The effects of a multiple-enzyme combination in maize-soya diets for broiler chickens

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Thesis presented in partial fulfillment of the requirements for the degree of
Master of Science in Agriculture

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March 2007

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Abstract

1. The use of a multiple-enzyme combination in maize-soya diets for broilers.

The effect of a multiple-enzyme combination in mash and pelleted vegetarian maize-soya diets for broilers was evaluated in terms of apparent excreta- and ileal nitrogen- and amino acid digestibility and production performance. Two separate digestibility trials and one performance trial were conducted. For Trial 1, the apparent nitrogen (N) - and amino acid (AA) digestibility was determined by the collection of the excreta (total collection method) and in Trial 2 from digesta collected at the terminal ileum (ileal digestibility method). Production performance was also recorded in Trial 1. In Trial 3, the effect of the multiple-enzyme combination in potentially improving performance of broilers in commercial conditions was evaluated. Broilers were fed a balanced- and low apparent energy (AME) vegetarian maize-soya diet with the addition of the multiple-enzyme combination. The addition of enzymes improved the apparent excreta- and ileal N digestibility of the mash diets during the period 14-21 d, and the ileal N-digestibility of the pelleted diets at 28 and 35 d of age. Conflicting results with regard to apparent excreta- and ileal AA digestibility were found. By both methods the digestibilities of threonine (Thr), methionine (Met) and phenylalanine (Phe) (14-21 d) and Cys (22-28 d and 29-35 d) were improved by the addition of the enzyme combination to the mash diets. Over the entire experimental period (14-35 d) the ileal digestibilities of histidine (His), Cys and leucine (Leu)of the mash diets were improved by 0.2 %, 0.2 % and 1.9 % respectively, following enzyme addition. By both methods the digestibilities of Thr, arginine (Arg), Met, Cys, Phe and Leu (14-21 d), serine (Ser), Arg, glutamic acid (Glu), Val, His, aspartic acid (Asp), lysine (Lys), proline (Pro), Met, tyrosine (Tyr), Phe and Leu (22-28 d), and Pro (29-35 d) were improved by the combination of enzymes and pelleting. For the entire experimental period (21-35 d), the ileal digestibilities of Ser, His, Lys, Met, Tyr, Cys, Phe and Leu was improved by the combination of enzymes and pelleting, indicating enzymatic activity was not destroyed by cold pelleting at 60 - 80º. The improvements in apparent nitrogen- and AA digestibilities were, in most cases, not reflected in production performance, although the combination of enzymes and pelleting resulted in improved body weight gain (BWG) for the first two weeks of chicks life and significantly improved the feed conversion ratio (FCR) during the second week of the chicks' life. The effect of the multiple-enzyme combination on the production performance of broilers on a low AME- and commercial diet was mostly non-significant except for a significantly lower feed

intake of the balanced diet for the fourth and fifth week of chick's life following enzyme addition. A financial calculation showed, however, that the enzyme combination might increase profitability of a nutritionally balanced vegetarian maize-soya diet for broilers.

Uittreksel

1. Die invloed van ‘n meervoudige ensiem-kombinasie in mielie-soya rantsoene vir braaikuikens

Die invloed van ’n meervoudige ensiem-kombinasie in meel- en verpilde mielie-soya rantsoene vir braaikuikens is geëvalueer in terme van skynbare fekale- en ileale stikstof- en aminosuur verteerbaarhede en produksie prestasie. Twee verterings- en een prestasie eksperiment is onderneem. Vir die eerste eksperiment is die skynbare stikstof (N)- en aminosuur verteringskoeffisiente vanuit die mis bepaal (fekale verteringskoeffisieënte) en in eksperiment twee deur gebruik te maak van die digesta wat uit die terminale ileum ingesamel is (ileale verteringskoeffisieënte). Die prestasie van die braaikuikens is ook in eksperiment een aangeteken. In die derde eksperiment is die prestasie van braaikuikens op ’n gebalanseerde en ’n lae skynbare metaboliseerbare energie (AME) rantsoen geëvalueer, soos beïnvloed deur die meervoudige ensiem-kombinasie. Die ensiem-kombinasie het die skynbare fekale- en ileale N-verteringskoeffisiënte van die meel dieëte verbeter maar slegs vir die periode 14-21 dae ouderdom. Die ensiem kombinasie in die verpilde dieëte get gelei tot verbeterde ileale N-verteerbaarhede by 28 en 35 dae ouderdom. Teenstrydige resultate met betrekking tot die skynbare fekale- en ileale aminosuurverteerbaarhede is gevind. Vir albei die metodes is die verteerbaarhede van Thr, Met en Phe (14-21 d) en Cys (22-28 d en 29-35 dae) verbeter deur die insluiting van die ensiem-kombinasie tot die meel dieët. Oor die volle eksperimentele periode (14-35 d), is die ileale verteerbaarhede van His, Cys en Leu in die meel dieëte verbeter met onderskeidelik 0.2 %, 0.2 % en 1.9 %, as gevolg van die toevoeging van die ensiem-kompleks. Vir albei metodes is daar gevind dat die skynbare verteerbaarheid van Thr, Arg, Met, Cys, Phe and Leu (14-21 d), Ser, Arg, Glu, Val, His, Asp, Lys, Pro, Met, Tyr, Phe en Leu (22-28 d) en Pro (29-35 d) verbeter kan word deur die insluiting van die ensiem-kombinasie voor verpilling. Vir die volle eksperimentele periode (14-35 d) is die skynbare ileale verteringskoeffisiënte van Ser, His, Lys, Met, Tyr, Cys, Phe en Leu verbeter deur die kombinasie van verpilling en ensieme wat dus aandui dat ensiematiese effektiwiteit nie betekenisvol beïnvloed is deur koue verpilling teen temperature van 60 – 80 ºC nie. Die verbetering in skynbare verteerbaarhede van stikstof en aminosure is egter nie weerspieël in die prestasie van die braaikuikens nie, alhoewel die kombinasie van ensieme en verpilling gelei het tot hoër ligaamsmassa toename in die eerste twee weke van kuikens se lewe en ook tot ’n betekenisvolle verbetering in voeromset

verhouding gedurende die tweede week van lewe. Die effek van die ensiem-kompleks op die prestasie van braaikuikens op ’n lae metaboliseerbare (AME) en gabalanseerde mielie-soya dieët was oor die algemeen nie betekenisvol nie, behalwe vir ’n betekenisvolle laer voerinname van die gebalanseerde rantsoen as gevolg van die toevoeging van die ensiem-kompleks vir die vierde en vyfde week van kuikens se lewe. ‘n Finansieële berekening het egter aangedui dat die toevoeging van die ensiem-kombinasie tot die gebalanseerde mielie-soya rantsoen vir braaikuikens mag lei tot verhoogde winsgewendheid.

Acknowledgements

- To my supervisor, Dr. Mariana Ciacciariello for her friendly guidance and motivation throughout my post-graduate studies.

- To my co-supervisor, Dr. Helet Lambrechts for her valuable input and comments

- To the Protein Research Trust who provided the financial support for my post-graduate studies.

- To Meadow Feeds for assisting in further financial assistance by means of the AgriSETA Financial Support Fund.

- To Dr. Erik van der Beke and Leni Kuterna at Aveve, Belgium for the generous donation of the enzyme-combination and the correspondence regarding the research project.

- To Michael, Selwyn, Martin, Lourens, Ruben and Dawid at Mariendahl research farm for their help in mixing the diets and looking after the experimental animals after hours.

- To Resia Swart for her assistance with the AA preparation and general laboratory work.

- To Gail Jordaan for her help with the statistical analysis of the data.

- To my friends and family for their love and support.

Contents

CHAPTER 1
GENERAL INTRODUCTION

The use of enzymes to enhance nutrient availability of animal feeds has been reported as far back as 1925 (Clicker and Follwell, 1925). The commercial application of feed enzymes as a feed additive however has a history of less than 20 years. The main focus of early enzymatic research with regards to monogastric nutrition was for the use of non-starch polysaccharide (NSP) -degrading enzymes such as β-glucanase and xylanase in barley, rye and wheat, in an attempt to alleviate the problems associated with increased digesta viscosity caused by the use of such viscous grains in animal diets. During the early 1990's research shifted and the main focus became to enhance nutrient availability (Choct, 2006). In this regard the use of phytase to release the phytate-bound phosphorous and possibly other nutrients in cereals was intensively investigated (Yi *et al*., 1996; Sebastian *et al*., 1997; Ravindran *et al*., 1999a; Touchburn *et al*., 1999). The use of phytase also reduces the phosphorous content of the excreta, thereby reducing the polluting load of phosphorous into the environment (Choct, 2006). It also became clear that the exogenous enzymatic disruption of NSP's may result in improved nutrient availability for broilers and many studies were conducted to investigate such assumptions (Annison and Choct, 1991; Cowan *et al*., 1996; Rebolé *et al*., 1999; Zanella *et al*., 1999). Due to the fact that many nutrients within cereal grains are not readily available to chickens due to a lack of certain digestive enzymes, especially early in chick development (Nitsan *et al*., 1991; Iji *et al*., 2001), the use of supplemental enzymes known to be limiting during the early stages of chick development such as proteases has also been investigated (Zanela *et al*., 1999; Kocher *et al*., 2002; Cowieson and Adeola, 2005; Odetallah *et al*., 2005).

Soya is considered a good source of protein and AAs and is probably the most popular vegetarian protein concentrate used in broiler feed. With the increased ban on animal-by products, the inclusion rates of soya into poultry diets has also increased during recent years. There is, however considerable variability between soyabean meal (SBM) samples. Ileal digestibilities of SBM samples obtained from the same mill may vary by as much as 1-5 % for Thr, 1-4 % for Val, 2-6 % for Met, 1-6 % for Isoleucine (Ile) and 2-5 % for Leu (Ravindran *et al.*, 1999b). As a result the use of these feedstuffs will depend not only on methods of improving the digestibility of nutrients therein to a level comparable to that of animal protein sources but also on reducing variability between feed samples, allowing for more accurate feed formulation and thus improved broiler performance. Exogenous feed enzymes may thus be used, not as a pro-nutrient, but rather as a means of improving homogeneity of broiler feed.

Maize is considered a homogenous commodity that is highly digestible for broilers (Bedford, 2002), although some reports have found considerable variation between samples (Classen, 1996)

and others that maize starch digestibility may be as low as 85 % at the terminal ileum (Noy and Sklan, 1994). Due to the fact that maize is a relatively poor source of protein and most amino acids, various enzymatic studies have been conducted in an attempt to increase the digestibility of nitrogenous substances and energy, mainly by degrading NSP's and thereby releasing nutrients embedded in the protein matrix of the cell wall (Zanella *et al*., 1999; Cowieson and Adeola, 2005; Meng and Slominski, 2005) with promising results.

To accurately evaluate the use of enzymes in broiler diets, not only potential improvements in broiler performance, but also improvements in nutrient digestibility (especially AA- and energy digestibility) should be considered. The earliest method to determine AA digestibility of feedstuffs for poultry was the method developed by Kuiken and Lyman (1948), which involves the determination of the difference between the proportions of AA's in the feed (AA input) and excreta (AA output) and thereby calculating the digestibilities of various AA's. Such early digestibility studies had the major advantage of great simplicity and no need to sacrifice birds after the trial period (McNab, 1995), but had the disadvantage of underestimated digestibilities due to the proportion of AA's from uric acid and the limited contribution of the caecal micro flora to the AA pool. Payne *et al.* (1968) were the first to suggest that protein- and AA digestibilities based on sampling of the ileal digesta is more accurate (Ravindran *et al*., 1999b) mostly because there is no contribution of uric acid in the digesta. For this method the bird has to be killed, the abdomen opened, and the digesta from the terminal ileum removed, freeze dried and analyzed.

The objective of this thesis is to evaluate the use of a commercial enzyme combination in nutritionally balanced and low AMEn vegetarian maize-soya broiler diets with the main emphasis on the potential improvements in N- and AA digestibilities and production performance of broiler chickens. The financial implications of the addition of such enzymes in broiler diets will also be discussed. Furthermore, a comparison will be drawn between two different methods of determining digestibility.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The use of exogenous enzymes to improve the digestibility and availability of nutrients in broiler diets has been extensively investigated in the past especially with regard to wheat-, barley-, and rye based diets (Brenes *et al*., 1993; Cowan *et al.*, 1996; Dänicke *et al*., 1999; Choct *et al*., 1999; Ravindran *et al.*, 1999a; Marron *et al*., 2001;). Exogenous phytase to improve the availability of phytate phosphorous is probably one of the most well researched feed enzymes in broiler nutrition not only due to significant performance improvements seen with the use of this enzyme but also due to the fact that phytase results in less phosphorous being excreted into the environment. The use of enzymes that target the NSP's, which are known to increase digesta viscosity and invariably affect nutrient absorption, is well documented (Choct and Annison, 1992; Almirall *et al*., 1995). The addition of enzymes to enhance the digestibility of NSP's in broiler chicken diets, such as xylanase and β-glucanase, has an extensive history spanning about 20 years (Choct, 2006).

The use of exogenous enzymes to improve the digestibility of maize-soya diets for broilers is less well documented. Neither maize nor soya is regarded as viscous feedstuffs even though they do contain appreciable amounts of NSP's (Bach Knudsen, 1996). The degradation of these NSP's may however not be sufficient to produce commercially viable improvements, therefore necessitating for multi-enzyme combinations to be used in these types of diets. Various reports suggest that significant improvements can be made with the use of such enzyme combinations (Zanella *et al*., 1999; Cowieson and Adeola, 2005; Meng and Slominski, 2005).

The improvement in the nutritive quality of feedstuffs and the reduction in environmental pollution are however not the only benefits associated with the use of feed enzymes. Other possible benefits include increased accuracy and flexibility in least-cost feed formulations, decreased variability of feed ingredients and improved well being of animals (Choct, 2006).

The objective of this chapter is to review the literature pertaining to the main anti-nutritional factors (ANF) associated with feedstuffs in broiler nutrition and the enzymes used to degrade them, with the main emphasis on enzymes for maize-soya diets. The effect of enzyme supplementation on nutrient digestibility, especially with regard to N- and AA digestibility will be discussed, followed by a review of the effect of processing on the nutritive value and enzymatic activity and the influences of age, sex and method of determination on AA digestibilities.

2.2 Anti-nutritive factors (ANF) in common raw materials in poultry diets

The main ANF associated with ingredients commonly used in poultry diets are listed in Table 2.1. Potential problematic compounds in maize include lectins, phytate, resistant starch and NSP's while oligosaccharides, NSP's, trypsin inhibitors, lectins and phytate are compounds that may

adversely affect digestion of diets based on soya. Heat labile ANF such as trypsin inhibitors and lectins in soyabeans can however be inactivated by heat processing (Kocher *et al*., 2002) making exogenous enzyme addition to degrade such compounds superfluous and unnecessary.

Table 2.1 Primary plant ingredients used in poultry diets and compounds in them which may cause adverse effects (Acomovic, 2001)

Ingredient	Some potential problem compounds
Maize	Lectins, phytate, resistant starch
Wheat	Arabinoxylans, wheat germ agglutinin, phytate, resistant starch
Barley	Beta-glucans, resistant starch
Rice	Phytate, arabinoxylans
Sorghum	Tannins, resistant starch
Rye	Arabinoxylans, polyphenols
Soyabean meal	Oligosaccharides and NSP's*, trypsin inhibitors, lectins
Peas	Resistant starch, proteins, saponins
Beans	Tannins, trypsin inhibitors, lectins, oligosaccharides, NSP's
Lupins	Oligosaccharides, NSP's, proteins
Rapeseed meal	Oligosaccharides, NSP's, tannins, glucosinolates
Sunflower meal	Oligosaccharides, NSP's

* Non-starch polysaccharides

2.2.1 Phytate

Phosphorous (P) is vital for skeletal development, maintaining homeostasis and in providing energy yielding substrates in the body for metabolic processes. Plant sources of P have been shown to be only 30 % available to monogastrics, in contrast to an availability approaching 100 % for synthetic P sources like mono- and dicalcium phosphates. The reason for the low availability of P in cereals and oilseeds is due to the fact that most of the P contained in these feedstuffs is in the form of phytic acid. Phytic acid (also known as phytate or myo-inositol hexaphosphate) is capable of binding or chelating divalent or trivalent cations, forming insoluble salts. Phytic acid also has the ability to bind proteins and arguably even digestive enzymes (Touchburn *et al*., 1999) and may adversely affect AA digestibility and nitrogen retention. This happens because at low pH, as in the monogastric stomach, proteins are positively charged and can form insoluble complexes with negatively charged phytate molecules due to strong electrostatic interactions (Yi *et al*., 1996). Phytic acid can thus be seen as an anti-nutritional factor (because of its chelate-forming properties) but also as a nutrient because it is a good source of phosphorous. Phytase enzymes release P from phytate in animal feeds. The effect of exogenous phytase, which releases phytate-bound phosphorous, from *Aspergillus ficuum* NRRL 3135 on dietary phosphorous utilization by chicks was first reported by Nelson, Shieh, Wodzinski and Ware in 1971 (Rosen, 2002) and is still receiving generous attention today. Even though poultry intestinal

mucosa secretes a phytase which is active at low inorganic phosphorous concentrations in the diet it is known that monogastrics elaborate very little phytase (Touchburn *et al*., 1999). By including exogenous phytase in broiler chicken diets, the organic phosphorous in the feed may thus be utilized to a greater extent and allows for lower rates of inclusion of more expensive inorganic phosphate sources.

2.2.1.1 Phytase and nitrogen (N) metabolism

Research done on the effect of phytase on N, protein and AA metabolism in poultry has produced somewhat contradictory results. Yi *et al*. (1996) studied the effect of microbial phytase on N and AA digestibility and N retention of turkey poults fed maize-soybean meal diets. These researchers suggested that the addition of phytase increased the apparent ileal digestibility (AID) of N and all of the EAA (Table 2.2). They have also found that the effect of phytase on the AID of N and AA were dependant on the dietary crude protein (CP) level and the non-phytate phosphorous (nP) level in the diet and generally showed an increase either in low nP diets with an adequate CP level or in adequate nP diets with a low CP level.

Similar research done on male and female broilers on a maize-soybean diet by Sebastian *et al*. (1997) showed that phytase supplementation in males increased the AID of CP but had no influence on AID of any AA except Met and Phe. In contrast, in the females, supplementary phytase increased the AID of all AA's except Lys, Met, Phe and Pro. It also seems that the magnitude of improvement of AA digestibilities by phytase supplementation varies depending on the feedstuff being evaluated (Ravindran *et al*., 1999a).

Recently Augspurger and Baker (2004) found that high dietary levels of efficacious phytase enzymes can release most of the P from phytate, but they do not improve protein utilization. They concluded that supplemental phytase, even at a very high dose level, does not improve the protein utilization of chicks fed diets based on ingredients first limiting in different AA's. Research also indicates that the protein efficiency ratio (PER) values for chicks (g weight gain per g protein intake) fed a range of different feedstuffs (casein, soybean meal, canola meal, wheat bran, meat and bone meal, and corn gluten meal) vary considerably and that phytase does not have a significant effect on the PER values for any of the feedstuffs evaluated except for casein (Boling-Frankenbach *et al*., 2001). Research thus indicates that a variety of factors such as sex, CP-level and nP-level in the diet may affect results obtained in protein digestibility studies with exogenous phytase. However, the use of exogenous phytases is beyond the scope of this thesis and therefore will not be discussed in detail.

2.2.2 Non-starch polysaccharides (NSP's)

Originally it was thought that NSP's did not significantly influence digestion as only limited fractions are fermented in the lower intestine. It is now known that, even at low levels (< 50g/kg), it

may exhibit an anti-nutritional effect (Annison and Choct, 1991). These NSP's are complex structures and are difficult to identify solely on their chemical composition.

Table 2.2 The effect of microbial phytase on improving availability of N and amino acids in poultry [1](Touchburn *et al*., 1999).

Species	CP (*g/kg*)	Phytase (*units/kg*)	Improvement in the availability of N and amino acids (*percentage units of control*)										Source
			N	Essential amino acids										
				Met	*Cys**	*Lys*	*Thr*	*Arg*	*His*	*Ile*	*Leu*	*Phe*	*Val*	
Turkey	225	750	1.0	0.6	2.1	0.7	1.0	0.6	0.8	0.7	0.9	0.8	0.8	Yi *et al. (1996)*
	280	750	1.5	0.5	3.1	1.3	1.2	1.1	1.3	1.9	1.5	1.6	1.8	Yi *et al. (1996)*
Broiler	220	600	1.3	0.3	-	0.6	4.3	1.8	0.3	3.1	1.9	1.1	2.5	Sebastian *et al.* (1997)
				Non-essential amino acids										
					Pro	*Asp*	*Ser*	*Glu*	*Gly*	*Ala*	*Try*			
Turkey	225	750			0.8	0.9	1.2	0.8	1.1	0.8	1.3			Yi *et al.* (1996)
	280	750			2.4	1.7	0.7	1.3	1.6	1.3	1.2			Yi *et al.* (1996)
Broiler	220	600			0.2	3.7	4.1	2.1	0.8	1.9	2.8			Sebastian *et al.* (1997)

* Semi-essential amino acid; CP: Crude protein

[1] Female birds

The main NSP's present in the raw materials commonly used in poultry nutrition are arabinoxylans, mixed-linked β-glucans, cellulose and pectins (Dalibard and Geraert, 2004). These NSP's require specific enzymes (Table 2.3) for depolymerisation, with the latter being specific with regard to main- and side chain structure.

2.2.2.1 Anti-nutritional effect of NSP's

It is generally assumed that NSP's exhibit their anti-nutritional effects in different ways. Firstly by increasing digesta viscosity through the release of soluble NSP's leached from cell walls (Chesson, 2001) which may result in reduced rates of diffusion of nutrients and/or reduced feed passage time (and thus ingestion)(Classen, 1996). This is also known as the viscosity model of nutrient inhibition by

Table 2.3 Main NSP's of raw materials used in poultry feed (Dalibard and Geraert, 2004).

	Structure		Required enzymes
Nature	Main chain	Side chain	
Arabinoxylans	$(\beta\ (1\rightarrow4)\ D\ Xylp)_n$	α(1→3) L Araf α(1→2) L Araf	Endo- 1,4-β-xylanase α-arabinofuranosidase, β-xylosidase, feruloyl esterase
Mixed linked β-glucans	$\{(\beta(1\rightarrow4)\ D\ glucp)_{2-5}$ $(\beta(1\rightarrow4)\ D\ glucp)_1\}_n$	-	Endo-1,3(4)-β-glucanase cellobio-hydrolase, β-glucosidase
Cellulose	$(\beta(1\rightarrow4)\ D\ glucp)_n$	-	Endo-1,4-β-glucanase, cellobiohydrolase, β-glucosidase

NSP's as proposed by Cowan *et al*. (1996). The absorption of large molecules is affected to a greater extent by increased viscosity than smaller molecules (Choct and Annison, 1992; Classen, 1996). In this regard the response in lipid digestion with enzyme supplementation is greater compared to protein digestion (Choct and Annison, 1992). This is most likely not the main reason for anti-nutritive activity in maize-soya diets as neither maize nor soya is known to increase digesta viscosity significantly.

Secondly it is thought that soluble NSP's increases the size and solidity of the unstirred layer at the mucosal surface of the digestive tract, resulting in limited contact between the digestive enzymes and the substrates (Chesson, 2001).

Thirdly, by encapsulating the starch, fat and protein in the feed (Cowan *et al.*, 1996) forming a physical barrier between the digestive enzymes and the substrates to be digested. Mainly due to under processing, large intact cells escape digestion and nutrients cannot be absorbed due to the fact that a variety of different enzymes and prolonged attack is needed to degrade the cell walls of such particles (Bedford, 2002). The encapsulation of these particles results in even less substrate available to enzymatic attack.

Fourthly, by altering the microbial profile of the digestive tract (Choct *et al*., 1999) and promoting bacterial proliferation to the detriment of digestive efficiency and bird health (Choct *et al*., 1996). This is most probably as a result of increased digesta viscosity. It is known, for example, that xylanase supplementation significantly reduces enterobacteria and total anaerobic microbes with a similar trend for gram-positive cocci and enterococci in rye based diets (Dänicke *et al*., 1999).

Lastly, by altering intestinal morphology. Mathlouthi *et al*. (2002) found that the addition of xylanase and β-glucanase to rye based diets increased ($P<0.05$) villi size and the villus height-to-crypt depth ratio, as well as the concentration of conjugated bile acids ($P<0.05$) in the small intestinal contents leading to increased nutrient absorption.

The anti-nutritional effect of NSP's is often confounded by the fact that the above mentioned factors rarely can be separated. It is more likely that a combination of these factors will result in the overall anti-nutritional effect associated with NSP's.

2.2.2.2 Mode of degradation of non-starch polysaccharide-degrading enzymes

Castañon *et al*., 1997 noted that there are two ways in which NSP-degrading enzyme preparations might act on cereal NSP's. Firstly, by transforming the existing insoluble NSP's to soluble NSP's and secondly by the hydrolysis of soluble NSP's. The enzymatically facilitated hydrolysis of the soluble NSP-fraction, especially in wheat, rye and barley based diets, reduces the anti-nutritional effect of the NSP's but may still only be seen as a restoration of expected performance and not an improvement (Chesson, 2001). In contrast, enzymes targeting the insoluble NSP's produce a much more limited response as the structural design and variability of the polysaccharides is the major controlling factor (Chesson, 2001).

Although the mode of action of NSP-degrading enzymes in viscous cereals is clear, the mode of action of such enzymes in non-viscous cereals such as maize is still unclear as limited research has paid any attention to this. One possibility is the proliferation of some ileal and caecal bacteria that compete with detrimental bacterial due to the rapid degradation of the cell wall components to produce sugars and oligomers as described by Bedford (2000). Another possible mode of action might be that the cell wall polysaccharides are made structurally unsound by the enzymes, resulting in increased substrate availability to endogenous amylase and proteases.

2.2.2.3 Non-starch polysaccharide-degrading enzymes and nitrogen metabolism

The degradation of cell wall components may not only release entrapped nutrients but also allow for better enzymatic degradation of these nutrients. Research conducted on pigs by Cowan *et al*. (1996) suggested that N-retention may be improved by the addition of a commercial enzyme preparation, although a clear dosage response could not be seen with all raw materials. Conversely, Rebolé *et al*. (1999) found no significant differences in apparent ileal CP- and AA digestibility in broilers using a similar enzyme preparation. More recently, Meng and Slominski (2005) found that a preparation of cell wall degrading enzymes with xylanase-, glucanase-, pectinase-, cellulose-, mannanase- and galactanase activity may improve ileal protein digestibility of a SBM based diet for broilers by up to 4.4 % ($P<0.05$). Using similar preparations, results by Zanella *et al.* (1999) showed improvements in overall CP digestibility of 2.9 % in a maize-soya diet for broilers ($P<0.05$). These researchers also found that the digestibilities of Lys, (Met) and Arg were not improved although that of Cys, Val and Thr was improved by 2.1, 2.3 and 3.0 % respectively ($P<0.05$). Conversely, Kocher *et al*. (2002) found that an enzyme combination containing mainly hemicellulase, pectinase, β-glucanase and protease activities significantly reduced ileal protein digestibility of SBM fed to broilers but did not affect broiler chicken performance ($P<0.05$). The abovementioned results suggest that in most cases positive responses in N- metabolism may be found when using NSP-degrading enzymes. The effect of enzyme supplementation on the digestibility of AA's needs further investigation as some essential amino acids (EAA) for broiler growth (Lys, Met and Arg) may arguably not be improved (Zanella *et al*., 1999).

2.3 Anti-nutritional factors in maize

Compared to other cereals such as wheat, rye and barley used in broiler chicken diets, the ANF's in maize pose less of an anti-nutritive threat. Perhaps the most important anti-nutrient in maize is phytate, which is beyond the purpose of this thesis, and resistant starch which will be discussed in this section.

2.3.1 Resistant starch

Starch is a mixture of glucans that plants synthesize as their principal energy reserve and consists of repeating α-amylose and amylopectin residues. Starch is the main polysaccharide of whole grain cereals ranging between 468 g.kg $^{-1}$ for oats to 690 g.kg $^{-1}$ for maize (Bach Knudsen, 1996). Maize is assumed to be a highly digestible cereal (Classen, 1996) and maize starch, in particular, is assumed to be completely digested by the time it exits the terminal ileum (Bedford, 2002). There is, however, some evidence suggesting that starch digestibility may be variable between grain samples, even of the same cultivar (Classen, 1996) although this is not the case for maize harvested dried as in South Africa. Noy and Sklan (1994) showed that maize starch digestibility at the terminal ileum may be as low as 85 %. This may be due to the fact that some forms of starch cannot be degraded by endogenous carbohydrases (Bedford, 2002) due to their different chemical structure and physical properties (Acamovic, 2001). Such starch is known as resistant starch and presents the opportunity for the use of exogenous feed enzymes. There are three classes of naturally occurring resistant starch, RS1, RS2 and RS3 (Brown, 1996): RS1 is based on the physical inaccessibility of the starch granules and is that proportion of the cereal endosperm cells that remains intact and undigested after processing. The starch contained in these cells pass through the digestive tract without being exposed to the digestive secretions and thus escapes digestion. Bedford (1996) suggested that an appreciable amount of starch escapes digestion by this route but is most probably fermented in the intestine or caeca. RS2 is that proportion of the maize starch that is not digested due to the physical and chemical structure of the native granule especially the structure of the α-1-4 glucose polymers that make up the starch itself (Bedford, 2002). The degree of resistance to digestion of a starch granule seems to be related to the structure and conformation of the starch granule. The linear α-1-4 glucose polymers can be classified into two patterns (A and B). In both patterns the starch β-glucan chains exist as left-handed, parallel-stranded double helices. The A pattern however has an additional helix, occupying the center of the hexagonal array. In the B pattern the center of the hexagonal array contains water. The A pattern is more rapidly digested than the B pattern which contains a greater amount of water (Bedford, 2002). While most cereals including wheat and maize posses the A pattern, it is known that high-amylose maize posses the B pattern and is thus more slowly digested (Bedford, 2002). A third pattern, also resistant to enzyme degradation, is called the C pattern and is considered a combination of the A and B patterns and are commonly found in legume starches for example pea starch. A second consideration with regards to starch granule structure is the proportion of amylose to amylopectin, the

latter being more easily digested (Bedford, 2002). A higher proportion of amylose results in a greater RS2 content and thus a slower rate of digestion.

When starches are processed at high temperatures, i.e. 154-171° C for high-amylose maize starch (Bach Knudsen, 1996), a proportion of the starch is gelatinized and no longer resistant to digestion. However, when these gelatinized structures are stored over a period of time, they can re-associate into crystalline complexes with proteins and cell wall structures and form indigestible retrograde starch known as RS3. Starches rich in amylose are more resistant to gelatinization than amylopectin-rich starches but are more likely to form retrograde starches. Even though RS3 is impervious to pancreatic amylases it is susceptible to fermentation in the large intestine and caeca (Bedford, 2002).

RS1 may thus be subject to exposure to attack by cell wall-degrading enzymes to produce digestible carbohydrates while RS2 and RS3 escapes digestion but are to a large extent exposed to attack by the caecal micro flora, possibly altering the caecal microbial populations (Bedford, 2002).

2.3.2 Non-starch polysaccharides

Arabinoxylans are plant carbohydrates with arabinose and xylose sugar components. These NSP's predominate in the maize endosperm, as with most other cereals although mixed linked β-glucans and cellulose, with glucose as sugar component, are also present (Chesson, 2001). The pericarp and seed coat are also rich in xylans, with xylose as the sugar component, and cellulose which unlike other cereals, are not extensively lignified (Chesson, 2001). Bach Knudsen (1996) evaluated the carbohydrate and lignin content of plant materials commonly used in animal feeding. The mean values of arabinose, xylose, β-glucans and cellulose for three maize samples was found to be 22 (3+19), 30 (2+28), 1 and 22 $g.kg^{-1}$ respectively (Table 2.4). The total NSP content of maize (97 $g.kg^{-1}$) is considerably lower compared to other cereals and less than half that of hulled oats (232 $g.kg^{-1}$). The ratio of insoluble non-cellulosic polysaccharides (I-NCP) to soluble non-cellulosic polysaccharides (S-NCP) was found to be much larger for maize than for any of the other cereals evaluated (wheat, rye, barley andoats)(Bach Knudsen, 1996). This is the reason why maize NSP is not considered to increase digesta viscosity in broilers as it is mainly soluble NSP that has water binding capacity which leads to increased viscosity. It is also clear that although β-glucans may be considered a problematic NSP's in maize, it only contributes to 0.1 % of the dry matter (DM) content of maize and to only about 1 % of the NSP content thereof. More problematic NSP's in maize are the I-NCP (6.6 % of DM and 68 % of total NSP) and cellulose (2.2 % of DM and ~23 % of total NSP).

Table 2.4 Carbohydrate and lignin content (g.kg^{-1} dry matter) (standard deviation) in whole grain cereals (Bach Knudsen, 1996).

	Maize	Wheat	Rye	Barley	Barley	Oats	Oats
	Mean	Mean	Mean	Hulled	Hulless	Hulled	Hulless
				Mean	Mean	Mean	Mean
Number of samples	3	5	7	10	6	3	4
LMW-sugars [a]							
Monosaccharides	4 (1)	3 (0)	6 (2)	4 (2)	n.m [f]	2 (1)	n.m
Sucrose	13 (1)	11 (2)	19 (3)	12 (7)	n.m	11 (2)	n.m
Raffinose	2 (0)	4 (1)	4 (1)	5 (1)	n.m	3 (1)	n.m
Stachyose	1 (0)	2 (0)	3 (1)	1 (1)	n.m	2 (1)	n.m
Total sugars	20 (2)	19 (1)	32 (3)	21 (7)	n.m	17 (4)	n.m
Starch	690 (18)	651 (27)	613 (5)	587 (31)	645 (17)	468 (25)	557 (38)
Fructan	6 (2)	15 (3)	31 (2)	4 (1)	n.m	3 (2)	n.m
NSP's [b]							
β-glucan	1 (1)	8 (1)	16 (2)	42 (5)	42 (6)	28 (3)	41 (8)
S-NCP [c]	9 (7)	25 (4)	42 (11)	45 (10)	50 (10)	40 (13)	54 (7)
Rhamnose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Arabinose	3 (2)	7 (2)	12 (2)	6 (1)	3 (1)	3 (1)	3 (1)
Xylose	2 (2)	9 (4)	20 (7)	6 (3)	4 (1)	2 (3)	2 (1)
Mannose	2 (1)	2 (1)	2 (1)	2 (1)	1 (<1)	2 (1)	1 (1)
Galactose	1 (1)	2 (1)	1 (1)	1 (1)	1 (<1)	2 (1)	2 (0)
Glucose	1 (1)	4 (3)	6 (4)	39 (7)	41 (8)	28 (5)	45 (7)
Uronic acids	1 (1)	1 (1)	1 (1)	2 (1)	1 (1)	3 (4)	2 (1)
I-NCP [d]	66 (11)	74 (6)	94 (9)	88 (10)	64 (11)	110 (9)	49 (10)
Rhamnose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Arabinose	19 (2)	22 (1)	24 (1)	22 (1)	17 (1)	15 (0)	10 (1)
Xylose	28 (3)	38 (3)	41 (4)	50 (4)	24 (4)	78 (8)	21 (7)
Mannose	1 (1)	1 (1)	3 (1)	2 (1)	3 (0)	1 (0)	2 (1)
Galactose	4 (1)	2 (1)	4 (1)	2 (1)	2 (0)	5 (0)	2 (0)
Glucose	9 (4)	7 (3)	20 (6)	8 (6)	17 (6)	5 (2)	11 (2)
Uronic acids	6 (1)	4 (1)	3 (0)	4 (0)	1 (0)	7 (0)	3 (1)
Cellulose	22 (3)	20 (4)	16 (3)	43 (5)	10 (3)	82 (5)	14 (6)
Total NSP's	97 (2)	119 (11)	152 (10)	186 (11)	124 (10)	232 (10)	116 (19)
Klason lignin	11 (2)	19 (2)	21 (2)	35 (3)	9 (2)	66 (9)	32 (6)
Dietary fibre	108 (4)	138 (10)	174 (10)	221 (13)	133 (12)	298 (19)	148 (23)
CHO [e] and lignin							
Analysed	823 (11)	823 (18)	850 (8)	834 (24)	n.m	787 (42)	n.m
Calculated	830 (5)	814 (14)	849 (11)	823 (20)	n.m	770 (14)	n.m

[a] Low molecular weight sugars; [b] Non-starch polysaccharides; [c] Soluble non-cellulosic polysaccharides; [d] Insoluble non-cellulosic polysaccharides; [e] Carbohydrates; [f] Not measured

2.4 Anti-nutritional factors in soya

Many ANF have been identified in soya, most notably trypsin inhibitors, lectins, NSP's and oligosaccharides. For the majority of these, their anti-nutritional effect may be minimized by heat processing. The more problematic compounds are those which are not destroyed by processing and will be discussed in this section.

2.4.1 Oligosaccharides

The main indigestible carbohydrate fraction in SBM is the oligosaccharides (60 g.kg^{-1}), which consist of mainly α-galactosides (Kocher *et al*., 2002). Although oligosaccharides are considered indigestible, the extent of bacterial degradation thereof in the hindgut may be extensive in broilers

(Carré *et al*., 1995). The energy obtained from bacterial fermentation, yielding mainly lactose and volatile fatty acids, is however, considerably less than that which would have been obtained if the oligosaccharides had been digested in the upper intestine producing glucose (Kocher *et al*., 2002). Coon *et al*. (1990) showed that the removal of oligosaccharides in SBM may lead to increased nitrogen corrected true metabolisable energy (TMEn) in adult roosters ($P<0.05$). Irish *et al*. (1995) however found no changes in TMEn, weight gain, feed efficiency, protein digestibility, or the digestible energy value of a corn-SBM diet supplemented with α-galactosidase ($P<0.05$) Their results also showed that the removal of up to 90 % of the alpha-galactosides (i.e. stachyose and raffinose) of sucrose did not result in any beneficial nutritive change in the SBM-based diets even though these fractions account for a large proportion of the sugars in SBM at 10 and 47 g kg^{-1} respectively ($P<0.05$)(Table 2.5).

It may thus be assumed that even though bacterial fermentation of the oligosaccharides of SBM may be substantial, the additional energy obtained thereof is not sufficient to induce significant responses in broilers.

2.4.2 Non-starch polysaccharides

Soyabean meal contains approximately 217 $g.kg^{-1}$ NSP's, of which 25 to 30 $g.kg^{-1}$ are soluble (Bach Knudsen, 1996). Soyabean cell walls contain a high proportion of pectic polysaccharides, which account for approximately half of the NSP's of the total seed (Chesson, 2001). The S-NCP make up approximately 29 % of the total NSP content of SBM with uronic acids (25 $g.kg^{-1}$), galactose (16 $g.kg^{-1}$) and arabinose (9 $g.kg^{-1}$) making the largest contributions to this fraction (Table 2.5). As with maize, the I-NCP and cellulose make up the bulk of the total NSP content in SBM, contributing approximately 42 % and 29 % respectively (Table 2.5). The I-NCP fraction in SBM is largely constituted by xylans (xylose) and xyloglucans (Bach Knudsen, 1996).

The pectic polysaccharide fraction of soya beans seems to be structurally unique. Huisman *et al*. (1998) found that 90 % of the uronic acids in SBM were recovered in the water-unextractable solid fraction which is indicative of more complex pectic substances compared to those isolated from other plant cells. The pectic backbone of soya has also been found to be structurally devoid of homogalacturonan and contains regions of mostly rhamnogalacturonan and xylogalacturonan (Huisman *et al*., 2001). Chesson (2001) suggests that this may be one of the reasons for the lack of response when polygalacturonase is used in enzyme preparations, as enzymes are very specific to substrate and side chain structure.

Table 2.5 Carbohydrate and lignin (g kg^{-1} dry matter) (standard deviation) in protein concentrates (Adapted from Bach Knudsen, 1996).

	Soybean	Rapeseed		Faba beans	White lupins
	Meal	Meal	Cake		
	Mean	Mean	Mean	Mean	Mean
Number of samples	6	4	3	6	3
LMW-sugars [a]					
Monosaccharides	7 (1)	8 (1)	4 (1)	5 (2)	5 (2)
Sucrose	70 (11)	58 (2)	68 (7)	27 (4)	24 (7)
Raffinose	10 (1)	4 (1)	3 (0)	4 (1)	10 (2)
Stachyose	47 (4)	12 (3)	13 (1)	16 (3)	53 (14)
Verbascose	3 (2)	0 (0)	0 (0)	34 (9)	14 (2)
Total sugars	137 (16)	82 (1)	90 (7)	86 (10)	104 (11)
Starch	27 (12)	18 (16)	15 (8)	407 (50)	14 (2)
NSP's [b]					
S-NCP [c]	63 (10)	55 (17)	43 (14)	50 (5)	134 (44)
Rhamnose	1 (1)	1 (1)	1 (1)	1 (0)	2 (1)
Arabinose	9 (2)	12 (4)	13 (3)	15 (2)	19 (4)
Xylose	2 (1)	4 (1)	2 (1)	1 (1)	0 (0)
Mannose	5 (1)	1 (1)	1 (0)	1 (0)	4 (1)
Galactose	16 (3)	6 (2)	5 (1)	4 (1)	80 (30)
Glucose	6 (3)	9 (11)	3 (3)	4 (2)	1 (1)
Uronic acids	25 (4)	22 (2)	18 (6)	24 (4)	27 (10)
I-NCP [d]	92 (9)	123 (5)	103 (21)	59 (45)	139 (21)
Rhamnose	2 (0)	2 (0)	2 (0)	0 (0)	1 (0)
Arabinose	17 (2)	31 (4)	31 (9)	9 (4)	24 (2)
Xylose	17 (3)	13 (3)	15 (5)	11 (3)	36 (1)
Mannose	8 (2)	5 (1)	4 (1)	1 (0)	5 (2)
Galactose	25 (3)	13 (1)	15 (4)	2 (1)	61 (16)
Glucose	1 (2)	12 (14)	5 (4)	28 (41)	1 (1)
Uronic acids	23 (3)	39 (7)	32 (1)	9 (5)	12 (5)
Cellulose	62 (18)	52 (3)	59 (11)	81 (12)	131 (31)
Total NSP	217 (27)	220 (20)	205 (20)	190 (50)	405 (54)
Klason lignin	16 (4)	134 (19)	90 (31)	20 (8)	12 (2)
Dietary fibre	233 (26)	354 (10)	295 (12)	210 (55)	416 (54)
CHO [e] and lignin					
Analysed	400 (15)	454 (17)	399 (13)	705 (31)	534 (47)
Calculated	416 (17)	457 (12)	n.m.[f]	665 (28)	498 (31)

[a] Low-molecular weight sugars; [b] Non-starch polysaccharides; [c] Soluble non-cellulosic polysaccharides; [d] Insoluble non-cellulosic polysaccharides; [e] Carbohydrates; [f] Not measured

2.5 Enzyme supplementation in maize-soya diets for broilers

2.5.1 Non-starch polysaccharide-degrading enzymes

The most abundant carbohydrates in maize and soya are cellulose, arabinoxylans, pectins, oligosaccharides and starch. Some of these compounds are relatively resistant to digestion such as cellulose, arabinoxylans and pectins. Many different primary enzymes are required to degrade these carbohydrates such as cellulase and cellobiohydrolyse to degrade cellulose, xylanase and arabinofuranosidase to degrade arabinoxylans, α-galactosidase to degrade the oligosaccharides and amylase to degrade starch.

2.5.1.1 Xylanase

Arabinoxylans occur as storage polysaccharides in legumes and can partially be solubilised by processing (Chesson, 2001). Arabinoxylans are primarily hydrolyzed by endo-1,4-β-xylanase activity, cleaving the (1,4)-linkages of the xylan backbone (Classen, 1996). Various reports in the literature suggest the efficacy of xylanase to improve nutrient digestibility and performance in rye (Dänicke *et al*., 1999; Silva and Smithard, 2002), wheat (Brenes *et al*., 1993; Choct *et al.*, 1999; Marron, *et al*., 2001;) and barley (Brenes *et al*., 1993) based diets for broilers. The use of xylanase in maize-soya based diets is less well documented. Zanella *et al*. (1999) found that an enzymatic cocktail exhibiting xylanase, protease and amylase activities improved overall CP digestibility by 2.9 % and Thr (Thr) and Val digestibility by 3 % and 2.3 % respectively. Using similar diets and enzymes, Cowieson and Adeola, (2005) found improvements in gain-to-feed ratio and body weight gain although the effect of the enzyme combination on ileal digestible energy (IDE) and the digestibility coefficients of N and dry matter (DM) were less dramatic. Meng and Slominski (2005) found that the ileal digestibility of starch and energy (AME_n) in maize based diets and the ileal digestibility of NSP's, AME_n and CP in SBM diets may be improved by a preparation of cell wall degrading enzymes supplying xylanase, glucanase, pectinase, cellulase, mannanase and galactanase activities. Undoubtedly these results suggest positive responses with xylanase although the improvements seen may be attributed to the combination of xylanase with other carbohydrases and protease.

2.5.1.2 β-Mannanase

β-mannans in SBM are linear polysaccharides composed of repeating β-1-4 mannose and α-1-6 galactose and glucose units attached to the β-mannan backbone (Jackson *et al.*, 2004). Various reports in the literature suggest that β-mannans are extremely antinutritional for humans (Morgan *et al*., 1985) and broilers (Ray *et al*., 1982; Verma and McNab, 1982). It has been suggested that the mode of action of β-mannanase is by decreasing digesta viscosity, as β-mannans are known to be highly viscous. Another possibility is that β-mannans decrease the energy availability (Rainbird *et al*., 1984).

A third mode of action may be a reduction in innate immune stimulation associated with a reduction in the β-mannan content entering the intestinal tract (Jackson *et al*., 2004). In a dose-response study with β-mannanase in broilers, Jackson *et al*. (2004) found that the addition of β-mannanase at 80 or 110 MU/ton (where 1 MU is 10^6 enzyme activity units) significantly improved the weight gain and FCR of male broilers during starter and grower phases. These researchers conclude that the improvements in FCR seen in their experiments can mainly be attributed to increased energy utilization and perhaps changes in intestinal viscosity (Jackson *et al*., 2004).

2.5.2 Oligosaccharidases

2.5.2.1 α-Galactosidases

Oilseeds such as soya are particularly rich in oligosaccharides such as raffinose, stachyose and verbascose, which are generally assumed to be indigestible by the birds but readily fermentable by the intestinal flora (Bedford, 2002). The use of α-galactosidases to degrade these oligosaccharides to their sugar components (glucose, fructose and galactose) may improve the availability of energy and possibly the nutritive quality of the diet for broilers. This is due to the fact that substrates that would normally have been indigestible such as oligosaccharides, yields energy providing sugars to the bird (glucose, fructose and galactose). The removal of the substrate by the addition of enzymes is also beneficial to the bird for various reasons (Bedford, 2002). Firstly, bacteria compete for nutrients in the ileum. The more substrate available, the greater the bacterial populations and the greater the bacterial challenge to the bird. Secondly, some bacteria secrete enzymes, which inactivate bile salts, lecithin and pancreatic enzymes and damage the small intestinal surface, compromising the digestive ability of the bird. Lastly, a costly immune response may be triggered if the population of a certain species reaches a significant level.

The extraction of oligosaccharides from SBM with ethanol has been shown to improve the digestibility thereof (Coon *et al*., 1990) and the addition of α-galactosidase has also been shown to increase the true metabolisable energy (TME) of SBM (Knap *et al*., 1997). In contrast, Irish *et al*. (1995) demonstrated that the addition of α-galactosidases did not significantly improve the growth rate or feed conversion efficiency in broilers. It can then be suggested, in agreement with as Bedford (2002), that although theoretically possible, it is not clear whether the oligosaccharides in oilseeds are actually detrimental to bird performance, especially when fed at commercial levels.

2.5.3 Proteases

Various reports in the literature suggest that the nutritive value of broiler diets may be enhanced by proteases when used in combination with other enzymes such as xylanases and amylase (Zanella *et al*., 1999; Cowieson and Adeola, 2005). Zanella *et al*. (1999), found that enzyme supplementation of a combination of protease, xylanase and amylase, significantly increased the overall CP digestibility by 2.9 % ($P<0.05$), but that the digestibility of Lys, Met and Arg were not improved by the enzyme product ($P<0.05$). Their results also suggest that the enzymes improved BW and the FCR by 1.9 % and 2.2 % respectively ($P<0.05$). These findings may however not be attributed solely to the effects of protease as the enzyme product contained appreciable xylanase and amylase activities. It does however suggest a positive interaction between these enzymes.

Recently Odetallah *et al*. (2005) investigated the effect of a protease containing feed additive in broiler diets consisting of mainly maize and soya. Their results indicate that enzyme supplementation improved the 22 d BW and FCR of the broilers. They concluded that protease supplementation of starter broiler diets resulted in improved market growth performance. Protease supplementation of

maize-soya diets may thus improve the nutritive value thereof for broilers, especially when used in combination with other cell wall degrading enzymes and amylase.

2.6 Thermal processing

2.6.1 The effect of thermal processing on the nutritional value of soya

Thermal processing of soya is necessary to inactivate heat labile ANF such as lectins and trypsin inhibitors. The nutritional value of the compact folded proteins in soya can also be increased by toasting and particularly extrusion (Marsman *et al*., 1997). The over processing of SBM may however affect the availability of certain AA's. Non-enzymatic browning reactions between Lys and oligosaccharides such as raffinose and stachyose, for instance may result in the formation of Maillard products (Parsons *et al*., 1992). Lys, Arg and tryptophan seems to be greatly affected (Clandinin, *et al*., 1947; Renner *et al*., 1953; Fernandez and Parsons, 1996) although the results obtained from earlier experiments with broilers (Clandinin, *et al*., 1947; Renner *et al*., 1953) may not have been definitive due to long autoclaving times and the microbiological assays used. More recently Parsons *et al.* (1992) found that the digestibility of several AA's, in particular Lys, Cys, His and Asp, in dehulled, solvent extracted SBM (DSBM) decreased following autoclaving. Pelleting temperature undoubtedly affects the availability of AA's especially under such harsh processing conditions as previously described. However, under lower pelleting temperatures (60° C) and reduced humidity the availability of AA's is affected to a lesser extent in pigs (Mavromichalis and Baker, 2000). The nitrogen corrected metabolisable energy (ME_n) (Renner and Hill, 1960) and TME (Sibbald, 1980) values of over processed SBM may also be reduced by over processing, although Parsons *et al*. (1992) found that autoclaving of DSBM resulted in no significant reduction in the TMEn.

2.6.2 The effect of thermal processing on enzymatic activity

Thermal processing affects the enzymatic activity not only in the feed but also at the site of digestion. Spring *et al*. (1996) investigated the effect of different pelleting temperatures of a wheat-barley-soya diet on the activities of cellulase, bacterial amylase, fungal amylase and pentosanase in broilers. They found that cellulase, fungal amylase and pentosanase maintained activity at pelleting temperatures of up to 80° C, and bacterial amylase up to 90° C. It is important to note that although pelleting temperature affects the stability of feed enzymes, the time during processing also is a vital variable to consider. In this regard Inborr and Bedford (1994) found that at processing temperatures of 75°C, the β-glucanase activity in a barley-based diet was reduced to 66% and 49% of the initial activity at processing times of 30 seconds and 15 minutes, respectively. Interestingly, despite this reduction in enzymatic activity, processing had no significant effect on chick performance.

2.7 Nitrogen and amino acid digestibility assays

Digestibility assays are good indicators of the quality of feedstuffs and also of the performance of broiler chickens on rations using these ingredients. In most cases, for example, increased N- and AA digestibility will lead to greater protein accretion and thus improved BW. In the section that follows, the different methods of determining digestibility will be discussed along with the influences that age and sex have on digestibility values.

2.7.1 Method of determination

In many previous studies, the digestibility of N and AA was determined from the collection of the excreta (Ravindran *et al*. 1999b; Kadim *et al*., 2002). The digestibility values obtained from such trials are considered to be inaccurate because in avian species the urine and faeces are excreted together, resulting in underestimated digestibility values. Furthermore, assuming that all nitrogenous substances are absorbed at the terminal ileum (Webb, 1990), the influence of microbial processes in the caeca, although limited (Ten Doeschate *et al*., 1993) may further lead to inaccurate estimations. The influence of microbial processes may be minimized by using caecectomized adult cockerels. Angkanaporn *et al*. (1997) found that caecectomy only affected the digestibility of His, Arg and Lys, the values for other AA being unaffected in SBM. Further complications in digestibility assays for N and AA are the estimation of correction factors for endogenous AA output. These endogenous AA may be accounted for by using a protein free diet (Kadim *et al*., 2002) and the digestibility values obtained thereof may be used to calculate the true digestibility values. The most accurate estimations for the digestibility of nitrogenous substances would therefore be based on sampling of the ileal contents at the terminal ileum. In a comprehensive study by Ten Doeschate *et al*. (1993), using a compound commercial diet, the digestibility coefficients of several AA were compared as determined from the ileal and total collection method. The apparent digestibility coefficients of six AA (i.e. Cys, Asp, Thr, Ser, Tyr and Arg) and N were shown to be distinctly higher when determined from excreta samples compared to those determined from ileal samples. Conversely, they also found the digestibility coefficients for Pro and alanine to be higher as determined by the ileal method. Similarly, Kadim *et al*. (2002) found that the apparent and in some cases the true digestibility values of Thr was higher by determination from sampling of the excreta for sorghum, wheat, SBM, fish meal, meat-and-bone meal and blood meal. In SBM the apparent and true excreta digestibility values for Pro were found to be higher which was in contrast to findings by Ten Doeschate *et al*. (1993). Such conflicting findings may be ascribed to the fact that the digestibility values of nitrogenous substances vary not only between the AA being evaluated, but also between feedstuffs and even between cereals (Ravindran *et al*., 1999b). Table 2.7 compares the digestibility values of AA's of various digestibility studies and the significant differences between the excreta and ileal digestibility values for maize and SBM.

2.7.2 Sex

It has previously been reported that there is no influence of sex on digestibility values (Sørensen *et al*., 1983). Ten Doeschate *et al.* (1993), however, found that the digestibility coefficients of a commercial diet were generally about 3 % higher for female broilers than for males. The digestibility of protein does not seem to be affected by sex (Larbier and Chagneau, 1992; Larbier *et al*., 1993) although Ten Doeschate *et al*. (1993) reported significant differences in N digestibility between males and females. These reporters however, concluded that the increased digestibility is most probably related to a higher FCR by female broilers.

2.7.3 Age

The digestive ability of older birds should theoretically be higher than for younger birds due to the lack of adequate intestinal enzymes in the early stages of life. Ten Doeschate *et al.* (1993) however found that the dry matter (DM) and N digestibility of broilers seems to decrease with age but that the AA digestibilities were highest between 41 and 43 d of age. This is in accordance with earlier work by Larbier and Chagneau (1992) who found that the true digestibility of protein decreases between the third and sixth week of development. A similar trend was found by these researchers for the digestibility of AA's.

2.8 Discussion

Previous research with the use of exogenous enzyme combinations in maize-soya diets has produced contradictory results, especially with regard to N and AA digestibility. It is difficult to explain such opposing arguments but a proportion of the variability may be attributed to the influence of sex (Ten Doeschate *et al*., 1993; Touchburn *et al*., 1999) genotype (Ten Doeschate *et al*., 1993), and method of determination (ileal vs. faecal digestibility) (Ten Doeschate *et al*., 1993; Ravindran *et al*., 1999b; Kadim *et al*., 2002). It is also known that specific enzymes with specific activities are required to degrade the very unique ANF, especially in SBM. In earlier enzyme research this was not taken into account when enzyme combinations for broiler feeds were produced. Various reports in the literature, however suggest that enzyme combinations in the past have been successful in increasing the digestibility of N in broilers for a variety of feedstuffs. This does not mean that all enzymes may yield the same results in maize-soya diets. Although maize, for example, is considered a homogenous commodity, sample variations do exist. Ideally enzyme combinations that work for every producers' very unique production conditions should be produced, or at least a combination that works for the local production area.

Table 2.7 Comparison of the apparent amino acid digestibility values of maize, SBM and a compound diet, as determined by the ileal or total excreta collection method.

	Maize[1]			SBM[2]			SBM[3]			SBM[4]			SBM[5]			Compound diet[6]		
	ID	ED		ID	ED		ID	ED		ID	ED		ID	ED		ID	ED	
Indispensable																		
Threonine	0.62	0.69	NS	0.77	0.79	*	0.74	0.79	*	0.75	0.78	NS	0.95	0.99	NS	0.77	0.81	***
Valine	0.82	0.76	**	0.84	0.79	*	0.83	0.81	NS	0.80	0.80	NS	0.93	0.96	NS	0.83	0.83	NS
Methionine	0.88	0.89	NS	0.89	0.88	NS	0.95	0.86	*	0.91	0.84	NS	0.98	0.96	NS	0.93	0.92	NS
Isoleucine	0.84	0.76	*	0.86	0.81	**	0.81	0.81	NS	0.80	0.80	NS	0.96	0.96	NS	0.86	0.87	NS
Leucine	0.91	0.90	NS	0.85	0.84	*	0.80	0.84	NS	0.82	0.83	NS	0.91	0.94	NS	0.86	0.87	*
Phenylalanine	0.87	0.84	NS	0.86	0.85	*	0.86	0.86	NS	0.85	0.84	NS	0.92	0.90	NS	0.86	0.87	NS
Histidine							0.84	0.84	NS	0.83	0.83	NS	0.93	0.96	NS	0.83	0.83	NS
Lysine	0.74	0.75	NS	0.86	0.86	NS	0.85	0.88	NS	0.86	0.84	NS	0.96	0.97	NS	0.87	0.88	NS
Arginine	0.86	0.85	NS	0.89	0.91	NS	0.88	0.92	*	0.89	0.91	NS	0.96	0.96	NS	0.89	0.91	***
Dispensable																		
Aspartic acid	0.76	0.76	NS	0.81	0.85	*	0.80	0.85	**	0.81	0.85	*	0.92	0.95	NS	0.79	0.84	***
Serine	0.75	0.80	NS	0.81	0.85	***	0.75	0.85	NS	0.79	0.84	NS	0.95	100	NS	0.80	0.84	***
Glutamic acid	0.90	0.88	*	0.87	0.87	NS	0.90	0.88	NS	0.88	0.87	NS	0.92	0.99	NS	0.88	0.89	*
Alanine	0.88	0.83	***	0.83	0.74	**	0.79	0.77	NS	0.81	0.77	*	0.91	0.97	NS	0.85	0.83	*
Tyrosine	0.78	0.79	NS	0.86	0.85	NS	0.86	0.87	NS	0.85	0.86	NS	0.92	0.96	NS	0.86	0.89	**
Proline													0.92	1.01	*			

ID Ileal digestibility coefficient

ED Excreta digestibility coefficient

[1] Ravindran *et al*., 1999b; [2] Kadim *et al*., 2002; [3] Ten Doeschate *et al*., 1993; NS Not significant; * P<0.05; ** P<0.01; *** P<0.001

The digestibility of NSP's in maize-soya diets may be improved by enzyme addition, although the NSP's in these ingredients do not exhibit their anti-nutritive effect in the same way as for wheat-, barley- and rye based diets i.e. by increasing digesta viscosity. The detrimental effects of NSP's are more likely due to changes in intestinal morphology, microbial profile and encapsulation of starch; fat and protein, associated with NSP's are reduced with NSP-degrading enzymes in maize-soya diets.

A combination of different enzymatic activities (such as a multicarbohydrase enzyme combined with amylase and protease activities) may be required in maize-soya diets for broilers based on the literature reviewed in this Thesis. Even if the multicarbohydrase enzyme cannot degrade the unique NSP's in maize and soya, some cell wall degradation may still occur which may lead to greater starch and protein digestibility by endogenous and exogenous amylase and proteases.

The thermal processing of maize-soya diets containing exogenous enzymes may produce interesting results. Although it is known that enzyme activity decreases with temperature and processing time, the availability of AA to the broiler does not seem to be affected to such a great extent, especially at lower pelleting temperatures (between 60 and 70° C). It also seems unlikely that the energy availability will be significantly affected by thermal processing at such low temperatures.

Various factors such as age, sex, genotype and method of determination seem to affect the digestibility coefficients for CP and AA's. The digestibility coefficients also seem to vary between feedstuffs and even within cereals and protein concentrates such as SBM. For this reason it is necessary to use well defined animals of similar age and sex (Ten Doeschate *et al*., 1993) to validate digestibility coefficients obtained from such experiments.

The objective of this thesis was to evaluate whether the digestibility of N and more specifically, that of individual AA of a balanced vegetarian maize-soybean diets and broiler chicken performance on a low AME diet may be improved by the addition of a commercial enzyme product. A second objective was to test whether the enzymatic activity of the compound used was affected by cold pelleting of the diets at 60-70° C.

CHAPTER 3

EXOGENOUS ENZYMES FOR BALANCED MAIZE-SOYA DIETS FOR BROILERS: EFFECTS ON APPARENT EXCRETA NITROGEN- AND AMINO ACID DIGESTIBILITY AND PRODUCTION PERFORMANCE

3.1 Introduction

Exogenous enzymes, such as phytases have been widely used in broiler feeds in the past few years. In a further attempt to improve the digestibility of nutrients in cereals, different enzymes are becoming readily available to the market. Most of these commercial enzyme combinations currently target the structural carbohydrate fraction (non-starch polysaccharide or NSP) of the cereal that cannot be digested by avian endogenous enzymes. These enzyme combinations are designed to target and break-down some of the potentially problematic compounds present in common ingredients for poultry feed namely lectins (maize, soya), arabinoxylans (rice, rye, and wheat), beta-glucans (barley), oligosaccharides and NSP's (lupins, rapeseed, sunflower, and soya) (Acamovic, 2001). Without the assistance of these exogenous enzymes, these compounds pass to the hindgut almost undigested resulting in a potential loss of energy and AA's. Many enzymes and their combinations that have been tested up to date have focused on NSP's in wheat and especially barley, with very controversial results (Brenes *et al*., 1993; Cowan *et al*., 1996; Choct *et al*., 1999; Dänicke *et al*., 1999; Ravindran *et al*., 1999a; Marron *et al*., 2001). Little is known what effect these enzymes may have in maize-soya diets which are the most commonly used diets for poultry in South-Africa. The use of commercial enzyme preparations for vegetarian maize-soya diets and more specifically the effect of these enzyme combinations on nitrogen and AA digestibility have not been intensively investigated. The objective of this trial is to evaluate the apparent nitrogen (N) and AA (AA) digestibility, of a vegetarian maize-soya diet for broilers, fed as a pellet or mash, either with or without the addition of a commercial enzyme preparation.

3.2 Materials and methods

Forty-eight day-old male broiler chicks (Cobb 500) were placed in wire-mesh cages (3 chicks per cage). The experimental units (cages) were allocated at random to the 4 experimental treatments (see Table 3.2) with 4 replicates per treatment. The experiment was a completely randomized design with main effect being dietary treatment. The birds were vaccinated at day-old against Newcastle Disease and Infectious Bronchitis. The environmental temperature within the house was 30° C for the first day and was then decreased by 0.5° C every second day to 20° C. Continuous light (24L: 0D) was provided for the duration of the trial. Birds were offered *ad libitum* access to food and water. Experimental procedures were approved by the University of Stellenbosch Animal Ethics Committee.

A starter (0 to 14 days) and a finisher (15 to 35 days) broiler diets were formulated using the primary breeder specifications (Cobb, 2004). A detail of the ingredients used in the diets and the formulated nutrient composition of the diets are shown in Table 3.1. The starter and finisher diets were fed as either a mash or a crumble/pellet (For starter and finisher respectively). A commercial enzyme product (AveMix XG 10, AVEVE, Belgium) was added to treatments C+ and Pe+ at the manufacturers' recommendation of 0.1g/kg. A summary of the dietary treatments is shown in Table 3.2. The enzyme combination contained guaranteed activities for xylanase and glucanase and significant activities of cellulase, xyloglucanase, endo-xylanase, galactomannanase, mannanase and pectinase. The starter diet was provided *ad libitum* from day one to day 13. At the beginning of the trial period on the morning of day 14 the birds were fasted for four hours, the excreta pans were placed to cover the bottom of the cages and the birds allowed *ad libitum* access to the finisher diet and fresh water. On days 21, 28 and 35, the excreta was weighed (as-is) and dried in a forced-air oven at 80° C for 24 hours (Ravindran *et al*., 1999b). Care was taken to avoid contamination with feathers, scales and debris. The dried excreta was weighed and grounded to pass through a 0.75 mm sieve. A representative sample from each cage was taken and the samples pooled together within treatments. Birds were weighed (per cage) on arrival and on days 7, 14, 21, 28 and 35. Food intake (per cage) was also measured for days 7, 14, 21, 28 and 35.

Duplicate samples of both the dietary treatments and the excreta samples were analyzed for N content with a Leco N analyzer (AOAC, 1990). Digesta and feed were hydrolyzed for 24 hours with 6 N hydrochloric acid at 110 ºC for the determination of AA by HPLC using the Pico-Tag system from Waters Chromatography Systems. Apparent excreta digestibility coefficients for N and individual AA of the dietary treatments for days 21, 28 and 35 were calculated using the following equations:

1. Apparent N digestibility (AND) = $\frac{\text{(N consumed – N excreted)}}{\text{N consumed}}$

where N consumed = Dry matter (DM) intake x N-content $_{feed}$

and N excreted = DM excreta output x N-content $_{excreta}$

2. Apparent AA digestibility (AAD) = $\frac{\text{(AA consumed – AA excreted)}}{\text{(AA consumed)}}$

where, AA consumed = AA concentration $_{feed}$ x DM intake

and AA excreted = AA concentration $_{excreta}$ x DM excreta output

Table 3.1 Composition of starter and finisher diets based on as fed basis (g/kg)

Ingredient	**Starter**	**Finisher**	
Maize	530.2	579.3	
Maize gluten 60	38.7	59.7	
Soybean 46	335.2	75.0	
Soybean full fat		198.1	
Choline chloride 60%		0.5	
DL-methionine	1.5	0.9	
L-threonine	0.9	7.6	
L-lysine HCl	4.5	3.7	
Limestone	17.3	17.7	
Salt	3.6	1.8	
Monocalcium phosphate	16.6	16.6	
Sodium bicarbonate		2.7	
Sunflower oil	50.0	35.0	
Vit + min premix	1.5	1.5	
	Calculated nutrient content (*)	*Calculated nutrient content (*)*	*Analysed nutrient content*
AMEn (MJ/kg)	12.63	13.00	
Crude protein (%)	22.95	21.44	18.09
Lysine (%)	1.37	1.14	1.74
Methionine (%)	0.48	0.41	0.33
Methionine + Cystine (%)	0.80	0.72	0.39
Threonine (%)	0.84	1.43	0.86
Tryptophan (%)	0.21	0.18	
Arginine (%)	1.36	1.16	1.64
Isoleucine (%)	0.92	0.82	0.72
Leucine (%)	2.00	2.00	2.28
Histidine (%)	0.54	0.49	0.65
Phenylalanine (%)	0.99	0.92	0.90
Tyrosine (%)	0.85	0.77	0.90
Phenylalanine+tyrosine (%)	1.83	1.68	1.80
Valine (%)	1.02	0.93	1.07
Calcium (%)	1.00	1.00	
Available Phosphorus (%)	0.50	0.50	

(*) Amino acids expressed on a digestible basis.

Digestibility coefficients for glycine were not determined because uric acid is non-quantitatively converted to glycine during acid hydrolysis (Kadim *et al*., 2002).

Table 3.2 A description of the dietary treatments for broiler chickens from 0-35 days of age

Treatment	Description
C+	Control + AveMix XG (0.1g/kg)
C	Mash diet (Control)
Pe+	Pelleted control diet + AveMix XG (0.1g/kg)
Pe	Pelleted control diet

All data were tested for homogeneity of variance using Levene's test and for normal distribution using the Kolmogorov-Smirnov test. All data was of equal variance and normal distribution and was analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0. Student's *t*-test was used to test for differences between treatment means when the treatment effect was significant. The probability level was set at 5 %.

3.3 Results and discussion

3.3.1 Digestibility assay

The apparent nitrogen digestibility (AND) coefficients for the respective determination periods are presented in Table 3.3. The AND was improved by enzyme addition (6 %), pelleting (5.9 %) and a combination of enzyme addition and pelleting (12.9 %), but only for the determination period between 14-21 days ($P<0.05$). The AND coefficient for treatment Pe+ was the highest for all determination periods but was only significantly higher during the period 14-21 days ($P<0.05$). This suggests that the combined effect of added exogenous enzyme and the pelleting process lead to the greatest AND during this period. This may be as a result of an increase in nutritional value of the compact folded soya protein as a result of processing (Marsman *et al*., 1997) and increased substrate availability for endogenous and exogenous digestive enzymes (Cowan *et al.*, 1996; Bedford, 2002). This effect may also be explained by the partial depolymerisation of galactan, arabinogalactan and possibly rhamnogalacturonan in the upper intestine, resulting in improved N digestibility, as suggested by Kocher *et al*. (2002). The positive response to the enzyme complex for only this period may be explained by the lower endogenous enzyme activity early in the chicks' life, thus maximizing the beneficial effect of exogenous enzyme addition (Nitsan *et al*., 1991; Iji *et al*., 2001). For treatments C+ and Pe+, with added enzymes, the AND was significantly improved ($P<0.05$) compared to treatments C and Pe respectively for this period indicating enzymatic efficacy in both the mash and pelleted diets. Furthermore, this indicates that pelleting did not destroy the efficacy of the exogenous enzyme complex. There were no significant differences in AND between treatments C+ and Pe indicating that the exogenous enzyme complex, when added to the mash diet, did not result in significantly higher AND coefficients compared to pelleting alone for the period 14 to 21 days. There were no significant differences in AND between treatments C and Pe for the last two experimental periods.

Table 3.3 Apparent Nitrogen Digestibility (AND) and standard error of nitrogen digestibility (SE) for the periods 14-21 days, 22-28 days and 29 to 35 days for broiler chickens fed a commercial diet supplemented with an enzyme combination

Period	14-21 Days			22-28 Days			29-35 Days		
Treatment	N	AND	SE[1]	N	AND	SE[1]	N	AND	SE[1]
C+	4	0.71[b]	0.006	4	0.71	0.003	4	0.72	0.018
C	4	0.65[c]	0.006	4	0.74	0.004	4	0.73	0.009
Pe+	4	0.78[a]	0.005	4	0.78	0.002	4	0.75	0.009
Pe	4	0.71[b]	0.044	4	0.70	0.009	4	0.70	0.043
LSD[2]		0.04			0.15			0.07	

[1] Standard error of N digestibility. [2] Least significant difference (P<0.05). [a-c] Means within a column with the same superscript are not significantly different (P<0.05)

Table 3.4 The apparent digestibility coefficients for individual amino acids for the determination period 14-21 days for broiler chickens fed a commercial diet supplemented with an enzyme combination

Amino acid	Treatment				LSD[1]	SEM[2] (N = 16)
	C+	C	Pe+	Pe		
Alanine	0.83[b]	0.84[ab]	0.86[a]	0.85[a]	0.020	0.004
Threonine	0.88[a]	0.86[b]	0.89[a]	0.88[a]	0.015	0.003
Serine	0.84[ab]	0.83[b]	0.84[ab]	0.85[a]	0.020	0.004
Arginine	0.88[c]	0.91[b]	0.92[a]	0.92[a]	0.010	0.004
Glutamic acid	0.91[ab]	0.90[b]	0.92[a]	0.92[a]	0.011	0.002
Valine	0.81[b]	0.82[ab]	0.84[a]	0.84[a]	0.021	0.005
Histidine	0.80[d]	0.86[b]	0.82[c]	0.89[a]	0.017	0.010
Aspartic acid	0.85[bc]	0.83[c]	0.86[ab]	0.87[a]	0.018	0.004
Lysine	0.88[a]	0.86[b]	0.89[a]	0.88[a]	0.015	0.003
Proline	0.87[bc]	0.86[c]	0.89[a]	0.88[ab]	0.016	0.004
Methionine	0.93[b]	0.92[c]	0.94[a]	0.93[bc]	0.001	0.002
Tyrosine	0.89	0.87	0.88	0.87	0.016	0.003
Cysteine	0.59[ab]	0.54[b]	0.63[a]	0.58[ab]	0.054	0.011
Isoleucine	0.83[b]	0.84[b]	0.88[a]	0.83[b]	0.022	0.006
Phenylalanine	0.90[a]	0.88[b]	0.91[a]	0.88[b]	0.015	0.004
Leucine	0.88[b]	0.89[b]	0.92[a]	0.91[a]	0.012	0.004

[1] Least significant difference (P<0.05). [2] Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05)

The lack of significant results beyond the age of 22 days of age suggests that pelleting did not have an effect on the degradation of nitrogenous substances during this period and therefore did not affect AND.

Table 3.5 The apparent digestibility coefficients for individual amino acids for the determination period 22-28 days for broiler chickens fed a commercial diet supplemented with exogenous enzymes.

	Treatment					
Amino acid	C+	C	Pe+	Pe	LSD[1]	SEM[2] (N = 16)
Alanine	0.83[c]	0.84[b]	0.90[a]	0.81[d]	0.010	0.008
Threonine	0.88[b]	0.88[b]	0.90[a]	0.88[b]	0.007	0.003
Serine	0.84[b]	0.85[b]	0.86[a]	0.84[b]	0.009	0.002
Arginine	0.91[c]	0.92[b]	0.92[a]	0.91[c]	0.005	0.001
Glutamic acid	0.91[b]	0.91[b]	0.94[a]	0.89[c]	0.006	0.004
Valine	0.81[c]	0.83[b]	0.88[a]	0.82[c]	0.010	0.008
Histidine	0.80[c]	0.88[b]	0.89[a]	0.89[a]	0.006	0.010
Aspartic acid	0.86[b]	0.86[b]	0.89[a]	0.84[c]	0.009	0.005
Lysine	0.87[b]	0.87[b]	0.90[a]	0.84[c]	0.008	0.006
Proline	0.87[c]	0.88[b]	0.92[a]	0.86[d]	0.007	0.005
Methionine	0.92[c]	0.93[b]	0.93[a]	0.91[d]	0.005	0.002
Tyrosine	0.89[b]	0.90[a]	0.90[a]	0.85[c]	0.008	0.005
Cysteine	0.70[a]	0.64[b]	0.60[c]	0.41[d]	0.029	0.029
Isoleucine	0.85[b]	0.85[b]	0.89[a]	0.81[c]	0.010	0.008
Phenylalanine	0.90[b]	0.89[b]	0.91[a]	0.85[c]	0.008	0.006
Leucine	0.91[c]	0.92[b]	0.93[a]	0.88[d]	0.006	0.004

[1] Least significant difference (P<0.05). [2] Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05)

The digestibility coefficients of individual AA for the determination period 14-21 days are presented in Table 3.4. The digestibility of four AA's was affected by enzyme addition alone, all of which are essential for poultry. The apparent AA digestibility (AAD) of Thr, Lys, Met and Phe was improved by enzyme addition compared to the control group (P<0.05). The results observed for Thr are in agreement with Zanella *et al.* (1999) for maize-soya diets supplemented with a commercial enzyme combination, while Lys and Met showed opposing results. The AAD coefficients for Arg and His were higher in the control mash diet (P<0.05), indicating a negative effect of the enzyme complex on the digestibility of these AA's during this determination period. For all other AA's no significant differences (P <0.05) were found between the control group and the group with added exogenous enzymes. Pelleting the control diet resulted in a larger improvement in AAD coefficients compared to those observed after exogenous enzyme addition, resulting in improved digestibility coefficients for Thr, Ser, Arg, glutamic acid, His, Asp, Lys, Pro and Leu (P<0.05). Similar to that observed for AND, these results could be attributed to the effects of processing on the structure of the protein in soya (Marsman *et al*., 1997). The combination of exogenous feed enzymes and pelleting (Pe+) improved the AAD of all AA's except that of alanine, Ser, Val and Tyr above that of the control group (C) (P<0.05). The AAD of His was negatively affected by the combination of exogenous feed enzymes

and pelleting (P<0.05). This result was unexpected and no reason for this could be found at this point. Tyr was the only AA that was unaffected by any exogenous enzymes, pelleting or a combination of enzymes and pelleting.

The AAD coefficients of individual AA's for the determination period 22-28 days are presented in Table 3.5. For this determination period the effect of enzyme addition was mostly not significant (Thr, Ser, glutamic acid, Asp, Lys, Ile and Phe) or negative (alanine, Arg, Val, His, Pro, Met, Tyr and Leu), the only exception being the AAD of cysteine which improved by 6.4 % following the addition of the enzyme complex (P<0.05). Pelleting, in contrast to enzyme addition, improved the digestibility of His by 1.5 % (P<0.05). The digestibility of all other AA's, however, was either unaffected (Thr and Ser) (P<0.05) or negatively affected (P<0.05) by the pelleting process which might be indicative of a degree of protein denaturation. This negative effect on AAD was counteracted by the enzyme complex, as is clear by comparing treatments Pe+ and C. The digestibility of all AA's improved by the combination of pelleting and exogenous enzymes during this determination period, except that of Tyr, which was not significantly affected (P<0.05), and cysteine which was found to be inferior compared to the control mash diet (P<0.05). This indicates that the disruption in structural stability of cysteine as a result of the pelleting process could not be improved by the enzyme complex and indicates greater sensitivity of cysteine to heat damage during pelleting.

The apparent digestibility coefficients for the determination period 29-35 days are presented in Table 3.6. The addition of the enzymatic complex improved the digestibility of the EAA Thr, Met and Phe, and also that of Cys (P<0.05). Zanella *et al.* (1999) reported similar results for Thr and cysteine and opposite results for Met when using a commercial enzyme combination with similar activities for maize-soya diets. The digestibility coefficients of all the other AA were either not significantly altered (Arg, glutamic acid, Val, Asp, Lys, Pro, Tyr, Ile and Leu) or found to be lower (alanine, Ser and His) following the addition of the enzyme complex (P<0.05). Pelleting generally lead to significantly lower AAD coefficients (P<0.05), except for that of Arg, Lys, Met, Ile, Phe and Leu, which remained unchanged following pelleting. Only the apparent digestibility of Thr seemed to be improved by the pelleting process (P<0.05). The combination of exogenous enzymatic activity and the pelleting process improved the digestibility of only Thr, Met and Phe, suggesting an inability of the enzyme complex to counteract the negative effects of heat damage caused by the pelleting process, except perhaps with regard to the aforementioned AA's.

3.3.2 Production performance

Throughout the trial period, enzyme addition did not significantly influence body weight gain (BWG) (Table 3.7). Similar results were reported by Marquardt *et al*. (1996). However, later research reports by Zanella *et al*. (1999) and Meng and Slominski (2005) showed otherwise. Pelleting resulted in improved BWG (P<0.05), but only for the period 8-14 d. The results presented in Table 3.7,

indicated that the combination of enzyme addition and pelleting resulted in improved BWG compared to the control mash diet, but only for the first two periods.

Table 3.6 The apparent digestibility coefficients for individual amino acids for the determination period 29-35 days for broiler chickens fed a commercial diet supplemented with an enzyme combination

	Treatment					
Amino acid	C+	C	Pe+	Pe	LSD[1]	SEM[2] (N = 16)
Alanine	0.89[b]	0.93[a]	0.86[bc]	0.85[c]	0.036	0.009
Threonine	0.97[a]	0.93[c]	0.94[b]	0.94[b]	0.014	0.004
Serine	0.86[b]	0.91[a]	0.81[bc]	0.79[c]	0.049	0.014
Arginine	0.95[a]	0.94[ab]	0.94[ab]	0.93[b]	0.017	0.003
Glutamic acid	0.94[a]	0.95[a]	0.93[a]	0.90[b]	0.023	0.006
Valine	0.89[ab]	0.92[a]	0.87[b]	0.86[b]	0.034	0.008
Histidine	0.91[b]	0.97[a]	0.86[c]	0.90[b]	0.026	0.010
Aspartic acid	0.90[ab]	0.92[a]	0.87[bc]	0.85[c]	0.036	0.008
Lysine	0.92[a]	0.91[ab]	0.91[ab]	0.89[b]	0.026	0.005
Proline	0.92[ab]	0.94[a]	0.90[bc]	0.89[c]	0.027	0.007
Methionine	0.96[a]	0.95[b]	0.97[a]	0.95[b]	0.013	0.003
Tyrosine	0.92[a]	0.93[a]	0.89[b]	0.87[b]	0.032	0.008
Cysteine	0.84[a]	0.70[b]	0.78[ab]	0.60[c]	0.091	0.026
Isoleucine	0.93[a]	0.90[ab]	0.91[a]	0.89[b]	0.029	0.006
Phenylalanine	0.96[a]	0.92[b]	0.95[a]	0.92[b]	0.020	0.005
Leucine	0.96[a]	0.94[ab]	0.94[ab]	0.93[b]	0.017	0.003

[1] Least significant difference (P<0.05). [2] Standard error of the mean. [a-c] Means within a row with common superscripts are not significantly different (P<0.05)

This effect may be explained by the partial depolymerisation of galactan, arabinogalactan and possibly rhamnogalacturonan in the upper intestine following processing (Kocher *et al*., 2002), resulting in improved N digestibility and thus growth. Feed intake (Table 3.7) was lower for the enzyme supplemented mash diets (C+) for the period 8-14 d, however these results are not unexpected. The effects of pelleting diets on broiler feed intake are well known. Notwithstanding, pelleting the experimental diets resulted in higher feed intake only for the period 15-21 days of age. Enzyme addition improved the FCR (Table 3.7) of the control mash diet for the period 8-14 d as a result of significantly lower feed intake during this period (P<0.05). This is in agreement with previous studies using similar diets (Zanella *et al*., 1999). Pelleting also significantly improved the FCR during this period (P<0.05). The results suggest that using a commercial enzyme combination of these characteristics in nutritionally balanced vegetarian maize-soya diets is most effective when such diets are subject to cold pelleting and the diet is fed during the early stages of the production cycle.

Table 3.7 Mean bodyweight gain (BWG)(kg), Feed intake (FI)(kg) and feed conversion ratio (FCR)(kg feed/kg BWG) of broiler chickens fed a diet supplemented with exogenous enzymes.

	0-7 d		8-14 d		15-21 d		22-28 d		29-35 d	
Treatment	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]
C+	0.099[b]	0.010	0.236[ab]	0.023	0.362	0.023	0.528	0.021	0.430	0.013
C	0.094[b]	0.007	0.213[b]	0.010	0.346	0.010	0.509	0.018	0.484	0.048
Pe+	0.121[a]	0.004	0.280[a]	0.016	0.395	0.020	0.540	0.006	0.443	0.020
Pe	0.124[a]	0.002	0.277[a]	0.012	0.399	0.015	0.503	0.033	0.394	0.057
LSD[1]	0.020		0.049		0.054		0.067		0.121	
Treatment	FI	SE[2]	FI	SE[2]	FI	SE[2]	FI	SE[2]	FI	SE[2]
C+	0.191	0.013	0.316[b]	0.010	0.578[ab]	0.039	0.867	0.051	0.979	0.019
C	0.175	0.010	0.368[a]	0.014	0.553[b]	0.013	0.868	0.023	1.016	0.049
Pe+	0.188	0.009	0.344[ab]	0.009	0.635[a]	0.025	0.930	0.020	0.975	0.017
Pe	0.189	0.004	0.333[ab]	0.019	0.649[a]	0.019	0.924	0.041	0.921	0.049
LSD[1]	0.029		0.042		0.080		0.111		0.113	
Treatment	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]
C+	2.023	0.330	1.371[b]	0.102	1.593	0.008	1.642[b]	0.074	2.281	0.086
C	1.892	0.152	1.748[a]	0.127	1.597	0.010	1.708[ab]	0.025	2.136	0.133
Pe+	1.562	0.036	1.242[b]	0.074	1.609	0.025	1.720[ab]	0.023	2.207	0.062
Pe	1.521	0.028	1.198[b]	0.025	1.630	0.012	1.848[a]	0.068	2.466	0.303
LSD[1]	0.565		0.278		0.047		0.163		0.534	

[1] Least significant difference. [2] Standard error. [a-b] Means within a column with common superscripts are not significantly different (P<0.05)

The effects would wear off as the chick matures. Neither enzyme addition nor pelleting consistently improved the digestibility of any of the AA's evaluated for all determination periods. A combination of exogenous enzymes and pelleting however, lead to improved digestibility coefficients for Thr, Met and Phe throughout the trial period, indicating that the digestibility of these AA's may significantly and consistently be improved by the addition of an enzyme compound of this characteristics.

CHAPTER 4

ENZYMES FOR BALANCED MAIZE-SOYA DIETS FOR BROILERS: EFFECT ON NITROGEN- AND AMINO ACID DIGESTIBILITIES

4.1 Introduction

Previous research with the use of exogenous enzyme combinations in maize-soya diets has produced conflicting results, especially with regard to N and AA digestibility. This may be partly explained by the influence of sex (Ten Doeschate *et al*., 1993; Touchburn *et al*., 1999) genotype and method of determination (ileal vs. faecal digestibility) (Ten Doeschate *et al*., 1993; Ravindran *et al*., 1999b; Kadim *et al*., 2002) on digestibility coefficients. Traditionally the digestibility of N and AA was determined from the collection of the excreta. The digestibility values obtained from such trials are considered underestimated because in avian species the urine and faeces are excreted together. Furthermore, assuming that all nitrogenous substances are absorbed before the terminal ileum (Webb, 1990), the influence of microbial processes in the caeca, although limited (Ten Doeschate *et al*., 1993) may further lead to inaccurate estimations. A more common method of determining digestibility has thus become to collect digesta at the terminal ileum, about 2 cm anterior to the ileo-caecal junction (Kadim *et al*., 2002). This ensures that there can be no contamination of the digesta with uric acid and that the influences of microbial processes are limited, thus improving the accuracy of digestibility coefficients of, especially, nitrogenous compounds. The objective of this trial was to evaluate the effect of a commercial enzyme combination in a mash and pelleted vegetarian maize-soya diet on the apparent ileal N- and AA digestibilities.

4.2 Materials and methods

Sixty four day-old male broiler chicks (Cobb 500) were placed in wire-mesh cages (4 chicks per cage). The experimental units (cages) were allocated at random to the 4 experimental treatments with 4 replicates per treatment. The experiment was a completely randomized design with main effect of dietary treatment. The birds were vaccinated at day-old against Newcastle Disease and Infectious Bronchitis. The environmental temperature within the house was 30° C for the first day and was then decreased by 0.5° C every second day to 20° C. Continuous light (24L: 0D) was provided for the duration of the trial. Birds were offered *ad libitum* access to feed and fresh water. Experimental procedures were approved by the University of Stellenbosch Animal Ethics Committee.

Starter (0 to 14 days) and a finisher (15 to 35 days) broiler diets were formulated using the primary breeder specifications (Cobb, 2004). A detail of the ingredients used in the diets and the formulated nutrient composition of the diets are shown in Table 4.1. The starter and finisher diets were fed as either a mash or a crumble/pellet respectively. A commercial enzyme product (AveMix XG 10. AVEVE, Belgium) was added to treatments C+ and Pe+ at the manufacturers' recommendation of

0.1g/kg. Celite® was included at 20g/kg as indigestible marker to all dietary treatments. A summary of the dietary treatments is shown in Table 4.2. The enzyme contained guaranteed activities for xylanase and glucanase and significant activities of cellulase, xyloglucanase, endo-xylanase, galactomannanase, mannanase and pectinase. The starter diet was provided *ad libitum* from day one to day 13 of the trial period.

On the morning of day 14, at the beginning of the pre-experimental/adaptation period all starter dietary treatments were removed and all finisher dietary treatments allocated to the respective cages. At the beginning of the trial period, on the morning of day 21, one bird per cage was stunned and killed by cervical dislocation. Immediately after death the abdomen was opened and the ileum exposed. A 15 cm segment of the terminal ileum, 2 cm anterior to the ileo-caecal junction to avoid contamination with urine, was removed and the segment emptied by gently squeezing the segment between the thumb and forefinger so as to prevent damage to the intestinal mucosa (Ten Doeschate *et al*., 1993). The segment was then gently flushed using distilled water from a sterile syringe. Digesta samples from individual birds were pooled within a treatment and immediately frozen at -20 °C to avoid bacterial contamination of the samples. The samples were then freeze-dried, finely ground to pass through a 0.75 mm sieve and stored at -20 °C awaiting chemical analysis. The same procedure was repeated on the morning of days 28 and 35 in order to obtain pooled ileal digesta samples for these different stages of digestive tract development.

Duplicate samples of both the dietary treatments and the excreta samples were analysed for N content with a Leco N analyzer (AOAC, 1990). Digesta and feed were hydrolyzed for 24 hours with 6 N hydrochloric acid at 110 ºC for the determination of AA by HPLC using the Pico-Tag system from Waters Chromatography Systems. Apparent excreta digestibility coefficients for N and individual AA of the dietary treatments for days 21, 28 and 35 were calculated using the following equations (Ravindran *et al*., 1999b)

1. Apparent N digestibility (AND) = $\frac{(N/AIA)_d - (N/AIA)_i}{(N/AIA)_d}$

Where $(N/AIA)_d$ = ratio of N to acid-insoluble ash in the diet
$(N/AIA)_i$ = ratio of N to acid-insoluble ash in ileal digesta

2. Apparent AA digestibility (AND) = $\frac{(AA/AIA)_d - (AA/AIA)_i}{(AA/AIA)_d}$

Where $(AA/AIA)_d$ = ratio of AA to acid-insoluble ash in the diet
$(AA/AIA)_i$ = ratio of AA to acid-insoluble ash in ileal digesta

Table 4.1 Composition of starter and finisher diets based on as fed basis (g/kg).

Ingredient	Starter	Finisher	
Maize	530.2	579.3	
Maize gluten 60	38.7	59.7	
Soybean 46	335.2	75.0	
Soybean full fat		198.1	
Choline chloride 60%		0.5	
DL-methionine	1.5	0.9	
L-threonine	0.9	7.6	
L-lysine HCl	4.5	3.7	
Limestone	17.3	17.7	
Salt	3.6	1.8	
Monocalcium phosphate	16.6	16.6	
Sodium bicarbonate		2.7	
Sunflower oil	50.0	35.0	
Vit + min premix	1.5	1.5	
Celite		20.0	
	Calculated nutrient content (*)	Calculated nutrient content (*)	Analysed nutrient content
AMEn (MJ/kg)	12.63	13.00	
Crude protein (%)	22.95	21.44	17.09
Lysine (%)	1.37	1.14	2.40
Methionine (%)	0.48	0.41	0.34
Methionine + Cystine (%)	0.80	0.72	
Threonine (%)	0.84	1.43	1.71
Tryptophan (%)	0.21	0.18	
Arginine (%)	1.36	1.16	2.66
Isoleucine (%)	0.92	0.82	0.91
Leucine (%)	2.00	2.00	3.08
Histidine (%)	0.54	0.49	0.75
Phenylalanine (%)	0.99	0.92	1.20
Tyrosine (%)	0.85	0.77	0.92
Phenylalanine+tyrosine (%)	1.83	1.68	2.12
Valine (%)	1.02	0.93	1.15
Calcium (%)	1.00	1.00	
Available Phosphorus (%)	0.50	0.50	

(*) Amino acids expressed on a digestible basis.

The percentage AIA was determined by the method described by Keulen and Young (1977).

Data were tested for homogeneity of variance using Levene's test and for normal distribution using the Kolmogorov-Smirnov test. All data was of equal variance and normal distribution and was analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0. The Student's *t*-test was used to test differences between treatment means when the treatment effect was significant. The probability level was set at 5 %.

Table 4.2 Description of dietary treatments used for broiler chickens from 0 to 35 days of age

Treatment	Description
C+	Control + AveMix XG (0.1g/1kg)
C	Mash diet (Control)
Pe+	Pelleted control diet + AveMix XG (0.1g/kg)
Pe	Pelleted control diet

4.3 Results and discussion

The apparent nitrogen digestibility (AND) for the respective periods are presented in Table 4.3. Enzyme addition improved the AND during the first period (21 d) but resulted in poorer AND in the last period (35 d) ($P<0.05$). The AND for the control diets was found to be higher for the first two periods and lower for the last period compared to the pelleted diet ($P<0.05$), indicating that pelleting only resulted in improved N digestibility later in chick life. The combination of enzymes and pelleting (Pe+) resulted in improved AND compared to the control group for two of the three periods (28 d and 35 d) but led to inferior AND for the first period (28 d) ($P<0.05$). The increased AND during the last two periods may be as a result of an increase in nutritional value of the compact folded soya protein as a result of processing (Marsman *et al*., 1997) and increased substrate availability for endogenous and exogenous digestive enzymes (Cowan *et al.*, 1996; Bedford, 2002). The apparent AA digestibilities (AAD) at 21 d are presented in Table 4.4. The AAD of Thr, Arg, His, Met, Tyr, Cys, Phe, Leu and Gly was improved by enzyme addition ($P<0.05$). Pelleting resulted in improved digestibilities of His, Lys, Met, Tyr, Cys, Phe, Leu and Gly. Enzymes and pelleting resulted in improved digestibilities of Thr, Arg, His, Met, Tyr, Cys, Phe, Leu and Gly ($P<0.05$). From Table 4.4 (Pe+) it is clear that the improved digestibilities of Thr and Arg was as a result of exogenous enzymes and not of pelleting, as these AA's digestibilities were not improved by pelleting alone.

The AAD at 28 d are presented in Table 4.5. The addition of the enzyme combination resulted in improved digestibilities of all AA's except Thr and Gly which had higher digestibilities in the C diets and that of Ala, which was not significantly different from the C group ($P<0.05$). Similar results were found between the Pe and C groups. The combination of enzymes and pelleting resulted in the greatest improvement in digestibilities, improving the digestibilities of all amino acids except Thr and Gly ($P<0.05$).

Table 4.3 Apparent Nitrogen Digestibility (AND) in broiler chickens of a diet supplemented with exogenous enzymes at 21 d, 28 d, 35 d respectively

Period	21 d		28 d		35 d	
Treatment	N	AND	N	AND	N	AND
C+	2	$0.984^{a} \pm 0.001^{*}$	2	$0.978^{b} \pm 0.000$	2	$0.978^{c} \pm 0.000$
C	2	$0.982^{b} \pm 0.001$	2	$0.978^{b} \pm 0.001$	2	$0.979^{b} \pm 6.684$
Pe+	2	$0.980^{c} \pm 0.000$	2	$0.984^{a} \pm 0.000$	2	$0.983^{a} \pm 0.001$
Pe	2	$0.976^{d} \pm 2.003$	2	$0.977^{c} \pm 0.000$	2	$0.984^{a} \pm 0.000$
LSD[1]		0.000		0.001		0.001

[*] Standard error of N digestibility. [1] Least significant difference (P<0.05). [a-d] Means within a column with the same superscript are not significantly different (P<0.05)

Table 4.4 Mean apparent digestibility coefficients and standard error of the mean for individual amino acids in broiler chickens on a diet supplemented with exogenous enzymes at 21 d

	Treatment				
	C+	C	Pe+	Pe	LSD[1]
Amino acid	Mash + AveMix	Control Mash	Pellet + AveMix	Control Pellet	
Alanine	$0.981^{c} \pm 0.001^{*}$	$0.988^{a} \pm 0.000$	$0.985^{b} \pm 0.001$	$0.988^{a} \pm 0.000$	0.002
Threonine	$0.987^{b} \pm 0.001$	$0.980^{c} \pm 0.000$	$0.995^{a} \pm 0.000$	$0.976^{d} \pm 0.001$	0.002
Serine	$0.985^{bc} \pm 0.001$	$0.987^{ab} \pm 0.000$	$0.987^{a} \pm 0.001$	$0.985^{c} \pm 0.000$	0.002
Arginine	$0.984^{a} \pm 0.001$	$0.970^{b} \pm 0.001$	$0.982^{a} \pm 0.001$	$0.971^{b} \pm 0.001$	0.003
Glutamic acid	$0.730^{c} \pm 0.011$	$0.961^{a} \pm 0.001$	$0.775^{b} \pm 0.011$	$0.747^{bc} \pm 0.007$	0.032
Valine	$0.976^{d} \pm 0.001$	$0.986^{a} \pm 0.000$	$0.980^{c} \pm 0.001$	$0.983^{b} \pm 0.000$	0.003
Histidine	$0.996^{b} \pm 0.000$	$0.994^{c} \pm 0.001$	$0.996^{b} \pm 0.000$	$0.997^{a} \pm 0.001$	0.001
Aspartic acid	$0.919^{c} \pm 0.003$	$0.986^{a} \pm 0.000$	$0.919^{c} \pm 0.004$	$0.930^{b} \pm 0.002$	0.011
Lysine	$0.944^{c} \pm 0.002$	$0.952^{b} \pm 0.001$	$0.952^{b} \pm 0.002$	$0.960^{a} \pm 0.001$	0.007
Proline	$0.967^{b} \pm 0.001$	$0.985^{a} \pm 0.000$	$0.971^{b} \pm 0.001$	$0.963^{c} \pm 0.001$	0.004
Methionine	$1.000^{a} \pm 0.002$	$0.999^{c} \pm 0.000$	$1.000^{a} \pm 0.002$	$0.999^{b} \pm 0.002$	0.000
Tyrosine	$0.988^{b} \pm 0.001$	$0.985^{c} \pm 0.000$	$0.989^{b} \pm 0.001$	$0.993^{a} \pm 0.000$	0.002
Cysteine	$0.995^{a} \pm 0.000$	$0.992^{b} \pm 0.000$	$0.995^{a} \pm 0.000$	$0.995^{a} \pm 0.000$	0.001
Isoleucine	$0.989^{b} \pm 0.000$	$0.990^{a} \pm 0.000$	$0.990^{ab} \pm 0.001$	$0.989^{ab} \pm 0.000$	0.001
Phenylalanine	$0.989^{a} \pm 0.000$	$0.980^{b} \pm 0.000$	$0.990^{a} \pm 0.001$	$0.989^{a} \pm 0.000$	0.002
Leucine	$0.936^{b} \pm 0.003$	$0.918^{c} \pm 0.001$	$0.945^{a} \pm 0.003$	$0.929^{b} \pm 0.002$	0.008
Glycine	$0.994^{b} \pm 0.000$	$0.984^{d} \pm 0.000$	$0.998^{a} \pm 0.000$	$0.985^{c} \pm 0.000$	0.001

[1] Least significant difference (P<0.05). [*] Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05)

The AAD at 35 d are presented in Table 4.6. Enzyme addition had a less dramatic effect during this period, resulting in improved digestibilities for only Pro and Cys (P<0.05). For the digestibilities of the other AA's, enzymes had either a negative effect (Ala, Thr, Ser, Arg, Val, His, Asp, Cys and Gly) or no significant effect (Glu, Lys, Met, Tyr, Ile, Phe and Leu).

Table 4.5 Mean apparent digestibility coefficients and standard error of the mean for individual amino acids in broiler chickens on a diet supplemented with exogenous enzymes at 28 days

	Treatment				
	C+	C	Pe+	Pe	LSD[1]
Amino acid	Mash + AveMix	Control Mash	Pellet + AveMix	Control Pellet	
Alanine	$0.984^{b} \pm 0.001$	$0.982^{b} \pm 0.000$	$0.988^{a} \pm 0.001$	$0.988^{a} \pm 0.000$	0.002
Threonine	$0.975^{c} \pm 0.000$	$0.989^{a} \pm 0.000$	$0.986^{a} \pm 0.001$	$0.979^{b} \pm 0.001$	0.003
Serine	$0.986^{b} \pm 0.001$	$0.978^{d} \pm 0.000$	$0.990^{a} \pm 0.001$	$0.983^{c} \pm 0.000$	0.002
Arginine	$0.979^{b} \pm 0.001$	$0.967^{c} \pm 0.001$	$0.991^{a} \pm 0.000$	$0.968^{c} \pm 0.001$	0.003
Glutamic acid	$0.757^{c} \pm 0.009$	$0.672^{d} \pm 0.005$	$0.841^{a} \pm 0.008$	$0.787^{b} \pm 0.005$	0.028
Valine	$0.981^{b} \pm 0.001$	$0.978^{c} \pm 0.000$	$0.986^{a} \pm 0.001$	$0.985^{a} \pm 0.000$	0.002
Histidine	$0.998^{a} \pm 0.000$	$0.994^{c} \pm 0.000$	$0.998^{a} \pm 0.000$	$0.997^{b} \pm 0.000$	0.000
Aspartic acid	$0.930^{b} \pm 0.003$	$0.886^{c} \pm 0.002$	$0.947^{a} \pm 0.003$	$0.944^{a} \pm 0.001$	0.009
Lysine	$0.949^{b} \pm 0.002$	$0.934^{c} \pm 0.000$	$0.965^{a} \pm 0.002$	$0.963^{a} \pm 0.001$	0.006
Proline	$0.972^{b} \pm 0.001$	$0.954^{d} \pm 0.001$	$0.978^{a} \pm 0.001$	$0.966^{c} \pm 0.001$	0.004
Methionine	$0.999^{c} \pm 0.000$	$0.999^{d} \pm 0.000$	$1.000^{a} \pm 0.000$	$0.999^{b} \pm 0.000$	0.000
Tyrosine	$0.989^{b} \pm 0.000$	$0.985^{c} \pm 0.000$	$0.992^{a} \pm 0.000$	$0.992^{a} \pm 0.000$	0.001
Cysteine	$0.995^{b} \pm 0.000$	$0.992^{c} \pm 0.000$	$0.996^{a} \pm 0.000$	$0.995^{ab} \pm 0.000$	0.001
Isoleucine	$0.990^{b} \pm 0.000$	$0.986^{c} \pm 0.000$	$0.992^{a} \pm 0.000$	$0.990^{b} \pm 0.000$	0.001
Phenylalanine	$0.988^{b} \pm 0.001$	$0.985^{c} \pm 0.000$	$0.991^{a} \pm 0.000$	$0.988^{b} \pm 0.000$	0.002
Leucine	$0.938^{b} \pm 0.002$	$0.907^{d} \pm 0.001$	$0.953^{a} \pm 0.002$	$0.927^{c} \pm 0.002$	0.008
Glycine	$0.988^{c} \pm 0.001$	$0.996^{a} \pm 0.000$	$0.993^{b} \pm 0.000$	$0.988^{c} \pm 0.000$	0.001

[1] Least significant difference (P<0.05). * Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05)

No significant differences were also observed between the C treatments and the Pe+ treatments for the digestibilities of several AA's (Ser, Glu, Val, His, Asp, Lys, Tyr and Leu), and the digestibility of several other were in fact found to be higher in the control diets (Ala, Thr, Arg, Met, Ile, Phe, Gly)(P<0.05). Only the digestibilities of Pro and Cys were improved by the combination of enzymes and pelleting (P<0.05). Pelleting on the other hand improved the digestibility of all AA's except Thr and Arg (P<0.05). From Table 4.6 it is thus clear that enzyme addition alone or in combination with pelleting has no beneficial effect on the digestibilities of most of the AA's. For this period the only significant treatment effect (P<0.05) with regards to AA digestibilities was when the control diet was pelleted. This is in agreement with a previous study by McCracken *et al*. (1999) with the study of pelleting of xylanase-supplemented diets.

Table 4.7 represents the AND and AAD for the entire experimental period (21-35 d). From these results it is clear that for the period 21-35 d enzymes increased the digestibilities of His (0.2%), Cys (0.2%) and Leu (1.9%) and decreased the digestibilities of Ala (0.3 %) and Val (0.4%) (P<0.05).

Table 4.6 Mean apparent digestibility coefficients (standard error) for individual amino acids in broiler chickens on a diet supplemented with exogenous enzymes at 35 d.

	Treatment				
	C+	C	Pe+	Pe	LSD[1]
Amino acid	Mash + AveMix	Control Mash	Pellet + AveMix	Control Pellet	
Alanine	0.984[d] ± 0.001[*]	0.989[b] ± 0.000	0.986[c] ± 0.001	0.992[a] ± 0.000	0.002
Threonine	0.980[c] ± 0.001	0.994[a] ± 0.000	0.984[b] ± 0.001	0.995[a] ± 0.000	0.002
Serine	0.989 [ab] ± 0.000	0.986[c] ± 0.000	0.987[bc] ± 0.001	0.989[a] ± 0.000	0.002
Arginine	0.985[b] ± 0.001	0.991[a] ± 0.000	0.973[d] ± 0.001	0.982[c] ± 0.001	0.003
Glutamic acid	0.789[b] ± 0.008	0.772[b] ± 0.003	0.792[b] ± 0.010	0.869[a] ± 0.003	0.027
Valine	0.981[c] ± 0.001	0.985[b] ± 0.000	0.983[b] ± 0.001	0.988[a] ± 0.000	0.002
Histidine	0.996[c] ± 0.000	0.997[b] ± 0.000	0.997[b] ± 0.000	0.998[a] ± 0.000	0.000
Aspartic acid	0.938[b] ± 0.002	0.922[c] ± 0.001	0.929[c] ± 0.004	0.958[a] ± 0.001	0.009
Lysine	0.955[b] ± 0.002	0.950[b] ± 0.001	0.952[b] ± 0.002	0.971[a] ± 0.001	0.006
Proline	0.974[b] ± 0.000	0.970[c] ± 0.000	0.975[b] ± 0.001	0.979[a] ± 0.001	0.003
Methionine	1.000[b] ± 0.000	1.000[b] ± 0.000	0.999[c] ± 0.000	1.000[a] ± 0.000	0.000
Tyrosine	0.990[b] ± 0.000	0.990[b] ± 0.000	0.990[b] ± 0.001	0.996[a] ± 0.000	0.001
Cysteine	0.996[b] ± 0.000	0.994[d] ± 0.000	0.994[c] ± 0.000	0.997[a] ± 0.000	0.001
Isoleucine	0.992[b] ± 0.000	0.991[b] ± 0.000	0.990[c] ± 0.001	0.994[a] ± 0.000	0.001
Phenylalanine	0.991[b]± 0.000	0.991[b]± 0.000	0.987[c]± 0.001	0.995[a] ± 0.000	0.001
Leucine	0.948[b] ± 0.002	0.943[bc] ± 0.001	0.940[c] ± 0.000	0.963[a] ± 0.001	0.008
Glycine	0.991[c] ± 0.000	0.998[a] ± 0.000	0.992[b] ± 0.000	0.998[a] ± 0.000	0.001

[1] Least significant difference (P<0.05). [*] Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05)

Pelleting increased the digestibilities of Ala (0.3%), His (0.2%), Lys (2%), Met (0.04 %), Cys (0.3 %), Phe (0.6 %) and Leu (1.8 %) (P<0.05). Pelleting did not have a significantly detrimental effect on the digestibility of any of the AA (P<0.05) suggesting that pelleting did not cause severe heat damage to nitrogenous substances and that greater benefit can be gained from pelleting alone than by enzyme addition alone (Table 4.7). The combination of enzymes and pelleting had the most beneficial effect however, resulting in improved digestibilities for Ser (0.4 %), His (0.2 %), Lys (1.1 %), Met (0.04%), Tyr (0.3%), Cys (0.2 %), Phe (0.4 %) and Leu (2.4 %) (P<0.05). Similar to pelleting, the combination of enzymes and pelleting did not significantly reduce the digestibility of any of the AA. No significant treatment effects were found for N digestibility.

By comparing the ileal digestibilities with the excreta digestibilities it was found that excreta digestibilities are mostly underestimated due to the contribution of uric acid in the excreta, the only exception being glutamic acid in both diets which were overestimated. As is clear from Table 4.8, the digestibility of cysteine especially was largely underestimated in both diets.

Table 4.7 Mean apparent nitrogen and amino acid digestibilities (standard error) for the entire experimental period (21-35 d).

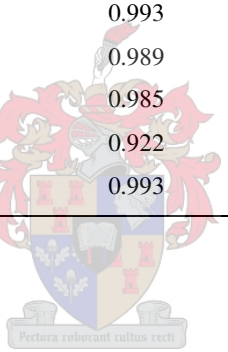
	Treatment				
	C+	C	Pe+	Pe	LSD[1]
Amino acids	Mash + AveMix	Control Mash	Pellet + AveMix	Control Pellet	
Alanine	0.983[c] ± 0.00[*]	0.986[b] ± 0.001	0.986[b] ± 0.001	0.989[a] ± 0.001	0.003
Threonine	0.981 ± 0.002	0.988 ± 0.003	0.988 ± 0.002	0.984 ± 0.004	0.008
Serine	0.987 [ab] ± 0.000	0.984[b] ± 0.002	0.988[a] ± 0.001	0.986[ab] ± 0.001	0.004
Arginine	0.983 ± 0.001	0.976 ± 0.005	0.982 ± 0.004	0.974 ± 0.003	0.010
Glutamic acid	0.759 ± 0.010	0.802 ± 0.054	0.803 ± 0.013	0.801 ± 0.023	0.090
Valine	0.979[b] ± 0.001	0.983[a] ± 0.002	0.983[a] ± 0.001	0.985[a] ± 0.001	0.004
Histidine	0.997[a] ± 0.000	0.995[b] ± 0.000	0.997[a] ± 0.000	0.997[a] ± 0.000	0.001
Aspartic acid	0.929 ± 0.000	0.931 ± 0.002	0.932 ± 0.005	0.944 ± 0.005	0.030
Lysine	0.949[bc] ± 0.002	0.945[c] ± 0.004	0.956[ab] ± 0.003	0.965[a] ± 0.002	0.009
Proline	0.972 ± 0.001	0.970 ± 0.006	0.975 ± 0.001	0.969 ± 0.003	0.010
Methionine	0.999[ab] ± 0.000	0.999[b] ± 0.000	0.999[a] ± 0.000	0.999[a] ± 0.000	0.000
Tyrosine	0.989[bc] ± 0.000	0.987[c] ± 0.001	0.990[b] ± 0.001	0.994[a] ± 0.001	0.002
Cysteine	0.995[a] ± 0.000	0.993[b] ± 0.000	0.995[a] ± 0.000	0.996[a] ± 0.000	0.010
Isoleucine	0.990 ± 0.001	0.989 ± 0.001	0.990 ± 0.001	0.991 ± 0.001	0.002
Phenylalanine	0.989[ab] ± 0.001	0.985[b] ± 0.002	0.989[a] ± 0.001	0.991[a] ± 0.001	0.004
Leucine	0.941[a] ± 0.003	0.922[b] ± 0.007	0.946[a] ± 0.000	0.940[a] ± 0.008	0.016
Glycine	0.991 ± 0.001	0.993 ± 0.003	0.994 ± 0.001	0.991 ± 0.002	0.001
Nitrogen	0.980[ab] ± 0.001	0.980[ab] ± 0.001	0.982[a] ± 0.001	0.979[b] ± 0.0016	0.003

[1] Least significant difference (P<0.05). [*] Standard error of the mean. [a-d] Means within a row with common superscripts are not significantly different (P<0.05).

Table 4.8 Comparison of excreta- (Ed) and ileal (Id) amino acid digestibilities of a mash and pelleted vegetarian maize soya diet for the period 21-35 d

Diet	Mash		Pellet	
Amino acids	Ed	Id	Ed	Id
Alanine	0.869	0.986	0.838	0.986
Threonine	0.889	0.988	0.901	0.988
Serine	0.862	0.984	0.828	0.988
Arginine	0.922	0.976	0.922	0.982
Glutamic acid	0.921	0.802	0.903	0.803
Valine	0.858	0.983	0.838	0.983
Histidine	0.900	0.995	0.892	0.997
Aspartic acid	0.870	0.931	0.850	0.932
Lysine	0.881	0.945	0.871	0.956
Proline	0.893	0.970	0.875	0.975
Methionine	0.929	0.999	0.928	0.999
Tyrosine	0.900	0.987	0.863	0.990
Cysteine	0.627	0.993	0.530	0.995
Isoleucine	0.860	0.989	0.838	0.990
Phenylalanine	0.898	0.985	0.880	0.989
Leucine	0.917	0.922	0.909	0.946
Glycine	*	0.993	*	0.994

* Could not be determined



CHAPTER 5

PRODUCTION PERFORMANCE OF BROILERS ON A LOW METABOLISABLE ENERGY (AMEn) SUPPLEMENTED WITH EXOGENOUS ENZYMES

5.1 Introduction

The application of exogenous enzymes for broiler diets has proven to be a successful means of improving production performance (Marquardt *et al*., 1996; Zanella *et al*., 1999; Augspurger *et al*., 2004; Dilger *et al*., 2004; Jackson, *et al*., 2004; Yu and Chung, 2004; Cowieson and Adeola, 2005; Meng and Slominski, 2005; Onyango *et al*., 2005) although linear improvements in performance with enzyme supplemented diets are often not seen (Irish *et al*., 1995; Peter and Baker, 2001). The use of enzymes in nutritionally balanced broiler diets have other benefits apart from an improvement in production performance, such as reduced variation between feed ingredients and less variation from flock to flock (Bedford, 2000). Other benefits include improved gut health (Dänicke *et al.*, 1999; Bedford, 2000; Sieo *et al*., 2005) and counteracting the effect of increased digesta viscosity, such as wet litter (Bedford, 2000), resulting from feeding cereal grains such as wheat, rye, triticale and barley (Almirall *et al*., 1995). Exogenous enzyme combinations also have the potential to increase the nutrient availability from nutritionally marginal diets, especially with regards to metabolisable energy (Yu and Chung, 2004; Cowieson and Adeola, 2005) and to reduce formulating costs by using less expensive ingredients (Bedford and Morgan, 1996).

The objective of this trial was to determine whether a commercial enzyme product had the potential to increase broiler performance on a low AMEn diet to that of a nutritionally balanced broiler diet. A cost calculation was also done to evaluate whether the addition of this enzyme combination may result in more profitable broiler production.

5.2 Materials and methods

Three hundred and eighty four day-old Cobb 500 broiler chicks (as-hatched) were placed in 24 floor pens, each pen containing 16 birds. The experimental units (pens) were allocated at random to four experimental treatments shown in Table 3.2, with six replicates per treatment. The experiment was a completely randomised design with main effect of dietary treatment. The birds were vaccinated at day-old against Newcastle Disease and Infectious Bronchitis. The environmental temperature within the house was 30° C for the first day and was then decreased by 0.5° C every second day to 20° C. Continuous light (24L: 0D) was provided for the duration of the trial. Birds were offered *ad libitum* access to feed and fresh water. Experimental procedures were approved by the University of Stellenbosch Animal Ethics Committee.

Control starter, low AMEn starter, control finisher and low AMEn finisher broiler diets were formulated using the primary breeder specifications. The low AMEn diets were formulated to be 10 %

below the minimum AMEn requirement for the specific strain. A detail of the ingredients used in the experimental diets and the formulated nutrient composition of the diets are shown in Table 5.1.

The starter and finisher diets were fed as crumbles and pellets respectively. A commercial enzyme product (AveMix XG, AVEVE, Belgium) was added to treatments C+ and L+ at the manufacturers' recommendation of 0.1g/kg. A summary of the dietary treatments is shown in Table 5.2. The enzyme contained guaranteed activities for xylanase and glucanase and significant activities of cellulase, xyloglucanase, endo-xylanase, galactomannanase, mannanase and pectinase. The starter diet was provided from day one to day 13 and the finisher diet from day 14 to the end of the trial at day 35. Birds were weighed (per pen) on arrival and on days 7, 14, 21, 28 and 35. Food intake (per pen) was also measured at days 7, 14, 21, 28 and 35 and mortality was recorded daily.

All data were tested for homogeneity of variance using Levene's test and for normal distribution using the Kolmogorov-Smirnov test. Data was of equal variance and normal distribution and was analysed by ANOVA using the GLM procedure of SAS Enterprise guide 3.0. The Student's *t*-test was used to test differences between treatment means when the treatment effect was significant. The probability level was set at 5 %.

5.3 Results and discussion

The treatments means for body weight (BW), body weight gain (BWG), food intake (FI) and feed conversion ratio (FCR) are presented in Tables 5.3- 5.7. Body weight was not significantly affected by enzyme supplementation during the first three periods (0-7 d, 8-14 d and 15-21 d). For the last two periods (22-28 d and 29-35 d) the L diet resulted in a significantly lower BW ($P<0.05$). There were no significant differences between the enzyme supplemented diets (L+ and C+) and their respective control groups (L and C) for all periods and for all traits (Table 5.3). When the enzyme was added to the low AMEn diet, the BW was still significantly lower compared to the nutritionally balanced control diet ($P<0.05$), indicating that enzyme addition could not improve AME levels, therefore impairing BW of birds fed on the low AMEn diet. The same effect was seen for BWG during the last two periods. These results are in disagreement with previous studies (Yu and Chung, 2004; Cowieson and Adeola, 2005). Feed intake was not significantly different during the first two weeks of life. For the period 15-21 d, FI was significantly higher in the L+ diet compared to all treatments ($P<0.05$). For the last two periods, the C+ diet resulted in lower FI ($P<0.05$) compared to the C diet. No significant differences on FI were however seen between for birds fed the L+ diet and the L diet during this period. The L+ diet reduced FI during the last two periods, but so did the L diet ($P<0.05$). This is in disagreement with a previous study using similar diets and enzymes having similar activities (Yu and Chung, 2004). The birds on the low AMEn diets also did not try to compensate for the lack of energy by increasing FI as might be expected and, in fact, had a significantly lower FI compared to the balanced diets for the last two periods ($P<0.05$).

Table 5.1 Composition of starter and finisher diets for broiler chickens based on an as fed basis (g/kg).

	Starter		Finisher	
Ingredient	Control	Low AMEn	Control	Low AMEn
Maize	549.85	594.59	708.73	664.53
Maize gluten 60		115.36		105.00
Soybean 46	270.00	238.86	251.34	186.14
Soybean full fat	125.23			
Choline chloride 60%	0.67		0.53	
DL-methionine	2.36	2.11	1.34	1.61
L-threonine	0.03	0.70		0.75
L-lysine HCl	7.68	6.67	1.46	5.48
Limestone	21.69	17.96	18.13	15.61
Salt	4.03	1.23	4.07	1.77
Monocalcium phosphate	11.78	16.45	7.41	13.31
Sodium bicarbonate	4.08	3.58	4.18	2.47
Vitamin E			0.40	
Vit + min premix	1.50	2.50	1.3	2.50
Stafac	0.1		0.1	
Salinocox	0.50		0.50	
Ronozyme	0.50		0.50	
Calculated nutrient content (*)				
AMEn (MJ/kg)	12.49	11.25	12.68	11.64
Crude protein (%)	22.00	21.41	17.50	19.04
Lysine (%)	1.27	1.37	0.95	1.15
Methionine (%)	0.56	0.60	0.41	0.52
Methionine + Cystine (%)	0.93	0.96	0.72	0.85
Threonine (%)	0.88	0.83	0.69	0.75
Tryptophan (%)	0.27	0.24	0.20	0.20
Arginine (%)	1.51	1.21	1.12	1.05
Isoleucine (%)	1.00	0.92	0.79	0.80
Leucine (%)	1.95	2.48	1.65	2.29
Histidine (%)	0.62	0.53	0.50	0.48
Phenylalanine (%)		1.08		0.96
Tyrosine (%)		0.92		0.82
Phenylalanine+tyrosine (%)	1.88	2.00	1.51	1.78
Valine (%)	1.05	1.06	0.84	0.95
Calcium	1.10	1.00	0.90	0.85
Available Phosphorus		0.50		0.42
Total Phosphorous	0.67		0.54	

(*) Amino acids expressed on a digestible basis.

Table 5.2 A description of the dietary treatments used throughout the trial

Treatment	Description
C+	Control + AveMix XG (0.1g/1kg)
C	Control
L+	Low ME control diet + AveMix XG (0.1g/kg)
L	Low ME control diet

Table 5.3 Mean body weight (BW) (g/bird) of the dietary treatments for all the trial periods.

Period		0-7 d		8-14 d		15-21 d		22-28 d		29-35 d	
Treatment	N	BW	SE[2]	BW	SE[2]	BW	SE[2]	BW	SE[2]	BW	SE[2]
C	6	155.10	0.88	399.17	3.38	791.88	4.04	1347.92[a]	13.51	1982.64[a]	20.66
C+	6	148.96	2.77	388.75	6.27	789.17	8.78	1331.25[a]	10.81	1958.54[a]	15.40
L	6	154.44	1.56	402.40	6.17	814.23	13.79	1190.78[b]	16.79	1567.40[b]	17.73
L+	6	151.46	3.23	392.71	6.10	798.82	5.40	1172.01[b]	11.03	1536.70[b]	14.85
LSD[1]		12.77		16.56		26.09		39.10		51.07	

[1] Least significant difference
[2] Standard error of the mean
[a-b] Means within a column with common superscripts are not significantly different (P<0.05)

Table 5.4 Mean body weight gain (BWG) (g/bird/period) of the dietary treatments for all the trial periods.

Period		0-7 d		8-14 d		15-21 d		22-28 d		29-35 d	
Treatment	N	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]	BWG	SE[2]
C	6	117.19	1.07	244.06	3.40	392.71	3.47	556.04[a]	12.76	634.72[a]	12.92
C+	6	111.17	2.64	239.79	4.11	400.42	6.30	542.08[a]	11.76	627.29[a]	8.26
L	6	116.86	1.52	247.97	5.22	411.82	8.16	376.55[b]	6.86	376.62[b]	17.83
L+	6	113.85	3.12	241.25	3.49	406.11	6.48	373.19[b]	6.09	364.69[b]	4.07
LSD[1]		12.74		12.15		26.09		28.96			

[1] Least significant difference
[2] Standard error of the mean
[a-b] Means within a column with common superscripts are not significantly different (P<0.05)

Table 5.5 Mean feed intake (FI) (g/bird/period) of the dietary treatments for all the trial periods.

Period		0-7 d		8-14 d		15-21 d		22-28 d		29-35 d	
Treatment	N	FI	SE[2]	FI	SE[2]	FI	SE[2]	FI	SE[2]	FI	SE[2]
C	6	183.33	4.43	402.92	4.86	673.96b	5.56	1022.92[a]	12.11	1261.21[a]	20.03
C+	6	190.63	2.83	397.71	6.06	679.17b	5.19	977.50[b]	10.48	1178.54[b]	14.28
L	6	181.18	2.30	404.71	11.12	679.38b	12.14	912.07[c]	14.11	953.50[c]	14.64
L+	6	171.35	2.84	402.19	5.73	727.26a	23.37	909.11[c]	6.37	953.04[c]	21.99
LSD[1]		35.78		21.71		40.43		32.85		53.24	

[1] Least significant difference
[2] Standard error of the mean
[a-c] Means within a column with common superscripts are not significantly different (P<0.05)

The excess of certain AA in these diets may however have been partly responsible for the lower FI. In other words to, prevent the potentially toxic effect of an excess of AA, FI is reduced.

FCR was only significantly affected in the last three periods. A higher FCR was seen for L+ diet compared to the L diet as a result of a significantly higher FI during this period. For the last two periods the FCR was significantly higher for the birds on the low ME diets (L+ and L) compared to their balanced counterparts (C+ and C). This is in accordance with Yu and Chung (2004) using a similar experimental design. Enzyme supplementation of the low AMEn diet did however not significantly improve FCR ($P<0.05$) as was seen with previous studies (Yu and Chung, 2004; Cowieson and Adeola, 2005) or even improve it to a level similar to the C diet. This may be explained by the variability in the chemical composition of especially soya and the inability of this specific product to target soya ANF's.

Table 5.6 Feed conversion ratio (FCR) (g intake per bird per period/g BWG per bird per period) of the dietary treatments for all the trial periods.

Period		0-7 d		8-14 d		15-21 d		22-28 d		29-35 d	
Treatment	N	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]	FCR	SE[2]
C	6	1.56	0.03	1.65	0.02	1.72[ab]	0.01	1.84[b]	0.02	1.99[b]	0.02
C+	6	1.72	0.04	1.66	0.02	1.70[ab]	0.03	1.81[b]	0.03	1.88[b]	0.02
L	6	1.55	0.02	1.64	0.05	1.65[b]	0.02	2.42[a]	0.03	2.55[a]	0.09
L+	6	1.51	0.05	1.67	0.03	1.79[a]	0.07	2.44[a]	0.03	2.61[a]	0.04
LSD[1]		0.41		0.11		0.12		0.09		0.15	

[1] Least significant difference
[2] Standard error of the mean
[a-b] Means within a column with common superscripts are not significantly different ($P<0.05$)

Table 5.7 presents a summary of the feed costs and relative financial benefit for each of the treatments. For the birds fed on the low AMEn diets, enzymes had no added financial benefit. Including the cost of the commercial enzyme complex, the L+ treatments was in fact R0.18 more expensive per chick housed to 35 d of age. The reason for this observation is increased FI (13.1 kg) in the L+ group compared to the L group and an inability to convert the feed more efficiently into BW. The same can be said for the R2.78 deficit between the L+ and the C group, although this difference may be partly explained by the higher inclusion rate of more expensive raw materials such as maize gluten 60 and synthetic AA's to the L diets, resulting in a much more expensive diet compared to the C diet.

Despite the more expensive C+ diet compared to the C diet, the relative financial benefit (R/c) was found to be R 0.32 more per bird housed for the enzyme supplemented group. This was a result of lower FI of the finisher diet and a higher BW per treatment for the C+ group.

In the current study it was shown that enzyme supplementation to a low AMEn diet did not result in a relative financial benefit comparable to a balanced maize-soya diet. This may be as a result

of an AA imbalance in the L diet, which in turn adversely affected the FI, FCR and therefore the BW. When these enzymes are, however added to a nutritionally balanced maize-soya diets the improvement in relative financial gain may be meaningful.

Table 5.7 Summary of the feed costs and the relative economic benefit of each dietary treatment based on current (October 2006) raw material prices.

Treatment	C+		C		L+		L	
Feed	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Price (R/kg)	1.86	1.56	1.84	1.55	1.88	1.81	1.87	1.79
Total feed consumption (kg)	56.48	272.18	56.28	280.19	55.06	241.59	53.86	229.69
Total feed cost/treatment (R)	105.05	424.60	103.56	434.29	103.51	437.28	100.72	411.15
Total BW/treatment (kg)	188.02		184.41		142.95		141.08	
Price/kg BW (R)	6.35		6.35		6.35		6.35	
Income generated (R)	1 193.93		1 171.00		907.73		895.86	
Relative financial benefit (R) *	6.92		6.60		3.82		4.00	

* Per bird housed excluding cost of labour, consumables and purchasing of day-old-chicks, but assumed to be equal for all treatments, and at current raw material costs.

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CHAPTER 6
GENERAL CONCLUSION

The primary objective of this thesis was to evaluate whether a commercial enzyme combination containing mainly NSP-degrading properties would improve the N and AA digestibility and thus performance of a commercial and low AMEn maize-soya broiler diet. Enzymes are proteins and may be denaturized at high temperatures. A secondary objective was thus to see whether cold pelleting at temperatures of up to 80 ºC would adversely affect enzymatic efficacy. Different methods of determining digestibility may also lead to contradicting results and therefore N and AA digestibilities were also determined by two different collection methods (excreta- and ileal digestibility).

Supplementation with exogenous enzymes resulted in improvements of the apparent excreta- and ileal N digestibility but only during one period (14-21 d), early in the chicks' life. Conflicting results with regards to AA digestibility was found for all periods. For both methods the digestibilities of Thr, Met and Phe (14-21 d) and Cys (22-28 d and 29-35 d) was improved. If the assumption is made that ileal digestibility coefficients are more accurate, then over the entire experimental period (14-35 d) (Table 4.7), the digestibilities of His, Cys and Leu were improved by 0.2 %, 0.2 % and 1.9 % respectively, by the addition of the exogenous enzymes. In some cases, enzymes adversely affected AA digestibility and in others the improvements in the unsupplemented pelleted diets were better than the enzyme supplemented mash diets.

The combination of enzymes and pelleting (Pe+) resulted in the greatest improvements in AA digestibilities as determined by both methods and in contrast to enzyme addition alone, the excreta- and ileal digestibilities were mostly in agreement. By both methods, the digestibilities of Thr, Arg, Met, Cys, Phe and Leu (14-21 d), Ser, Arg, Glu, Val, His, Asp, Lys, Pro, Met, Tyr, Phe and Leu (22-28 d), and Pro (29-35 d) were improved by enzyme addition and pelleting, most likely as a result of greater substrate availability post-pelleting for the activity of endogenous and exogenous enzymes (Cowan *et al*., 1996; Bedford, 2002). This furthermore indicates that pelleting did not destroy enzymatic efficacy, and that the combination of enzymes and cold pelleting was indeed beneficial for the chick's development. For the entire experimental period (14-35 d), the digestibilities Ser, His, Lys, Met, Tyr, Cys, Phe and Leu were improved by the combination of enzymes and pelleting. The greatest benefit of exogenous enzymes for pelleted diets was thus found to be for the period 22-28 d, although many AA's digestibilities may be improved over the entire period.

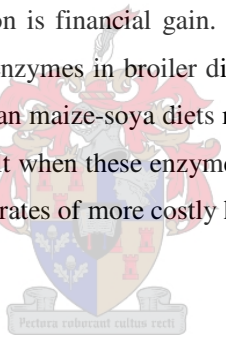
In contrast to enzyme addition alone, no negative effect was found in supplemented pelleted diets for the ileal digestibilities of any AA over the whole experimental period. Thus, even if enzymatic efficacy is greatest during the period 22-29 d, there can be no harm in including such products throughout the period 14-35 d. Due to the fact that the N digestibility was not greatly affected by the combination of enzymes and pelleting but many AA digestibilities were improved, the

assumption may be made that N digestibility alone is an inaccurate means of determining the digestibility of nitrogenous substances.

Assuming ileal AA digestibilities are more accurate, the results of this research suggest that when enzymes are added to mash diets, this method should be used as conflicting results between ileal- and faecal digestibilities exists. In pelleted diets which are supplemented with enzymes both methods may be used as greater similarities in AA digestibilities were found in these experiments, although theoretically ileal digestibility coefficients should be more accurate.

Negligible significant improvements in production performance were seen with regards to enzyme addition in the excreta digestibility study, a performance trial was conducted using a balanced and a low AMEn diet, with or without enzymes. In the low AMEn diet, the enzymes did not significantly improve BW or BWG to that of the balanced control diets or even significantly improve it compared to the unsupplemented low AMEn diet. Enzymes significantly reduced FI in the control balanced diet for the period 22-35 d, although this did not lead to significantly improved FCR. The FCR of birds fed the enzyme supplemented low AMEn diet was higher compared to the control group, but only for the period 15-21 d.

The main goal of broiler production is financial gain. As a result a calculation was done too evaluate the relative financial benefit of enzymes in broiler diets. The results of this research suggest that enzyme addition to balanced vegetarian maize-soya diets may result in significant financial gains. Financial losses may however be the result when these enzyme combinations are added to low AMEn diets in an attempt to reduce the inclusion rates of more costly high-energy raw materials.



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