout the duration of the trial. The chicks received a maize-soya based starter diet from day-old until 14 days-of-age and a grower diet from 15 days-of-age until 28 days-of-age. A finisher diet was fed from 29 days until the end of the trial, 35 days-of-age. The starter diet was fed as crumbles whilst the grower and finisher diet were fed as pellets. There was a total of 11 treatments. A typical maize soya diet was fed to simulate commercial conditions. The feed ingredient composition and calculated nutrient composition are shown in Table 3.4, Table 3.5 and Table 3.6. A positive control (PC) diet was formulated using standard commercial energy and amino acid levels. Three negative control (NC) diets were included either with 0.418 MJ/kg less energy (NC1), 4% lower amino acid levels (NC2) or lower in both energy and amino acid levels (NC3). Three concentrations of xylanase (Xygest HT) (10 mg/kg, 15 mg/kg and 30 mg/kg) were added to NC1, Kemzyme protease was added to NC2 at two concentration levels (150 mg/kg and 300 mg/kg) as well

as Proact protease (200 mg/kg). Both Kemzyme (300 mg/kg) and Xygest HT (30 mg/kg) were added to NC3 to form the last treatment (Table 3.3).

Table 3.3. Treatment diets and corresponding treatment

Treatment	Treatment description
PC	Positive Control
NC1	Negative Control 1 (PC-0.418 MJ/kg)
Xygest HT(10 mg/kg)	NC1 + 10 mg/kg Xygest HT
Xygest HT(15 mg/kg)	NC1 + 15 mg/kg Xygest HT
Xygest HT(30 mg/kg)	NC1 + 30 mg/kg Xygest HT
NC2	Negative Control 2 (NC2) (PC-4% amino acids)
Kemzyme (150 mg/kg)	NC2 + 150 mg/kg Kemzyme Protease
Kemzyme (300 mg/kg)	NC2 + 300 mg/kg Kemzyme Protease
Proact (200 mg/kg)	NC2 + 200 mg/kg DSM Proact Protease
NC3	Negative Control 3 (NC3) (PC-0.418 MJ/kg and -4% amino acids)
Kemzyme (300 mg/kg) + Xygest HT(30 mg/kg)	NC3 + 300 mg/kg Kemzyme Protease + 30 mg/kg Xygest HT

All diets contained fine phytase (1000 FTUs) and a coccidiostat (salinomycin at a rate of 500 mg/kg of feed) as this is standard practice for commercial broiler producers. An antibiotic growth promoter was not included, despite being standard practice for commercial broiler production in South Africa, to align the study to European regulations.

Two samples from each type of feed and batch were taken. One set was dispatched to an external laboratory and the second was sent to Kemin Industries. Feed samples were analysed by wet chemistry according to standard methods described by the Association of Official Analytical Chemists (AOAC, 2000) to determine the following components: DM content, CP content, crude fibre, ether extract, calcium, phosphorus, neutral detergent fibre and acid detergent fibre as can be observed in Table 3.7, Table 3.8 and Table 3.9.

The DM content and CP content were determined by using the Official Methods of Analysis as described previously.

The ash content of the feed was obtained according to AOAC's Official Method of Analysis (AOAC., 2000, Official Method of Analysis 942.05). Once the weight of the oven dried crucibles with samples was known, the crucibles and dried samples were placed in a muffle

furnace to be incinerated for 2 hours at 250˚C and 4 hours at 600˚C. Thereafter, the crucibles and ash were left in the furnace another 2 hours to cool before being placed in the desiccator for at least 30 minutes to cool further and then weighed. The ash percentage was determined using the following formula:

$$\%\ Ash = \frac{mass\ of\ crucible\ and\ ash\ sample}{mass\ of\ crucible\ and\ sample\ after\ drying}\ x\ 100$$

The crude fibre was determined by using the AOAC's Official Method of Analysis (AOAC., 2000, Official Method of Analysis 962.09). 1.0 g of sample, 150 mL of hot sulphuric acid and three drops of n-octanol was added to glass crucibles. The glass crucibles were boiled for 30 minutes in a hot extraction unit and then rinsed three times with 30 mL of hot distilled water. Three more drops of n-octanol were added to each crucible along with 150 mL of a sodium hydroxide solution. The crucibles were placed back into the hot extraction unit and boiled for another 30 minutes before being rinsed by hot distilled water. The crucibles were placed into a drying oven, cooled in a desiccator overnight and weighed. Ash samples were created by putting the crucibles into a furnace oven for 3 hours at 550˚C and then slowly cooled to a temperature of 250˚C before being placed into a desiccator and weighed. The CF percentage was determined by the following formula:

$$\%\ CF = \frac{mass\ of\ crucible\ \ and\ sample\ after\ boiling - mass\ of\ crucible\ and\ ash\ sample}{mass\ of\ crucible\ and\ sample\ before\ boilling}\ x\ 100$$

Crude fat was determined using the Foss Soxtec method according to the AOAC's Official Method of Analysis (AOAC., 2000, Official Method of Analysis 920.39). 2 g of sample was placed into a folded filter paper (to prevent any sample from spilling) and placed into a marked extraction thimble. The marked thimbles were placed into extraction tube, 40 mL of petroleum ether at a temperature of 60˚C -80˚C was added and allowed to boil and condense for 2.5 hours. The remaining petroleum ether was collected for 30 minutes and then the beakers were put into the drying oven for a minimum of 2 hours before being weighed. The below formula was used to determine the percentage of crude fat present in the feed sample.

$$\%\ Crude\ fat = \frac{mass\ of\ crude\ fat}{mass\ of\ sample}\ x\ 100$$

Table 3.4. The feed ingredient composition (%) and calculated nutrient composition (%) of broiler starter diets fed from 0-14 days-of-age in the performance trial

	Positive Control (PC)	Negative Control 1 [1]	Negative Control 2 [2]	Negative Control 3 [3]
Feed ingredient composition (%)				
Maize	53.20	55.10	56.20	57.60
Soya oil cake (46.5%)	31.40	32.60	31.40	30.50
Full fat soya	5.00	2.00	2.00	2.00
Soya oil (Crude)	3.25	2.01	3.25	1.62
Sunflower oil cake (34.7%)	3.00	4.00	3.00	4.00
Limestone	1.49	1.49	1.49	1.49
Dicalcium phosphate	1.08	1.07	1.11	1.10
Salt	0.24	0.23	0.23	0.23
Sodium bicarbonate	0.23	0.24	0.24	0.25
Methionine (DL 98%)	0.33	0.33	0.31	0.30
Lysine HCl (78%)	0.29	0.31	0.29	0.30
Threonine (98%)	0.13	0.13	0.13	0.13
Valine	0.03	0.03	0.02	0.02
Vitamin and mineral premix	0.25	0.25	0.25	0.25
Toxfin dry	0.10	0.10	0.10	0.10
Salinomycin 12%	0.05	0.05	0.05	0.05
AxtraPHY 100g (10 000ftu) Tpt	0.01	0.01	0.01	0.01
Nutrient composition (%)				
Dry matter	90.10	90.10	90.10	89.90
AME Broiler (MJ/kg)	12.04	11.65	12.05	11.65
Crude protein	22.74	22.78	21.90	21.98
Crude fat	6.16	4.44	5.68	4.11
Fat	6.79	5.08	6.30	4.75
Crude fibre	3.33	3.44	3.23	3.40
Ash	6.01	6.03	5.93	5.96
Calcium	0.96	0.96	0.96	0.96
Phosphorus (total)	0.81	0.81	0.80	0.81
Digestible phosphorus	0.48	0.48	0.48	0.48
Sodium	0.16	0.16	0.16	0.16
Chloride	0.23	0.23	0.23	0.23
Potassium	1.01	1.01	0.97	0.97
Calcium: Available phosphorus	2.00	2.00	2.00	2.00
Dietary cation anion balance (mEq/kg)	249.90	248.70	238.80	238.90
Digestible lysine	1.25	1.25	1.20	1.20
Digestible methionine	0.63	0.63	0.60	0.60
Digestible cysteine	0.30	0.30	0.29	0.29
Digestible methionine + cysteine	0.93	0.93	0.89	0.89
Digestible threonine	0.84	0.84	0.81	0.81
Digestible tryptophan	0.23	0.23	0.22	0.22
Digestible isoleucine	0.84	0.84	0.81	0.81
Digestible arginine	1.36	1.37	1.30	1.31
Digestible valine	0.94	0.94	0.90	0.90
Non-starch polysaccharides	14.91	15.16	14.60	14.99

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3] Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

Table 3.5. The feed ingredient composition (%) and calculated nutrient composition (%) of broiler grower diets fed from 14-28 days-of-age in the performance trial

	Positive Control	Negative Control 1 [1]	Negative Control 2 [2]	Negative Control 3 [3]
Feed ingredient composition (%)				
Maize	58.60	58.80	60.80	61.10
Soya oil cake (46.5%)	30.10	28.10	28.20	26.20
Soya oil (Crude)	4.54	3.30	4.18	2.94
Sunflower oil cake (34.7%)	3.00	6.00	3.00	6.00
Limestone	1.36	1.36	1.37	1.36
Dicalcium phosphate	0.87	0.85	0.89	0.87
Salt	0.24	0.22	0.24	0.22
Sodium bicarbonate	0.23	0.25	0.24	0.25
Methionine (DL 98%)	0.29	0.28	0.27	0.26
Lysine HCl (78%)	0.27	0.30	0.28	0.30
Threonine (98%)	0.11	0.11	0.10	0.10
Valine	0.02	0.01	0.01	0.004
Vitamin and mineral premix	0.25	0.25	0.25	0.25
Toxfin dry	0.10	0.10	0.10	0.10
Salinomycin 12%	0.05	0.05	0.05	0.05
AxtraPHY 100g (10 000ftu) Tpt	0.01	0.01	0.01	0.01
Nutrient composition (%)				
Dry matter	87.50	88.00	87.50	88.00
AME Broiler (MJ/kg)	20.70	21.00	20.15	20.24
Crude protein	20.87	5.47	6.35	5.17
Crude fat	7.26	6.08	6.96	5.78
Fat	3.11	3.57	3.08	3.54
Crude fibre	3.17	3.53	3.11	3.49
Ash	5.42	5.49	5.36	5.43
Calcium	0.87	0.87	0.87	0.87
Phosphorus	0.74	0.76	0.74	0.76
Digestible phosphorus	0.44	0.44	0.44	0.44
Sodium	0.16	0.16	0.16	0.16
Chloride	0.23	0.23	0.23	0.23
Potassium	0.91	0.91	0.88	0.88
Calcium: Available phosphorus	2.00	2.00	2.00	2.00
Dietary cation anion balance (mEq/kg)	224.60	224.30	215.60	215.40
Digestible lysine	1.12	1.12	1.08	1.08
Digestible methionine	0.57	0.57	0.54	0.54
Digestible cysteine	0.28	0.28	0.27	0.27
Digestible methionine + cysteine	0.85	0.85	0.81	0.81
Digestible threonine	0.75	0.75	0.72	0.72
Digestible tryptophan	0.21	0.20	0.20	0.20
Digestible isoleucine	0.76	0.76	0.73	0.73
Digestible arginine	1.23	1.24	1.18	1.19
Digestible valine	0.85	0.85	0.81	0.81
Non-starch polysaccharides	14.20	15.00	14.0	14.80

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3] Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

Table 3.6. The feed ingredient composition (%) and calculated nutrient composition (%) of broiler finisher diets fed from 28-35 days-of-age in the performance trial

	Positive Control	Negative Control 1 [1]	Negative Control 2 [2]	Negative Control 3 [3]
Feed ingredient composition (%)				
Maize	62.30	61.90	64.30	64.00
Soya oil cake (46.5%)	26.00	23.40	24.30	21.70
Soya oil (Crude)	5.32	4.28	4.99	3.95
Sunflower oil cake (34.7%)	3.00	7.00	3.00	7.00
Limestone	1.25	1.25	1.26	1.25
Dicalcium phosphate	0.68	0.65	0.70	0.67
Salt	0.25	0.23	0.25	0.23
Sodium bicarbonate	0.22	0.25	0.23	0.25
Methionine (DL 98%)	0.26	0.25	0.24	0.23
Lysine HCl (78%)	0.25	0.29	0.25	0.29
Threonine (98%)	0.08	0.08	0.08	0.08
Vitamin and mineral premix	0.25	0.25	0.25	0.25
Toxfin dry	0.10	0.10	0.10	0.10
Salinomycin 12%	0.05	0.05	0.05	0.05
AxtraPHY 100g (10 000ftu) Tpt	0.01	0.01	0.01	0.01
Nutrient composition (%)				
Dry Matter	90.00	89.90	89.90	89.80
AME Broiler (MJ/kg)	13.30	12.90	13.30	12.90
Crude protein	19.00	19.20	18.40	18.50
Crude fat	7.52	6.53	7.25	6.25
Fat	8.12	7.13	7.84	6.85
Crude fibre	3.01	3.61	2.98	3.58
Ash	4.91	5.00	4.86	4.94
Calcium	0.79	0.79	0.79	0.79
Phosphorus	0.69	0.70	0.68	0.70
Digestible phosphorus	0.40	0.40	0.40	0.40
Sodium	0.16	0.16	0.16	0.16
Chloride	0.23	0.23	0.23	0.23
Potassium	0.83	0.84	0.80	0.81
Calcium: Available phosphorus	2.00	2.00	2.00	2.00
Dietary cation anion balance (mEq/kg)	204.20	203.80	196.10	195.70
Digestible lysine	1.00	1.00	0.96	0.96
Digestible methionine	0.52	0.52	0.50	0.49
Digestible cysteine	0.26	0.26	0.25	0.25
Digestible methionine + cysteine	0.78	0.78	0.75	0.75
Digestible threonine	0.67	0.67	0.64	0.64
Digestible tryptophan	0.18	0.18	0.18	0.18
Digestible isoleucine	0.69	0.69	0.66	0.66
Digestible arginine	1.11	1.13	1.06	1.08
Digestible valine	0.77	0.77	0.74	0.74
Non-starch polysaccharides	13.70	14.70	13.60	14.60

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3]Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

Table 3.7. The nutrient composition (%) as formulated and the nutrient composition (%) as analysed of broiler starter diets fed from 0-14 days-of-age in the performance trial

	Positive Control		Negative Control 1 [1]		Negative Control 2 [2]		Negative Control 3 [3]	
	Formulated	Actual	Formulated	Actual	Formulated	Actual	Formulated	Actual
Dry matter	90.10	89.30	90.10	88.50	90.10	89.00	89.90	88.50
Crude protein	22.70	23.50	22.80	22.50	21.90	21.90	22.00	21.50
Fat	6.16	6.05	4.44	4.95	5.68	7.51	4.11	5.94
Crude fibre	3.33	4.30	3.44	4.69	3.23	4.63	3.40	4.92
Ash	6.01	5.40	6.03	5.58	5.93	5.48	5.96	5.65
Calcium	0.96	0.84	0.96	1.06	0.96	0.85	0.96	0.96
Phosphorus	0.48	0.43	0.48	0.64	0.48	0.60	0.48	0.60

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3] Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

Table 3.8. The nutrient composition (%) as formulated and as analysed (%) of broiler grower diets fed from 14-28 days-of-age in the performance trial

	Positive Control		Negative Control 1 [1]		Negative Control 2 [2]		Negative Control 3 [3]	
	Formulated	Actual	Formulated	Actual	Formulated	Actual	Formulated	Actual
Dry matter	87.50	88.60	88.00	88.50	87.50	89.60	88.00	88.40
Crude protein	20.87	20.10	21.00	20.60	20.15	20.00	20.24	20.00
Fat	7.26	7.61	6.08	6.62	6.96	7.32	5.78	5.94
Crude fibre	3.17	4.24	3.53	4.92	3.11	4.24	3.49	5.26
Ash	5.42	5.10	5.49	5.08	5.36	4.85	5.43	4.90
Calcium	0.87	0.85	0.87	0.88	0.87	0.85	0.87	0.62
Phosphorus	0.44	0.56	0.44	0.57	0.44	0.53	0.44	0.47

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3] Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

Table 3.9. The nutrient composition (%) as formulated and as analysed (%) of broiler finisher diets fed from 28-35 days-of-age in the performance trial

	Positive Control		Negative Control 1 [1]		Negative Control 2 [2]		Negative Control 3 [3]	
	Formulated	Actual	Formulated	Actual	Formulated	Actual	Formulated	Actual
Dry matter	90.00	88.80	89.90	88.40	89.90	89.00	89.80	88.80
Crude protein	19.00	18.60	19.20	18.80	18.40	18.40	18.50	18.80
Fat	7.52	8.17	6.53	5.80	7.25	6.69	6.25	6.03
Crude fibre	3.01	4.64	3.61	4.17	2.98	4.02	3.58	5.09
Ash	4.91	4.63	5.00	4.63	4.86	4.48	4.94	4.55
Calcium	0.79	0.57	0.79	0.62	0.79	0.56	0.79	0.62
Phosphorus	0.66	0.48	0.70	0.47	0.68	0.46	0.70	0.52

[1] Negative Control 1 (NC1): PC – 0.418 MJ/kg
[2] Negative Control 2 (NC2): PC – 4% amino acids
[3] Negative Control 3 (NC3): PC – 0.418 MJ/kg and – 4% amino acids

3.2.3 **Performance parameters**

At placement and at 7, 14, 21, 28, and 35 days-of-age, all birds were weighed in each pen, and weight of residual feed was taken. To obtain the individual body weight of the broilers, birds from the same pen were placed in a crate with a known weight. The total weight of the birds and crate was recorded. The weight of the crate was subtracted from the total weight and then the total weight was divided by the number of broilers that were placed in the crate to determine the average individual body weight of the broilers per pen.

The weekly feed intake was measured by weighing out an amount of feed at the start of a week and then weighing the amount of feed remaining after one week. The remaining weight of the feed was subtracted from the initial weight of the feed to determine the amount of feed eaten by the broilers per pen in one week. The cumulative feed intake was the summation of the weekly feed intakes.

These measurements were used to calculate the FCR per period. The FCR was determined by dividing the body weight gained by the feed intake and was corrected for mortality by adding the weight of the mortalities to the final end weight of the associated pen. The cumulative FCR was determined by dividing the total feed intake per pen by the total body weight gained gained per pen.

The portion yield was also determined by taking two samples per pen. On day 35, two birds per pen which represented the average weight of birds in the respective pen were slaughtered using a conventional slaughter technique. First the birds were weighed to determine their live weight. Then the birds were stunned by electrical stunning rendering them unconscious thereafter they were bled out from a single laceration to the neck. Once the birds had finished bleeding out, they were scalded in 60˚C water for 1 minute and then defeathered in a rotary drum mechanical picker. The carcass weight of each bird was measured. The breast, drumstick and thigh from each bird was collected and weighed to determine the portion yield of each treatment group.

Number, age and weight of birds that died during the trial was recorded to calculate mortality rate. Dead birds were removed from pens in a timeous manner, their weight, treatment number, pen number and suspected cause of death was recorded in a daily logbook.

3.2.4 **Statistical analysis**

The trial was designed as a complete randomised block design with eleven treatments and eight cage replicates per treatment. Blocking was done to minimise possible environmental effect on variance between treatments. The broiler house was divided into eight blocks. Treatment replications were randomised and repeated once within a block.

Data was analysed by ANOVA (Analysis of Variance) using a general linear model (GLM) as described by the below equation:

$$Y = \mu + Ti + Lj + TLij + eij$$

Where:
Y= variable studied during the period
μ=overall mean of the population
Ti = effect of the ith treatment
Li = effect of the jth source
TLij = effect of the kth level
eij = error associated with each Y

Means and standard error of mean (SEM) were calculated and significance of difference ($P < 0.05$) between means was determined by Fischer's test (Samuels, 1989).

CHAPTER 4. RESULTS

4.1 Protease digestibility trial

4.1.1 Effect of protease on the ileal crude protein digestibility and ileal dry matter digestibility

The effect of protease supplementation at various levels on the digestibility of ileal CP and dry matter is summarised in Table 4.1.

The CP digestibility of the NC group was significantly higher ($P<0.05$) than Kemzyme (150 mg/kg) and the Kemzyme (400 mg/kg) groups. The CP digestibility was the lowest at 84.9% when the birds were supplemented with 150 mg/kg Kemzyme protease. This is significantly lower than the CP digestibility found for the NC, the Kemzyme (200 mg/kg) and the Proact groups. The CP digestibility was the highest, 86.6%, when the birds were supplemented with 200 mg/kg Kemzyme protease. This is significantly higher than the Kemzyme (150 mg/kg), Kemzyme (300 mg/kg) and Kemzyme (400 mg/kg) groups. The addition of protease at both 150 mg/kg Kemzyme or 400 mg/kg Kemzyme did not significantly ($P<0.05$) improve the CP digestibility compared to the negative control.

In terms of dry matter digestibility, the NC group showed a significantly ($P<0.05$) higher DM digestibility than the Kemzyme (150 mg/kg) group. The lowest DM digestibility, 74.6%, was observed when 150m mg/kg Kemzyme protease was supplemented. This is significantly ($P<0.05$) lower than the NC and the Kemzyme (200 mg/kg) groups. The addition of 300 mg/kg and 400 mg/kg of Kemzyme and 200 mg/ton of Proact had no significant effect of the DM digestibility.

Table 4.1. Effect of increasing doses of dietary protease on the ileal crude protein digestibility (%) and ileal dry matter digestibility (%)

Treatment	CP Digestibility	DM Digestibility
Negative Control (NC)	86.2 ^{AB}	76.3 ^{A}
Kemzyme (150 mg/kg)	84.9 ^{D}	74.6 ^{B}
Kemzyme (200 mg/kg)	86.6 ^{A}	76.6 ^{A}
Kemzyme (300 mg/kg)	85.2 ^{BCD}	75.8^{AB}
Kemzyme (400 mg/kg)	85.0 ^{D}	75.3 ^{AB}
200 mg/kg Proact (200 mg/kg)	86.4 ^{AC}	75.9 ^{AB}
SEM[1]	0.0040	0.0049

$^{A-D}$Means within a column without a common superscript are significantly different (P<0.05)
[1]Standard error of means

4.1.2 The effect of protease supplementation on the ileal amino acid digestibility of broilers

The effect which dietary supplementation of protease at various levels (150 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg) had on the ileal amino acid digestibility is summarised in Table 4.2.

The addition of protease resulted in no significant improvement (P<0.05) on the ileal digestibility of tryptophan except in the Kemzyme (400 mg/kg) group. However, the protease (300 mg/kg) group significantly reduced (P<0.05) the ileal digestibility of tryptophan. The ileal digestibility of arginine, serine, glycine, threonine, alanine and glutamic acid did not significantly improve (P<0.05) when a protease was supplemented compared to the negative control. On the contrary, the ileal digestibility of aspartic acid significantly improved (P<0.05) in all treatments compared to the NC group. The addition of protease did not significantly improve (P<0.05) the ileal digestibility compared to the NC group for the following amino acids: tyrosine, proline, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine. The addition of Kemzyme (150 mg/kg) resulted in a significant (P<0.05) decrease in the ileal digestibility of proline, methionine, valine, phenylalanine, leucine and lysine when compared to the ileal amino acid digestibility noted for the NC group.

Table 4.2. The effect of increasing doses of dietary protease on the ileal amino acid digestibility of broiler chickens

Treatment	Negative Control (NC)	Kemzyme (150 mg/kg)	Kemzyme (200 mg/kg)	Kemzyme (300 mg/kg)	Kemzyme (400 mg/kg)	Proact (200 mg/kg)	SEM[1]
Tryptophan	86.1 ^{B}	85.4 ^{B}	85.6 ^{B}	83.8 ^{C}	89.8 ^{A}	85.9 ^{B}	0.489
Arginine	90.9 ^{A}	87.2 ^{E}	89.5 ^{AC}	89.3 ^{D}	88.8 ^{BCDE}	89.7 ^{AB}	0.563
Serine	87.1 ^{A}	82.0 ^{B}	85.1 ^{A}	84.9 ^{A}	82.1 ^{B}	85.2 ^{A}	0.803
Aspartic acid	81.9 ^{E}	85.1 ^{BCD}	86.8 ^{AB}	85.8 ^{AD}	86.7 ^{AC}	87.7 ^{A}	0.675
Glutamic acid	91.7 ^{AC}	91.4 ^{BCD}	92.5 ^{AB}	91.6 ^{BCD}	91.6 ^{AD}	92.7 ^{A}	0.388
Glycine	84.6 ^{A}	79.6 ^{E}	82.5 ^{BC}	82.3 ^{BD}	81.2 ^{CDE}	83.2 ^{AB}	0.647
Threonine	84.3 ^{A}	81.3 ^{D}	83.9 ^{AC}	83.3 ^{AE}	82.3 ^{BCDE}	84.0 ^{AB}	0.585
Alanine	90.3 ^{AB}	88.9 ^{BC}	90.7 ^{A}	89.6 ^{AC}	88.3 ^{C}	90.7 ^{A}	0.575
Tyrosine	86.3 ^{AC}	85.3 ^{BCD}	89.1 ^{A}	83.0 ^{D}	88.5^{AB}	86.1 ^{AD}	1.14
Proline	88.1 ^{A}	83.7 ^{C}	87.4 ^{A}	87.1 ^{AB}	85.2 ^{BC}	87.7 ^{A}	0.746
Methionine	94.6 ^{A}	90.0 ^{C}	94.0 ^{A}	91.4 ^{BC}	91.0 ^{C}	93.3 ^{AB}	0.793
Valine	85.5 ^{AB}	82.8 ^{DE}	85.2 ^{AC}	83.7 ^{CE}	84.3 ^{BCD}	86.0 ^{A}	0.560
Phenylalanine	89.2 ^{AB}	87.3 ^{C}	89.6 ^{A}	87.1 ^{C}	88.2 ^{BC}	89.8 ^{A}	0.447
Isoleucine	86.1 ^{AC}	84.7 ^{CE}	87.1 ^{AB}	84.6 ^{DE}	85.9 ^{BCD}	87.5 ^{A}	0.511
Leucine	88.8 ^{A}	86.9 ^{B}	89.3 ^{A}	87.4 ^{B}	87.3 ^{B}	89.3 ^{A}	0.431
Histidine	76.1 ^{A}	68.9 ^{AD}	72.0 ^{AB}	71.2 ^{AC}	75.2 ^{A}	65.2 ^{BCD}	2.89
Lysine	88.9 ^{A}	86.9 ^{B}	89.8 ^{A}	84.9 ^{C}	89.3 ^{A}	89.2 ^{A}	0.616

$^{A-E}$Means within a row without a common superscript are significantly different (P<0.05)
[1]Standard error of means

4.2 Protease and xylanase performance trial

Although the trial was designed as a single experiment, results are presented separately to evaluate the effects of xylanase, protease and the combination thereof on broiler performance.

4.2.1 The effect of xylanase on weekly and cumulative broiler performance

The body weight of the broilers was not significantly affected by the addition of Xygest HT until day 21, as indicated in Table 4.3. There was also no significant difference between the NC and the PC groups until day 21. On day 21, the body weight of the broilers from the PC group was noted to be significantly higher ($P<0.05$) than those in the negative control group and the addition of Xygest HT did not result in a significant increase ($P<0.05$) in body weight compared to both the NC and PC groups. The addition of Xygest HT (30 mg/kg) resulted in a significant decrease ($P<0.05$) in body weight on day 21. On day 28, the body weight of the broilers which were part of the PC group was significantly higher ($P<0.05$) than the body weight of the broilers from the NC group and the three treatments containing Xygest HT. The addition of Xygest HT (15 mg/kg) and of Xygest HT (30 mg/kg) led to a significantly ($P<0.05$) lower body weight compared to both the NC and the PC groups on day 28. From day 28 until day 35, the addition of Xygest HT resulted in no significant increase in the body weight of broilers. However, the addition of Xygest HT (30 mg/kg) led to a significantly ($P<0.05$) lower body weight compared to body weight noted for the PC and NC groups.

Table 4.3. The effect of increasing doses of xylanase on the weekly average body weight (g) of broilers

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Positive Control (PC)	43.10	186.2	530.2	1160 ^{A}	1964 ^{A}	2728 ^{A}
Negative Control 1 [1]	42.93	179.9	514.1	1115 ^{BC}	1921 ^{B}	2725 ^{A}
Xygest HT (10 mg/kg)	43.26	184.7	525.2	1136 ^{AC}	1882 ^{BC}	2734 ^{A}
Xygest HT (15 mg/kg)	43.15	184.0	523.1	1146 ^{AB}	1867 ^{C}	2732 ^{A}
Xygest HT (30 mg/kg)	43.21	182.5	520.8	1117 ^{BC}	1824 ^{D}	2657 ^{B}
SEM[2]	**0.014**	**0.014**	**0.014**	**0.014**	**0.014**	**0.014**

$^{A-D}$ Means within column without same superscripts are significantly different ($P<0.05$)

[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg

[2]Standard error of means

The addition of exogenous Xygest HT at various doses did not result in a significant difference (P<0.05) in the weekly feed intake of broilers from day 0 until day 21 (Table 4.4). The lowest weekly feed intake from day 21 to day 28, 1002 g, was from the Xygest HT (30 mg/kg) treatment group. There was no significant difference in the weekly feed intake for the PC, NC1, Xygest HT (10 mg/kg) and Xygest HT (15 mg/kg) groups between day 21 and day 28. From day 28 until day 35, the weekly feed intake of the NC group was significantly (P<0.05) lower than that of the PC group. The lowest weekly feed intake for day 28 to day 35 (1361 g) was observed for the PC group. This was significantly lower (P<0.05) than both the NC1 group and the group that received feed with 15 mg/kg of xylanase added.

Table 4.4. The effect of increasing doses of xylanase on the weekly average feed intake (g) of broilers

	Day 0-7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	178.6	392.6	778.0	1060 ^{A}	1361 ^{BC}
Negative Control 1 [1]	178.2	393.3	746.7	1061 ^{A}	1413 ^{A}
Xygest HT (10 mg/kg)	178.7	400.6	751.9	1028 ^{AC}	1373 ^{AC}
Xygest HT (15 mg/kg)	187.8	403.0	759.7	1035 ^{AB}	1417 ^{A}
Xygest HT (30 mg/kg)	177.7	401.3	749.9	1002 ^{BC}	1384 ^{AB}
SEM[2]	**0.018**	**0.018**	**0.018**	**0.018**	**0.018**

$^{A-C}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg

[2]Standard error of means

The effects of increasing doses of xylanase on broiler weekly FCR is summarised in Table 4.5. No significant difference was observed between the PC group's weekly FCR and the NC group's weekly FCR throughout the trial. Dietary Xygest HT fed at various levels resulted in no reduction in the weekly FCR for the first three weeks (Day 0 until day 21). The weekly FCR, between day 21 and day 28, was significantly reduced (P<0.05) with the addition of Xygest HT (10 mg/kg) when compared to the weekly FCR of the broilers from the PC treatment group. During the last week, day 28 until day 35, the broilers from the treatment containing Xygest HT (10 mg/kg) had the lowest weekly FCR. This was significantly lower (P<0.05) than the weekly FCR noted for the NC group.

Table 4.5. The effect of increasing doses of xylanase on the weekly feed conversion ratio of broilers

	Day 0-7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	1.24 ^{BCD}	1.14	1.25	1.46 ^{A}	1.60 ^{AD}
Negative Control 1 [1]	1.31 ^{AB}	1.18	1.29	1.39 ^{AD}	1.66 ^{A}
Xygest HT (10 mg/kg)	1.26 ^{AC}	1.17	1.26	1.36 ^{BCD}	1.57 ^{BCD}
Xygest HT (15 mg/kg)	1.34 ^{A}	1.18	1.26	1.42 ^{AC}	1.63 ^{AC}
Xygest HT (30 mg/kg)	1.28 ^{AD}	1.19	1.27	1.43 ^{AB}	1.64 ^{AB}
SEM[2]	**0.028**	**0.028**	**0.028**	**0.028**	**0.028**

$^{A-D}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg

[2]Standard error of means

Throughout the first three weeks, day 0 until day 21, there were no significant differences regarding cumulative feed intake for any of the treatment groups. The cumulative feed intake for day 0 until day 28 for the treatment containing 30 mg/kg of xylanase was significantly lower than the cumulative feed intake for the boilers being fed the PC diet. Throughout the trial, day 0 until day 35, the cumulative feed intake was the lowest for the Xygest HT (30 mg/kg)) group. This was a significantly lower (P<0.05) than the cumulative feed intake observed for both the NC group and the Xygest HT (15 mg/kg) group (Table 4.6).

Table 4.6. The effect of increasing doses of xylanase on the cumulative feed intake (g) of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	178.6	571.2	1349	2410^{A}	3771^{AB}
Negative Control 1 [1]	178.2	571.5	1318	2380^{AC}	3793^{A}
Xygest HT (10 mg/kg)	179.7	579.3	1331	2360^{AD}	3734^{AC}
Xygest HT (15 mg/kg)	187.8	590.8	1351	2386^{AB}	3804^{A}
Xygest HT (30 mg/kg)	177.7	579.0	1329	2332^{BCD}	3716^{BC}
SEM[2]	**0.0269**	**0.0269**	**0.0269**	**0.0269**	**0.0269**

$^{A-C}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg

[2]Standard error of means

During the first week of the trial (day 0 to day 7) the cumulative FCR for the PC group was noted to be the highest, 0.949, as can be seen in Table 4.7. The cumulative FCR from day 0 to day 14 was the lowest, 1.07, in the PC group; this was significantly (P<0.05) lower

than all other treatment groups. No significant differences were noted between the treatment groups for day 0 to day 21, day 0 to day 28 and day 0 to day 35.

Table 4.7. The effect of increasing doses of xylanase on the cumulative feed conversion ratio of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	0.949 ^{B}	1.07^{BCD}	1.17	1.25	1.36
Negative Control 1 [1]	0.986^{AB}	1.11^{AC}	1.20	1.28	1.39
Xygest HT (10 mg/kg)	0.965^{B}	1.10^{AD}	1.18	1.25	1.35
Xygest HT (15 mg/kg)	1.02^{A}	1.13^{A}	1.20	1.28	1.39
Xygest HT (30 mg/kg)	0.971^{AB}	1.11^{AB}	1.19	1.2854	1.39
SEM[2]	**0.016**	**0.016**	**0.016**	**0.016**	**0.016**

$^{A-D}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg

[2]Standard error of means

4.2.2 The effect of protease on weekly and cumulative bird performance

The effect of various does of exogenous protease on the weekly and cumulative bird performance is summarised in Table 4.8, Table 4.9, Table 4.10, Table 4.11 and Table 4.12.

From day 0 until day 14 the weekly average body weight of the broilers did not differ significantly regardless of the treatment group. For both day 21 and day 28, the body weight of broilers from the PC group were significantly greater (P<0.05) than the body weight observed for the broilers from the NC group. On day 28 the body weight of the broilers receiving 300 mg/kg Kemzyme was significantly greater (P<0.05) than those on the NC diet.

Table 4.8. The effect of increasing doses of protease on the weekly average body weight (g) of broilers

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Positive Control (PC)	43.10	186.2	530.2	1160 ^{A}	1964 ^{A}	2728 ^{A}
Negative Control 2[1]	43.53	187.1	530.7	1097 ^{B}	1785 ^{C}	2706 ^{AB}
Kemzyme (150 mg/kg)	42.66	184.1	523.8	1110 ^{B}	1729 ^{D}	2590 ^{C}
Kemzyme (300 mg/kg)	43.48	184.1	528.9	1116 ^{B}	1838 ^{B}	2686 ^{B}
Proact (200 mg/kg)	43.10	180.9	520.2	1089 ^{B}	1805 ^{BC}	2623 ^{C}
SEM[2]	**0.0146**	**0.0146**	**0.0146**	**0.0146**	**0.0146**	**0.0146**

$^{A-D}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 2 (NC2) PC-4% amino acids

[2]Standard error of means

The average weekly feed intake of broilers was not significantly impacted by Kemzyme supplementation for the first three weeks of the trial. Between day 21 and day 28 the feed intake of the positive control group broilers was significantly (P<0.05) higher than that of all the other treatments. During the final week of the trial, day 28 to day 35, the weekly feed intake of the broilers did not differ significantly between the NC group and the PC group. It was observed that the Kemzyme (300 mg/kg) group had a significantly (P<0.05) higher weekly feed intake than both the PC group as well as the Proact (200 mg/kg) treatment group for day 28 to day 35.

Table 4.9. The effect of increasing doses of protease on the weekly average feed intake (g) of broilers

	Day 0-7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	178.6	392.6	778.0	1060^{A}	1361^{BC}
Negative Control 2[1]	177.6	403.7	735.8	1001^{B}	1399^{AB}
Kemzyme (150 mg/kg)	174.9	398.9	741.0	959.4^{B}	1383^{AC}
Kemzyme (300 mg/kg)	177.4	401.5	750.8	1000^{B}	1429^{A}
Proact (200 mg/kg)	177.3	403.1	750.4	988.3^{B}	1352^{BC}
SEM[2]	**0.0209**	**0.0209**	**0.0209**	**0.0209**	**0.0209**

$^{A-C}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 2 (NC2): PC-4% amino acids

[2]Standard error of means

The weekly FCR of the broilers between different treatments did not differ significantly for the first four weeks of the trial, day 0 until day 28. The weekly FCR for the negative control group was significantly lower (P<0.05) than the positive control group for the last week of the trial, 28 to day 35.

Table 4.10. The effect of increasing doses of protease on the weekly feed conversion ratio of broilers

	Day 0-7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	1.24	1.14	1.25	1.46	1.60 ^{A}
Negative Control 2[1]	1.24	1.17	1.30	1.48	1.50^{BC}
Kemzyme (150 mg/kg)	1.23	1.17	1.28	1.49	1.57 ^{AB}
Kemzyme (300 mg/kg)	1.27	1.16	1.31	1.42	1.63 ^{A}
Proact (200 mg/kg)	1.28	1.19	1.33	1.46	1.57 ^{AC}
SEM[2]	**0.033**	**0.033**	**0.033**	**0.033**	**0.033**

$^{A-C}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 2 (NC2): PC-4% amino acids

[2]Standard error of means

The cumulative feed intake was not significantly affected by the treatment groups for the first three weeks namely day 0 to day 7, day 0 to day 14 and day 0 to day 28. It was observed that the NC group had a significantly lower feed intake than the PC group from day 0 to day 28. The cumulative feed intake measured throughout the trial (day 0 to day 35) for the broilers both the Kemzyme (150 mg/kg) group and the Proact (200 mg/kg) group were significantly (P<0.05) lower than those of the PC group.

Table 4.11. The effect of increasing doses of protease on the cumulative feed intake (g) of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	178.6	571.2	1349	2410^{A}	3771^{A}
Negative Control 2[1]	177.6	581.3	1317	2318^{B}	3717^{AB}
Kemzyme (150 mg/kg)	174.9	573.8	1315	2274^{B}	3657^{B}
Kemzyme (300 mg/kg)	177.4	578.9	1330	2330^{B}	3759^{A}
Proact (200 mg/kg)	177.3	580.4	1331	2319^{B}	3671^{B}
SEM[2]	**0.0262**	**0.0262**	**0.0262**	**0.0262**	**0.0262**

$^{A-B}$ Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 2 (NC2): PC-4% amino acids

[2]Standard error of means

The supplementation of increasing doses of exogenous protease had no significant effect on the cumulative FCR for days 0 to day 7 and day 0 to day 14. From day 0 to day 21 the Proact (200 mg/kg) treatment group had a significantly higher (P<0.05) cumulative FCR when compared to the PC group. It was noted that the NC group had a significantly higher (P<0.05) cumulative FCR compared to the PC group from day 0 to day 28. From day 0 until

day 35 the cumulative FCR for the PC group was significantly lower (P<0.05) than the cumulative FCR noted for the Kemzyme (150 mg/kg) group and the Proact (200 mg/kg) group.

Table 4.12. The effect of increasing doses of protease on the cumulative feed conversion ratio of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	0.949	1.07	1.17^{BCD}	1.25^{B}	1.36^{BCD}
Negative Control 2[1]	0.944	1.09	1.20^{AC}	1.31^{A}	1.37^{AD}
Kemzyme (150 mg/kg)	0.945	1.09	1.19^{AD}	1.32^{A}	1.40^{A}
Kemzyme (300 mg/kg)	0.965	1.09	1.20^{AB}	1.29^{AB}	1.39^{AC}
Proact (200 mg/kg)	0.976	1.11	1.23^{A}	1.31^{A}	1.39^{A}
SEM[2]	**0.0162**	**0.0162**	**0.0162**	**0.0162**	**0.0162**

[A-D] Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 2 (NC2): PC-4% amino acids

[2]Standard error of means

4.2.3 The effect of a combination of protease and xylanase on weekly and cumulative bird performance

There was no significant effect of a combination of xylanase and protease on the weekly average body weight of broilers for the first three weeks of the trial (Day 0, day 7, day 14 or day 21). On day 28 and on day 35, the average body weight of the broilers in the PC group was significantly (P<0.05) higher than those in the NC group and the Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg) treatment group. These results are summarised in Table 4.13.

Table 4.13. The effect of a combination of xylanase and protease on the weekly average body weight (g) of broilers

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Positive Control (PC)	43.10	186.2	530.2	11600	1964 [A]	2728 [A]
Negative Control 3 [1]	43.42	184.3	519.0	1090	1789 [B]	2657 [B]
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	43.26	179.3	522.0	1109	1822 [B]	2679 [B]
SEM[2]	**0.0142**	**0.0142**	**0.0142**	**0.0142**	**0.0142**	**0.0142**

[A-B] Means within column without same superscripts are significantly different (P<0.05)

[1]Negative Control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids

[2]Standard error of means

A combination of Xygest HT (30 mg/kg) and Kemzyme (300 mg/kg) had no significant effect on the weekly average feed intake (g) of broilers (Table 4.14). There was also no significant difference between the weekly FI of the PC group and the NC group.

Table 4.14. The effect of a combination of Xylanase and Protease on the weekly average feed intake (g) of broilers

	Day 0-7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	178.6	392.6	778.0	1060	1361
Negative Control 3 [1]	184.1	401.7	730.5	1007	1380
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	177.1	411.5	761.1	1018	1375
SEM[2]	**0.0212**	**0.0212**	**0.0212**	**0.0212**	**0.0212**

[1]Negative Control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids

[2]Standard error of means

A combination of Xygest HT (30 mg/kg) and Kemzyme (300 mg/kg) had no significant effect on the weekly FCR of broilers or the cumulative feed intake of broilers as can be seen in Table 4.15 and Table 4.16, respectively.

Table 4.15. The effect of a combination of xylanase and protease on the weekly feed conversion ratio of broilers

	Day 0 -7	Day 7-14	Day 14-21	Day 21-28	Day 28-35
Positive Control (PC)	1.24	1.14	1.25	1.46	1.60
Negative Control 3 [1]	1.31	1.20	1.30	1.47	1.54
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	1.30	1.20	1.32	1.45	1.59
SEM[2]	**0.0298**	**0.0298**	**0.0298**	**0.0298**	**0.0298**

[1]Negative control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids

[2]Standard error of means

Table 4.16. The effect of a combination of xylanase and protease on the cumulative feed intake (g) of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	178.6	571.2	1349	2410	3771
Negative Control 3 [1]	184.1	585.9	1316	2324	3704
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	177.1	588.6	1350	2368	3742
SEM[2]	**0.0269**	**0.0269**	**0.0269**	**0.0269**	**0.0269**

[1]Negative control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids

[2]Standard error of means

The cumulative FCR of the broilers for day 0 to day 35 was not significantly affected by the combination of Xygest HT (30 mg/kg) and Kemzyme (300 mg/kg) as depicted in Table 4.17. From day 0 to day 7 the NC group's cumulative FCR was significantly higher (P<0.05) than the cumulative FCR noted for the PC group. For day 0 to day 21 and day 0 to day 28 the PC group had a significantly lower (P<0.05) cumulative FCR compared to both the NC groups and the treatment group containing a combination of Xygest HT (30 mg/kg) and Kemzyme (300 mg/kg).

Table 4.17. The effect of a combination of xylanase and protease on the cumulative feed conversion ratio of broilers

	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Positive Control (PC)	0.949^{B}	1.07^{B}	1.17^{B}	1.25^{B}	1.36
Negative Control 3 [1]	0.997^{A}	1.13^{A}	1.22^{A}	1.31^{A}	1.39
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	0.983^{AB}	1.13^{A}	1.23^{A}	1.31^{A}	1.40
SEM[2]	**0.0156**	**0.0156**	**0.0156**	**0.0156**	**0.0156**

[A-B]Means within column without same superscripts are significantly different (P<0.05)

[1]Negative control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids

[2]Standard error of means

4.2.4 The effect of xylanase and protease on dressing percentage and portion yield

Both the drumstick portion yield (PY) and the wing PY of 35-day old broilers was not significantly affected by exogenous supplementation of increasing doses of Xygest HT or by the NC group. Broilers from the Xygest HT (30 mg/kg) group had a significantly higher (P<0.05) dressing % than the NC group and the Xygest HT (10 mg/kg) group. The breast PY of 35-day old broilers supplemented with Xygest T (30 mg/kg) was significantly lower (P<0.05)

than the PC groups breast PY. The effect of xylanase and protease on dressing percentage and portion yield is summarised in Table 4.18.

Table 4.18. The effect of increasing doses of xylanase on the dressing percentage and portion yield (%, PY) of 35-day old broilers

	Dressing %	Breast PY	Drumstick PY	Wing PY
Positive Control (PC)	77.2 ^{AC}	31.2 ^{A}	26.0	9.67
Negative Control 1[1]	77.0 ^{BC}	30.5 ^{AB}	26.8	9.69
Xygest HT (10 mg/kg)	76.8 ^{BC}	30.3 ^{AC}	26.7	9.53
Xygest HT (15 mg/kg)	77.8 ^{AB}	30.0 ^{AD}	27.0	9.56
Xygest HT (30 mg/kg)	79.2 ^{A}	29.7 ^{BCD}	26.5	9.85
SEM[2]	**0.699**	**0.466**	**0.464**	**0.139**

$^{A-D}$ Means within column without same superscripts are significantly different (P<0.05)
[1]Negative Control 1 (NC1): PC- 0.418 MJ/kg
[2]Standard error of means

Increasing doses of Kemzyme and the NC diet did not have a significant effect on the dressing percentage, drumstick portion yield and wing portion yield of 35-day old broilers (Table 4.19). The breast portion yield of 35-day old broilers from the NC group and the Kemzyme (300 mg/kg) group was significantly lower (P<0.05) than the breast portion yield of the PC group.

Table 4.19. The effect of increasing doses of protease on the dressing percentage and portion yield (%, PY) of 35-day old broilers

	Dressing %	Breast PY	Drumstick PY	Wing PY
Positive Control (PC)	77.2	31.2 ^{A}	26.0	9.7
Negative Control [1]	77.8	29.4 ^{BC}	26.6	9.7
Kemzyme (150 mg/kg)	76.2	30.3 ^{AC}	25.9	9.8
Kemzyme (300 mg/kg)	77.2	29.8 ^{BC}	26.2	9.7
Proact (200 mg/kg)	76.7	30.6 ^{AB}	27.0	9.7
SEM[2]	**0.630**	**0.422**	**0.596**	**0.186**

$^{A-C}$ Means within column without same superscripts are significantly different (P<0.05)
[1] Negative Control 2 (NC2): PC-4% amino acids
[2]Standard error of means

The combination of Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg) did not have a significant effect on the dressing percentage, drumstick portion yield or wing portion yield of 35-day old broilers, as described in Table 4.20. There was no significant difference between the PC group and the NC group's dressing percentage and percent portion yield. The breast PY of the PC group was significantly higher (P<0.05) than the breast portion yield noted for the Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg) treatment group.

Table 4.20. The effect of a combination of xylanase and protease on the dressing percentage and portion yield (%, PY) of 35-day old broilers

	Dressing %	Breast PY	Drumstick PY	Wing PY
Positive Control (PC)	77.2	31.2 [A]	26.0	9.67
Negative Control [1]	77.9	30.1 [AB]	26.8	9.77
Kemzyme (300 mg/kg) + Xygest HT (30 mg/kg)	78.0	29.9 [B]	26.8	9.66
SEM[2]	**0.756**	**0.358**	**0.343**	**0.160**

[A-B] Means within column without same superscripts are significantly different (P<0.05)
[1]Negative Control 3 (NC3): PC-0.418 MJ/kg and -4% amino acids
[2]Standard error of means

CHAPTER 5. DISCUSSION

Dietary enzymes have extensively been used to improve broiler performance. The main aim of providing dietary enzymes is to improve performance by enhancing the feed efficiency (Stefanello *et al*., 2016). These two trials focused specifically on the effect of protease and xylanase on broilers in terms of amino acid digestibility and performance. Although most of the results received from the two trials pointed towards these two enzymes not having a significantly beneficial effect on broiler performance, this is not entirely unusual. Due to the many factors which can influence the efficacy of an exogenous enzymes there are several studies which report similar findings.

5.1 Protease digestibility trial

During this trial, the supplementation of protease in broiler diets did not result in significantly beneficial results in terms of CP digestibility, DM digestibility or the ileal digestibility of the majority of amino acids. This is contrary to what Dos Santos *et al*. (2017) found. Dos Santos *et al*. (2017) noted that when broilers were supplemented with an exogenous protease the apparent ileal digestibility of amino acids was enhanced. However, literature reports inconsistent results when it comes to ileal amino acid and crude protein digestibility. Some trials report an enhanced amino acid digestibility for all amino acids (Stefanello *et al*., 2016) whilst others report that only some amino acid digestibilities improve with exogenous enzyme supplementation (Bertechini, 2009). On the contrary, numerous trials report no effect and in some instances a decreased digestibility was noted (Rada *et al*., 2016; Walk *et al*., 2019). No dose response was observed as expected. The treatment group being fed a standard commercial broiler diet had significantly ($P<0.05$) better digestibility than those treated with protease. These results could be due to factors influencing enzyme efficacy such as the dose of exogenous enzyme supplemented, or the type of diet being fed. When looking at the digestibility of the standard commercial diet used in the trial, the amino acid digestibility was already very high with most of the ileal amino acid digestibility values over 80% (Table 4.1). This may have contributed to the lack of effect of the exogenous protease supplementation on the ileal amino acid digestibility, as the higher the digestibility, the less substrate there is available for the enzyme; thereby reducing the potential impact of the exogenous enzyme (Selle *et al*., 2009).

It is interesting to note that both the highest dose of protease (400 mg/kg) and the lowest dose of protease (150 mg/kg) resulted in the lowest CP digestibility which was

significantly lower than the NC. The over supplementation of exogenous enzymes is reported to interfere with the animals own capabilities to produce endogenous enzymes therefore hampering the animals innate ability to digest feed, whilst under supplementing an exogenous enzyme is insufficient to produce a response (Khusheeba & Sajid, 2013). This appears to be what happened in this instance.

The results from the protease digestibility trial are unpredictable and no clear trend can be seen. This contradicts several protease digestibility studies, such as Angel *et al*. (2011), who found that a supplement of 200 mg/kg of protease to broilers on a low protein diet resulted in enhanced amino acid digestibility, which led to a similar FCR as birds on a high protein diet. Olukosi *et al*. (1997) reported similar findings to Angel *et al*. (2011) that dietary enzyme supplementation leads to improved nutrient digestibility.

5.2 Protease and xylanase performance trial

Dietary enzymes have extensively been used to improve broiler performance. This trial focused specifically on the effect of protease and xylanase on broiler performance. The results from these two studies indicated that the addition of exogenous enzymes does not consistently give beneficial effects as expected. Bao *et al*. (2013) similarly stated in a review paper on exogenous enzymes that when proteases and xylanases are used in broiler diets, the results are often inconsistent and therefore unpredictable. Incorrect nutrient specifications and inherent variation in ingredient quality were some of the reasons given for this inconsistency. Boa *et al.* (2013) further described that the efficacy of an enzyme is highly dependent on factors such as substrate concentration, anti-nutritional factors and the interaction between different nutrients in the diet.

5.2.1 Production parameters

For the first three weeks of the trial, exogenous xylanase supplementation had no significant effect on broiler production parameters. Feed conversion ratio is often used as an overall performance indicator when broiler producers want to enhance efficiency. A bird that eats more feed but gains less weight is not desirable and the same is true for a bird that eats less feed but weighs less. The addition of 10 mg/kg of xylanase significantly reduced the weekly FCR during the last two weeks of the trial, however no other beneficial effects of xylanase supplementation were noted throughout the study for the weekly or cumulative FCR. The weekly feed intake was not impacted by the addition of exogenous xylanase for the first three weeks.

The addition of xylanase showed no predictable benefit on the weekly or cumulative bird performance. This is contrary to the beneficial responses to xylanase that are frequently reported in literature (Marron *et al*., 2001; Cowieson *et al*., 2010; Barekatain *et al*., 2013). This may be due to the theory that broilers are becoming more adapted to the high grain diets that are being fed (Cowieson *et al*., 2010). The more adapted broilers become to the typical commercial diet that is fed, the less relevant exogenous enzymes will become as the gap for improvement will become smaller and smaller. Often the enhanced performance results of broilers being supplemented with an NSP degrading enzyme are reported on a wheat-based diet which have higher levels of NSP (Rios *et al*., 2017). A factor which influences the efficacy of an enzyme is the level of ANF present in the diet (Sheppy, 2003). Thus, it could also be that the lack of effect of exogenous xylanase on broiler performance could be attributed to the relatively low levels of NSPs in a maize based diet when compared to a wheat-based diet. A lower NSP level will induce a smaller enzyme response.

No significant effect was seen for all performance parameters, both weekly and cumulative, when protease was supplemented for at least the first 14 days of the trial. On day 28 those given 300 mg/kg of protease were significantly heavier than the group being fed an amino acid deficient diet. Furthermore, two treatment groups, 200 mg/kg ProAct and 150 mg/kg Kemzyme protease, had significantly lower cumulative FI than the standard commercial broiler diet for day 0 to day 35. However, the addition of protease did not result in any beneficial reduction in the FCR, both cumulative and weekly, for the duration of the trial. This is not what was anticipated, however, both similar and contradictory reports can be found in literature. Several reports found an improved broiler performance (Xu *et al.*, 2017) whilst others found improved performance only under specific conditions (Angel *et al*., 2011). Furthermore, numerous trials have reported no significantly beneficial results from exogenous protease supplementation (Barekatain *et al*., 2013; Walk *et al*., 2019). Mahmood *et al*. (2017) found that the beneficial effects of protease were only present when the broilers were fed a diet containing poorly processed soybean meal. The soybean meal used in this study was processed well and this could have contributed to the lack of results seen when exogenous protease was supplemented.

Exogenous enzymes are often used in an enzyme cocktail with several enzymes used in combination. However, the interaction between protease and xylanase is poorly understood and requires further research to fully understand the interaction. The combination of protease and xylanase did not have a synergistic or additive effect in these trials. The effects of the combination of protease and xylanase being supplemented resulted in no significantly beneficial impact on the broiler performance parameters including both weekly and cumulative

measures. Two factors which could have yielded these insignificant results are the interaction of the various enzymes used in the diet and the amount of substrate available for the enzymes (Barekatain *et al*., 2013). Due to the lack of understanding regarding the modes of action of xylanase and protease, it is not clear how the two enzymes interact.

5.2.2 **Ration evaluation**

For many of the performance measures there was no significant difference between the PC and the NC groups. The weekly FCR for xylanase showed no significant difference between the group being fed a low energy diet and the group being fed a standard commercial diet for the first three weeks of the trial. No difference was observed in terms of broiler performance parameters for the first three weeks between the standard commercial diet treatment and the treatment diet which was deficient in amino acids. Both of these results could indicate that the nutrient specifications of the NC diets were still too high. The efficiency of an exogenous enzyme is dependent on several factors including the nutrient density of the diet. If a diet is too nutrient dense and still meets the nutritional requirements of the broiler, it leaves little to no room for improvement when an enzyme is supplemented (Selle *et al*., 2009).

Enzyme recovery was not possible throughout the trial due to long international turn-a-round times. This meant that the dose and activity of the exogenous enzyme could not be verified which may have impacted the trials' findings. The ability of an enzyme to have a positive influence on broiler performance and nutrient digestibility is dependent on these two factors. There is therefore no way to confirm that the dietary enzymes were not altered or damaged during the feed processing process, by the broiler's innate enzymes or the various pH's found in the broiler's digestive tract (Khusheeba & Sajid, 2013; Ravn *et al*., 2018).

CHAPTER 6. CONCLUSION

6.1 Conclusion

Exogenous enzymes have the potential to significantly reduce costs and improve performance of broiler chickens. However, to achieve this potential may be more challenging than anticipated. In the two trials conducted, the use of exogenous enzymes, alone and in combination, did not lead to the expected benefits of a xylanase and a protease. This could be due to the numerous factors influencing enzyme efficacy.

In nutrient deficient diets, the application of dietary enzymes is more relevant. The greater the deficit the larger the response to the enzyme. This can lead to a lower feed cost as well as the exploration of alternative feed in terms of ingredients which were previously avoided due to low nutrient values. The diets fed during the trials could have been too nutrient dense to illicit a response to the enzyme supplementation. Whilst the diets were reduced to below broiler nutrient requirements, perhaps a response was not seen as broilers themselves are becoming more adapted to the high grain diets being fed. As discussed, the digestibility of the diets used in the digestibility trial was already high. All of these factors could have reduced the potentially beneficial impact of exogenous enzymes on broiler performance.

The level of ANF influences the response to exogenous enzymes. Whilst the feed was not analysed for ANFs, it is well known that wheat has a higher level of ANFs than maize. A large portion of research conducted on enzymes is on wheat-based diets and not on the typical maize based diet used in South Africa. By this logic it is understandable that literature on wheat-based diets shows beneficial responses to exogenous enzymes. An area which warrants further investigation is the effect of exogenous enzymes on broiler performance on lower nutrient dense maize based diets.

An area for further research is to fully understand the mechanisms behind an enzyme's mode of action. A better understanding of this can result in a better application of the enzyme and therefore a better result should be seen. Whilst in this trial no additive or synergistic effect was seen, the interaction between enzymes is a key point for further research as many commercial broiler producers use an enzyme combination to target various substrates at once. It is thought that the two enzymes may have competed against one another leading to a lack of substrate for a sufficient enzyme response.

These studies concluded that to achieve the benefits of an exogenous enzyme requires specific circumstances.

6.2 Critical review

Observations made during the trial which could have affected the results:

There was not a consistent significant difference seen between the positive control diet and the negative control diet. This could indicate that the negative control diet was too nutrient dense which would have a negative implication on the potential benefits that the exogenous enzymes can provide. To avoid this from occurring in future studies it is suggested that the nutrient specifications of the negative control diet should be lowered further. Before commencement of the trial, perhaps a pilot trial can be conducted to assess whether there will be a growth difference between the negative control diet group and the positive control group.

Unfortunately, enzyme recovery was not possible to verify the amount of exogenous enzyme supplemented in each diet. This is often a crucial step in enzyme studies which could not be completed due to long turnaround times at international laboratories and a lack of procedure/routine analysis from the enzyme supplier regarding protease recovery.

During the performance trial, several birds contracted *E. coli*, as diagnosed by the trial veterinarian, during the first two weeks which could have impacted the trial and results. The infection was evenly spread between treatment groups. It was suspected that the poor chick health problem originated in the hatchery which affected general chick health and quality. The birds were treated in a timeous manner and due to it affecting all treatments, the trial continued. Despite this challenge, the overall performance of the broilers were good and well above the production standard described for Ross 308 broilers in the Ross broiler management guide (Aviagen, 2018).

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