The use of fibrolytic enzymes in maize-soya based broiler diets

by

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Abstract

A growth and digestibility trial was conducted comparing the effect of an experimental enzyme at three different inclusions. A negative control containing no enzyme additions and a positive control containing a proven commercial enzyme were compared in a maize-soybean diet noting the performance of broilers and the digestibility of the grower feed. The commercial enzyme was a granular product with a xylanase activity of 38114.29 nkat/g and the second enzyme (ABO374) was a liquid experimental product with a xylanase activity of 1426.86 nkat/ml. Five diets were used i.e. control basal diet without enzyme supplementation (negative control), basal diet supplemented with the commercial enzyme (positive control) and three basal diets supplemented with the test enzyme at various inclusion levels (ABO 50, ABO 100 and ABO 200). The positive control was supplemented with 200 g/ton of the commercial enzyme; ABO 50 was supplemented with ABO374 at an inclusion level of 2671 ml/ton, ABO 100 with 5342 ml/ton and ABO 200 with 10684 ml/ton. Supplementation with the test enzyme (ABO 50) significantly improved BW at 23 days of age by 4.6 % (1107.4 g *vs* 960.96 g) and at 37 days of age by 3.2 % (2311.75 g *vs* 2237.81 g) over the negative control. Body weight gain for the total period of the trial was significantly improved by 3.24 % (64.32 g/bird/day *vs* 62.24 g/bird/day) the test enzyme supplementation (ABO 50) when compared to the negative control. During the starter phase, test enzyme supplementation (ABO 50) led to an improvement of 4.58 % (1.25 *vs* 1.31) in FCR in comparison with the negative control. The FCR for the total trial obtained by the test enzyme supplementation was significantly lower than the FCR obtained by the positive control. The highest EPER obtained for this trail was by the test enzyme supplemented diets and this was significantly higher than the EPER obtained by the positive control. It is clear from this growth trial that the test enzyme (ABO374) at an inclusion level of 2671 ml/ton outperformed the commercial enzyme and that it has the potential to improve the production performance of broilers on a maize-SBM based diet.

The total tract digestibility method and total collection method was used to conduct the digestibility trial. The total tract digestibility method measures the difference between the amounts of each nutrient consumed from the amounts of each nutrient excreted in faeces. Only apparent digestibilities are reported for the digestibility trial. Apparent digestibility does not take the endogenous protein fraction in the faeces into account. The endogenous protein fraction is derived from digestive enzymes and proteins from the intestinal walls that are secreted into the digestive tract. The grower negative control, positive control, ABO 50, ABO 100 and ABO 200 diets used in the production trial were also used in the digestibility trial. Supplementation with the test enzyme showed no significant improvements on the apparent digestibility of dry matter, organic material, ash, crude protein, metabolisable energy or crude fibre. No significant improvements in the apparent digestibility of the amino acids (threonine, arginine, valine, lysine, methionine, cysteine and isoleucine) were noticed

either and thus the digestibility of the grower feed were not influenced by the addition of enzymes due to the supplementation of the test enzyme ABO374.

Pelletisation of the grower diets could have lead to the inactivation of the enzyme due to the high temperature at which pelletisation takes place. Another possible reason why enzyme supplementation did not increase nutrient digestibility, may be that the breakdown of non-starch polysaccharides by the enzymes led to an increase in the concentration of oligosaccharides in the small intestine of the birds, thus leading to the decrease in nutrient absorption

Key words: body weight, body weight gain, feed conversion ratio, European production efficiency ratio, maize, soybean meal, apparent digestibility, dry matter, organic material, ash, crude protein, metabolisable energy, crude fibre, xylanase, pellitisation

Uitreksel

‘n Groei en vertering studie was uitgevoer om die effek van ‘n eksperimentele ensiem wat teen drie verskillende insluitingsvlakke by ‘n basale dieët bygevoeg is te vergelyk met ‘n negatiewe kontrole wat geen ensiem bevat het nie en met ‘n positiewe kontrole wat ‘n kommersiële ensiem bevat in ‘n miele-sojaboon oliekoek dieët op die produksie vermoë van braaikuikens en die verteerbaarheid van die groei voer. Die kommersiële ensiem was ‘n granulêre produk met ‘n xylanase aktiwiteit van 38114.29 nkat/g en die eksperimentele ensiem (ABO374) was ‘n vloeistof produk met ‘n xylanase aktiwiteit van 1426.86 nkat/ml. Vyf diëte was gebruik nl. ‘n basale dieët met geen ensiem byvoeging (negatiewe kontrole), basale dieët met die byvoeging van die kommersiële ensiem (positiewe kontrole) en drie basale diëte wat met die byvoeging van die eksperimentele ensiem teen drie verskillende insluitings vlakke (ABO 50, ABO 100 and ABO 200). Die kommersiële ensiem was by die positiewe kontrole bygevoeg met ‘n insluitings vlak van 200 g/ton, ABO374 was bygevoeg by ABO 50 met ‘n insluitings vlak van 2671 ml/ton, ABO 100 met 5342 ml/ton en ABO 200 met 10684 ml/ton. Die byvoeging van die eksperimentele ensiem (ABO 50) het gelei tot die betekenisvolle verbetering van die liggaamsmassa van die voëls by die ouderdom van 23 dae met 4.6 % (1107.4 g teenoor 960.96 g) en by die ouderdom van 37 dae met 3.2 % (2311.75 g teenoor 2237.81 g) teenoor die negatiewe kontrole. Liggaams massa toename vir die hele periode van die studie was betekenisvol verhoog met 3.24 % (64.32 g/kuiken/dag teenoor 62.24 g/kuilen/dag) met die byvoeging van die eksperimentele ensiem (ABO374) teenoor die negatiewe kontrole. Voeromset verhouding was betekenisvol verbeter met 4.58 % (1.25 teenoor 1.31) toe die kommersiële ensiem bygevoeg was teenoor die negatiewe kontrole. Die hoogste europese produksie effektiwiteits verhouding wat verkry is vir die hele studie periode is deur die byvoeging van die eksperimentele ensiem (ABO374). Hierdie groei studie dui dus duidelik aan dat die gebruik van die eksperimentele ensiem (ABO374) baie beter resultate as die kommersiële ensiem opgelewer het teen ‘n insluitings vlak van 2671 ml/ton, dus het ABO374 die potensiaal om die produksie potensiaal van braaikuikens op ‘n mielie-sojaboonoliekoek dieët te verbeter.

Die totale spysverteringskanaal verteerbaarheid metode was gebruik om die verteerbaarheid studie uit te voer. Die totale spysverteringskanaal verteerbaarheid metode meet die verskil tussen die nutriënt inhoud van die voer en die nutriënt inhoud van die mis. Slegs die skynbare verteerbaarheid van nutriënte word vir hierdie verteerbaarheidstudie gerapporteer. Skynbare verteerbaarheid sluit nie die endogene proteïenfraksie wat afkomstig is van verteringsensieme of die proteïene afkomstig vanaf die spysverteringskanaal se intestinale wande af in nie. Die negatiewe kontrole, positiewe kontrole, ABO 50, ABO 100 en ABO 200 groei diëte gebruik in die produksie studie is gebruik vir die verteringsstudie. Die byvoeging van die eksperimentele ensiem het geen betekenisvolle resultate opgelewer ten opsigte van droë materiaal, organiese material, as, ru-proteïen, ru-vesel of metaboliseerbare energie nie. Daar was ook geen betekenisvolle resultate opgelewer wanneer die eksperimentele ensiem bygevoeg was nie ten opsigte van die verteerbaarheid vir aminosure (treonien, arginien, valien, metionien, sisteïen en isoleosien) nie en dus is die verteerbaarheid van die groeivoer glad nie beïnvloed deur die byvoeging van die eksperimentele ensiem nie.

Die verpilling van die groei voer mag dalk gelei het tot die inaktivering van die eksperimentele ensiem deur dat dit blootgestel was aan hoë temperature. ‘n Ander moontlike rede vir die mislukking van die ensiem kon gewees het dat die afbreking van die nie-stysel polisakkariedes deur die ensiem kon gelei het tot die verhoging van die oligosakkariede konsentrasie in die laer spysverterings kanaal en dus kon dit lei tot ‘n verhoogde deurvloeitempo, gevolg deur ‘n afname in die absorpsie van nutriënte.

Sleutel woorde: Liggaamsmassa, liggaamsmassa toename, voeromsetverhouding Europese produksie effektiwiteits verhouding, mielie, sojaboonoliekoek, skynbare verteerbaarheid, droëmateriaal, organiesematerial, as, ru-proteïen, ru-vesel, metaboliseerbare energie, verpilling

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List of Abbreviations

AA	Amino acids
ADF	Acid-detergent fibre
ADIN	Acid-detergent insoluble nitrogen
AFMA	Animal Feed Manufacturers Association
AGP	Antimicrobial growth promoters
AME	Apparent metabolisable energy
ANFs	Anti-nutritional factors
BW	Body weight
BWG	Body weight gain
Ca	Calcium
CCPR	Crypt cell proliferation rate
CF	Crude fibre
Co	Cobalt
CP	Crude protein
DM	Dry matter
EE	Ether extract
EPER	European production efficiency ratio
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FI	Feed intake
I-NCP	Insoluble non-cellulosic polysaccharide
Mg	Magnesium
ME	Metabolisable energy
Mn	Manganese
Na	Potassium
NDF	Neutral-detergent fibre
NSP	Non-starch polysaccharide
NSPs	Non-starch polysaccharides
P	Phosphorous
SAPA	South African Poultry Association

SBM	Soybean meal
Se	Selenium
S-NCP	Soluble non-cellulosic polysaccharide
SU	Stellenbosch University
VFA	Volatile fatty acids
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The application of feed enzymes to poultry diets for the enhancement of nutrient availability had been reported as far back as 1926 (Clickner & Follwell, 1926). Early research conducted on feed enzymes in poultry nutrition focused on non-starch polysaccharide (NSP) degrading enzymes, specifically xylanase and β-glucanase, in diets containing wheat, rye and barley (Choct, 2006).

Non-starch polysaccharides are complex sugars that occur in the cell walls of cereal grains (Bedford & Schulze, 1998). Non-starch polysaccharides include β-glucans, found in barley and oats, and arabinoxylans in rye and wheat (Beg *et al*., 2001). Enzymes such as xylanase and β-glucanase, have the ability the break down these structural polysaccharides, making the nutrients available to the animal (Bedford, 2000; Choct, 2006).

Diets containing high levels of cereal grains, due to high fibre content, can affect the production efficiency of monogastric animals such as poultry and pigs. When monogastric animals are fed a diet with a high fibre content, it can have a negative influence on nutrient utilisation, resulting in metabolic disorders (Nnenna *et al*., 2006). In high fibre diets, a large component of the digested matter consists of undigested NSP, which can cause intestinal disturbances, characterised by sticky droppings, and poor growth in young animals. Non-starch polysaccharides have a tendency to form high molecular weight viscous aggregates in the gastrointestinal tract, which considerably decreases the passage rate of digesta through the gastrointestinal tract. A decrease in the passage rate of digesta also causes a reduction in the diffusion of digestive enzymes, and stimulates the proliferation of bacteria inside the gastrointestinal tract (Bedford & Schulze, 1998).

Digestibility studies on the use of NSP-degrading enzymes in broilers where the diets were based on wheat, rye and barley have revealed positive results in the production performance of the broilers and the nutrient digestibility of the feed (Bedford & Schulze, 1998; Bedford, 2000; Choct, 2006).

Modern commercial broiler diets are formulated to contain maize and soybean meal. Soybean-meal (SBM) is a good source of protein, but there is considerable variation between the nutrient content of SBM samples (Knudsen, 1997). Soybean-meal also contains a considerable amount of oligosaccharides, a high concentration of oligosaccharides present in the digestive system of a broiler can lead to a higher feed passage rate and then decrease nutrient absorption in the gut (Coon *et al*., 1990). Maize is used as a main energy source, and has been considered to be a homogenous commodity in terms of its nutrient content. However, researchers have shown that there is considerable variation between the nutrient content of maize samples, especially in the amount of energy. Maize also contains a small amount of non-starch polysaccharides (NSPs) (Classen, 1996;

Knudsen, 1997; McNab & Boorman, 2002). Researchers have therefore changed their focus to the inclusion of feed enzymes in maize-SBM based diets for poultry to improve nutrient digestibility, broiler production performance, as well as reducing the variability in the nutrient content between feed samples (Bedford, 2000; Choct, 2006).

The objective of this thesis is to evaluate the inclusion of an experimental xylanase enzyme product (ABO374) in a nutritionally balanced maize-SBM broiler diet, and to study it's effect on nutrient digestibility, and broiler production performance.

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CHAPTER 2

LITERATURE REVIEW

2.1 Broiler production in South Africa

The poultry industry is currently the largest agriculture sector in South Africa. In financial terms the industry makes up 24 % of all the agricultural production and 48 % by animal volume production. The industry also supplies 63.6 % of the animal protein consumed in South Africa (Esterhuizen, 2010; Vauqulin, 2010) Broiler production has increased ever since intensive broiler production started in South Africa. According to the Broiler Organisation Committee of the South African Poultry Association (SAPA) (2009) growth of 30,6 % was achieved for the period from 2004 to 2008 and for the period of 2008 to 2009, 0,8 % (Vauqulin, 2009a; Vauqulin, 2009b; Vauqulin, 2010).

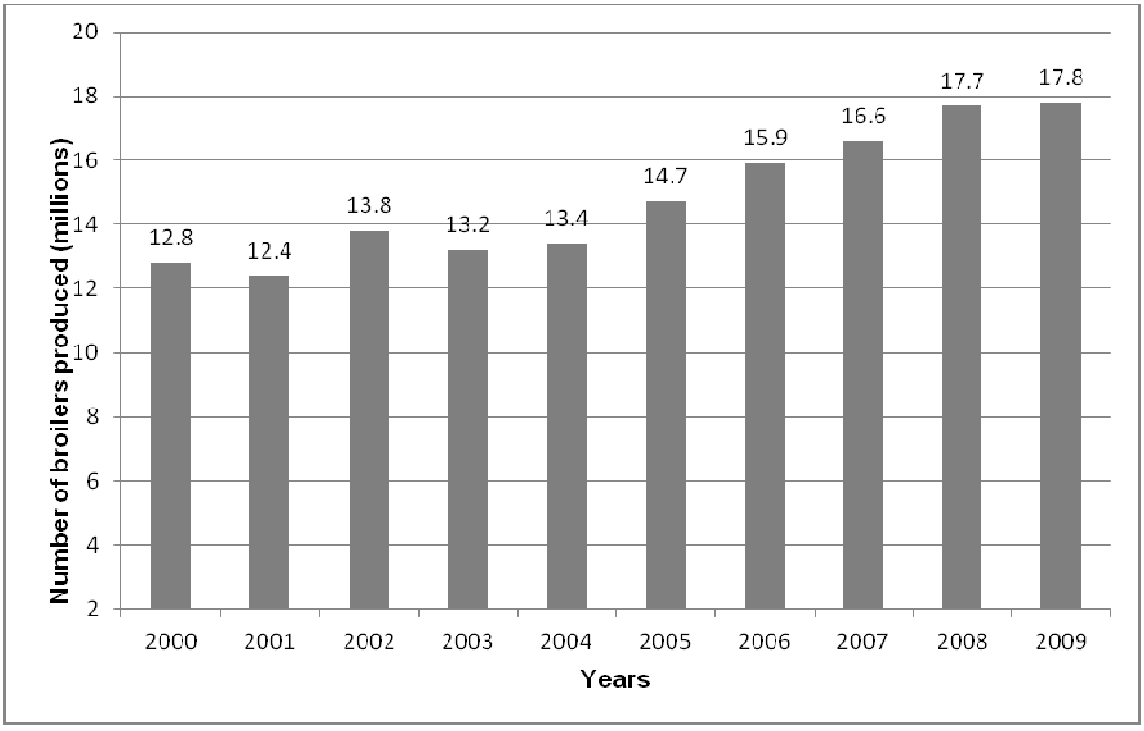

Figure 1 Average broiler production per week in South Africa (adapted from Esterhuizen, 2010; Vauqulin, 2010)

The total broiler production per week for 2009 was 17,8 million broilers and the production for the whole year of 2009 was 931,4 million broilers. A total of 1,569,978 tonnes of poultry meat was supplied by the industry in 2009, 19,068 tonnes were exported and 231,303 tonnes of poultry meat were imported. The largest amount of imported poultry meat originates from Brazil (Esterhuizen, 2010; Vauqulin, 2010). The provincial distribution of broiler producers is presented in Table 1.

Table 1 Distribution of broiler farms in South Africa showing number of broilers produced per year per province and percentage share in total broiler production (adapted from Vauqulin, 2010)

Province	Broilers	% of Broilers
Eastern Cape	6 686 270	6.6
Freestate	5 640 563	5.5
Gauteng	5 608 567	5.5
Kwazulu Natal	15 910 666	15.6
Limpopo	2 511 381	2.5
Mpumalanga	17 908 594	17.6
North West	25 556 927	25.1
Northen Cape	70 000	0.1
Western Cape	22 087 800	21.7
Total	101 980 768	100.0

2.1.1 Feeding of broilers

Broiler feed contributes 50 – 60 % of the total production cost of poultry meat (Henry & Rothwell, 1995; Parsippany, 2008). In 2009 the Animal Feed Manufacturer's Association (AFMA) reported that the poultry industry used 68 % of the feed produced by their members.

An average broiler feed price of R3,326 per ton for 2009 was obtained, this was 5% lower than the feed price of R3502 for the year 2008. The determining factor regarding the price of broiler feed in South Africa is the cost of raw materials, with maize and soya being the two major raw materials used in broiler feeds (Gous, 1998; Kleyn, 2005; Esterhuizen, 2010). Broiler producers therefore have to overcome these higher input costs for production to remain profitable, let alone to survive in the industry (Vauqulin, 2009a; Vauqulin, 2009b).

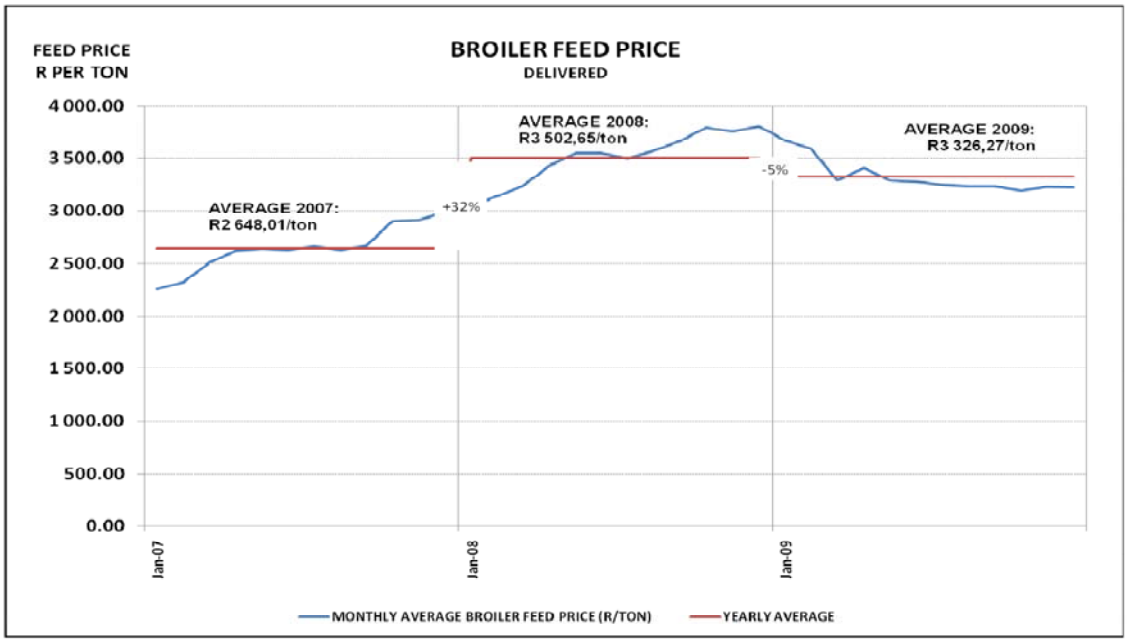

Figure 2 Average monthly broiler feed price per year showing changes from 2007 to 2009 (adapted from Esterhuizen, 2010)

The increase in the cost of raw materials has put pressure on feed manufacturing companies to increase the nutrient utilisation efficiency of their feed (Bedford, 2009). According to Bedford (2009) the demand for bio-fuels and the demand for poultry meat in third world countries will not only lead to the increase in the cost of grains, but will also lead to a decrease in the supply of grains. It is estimated that 36 % of the maize production in the United States (US) will be used for ethanol production in the year 2015 (Vauqulin, 2009b). Considering this, the feed manufacturing companies are turning their interests to feed additives like exogenous enzymes to improve the efficiency of nutrient utilisation of their feed (Bedford, 2009).

Soybean meal is the major protein source used internationally in broiler diets (Henry & Rothwell, 1995; Ziggers, 2009; McDonald *et al.*, 2002). The protein source used in the diet can contribute 30 – 35 % of the ration cost (Henry & Rothwell, 1995; Ziggers, 2009). The major grain used as an energy source is maize, wheat is used when maize is not available and barley is used to a lesser extent (Henry & Rothwell, 1995). The contribution of grain towards the cost of a broiler diet is typically around 60 %. Other ingredients used in a broiler diet contribute around 5 – 10 % of the diet cost (Henry & Rothwell, 1995).

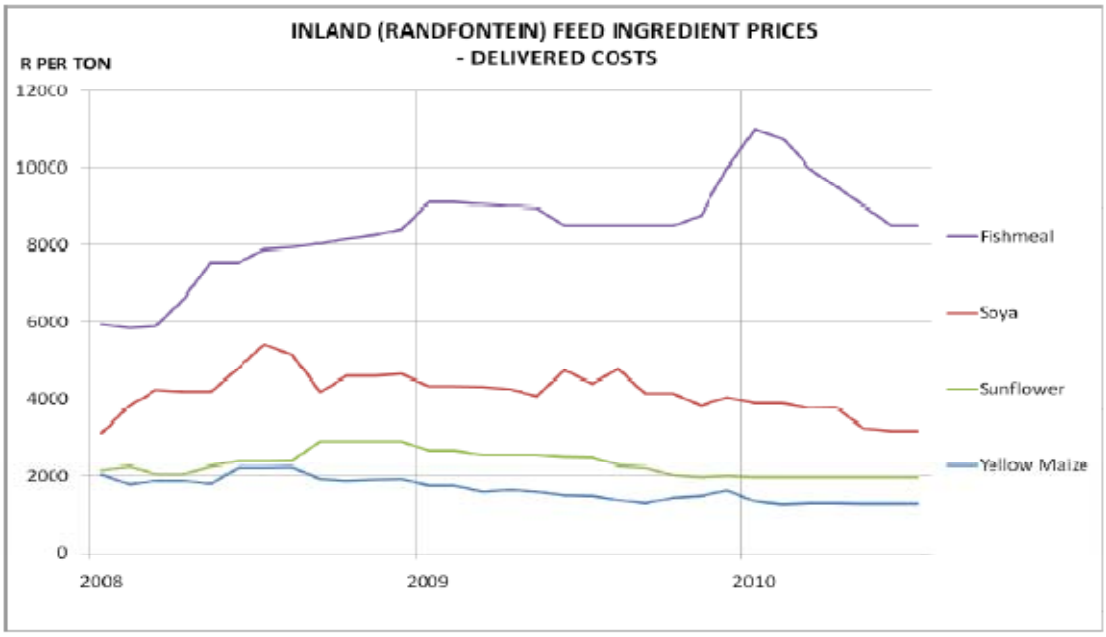

Figure 3 Monthly average real raw material cost per year showing changes from 2008 to 2010 (Vauqulin, 2010)

2.1.1.1 Raw materials used in broiler feed

Maize (*Zea mays)* is used in poultry feed because it is an excellent source of digestible energy. Only yellow maize was used in broiler feed up to 2007 and since then white maize has also been included (Vauqulin, 2009b). In 2008 the total consumption of white and yellow maize was 9 million tonnes, 25 % was consumed by the poultry industry (Department of Agriculture, 2009; Vauqulin, 2009b;). White maize is used in broiler diets and not in laying hen diets. The reason being that yellow maize contains the pigment cryptoxanthin, this pigment is responsible for the yellow colour of the egg yolk and for carcass fat colour (McDonald *et al.*, 2002). White maize does not contain the cryptoxanthin pigment (McDonald *et al.*, 2002; Cowieson, 2005).

Maize has a low concentration of soluble non-starch polysaccharides (NSP), it contains 1 g/kg water soluble NSP with arabinoxylan being the major NSP (Cowieson, 2005). Lectins, trypsin inhibitors and phytin, classified as anti-nutritional factors (ANFs), are also found in maize at low concentrations (Cowieson, 2005). Maize is generally considered to be well digested by broilers, but the variety in maize cultivars leads to a variation in the energy content of maize. Researchers suggest that the resistant starches found in maize are responsible for the limitations of the energy value of maize (Cowieson, 2005).

Soybean meal is obtained after the extraction of oil from soybeans, the remaining material is then roasted and ground (McDonald *et al.*, 2002). Soybean meal is primarily used in broiler diets as an energy and protein source (Zanella *et al.*, 1999).

However, the feeding of raw soybeans has negative effects on the performance of broilers due to the presence of anti-nutritional factors (Zanella *et al.*, 1999; Vauqulin, 2009b).

The anti-nutrional factors present in soybeans are protease inhibitors, non-starch polysaccharides, haemagglutins or lectins, saponins, allergenic-, goitrogenic- and rachitogenic factors (McDonald *et al.*, 2002; Ziggers, 2009. The Kunitz anti-trypsin factor and the Bowman-Birk chymotrypsin inhibitor are the two most important protease inhibitors regarding animal nutrition (McDonald *et al.*, 2002; Ziggers, 2009). According to Choct (1997), the total NSP content of soybeans is 19,2 % of the dry matter (DM). The ANF present in soybeans can be divided into two categories, namely heat labile and heat stable components. Trypsin inhibitors and lectins are the two most important heat labile ANFs which can reduce the digestibility and utilization of nutrients. Proteins exhibiting antigenic effects (soy antigens) and oligosaccharides are the most important heat stable ANFs. In the processing of soybean meal, only heat labile ANFs are inactivated. Soybean meal therefore still contains a high amount of heat stable ANFs, like oligosaccharides and soluble NSP (McDonald *et al.*, 2002; Ziggers, 2009).

Table 2 Chemical composition (g/kg) of common raw materials used in broiler feed expressed on a dry matter basis (adapted from Thomas *et al.*, 2000; McDonald *et al.*, 2002)

Cereal	DM g/kg	CF	EE	Ash	CP	NDF	ADF	ADIN	Starch & sugar	ME (MJ/kg)
Maize	860	24	42	13	98	117	28	1.3	717	14.2
Soybean meal	900	58	17	62	503	125	91	2.2	124	13.3
Barley	860	53	17	26	108	201	64	0.4	599	12.8
Wheat	860	26	19	21	124	124	30	0.4	701	10.1
Rye	860	26	19	21	124	357	-	-	-	13.4
Oats	860	105	49	33	109	310	149	0.4	482	12

CF- Crude fibre; EE- Ether extract; CP- Crude protein (N x 6.25); NDF- Neutral-detergent fibre; ADF- Acid-detergant fibre; ME- Metabolisable energy

Whole grain wheat (*Triticum aestivum*) can be utilised by broilers due to their ability to generate high grinding pressure and abrasive action within their gizzards (Rose, *et al.*, 1995; Rose, 1996; McDonald *et al.*, 2002). The nutritional value of wheat is however very variable, for instance the crude protein content can vary from 60 to 220 g/kg DM (McDonald *et al.*, 2002; Svihus & Gullord, 2002). The variability of the chemical composition of wheat is affected by both the variety of wheat and the environment (Rose, 1996). According to Choct *et al.* (1996) and Choct *et al.* (1999) the variability of the apparent metabolisable energy content of wheat is related to the anti-nutritional effects of the non-

starch polysaccharides (NSPs) present in the wheat. The total NSP content of wheat is 119 g/kg DM (Knudsen, 1997).

The mixture of proteins present in the endosperm of wheat is referred to as gluten and gluten has the property of elasticity. This property of elasticity is the main reason why wheat is unpalatable to broilers when fed as finely ground wheat, it is therefore recommended that wheat is fed as whole grains (McDonald *et al.*, 2002). The price and availability is the determining factors regarding the use of wheat in broiler diets.

The use of barley (*Hordeum sativum)* in broiler diets is limited by the high amount of NSPs present in barley and it's deficiency in the essential amino acid lysine (Jeroch & Dänicke, 1995; McDonald *et al.*, 2002). The total NSP content of hulled barley is 186 g/kg DM and 124 g/kg DM for dehulled barley (Jeroch & Dänicke, 1995a; Knudsen, 1997; McDonald *et al.*, 2002; Svihus & Gullord, 2002). The largest concentration of insoluble NSPs are found in the hulls of barley and thus it is recommended that dehulled barley be used instead for broiler diets. The successful growing of hulless barley and the use of NSP-degrading enzymes has made the use of barley in broiler diets more viable (Jeroch & Dänicke, 1995a; Svihus & Gullord, 2002).

The use of oats (*Avena sativa*l) and rye (*Secale cereal*) in broiler diets are limited due to high NSP content (McDonald *et al.*, 2002). The total NSP content of oats is 232 g/kg DM and of rye is 152 g/kg (Knudsen, 1997). According to McDonald *et al.* (2002) studies done on the use of rye in broilers has revealed that rye contains two detrimental factors. These factors are an appetite-depressing factor which is located in the bran of rye and a growth-depressing factor found in all parts of the rye grain.

Table 3 Mineral content of common raw materials used in broiler feed expressed on a dry matter basis (adapted from McDonald *et al.*, 2002)

Cereal	Ca g/kg	P g/kg	Mg g/kg	Na g/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg	Co mg/kg	Se mg/kg
Maize	0.3	2.7	1.1	0.2	2.5	6	16	0.02	0.02
Soybean meal	3.5	6.8	3.0	0.4	25.0	32	61	0.20	0.55
Barley	0.5	4.0	1.3	0.2	4.8	18	19	0.04	0.02
Wheat	0.5	3.5	1.2	0.1	5.0	42	50	0.05	0.02
Rye	0.7	3.7	1.4	0.3	8.0	66	36	-	-
Oats	0.8	3.7	1.3	0.2	3.6	42	41	0.04	0.03

Ca- Calcium; P- Phosphorous; Mg- Magnesium; Na- Sodium; Mn- Manganese; Zn- Zinc; Co- Cobalt; Se- Selenium

2.1.1.2 Non-nutritive feed additives commonly used in broiler feed

Feed additives are non-nutritive and are included in broiler feed with the aim to improve the digestibility of feed, thereby improving the growth efficiency and feed utilisation and preventing disease. Feed additives commonly used include antimicrobials, antioxidants, emulsifiers, binders and enzymes (Mathlouthi *et al*., 2003; Aviagen Inc., 2002; Pollmann *et al*., 1980; Acamovic, 2001; Choct, 2006; Huff *et al.,* 1998).

Antimicrobial growth promoters (AGPs) are antibiotics used at sub therapeutic levels in poultry feed to enhance growth. AGPs decrease infectious pathogenic microorganisms in broilers and thereby increases the health of the animal, and also reduces the incidences of disease (Aviagen Inc., 2002; Mathlouthi *et al*., 2003). Prebiotics are non-digestible food ingredients that stimulate the growth or activity of beneficial bacteria in the digestive system (Aviagen Inc., 2002; Partanen & Mroz, 2008). A probiotic is a live beneficial microbial feed additive given to monogastric animals to improve the intestinal balance of micro organisms in the animal. The increase in beneficial bacteria in the digestive system of monogastric animals leads to a decrease in non-beneficial bacteria that are competing with the animal for nutrients (Pettersson & Åman, 2007). This effect leads to an improvement in feed efficiency (Pettersson & Åman, 2007; Partanen & Mroz, 2008). Mycotoxin binders are substances which bind, adsorb, inactivate or change mycotoxins inside the digestive system of broilers. Mycotoxin binders are only used when there is a risk of mycotoxin contamination of the feed or raw materials (Huwig *et al.*, 2001). Organic acids are organic compounds with acidic properties, used in the animal feed industry as feed preservatives to reduce bacterial contamination of feed and to improve the development of beneficial microflora in the digestive tract of the broiler (Aviagen Inc., 2002)

The supplementation of exogenous enzymes in poultry diets are mainly for the improvement of production efficiency and nutrient utilisation (Pollmann *et al.*, 1980; Huff *et al.,* 1998; Acamovic, 2001; Aviagen Inc., 2002; Choct, 2006). Enzyme supplementation of monogastric diets have been increasingly investigated and have shown to exert beneficial effects (Acamovic, 2001). The beneficial effects of exogenous enzyme supplementation depends on a number of factors. These factors include the dietary components, processing of the diet and the type of enzymes used (Acamovic, 2001). The major types of enzymes used for the supplementation of monogastric diets can be seen in Table 4. Phosphorus is stored as phytate in plants. Phytate binds certain minerals, amino acids and energy, reducing their availability for digestion or absorption. The use of phytase in broiler feed increases the digestibility of phytate (Aviagen Inc., 2002; Choct, 2006).

Table 4 Enzymes used as dietary additives with their substrates (adapted from Acamovic, 2001)

Carbohyrases	Substrates
Amylases	Starch
Pectinases	Pectins
B-Glucanases	B-glucans
Arabinoxylanases	Arabinoxylans
Cellulases	Cellulose, hemicelluloses
Hemicellulases	Hemicellulose
Acid proteases	Proteins
Alkaline proteases	Proteins
Phytases	Phytic acid esters
Esterases	Fats, esters
Lipases	Fats, esters

2.2 Non-starch polysaccharides (NSPs)

Carbohydrate is the major component of a poultry diet, it can contribute 60 – 70% of the diet (Bedford, 1993; Williams *et al.,* 1997). Carbohydrates found in raw materials used for broiler diets are grouped into three groups. The first group is monosaccharides such as glucose, fructose, galactose, xylose and ribose. The second, carbohydrates are oligosaccharides. Oligosaccharides are saccharide polymers consisting of three or more monosaccharides. Polysaccharides are the third group of carbohydrates found in raw materials. Polysaccharides are macromolecular polymers of monosaccharides linked by glycosidic bonds (Williams *et al.,* 1997). Choct (1997) defined non-starch polysaccharides (NSP) as polymeric carbohydrates with different compositions and structures than amylase and amyl pectin.

Starch is the greatest nutrient of importance in cereal grains and it accounts for 60 – 70% of a grain's weight (Bedford, 1993). Starch is a polysaccharide made up of glucose units linked by α-(1-4) bond with a few α-(1-6) bonds (Williams *et al.,* 1997). The term NSP covers a large variety of polysaccharide molecules. The type and amount of NSPs present in raw materials vary considerably. Not only do they vary between different raw materials, but also within the same raw material due to different geographical production locations and varieties.

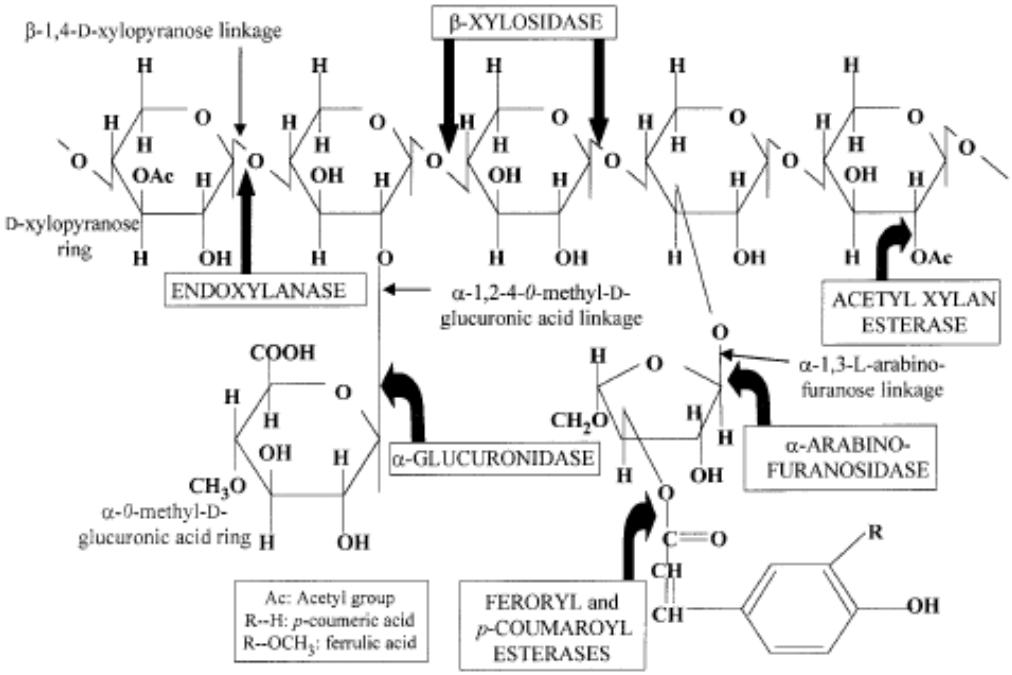

Figure 4 A hypothetical plant xylan structure showing different substituent groups with sites of attack by microbial xylanase (Beg *et al.,* 2001)

Non-starch polysaccharides include cellulose, hemicelluloses, oligosaccharides and pectins (Williams *et al.,* 1997; Parsippany, 2008). Non-starch polysaccharides (NSPs) can be divided into two groups, soluble and insoluble NSPs (Castanon *et al*, 1997; Williams *et al.,* 1997). Starch can function as a major nutritional component of the diet, but a number of non-starch polysaccharides can have negative effects on poultry. Poultry cannot digest the insoluble NSPs, only soluble NSPs have the potential to be digested by poultry (Williams *et al.,* 1997; Parsippany, 2008). According to Carre *et al.* (1995) the degradation of soluble NSP can be as high as 80-90 %, while the insoluble NSPs remain un-digested. A detailed classification of NSPs is shown in Figure 5.

Even if soluble NSPs are digestible by poultry, they still have anti-nutritional properties. They encapsulate other nutrients in the gut and thereby decrease nutrient digestibility. The decrease in nutrient digestibility results in a decrease of the apparent metabolisable energy (AME) of the diet and thereby leading to poorer feed conversion ratio (FCR) (Bedford, 1993; Choct *et al.,* 1995; Castanon *et al*, 1997; Marquardt & Han, 1997; Williams *et al.,* 1997; Bedford & Schulze, 1998; McNab & Boorman, 2002; Hetland *et al.*, 2004).

Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed-linked β-glucans and arabinoxylans (Parsippany, 2008)(Table 5). Depolymerisation of these NSPs requires specific enzymes, these enzymes are specific to the main and side chain structure of the NSP (Bedford, 1993; Henry & Rothwell, 1995; Castanon *et al*, 1997; Bedford & Schulze, 1998; Andersson *et al.,* 2003; Bhat, 2000; Dalibard & Geraert, 2004).

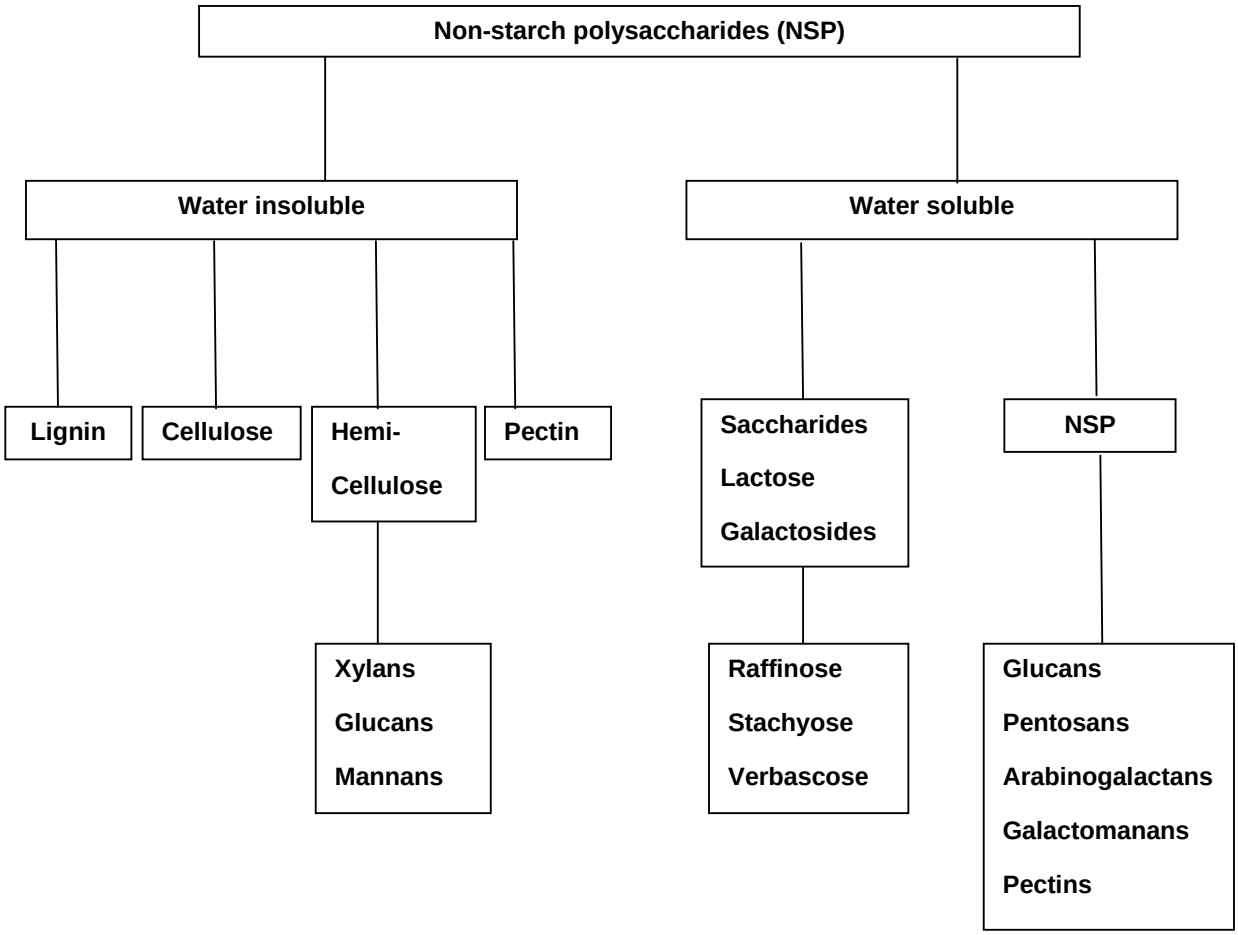

Figure 5 Classification of non-starch polysaccharides (Liang, 2000)

Table 5 Main non-starch polysaccharides commonly found in raw materials used in poultry feed (Dalibard & Geraert, 2004)

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

2.2.1 Diversity in the source and characteristics of non-starch polysaccharides

Xylans and β-glucans exist in nature as the principal components of the cell wall material of plants (Bedford & Schulze, 1998). Organisms derive energy from these substrates through the metabolism of the sugars xylose, glucose and arabinose (Bedford & Schulze, 1998).

Xylans or hemicelluloses in plants are situated between the lignin and cellulose fibres (Beg *et al.,* 2001). Xylans are bound to the cellulose fibrils via hydrogen bonds (Selinger *et al.*, 1996). Xylan polymers can also be cross linked with other hemicellulose backbones or to lignin through the residues of 4-*O*-methyl-α-D-glucuronic acid or ferulic acid (Selinger *et al.*, 1996). The structure of most xylans are similar, namely a β-1,2 linked backbone of D-xylose (five-carbon sugar) residues (Bedford & Schulze, 1998; Beg *et al.,* 2001). β-xylopyranose residues makes up the main chain of xylan (Beg *et al.*, 2001). The variety of xylans are due to the source of xylan which determines the size of the backbone, type and degree of substitutions from the backbone (Bedford & Schulze, 1998).

Xylan varies significantly from source to source due to the substitution pattern (Bedford & Schulze, 1998). The most common substitutes are α-4-*0*-methylglucuronic acid, arabinose, acetic acid, and various phenolics that are linked through substituent sugars. Xylans that originate from cell walls that are more lignified are far less branched than other xylans. The reason for the xylan being less branched is the substitution of arabinose, which is the major side chain, with glucuronide or with the methyl derivative of glucuronide (Chesson, 2001).

The β-glucan structure in plants also varies (Bedford & Schulze, 1998). The principal backbone, β-1,4 glucose backbone, varies in length and in the degree and pattern of β-1,3 linkage substitution (Bedford & Schulze, 1998). A mixed-linkage of 1-3 or 1-4 β-D-glucan dominates in the endosperm cell walls of grains, with β-glucan making up 3 – 5% of the kernel weight and consisting of 30% 1-3 linkages and 70% 1-4 linkages (Selinger *et al.*, 1996). A simple definition of β-glucan is that it is a homopolymer of D-glucose linked in a β-configuration consisting of a variety of linkages in a linear or branched chain form (Marquardt & Han, 1997). Arabinoxylans are branched polysaccharides which are extensively substituted with side chains containing one or more arabinose residues (Marquardt & Han, 1997; Chesson, 2001).

The physical properties such as solubility, viscosity and water binding capacity of xylans and β-glucans get altered by the differences in substitution patterns (Bedford & Schulze, 1998). Smith and Annison (1996) stated that the solubility of a NSP is affected by the size of the molecule, whether the polysaccharide is linear or branched, presence of charged groups and the surrounding structures. The susceptibility to be attacked by enzymes also gets altered (Bedford & Schulze, 1998). According to Izydorczyk and Biliaderis (1995) polysaccharides with a low degree of branching and high ferulic acid content can lead to the highest increase in the viscosity of a solution.

Table 6 Classification of common non-starch polysaccharides (adapted from Knudsen, 1997; Stephen, 1995)

Non-starch polysaccharide	Constituent monomers	Solubility, water holding capacity	Common sources
Cellulose	Glucose	-	Most cereals, legumes and forages, plant cell wall
Hemicellulose	Glucose, rhamnose, xylose, galactose, fucose, arabinose	+/-	Cereal, legume hulls
β-glucans	Glucose	+	Barley, oats, rye
Pectins	Uronic acids	+	Fruits, chicory and sugar beet pulp
Fructans and inulins	Fructose, glucose	+	Yam, rye, Jerusalem artichoke, chicory

+ Soluble, - Insoluble

Due to the diversity in the structure of the plant cell wall and the complex chemical nature of hemicelluloses, it requires a complex of several hydrolytic enzymes (Bedford & Schulze, 1998); (Bedford, 1993; Beg *et al.*, 2001). The xylanolytic enzyme system necessary for the hydrolysis of xylan comprises of β-1,4-endoxylanase, β-D-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase and phenolic acid esterase (Bedford, 1993; Bedford & Schulze, 1998; Beg *et al.*, 2001). β-1,4-endoxylanase cleaves the internal linkages of the xylan backbone, while β-D-xylosidase hydrolyses the short xylo-oligosaccharides from the non-reducing end to release xylose (Bedford & Schulze, 1998).

2.2.2 Anti-nutritive effects of non-starch polysaccharides

Animals do not synthesize the enzymes necessary for the digestion of cellulose, β-glucans, arabinoxylans or pectins (Bedford & Schulze, 1998).

The anti-nutritive effects of NSPs in broiler diets are determined by the solubility of the NSP. Soluble NSPs has the ability to interact with other dietary components, insoluble NSPs are inert and do not posses this ability. According to Williams *et al.* (1997) most of the anti-nutritive effects of NSPs are directly attributed to soluble NSPs. A viscous solution is obtained when soluble NSPs are dissolved in water (Bedford, 1993; Classen, 1996; Williams *et al.,* 1997; Bedford & Schulze, 1998; Chesson, 2001; Hetland *et al.*, 2004).

After the ingestion of NSPs, the NSPs are solubilised and this leads to an increase in the viscosity of the digesta (Classen, 1996). This increase in the intestinal viscosity leads to a decreased rate of

digestion, which in turn leads to a reduction in performance (Bedford, 1993; Classen, 1996; Williams *et al.,* 1997; Chesson, 2001). Figure 6 shows the effect of an increase in gut viscosity on feed conversion efficiency (FCE) and weight gain of broilers, Figure 7 shows the relationship between the digestibility of energy and the concentration of dietary NSPs.

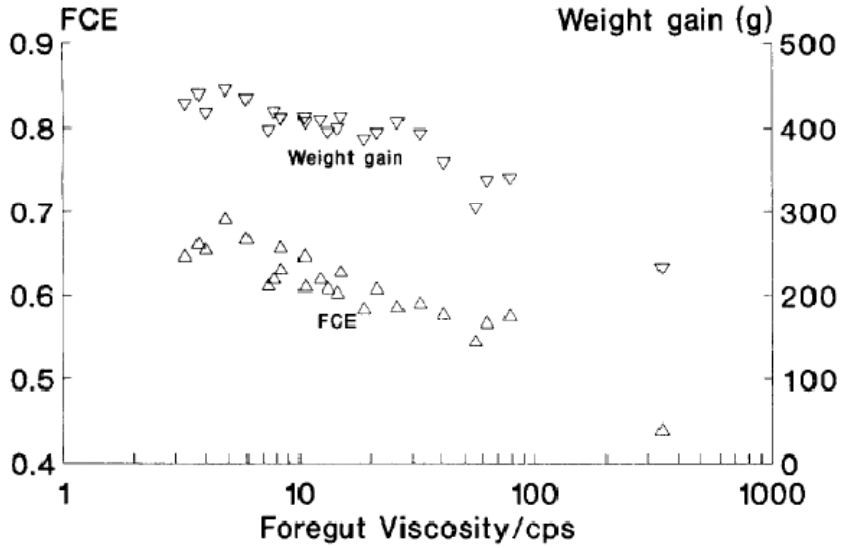

Figure 6 Effect of increased gut viscosity due to non-starch polysaccharides on weight gain and FCE in broilers (Bedford, 1993)

The digestion and absorption rate of a feed depends on a complex formation between the substrate and its digestive enzyme. It also depends on the release of the substrate-enzyme product (Bedford, 1993). The end product of digestion must then be passed on to the enterocytes through the intestinal lumen for absorption (Bedford, 1993; Bedford, 2000).

Thus, for a rapid digestion it is essential for enzymes, their substrates and end products to move freely through the gut by means of diffusion. However, diffusion decreases as the viscosity of the gut increases (Bedford, 1993; Bedford, 2000; Choct & Annison, 2007). An increase in the production of digestive enzymes can compensate for the reduction in the diffusion of enzymes. Researchers have found that diets containing a high concentration of NSPs leads to the enlargement of the bird's pancreas. This indicates an increased secretion of endogenous enzymes by the pancreas (Hetland *et al.*, 2004). This compensation method is however limited in young animals (Nitsan *et al.,* 1991; Bedford, 1993).

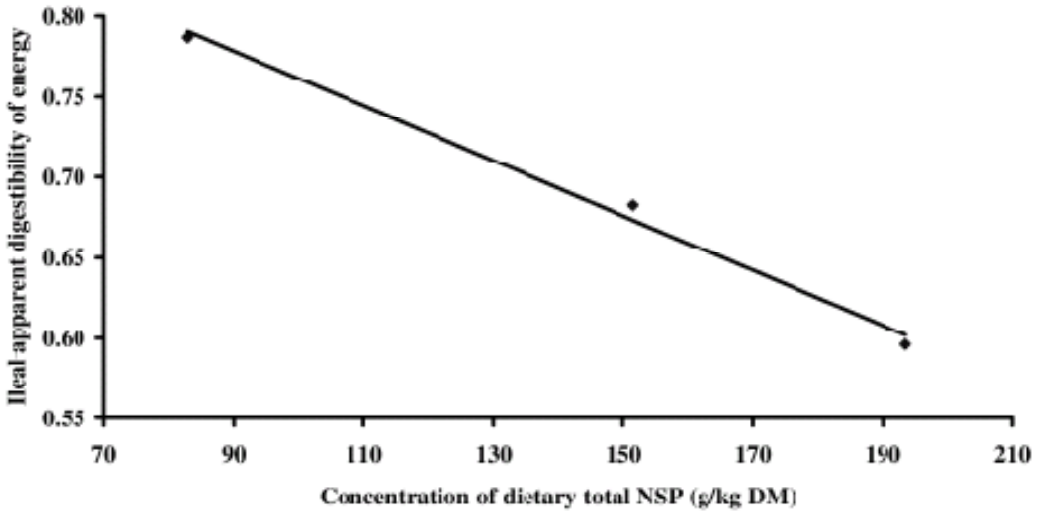

Figure 7 Relationship between ileal apparent digestibility of energy and dietary total non-starch polysaccharides (Yin *et al.,* 2000)

The reduction in digestion rate is not the only problem resulting from an increased gut viscosity. The increase in viscosity also leads to a reduced rate of feed passage in the gut. This further leads to a reduced throughput and feed intake, which then limits the assimilation rate of nutrients. An increase in digesta viscosity due to the intake of NSPs must be considered only as a useful indicator of the anti-nutritive effects of NSPs and not the mechanism of action by which NSPs exert their anti-nutritive effects (Williams *et al.,* 1997). Because of this slower passage rate, there is a proliferation of bacteria in the gut. These bacteria then migrate to the upper region of the small intestine (Bedford, 1993; Bedford, 2000). The competition between the host and bacteria for nutrients thus increases, especially nutrients like starch and proteins. Bile acid degrading enzymes are produced by some of the intestinal bacteria and this reduces the capabilities of the host for lipid digestion (Bedford, 1993; Bedford, 2000). Not only does this effect lipid digestion, but also protein digestion. Bile acids stabilize pancreatic proteases in the lumen of the intestine, but due to the lack of bile acids, protein digestion is compromised (Bedford, 1993; Bedford, 2000).

Not only do NSPs increase the viscosity of the digesta, but it also acts as a physical barrier to endogenous enzymes. Not all the cell walls of the grain's endosperm is broken open during feed processing like grinding and pelleting (McNab & Boorman, 2002). Digestibility of feed is reduced by the NSPs encapsulating the material inside the endosperm of the grain and thus reducing the utilisation of starch and proteins (Bedford, 1993; Classen, 1996).

The increase in digesta viscosity due to the NSPs content of a diet also leads to the increase in the size and stability of the unstirred layer at the mucosal surface of the broiler's digestive tract. The swelling of the digestive tract leads to an impaired digestibility due to reduced contact between

digestive enzymes and their substrates and a slower uptake in the foregut of released sugars, amino acids and lipids (Chesson, 2001).

The performance parameters of broilers which are affected significantly by NSPs are growth rate and FCR (Bedford, 1993; Bhat, 2000). Intestinal disturbances due to the inclusion of NSP in monogastric diets can be identified by sticky droppings (Selinger *et al.*, 1996; Bedford & Schulze, 1998; Bhat, 2000; Hetland *et al.*, 2004; Nnenna *et al.*, 2006). Sticky droppings can easily be distinguished from normal droppings based on colour variation. Normal droppings are brown with a white cap of uric acid, while sticky droppings are dark brown (McIlroy *et al.,* 1993; Thomas *et al.,* 2004; Miles & Johnson, 2009). NSPs binds to the water in the intestinal tract of the bird due to the water-holding capacity of the soluble fibres, this leads to an increase in the thickness of the intestinal contents and result in gelatinous droppings (sticky droppings) (Selinger *et al.*, 1996; Bedford & Schulze, 1998; Bhat, 2000; Hetland *et al.*, 2004; Nnenna *et al.*, 2006). The moisture content of litter is increased by the sticky droppings and this leads to wet litter. Studies done on the effects of wet litter on broiler performance have shown that the presence of wet litter in broiler production units can lead to an ulcerative condition of the skin which affects the plantar surface of the feet, the hock and the breast. Pain is experienced by the bird due to the tissue trauma. These conditions are commonly called foot pad dermatitis, hock burn and breast burn, they can occur separately or together in a single bird (Tucker & Walker, 1992; Haslam *et al.,* 2007). Tucker and Walker (1992) reported that these conditions are caused by the combination of the moisture from litter and the chemical burning effect of ammonia from the urea present in the litter. The studies of McIlroy *et al*. (1993) revealed that the ulcerative condition caused by wet litter can result in poor feed conversion ratios which in turn lead to a decrease in average daily gain. Thomas *et al*. (2004) concluded in their study that occurrence of breast burns has a negative effect on the carcass quality and can lead to carcass condemnation.

2.2.3 Mechanism of non-starch polysaccharide-degrading enzyme action

Over the past 10 years researchers have debated over the mechanism by which the exogenous enzymes xylanase and β-glucanase exert their effect in poultry (Bedford, 1993; Bedford & Schulze, 1998). There are two possible ways by which these enzymes exert their effect (Bedford, 1993; Bedford & Schulze, 1998). One of these methods is based on the encapsulation of the cereal endosperm by the cell wall (Bedford, 1993; Bedford & Schulze, 1998). This encapsulation leads to the non-availability of starch and protein inside the endosperm (Bedford & Schulze, 1998). The cell wall can be removed with the supplementation of xylanase and β-glucanase, thereby facilitating the digestion of the starch and protein trapped inside the endosperm (Bedford, 1993, Bedford & Schulze, 1998; Bedford, 2000). This theory is however difficult to support due to digestibility data and enzyme kinetics (Bedford & Schulze, 1998).

Chesson and Travis (1997) suggested that the pore size in the cell walls is too small for the penetration of bacteria, xylanase or amylase. A demonstration where cellulolytic bacteria was used to degrade the cell wall revealed that there was no increase in cell pore size, only a reduced soluble biomass was observed (Chesson & Travis, 1997; Bedford & Schulze, 1998). It is hence unlikely for cell wall degradation to occur (Chesson & Travis, 1997; Bedford & Schulze, 1998). However, microscopic analysis done by Bedford and Autio, (1996) on the broiler's intestinal contents has revealed that there is damage to the structure of the endosperm cell wall. They found that this was most obvious at the level of the gizzard (Bedford & Autio, 1996). Choct *et al.* (1996) stated that this is not due to the direct effect of the enzyme, but due to its indirect effect in producing soluble, fermentable oligomers. These oligomers then enter the ceca, which in turn enhances fermentation (Choct *et al.*, 1996).

The enhancement in fermentation elevates the enteroglucagon concentrations which reduces the gastrin concentrations markedly (Bedford & Schulze, 1998). This leads to the reduction of gastric emptying and an increase in motility. Thus, xylanase indirectly enhances the degradation of the cell wall by stimulating gizzard or stomach function (Bedford & Schulze, 1998).

The alternative way by which these enzymes exert their effect is based on the fact that some components of the cell wall dissolve in the digestive tract to form viscous aggregates (Choct *et al.*, 1995;Bedford & Schulze, 1998; Bedford, 2000). To reduce the viscosity, a well targeted hydrolytic event needs to take place (Bedford, 1993; Bedford & Schulze, 1998). The reason for this is that the molecular size and concentration of the viscous polymer is exponentially related to the subsequent solution viscosity (Castanon *et al*, 1997; Bedford & Schulze, 1998).

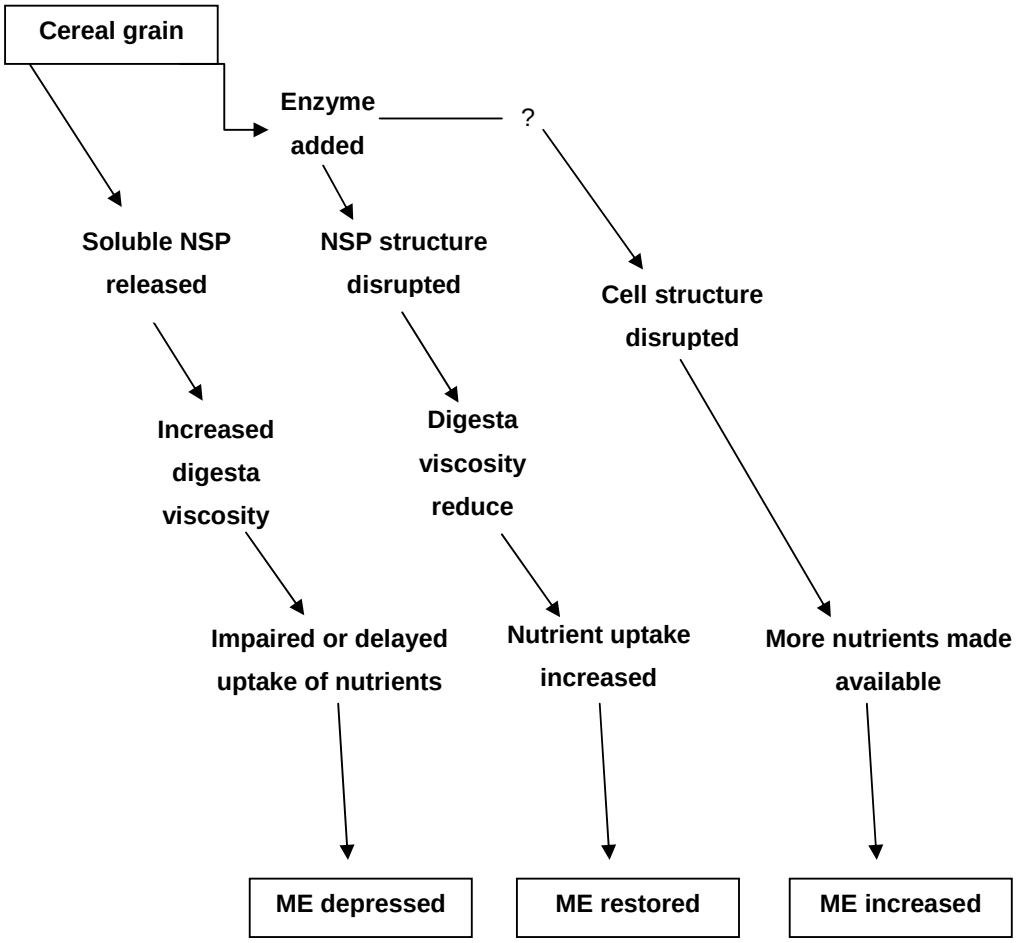

Figure 8 Summary of the possible effects of the addition of polysaccharidase-rich enzyme preparations to poultry diets containing NSPs (Chesson, 2001)

Another demand for the reduction in viscosity is that the events of the enzymes need to be aimed at the centre of the polymer, namely the endo-acting enzymes. The efficacy of the enzymes achieving this objective will differ due to the different sources of enzymes (Bedford, 1993; Bedford & Schulze, 1998). The polysaccharide only needs to be cleaved at a few places to reduce the viscosity of the digesta and thereby increasing the nutritive value of the feed (Williams *et al.*, 1997). Table 7 shows the non-starch polysaccharide-degrading enzymes commonly used in with their corresponding substrates.

Table 7 Non-starch polysaccharide-degrading enzymes and their corresponding substrates commonly used in the poultry industry (adapted from Remus, 2008)

Antinutrient	Where found	Problem	Content	Enzyme
Arabinoxylans	Cell wall of plant based ingredients	Relatively resistant to digestion and may reduce nutrient digestibility; soluble causes viscosity	Moderate to low – depending on ingredient	Xylanase Arabinofuranosidase
β-glucans	Cell wall of cereals	Soluble form causes extremely high viscosity	Moderate to low and not found in maize	β-glucanase
Mannans	Soya bean meal, yeast cell walls	Resistant to digestion	Variable	β-mannanase
Oligosaccharides	Soya bean meal, etc.	Resistant to digestion	Variable	α-galactosidase
Cellulose	Plant ingredients	Insoluble and resistant to digestion	High	Cellulase Cellobiohydrolase
Starch	Cereals, cereal by-products primarily	Structural resistance, retrogradation or protein binding	High	Amylase Debranching activities (protease)

2.3 Non-starch polysaccharides in maize

The cell wall composition of maize is similar to that of wheat, but the cellular endosperm is surrounded by a thinner endosperm cell wall (Chesson, 2001). The endosperm of maize is dominated by arabinoxylans and small amounts of cellulose. Mixed linked glucans are also present (Saulnier *et al*., 1995b; Cowieson, 2005). The pericarp and seed coat, the cell walls forming the outer layers of the maize kernel, are not so extensively lignified as the other cereals. However, they do contain similar concentration levels of cellulose and xylans as other cereals (Saulnier *et al*., 1995b). The amount of

NSPs leaching from the endosperm and other cell walls due to the relatively low cell wall content of maize is insufficient to have an effect on intestinal viscosity (Chesson, 2001).

According to Chesson (2001) there is no theoretical basis for the use of NSP degrading enzymes in maize-based diets. Gill (2001) however stated that the variation in maize is much greater than usually taken into consideration when formulating diets. This statement, with the fact that the structure of maize is rich in glucuronoarabinoxylans (compared to other cereals), may mean that the use of exogenous NSP degrading enzymes may be necessary in maize-based broiler diets (Huisman, Schols & Voragen, 2000; Gill, 2001). Another reason why maize NSPs are not considered to increase the viscosity of digesta is the fact that the ratio of insoluble non-cellulosic polysaccharide (I-NCP) to soluble non-cellulosic polysaccharide (S-NCP) of maize is much larger than that of any other cereals (oats, rye, wheat and barley) (Knudsen, 1997; Fourie, 2007).

β-glucans are considered the problematic NSP in maize, however it only contributes to 1 % of the total NSP content of maize and 0,1 % of the DM content of maize (Knudsen, 1997). According to Knudsen (1997) the NSPs of concern in maize is the I-NCP and cellulose. The I-NCP content of maize is 66 g/kg of the DM content of maize which is 68 % of the total NSP content of maize. Cellulose contributes 23 % to the total NSP content of maize and 2.2 % of the DM content of maize (Saulnier *et al.,* 1995 a; Knudsen, 1997). The total NSP content of maize according to Knudsen (1997) is 97 g/kg, which is considerably lower than that of other cereals.

2.4 Non-starch polysaccharides in rye, wheat, barley and oats

The major NSP found in rye and wheat is arabinoxylans and it is the major contributor to the soluble NSP fraction of wheat and barley (Beg *et al.*, 2001; Chesson, 2001). The endosperm and the bran of wheat is the major source of soluble arabinoxylans. Arabinoxylan from the bran contributes 33.3 % to the total soluble arabinoxylan content of wheat and bran contributes 64.2 % (Chesson, 2001). The NSP content of wheat according to Knudsen (1997) is 119 g/kg, 25 g/kg of the total NSPs are soluble. The largest contributors to the S-NCP fraction of wheat are xylose, arabinose and glucose at 9 g/kg, 7 g/kg and 4 g/kg respectively (Knudsen, 1997; Fourie, 2007). According to Knudsen (1997) the NSP of concern in wheat is the I-NCP and cellulose. The I-NCP content of wheat is 74 g/kg of the DM content of wheat, 62 % of the total NSP content of wheat. Cellulose contributes 17 % to the total NSP content of wheat and 2 % of the DM content of wheat (Saulnier *et al.,* 1995a; Knudsen, 1997). Rye contains 152 g/kg NSPs, 42 g/kg of the NSP content is S-NCP and 94 g/kg is I-NCP (Knudsen, 1997). The soluble NSP fraction of rye is 34 g/kg of the total dry matter of rye and the insoluble NSP fraction is 55 g/kg (Choct, 1997). The carbohydrate and lignin content of maize, wheat, barley, oats and rye is given in Table 8.

Two types of water soluble non-starch polysaccharides are present in wheat and rye, linear arabinoxylans and highly branched arabinogalactans. The linear arabinoxylans contain D-xylose units that are linked 13-(1-4) glycosidically, this linkage forms a long xylan backbone. The high viscosity of

digesta obtained by feeding wheat and rye to broilers is due to the linear arabinoxylans present. It is estimated that wheat pentosans can bind to 23 % of the water present in the digestive tract of the bird (Girhammar & Nair, 1992).

Table 8 Carbohydrate and lignin content (g/kg dry matter) in maize, wheat, barley, rye and oats (adapted from Knudsen, 1997)

	Maize	Wheat	Rye	Barley	Oats
Number of samples	3	5	7	10	3
LMW-sugars [a]					
Monosaccharides	4	3	6	4	2
Sucrose	13	11	19	12	11
Raffinose	2	4	4	5	3
Stachyose	1	2	3	1	2
Total sugars	20	19	32	21	17
Starch	690	651	613	587	486
Fructan	6	15	31	4	3
NSPs [b]					
β-glucan	1	8	16	42	28
S-NCP [c]	9	25	42	56	40
Rhamnose	0	0	0	0	0
Arabinose	3	7	12	6	3
Xylose	2	9	20	6	2
Mannose	2	2	2	2	2
Galactose	1	2	1	1	2
Glucose	1	4	6	39	28
Uronic acids	1	1	1	2	3
I-NCP [d]	66	74	94	88	110
Rhamnose	0	0	0	0	0
Arabinose	19	22	24	22	15
Xylose	28	38	41	50	78
Mannose	1	1	3	2	1
Galactose	4	2	4	2	5
Glucose	9	7	20	8	5
Uronic acids	6	4	3	4	7
Cellulose	22	20	16	43	82
Total NSPs	97	119	152	186	232
Klason lignin	11	19	21	35	66
Dietary fibre	108	138	174	221	298
CHO[e] and lignin:					
Analysed	823	823	850	834	787
Calculated	830	814	849	823	770

[a] Low molecular weight sugars; [b]Non-starch polysaccharides; [c]Soluble non-cellulosic polysaccharides; [d] Insoluble non-cellulosic polysaccharides; [e] Carbohydrates

β-glucans are the major NSP found in barley and oats (Jeroch & Dänicke, 1995; Choct, 1997; Beg *et al.*, 2001). The soluble NSP fraction of barley mainly consist of mixed-linked (1-3)(1-4)-β-glucans and it is responsible for the increase in gut viscosity when barley is fed to broilers (Jeroch & Dänicke, 1995; Choct, 1997; Bennett, Classen & Riddell, 2002; Svihus *et al*., 2005). According to Jeroch and Dänicke (1995) the β-glucan content of barley can vary from 24 to 80 g/kg of the total DM of barley.

They ascribe this variation in the β-glucan content of barley to the differences in genetic factors (type of barley), climatic factors (area of production), stage of the plant maturity, use of nitrogen fertilisers for barley production and the amount of time which barley is stored after harvesting. According Svihus and Gullord (2002) the majority of the insoluble fibre fraction is found in the hull of barley, therefore it is better to feed hulless barley to poultry. The study of Knudsen (1997) revealed that the total NSP content of barley is 186 g/kg of the total dry matter of barley, 30 % of this is S-NCP and 47 % is I-NCP. The total NSP content in oats is 232 g/kg of the total dry matter of oats and consist of mixed-linked (1-3)(1-4)-β-glucans, arabinoxylans and cellulose (Knudsen, 1997). Oats have the highest NSP, cellulose and lignin content when compared to all the other cereals used in broiler diets (Knudsen, 1997). According to Svihus and Gullord (2002) more than 67 % of the NSPs present in oats occur in the hulls of oats.

2.5 Non-starch polysaccharides in soya

The NSPs content of soybean meal (SBM) according to Knudsen (1997) is 217 g/kg while 30 g/kg of the total NSPs are soluble. The largest contributors to the S-NCP (29 % of the total NSP content of SBM) fraction of SBM are uronic acid, galactose and arabinose at 25 g/kg, 16 g/kg and 9 g/kg respectively (Knudsen, 1997; Fourie, 2007). The I-NCP fraction of SBM is 42 % of the total NSP content and the fraction of cellulose is 29 % of the total NSP content. The bulk of the total NSPs of SBM is I-NCP and cellulose, the same as with maize (Knudsen, 1997). The largest contributors to the I-NCP fraction of SBM are xylans (xylose) and xyloglucans. The contribution of xylose to the I-NCP fraction is 17g/kg (Knudsen, 1997).

The cell wall of soybean contains a large amount of pectic polysaccharides like galacturonans and associated arabinogalactans. These pectic polysaccharides make up half of the total NSP content of soybeans (Chesson, 2001). According to Huisman *et al.* (1998) the pectic polysaccharide fraction in soybeans are structurally unique. A study done by Huisman *et al.* (1998) revealed that only 90 % of the uronic acids can be recovered in the water-unextractable solid fraction of SBM. When this fraction was compared to the fractions isolated from other plant cells, it indicated that the pectic substances in SBM are more complex than those of other plant cells (Huisman *et al.*, 1998). Another study done by Huisman *et al.* (2001) revealed that the pectic backbone of soybeans is structurally free of homogalacturon. Rhamnogalacturon and xylogalacturon are mostly found in regions of the pectic backbone (Huisman *et al.,* 2001). The carbohydrate and lignin content of soybean is given in Table 9.

Chesson (2001) suggested that the lack of homogalacturon in the pectic backbone of soybeans may be the reason why the use of polygalacturonase in enzyme preparations for SBM-based diets is unsuccessful. Chesson (2001) based his suggestion on the findings of Huisman *et al.* (1998) and Huisman *et al.* (2001) and the fact that enzymes are very specific to the substrate and the side chain structure of the substrate (Bedford, 1993; Castanon *et al*, 1997; Chesson, 2001).

Table 9 Carbohydrate and lignin content (g/kg dry matter) of soybean meal (adapted from Knudsen, 1997)

	Soybean meal	
	Mean	**SD [f]**
Number of samples	6	
LMW-sugars [a]		
Monosaccharides	7	1
Sucrose	70	11
Raffinose	10	1
Stachyose	47	4
Verbascose	3	2
Total sugars	137	16
Starch	27	12
NSPs [b]		
S-NCP [c]	63	10
Rhamnose	1	1
Arabinose	9	2
Xylose	2	1
Mannose	5	1
Galactose	16	3
Glucose	6	3
Uronic acids	25	4
I-NCP [d]	92	9
Rhamnose	2	0
Arabinose	17	2
Xylose	17	3
Mannose	8	2
Galactose	25	3
Glucose	1	2
Uronic acids	23	3
Cellulose	62	18
Total NSPs	217	27
Klason lignin	16	4
Dietary fibre	233	26
CHO[e] and lignin:		
Analysed	400	15
Calculated	416	17

[a] Low molecular weight sugars; [b] Non-starch polysaccharides; [c] Soluble non-cellulosic polysaccharides; [d] Insoluble non-cellulosic polysaccharides; [e] Carbohydrates; [f] Standard deviation

2.6 Non-starch polysaccharide-degrading enzymes in broiler diets

The first development and use of NSP-degrading enzymes was in barley and then in wheat based broiler diets. Researchers found that the use of NSP-degrading enzymes in barley and wheat based broiler diets improved litter quality and performance. The use of NSP-degrading enzymes in wheat and barley based diets for broilers are therefore well established and accepted (McNab & Boorman, 2002; Bedford, 2009).

Pettersson and Aman (2007) tested the addition of an enzyme cocktail, consisting of xylanase and β-glucanase to an unpelleted poultry diet containing rye and wheat. The enzyme cocktail was added at various inclusion levels. The addition of the enzyme cocktail in Pettersson and Aman's (2007) trail resulted in a significant increase in body-weight and feed intake. When Choct *et al.* (1995) tested the

supplementation of fibrolytic enzymes to broiler diets containing low apparent metabolisable wheat, they also observed positive results. Their enzyme cocktail was added at 1 g per kg of feed and consisted of xylanase, β-glucanase and pectinase. A 24.3 % increase in apparent metabolisable energy was observed when the enzyme cocktail was added to the low apparent metabolisable energy (AME) wheat diet (Choct *et al.*, 1995). The most interesting observation in this trail was the improvement of 34.1 % in the feed conversion efficiency (Choct *et al.*, 1995). The supplementation of the enzymes also led to a significant decrease in gut viscosity (Choct *et al.,* 1995). The most important characteristic of low apparent metabolisable wheat is the incomplete starch digestion with the excretion of starch in the faeces as a consequence. However, starch digestion in the small intestine of broilers is increased with enzyme supplementation (Choct *et al.*, 1995). Anderson *et al.* (2003) tested the effect of an endo-xylanase-containig enzyme on the solubility of rye. Rye bran was incubated with different amounts of enzyme preparations. Their results showed that the use of the enzyme resulted in an increase in the amount low-molecular-weight arabinoxylan fragments with 26.3 %, while the amount high-molecular-weight arabinoxylan fragments decreased by 1.4 %. The ratio of arabinose to xylose also decreased from 1.1 to 0.3 (Andersson *et al.,* 2003). This is one of the possible reasons why enzyme supplementation increase the digestibility of broiler diets based on wheat or rye. The reduction in the amount of high-molecular-weight arabinoxylan fragments leads to a decrease in gut viscosity and therefore an increase in the digestion and uptake of nutrients. The study of Choct *et al.* (1999) regarding the use of a commercial xylanase product in wheat based broiler diets revealed that the use of xylanase significantly ($P<0.001$) increased the AME of the wheat and starch digestibility in the jejunum and ileum of the bird. An increase of 6 % for starch digestibility in the jejunum and 3n% in the ileum was observed, the AME increased from 13.7 to 14.5nMJ/kg DM (Choct *et al.*, 1999). The use of xylanase also reduced ($P<0.01$) the digesta viscosity in the bird. Duodenal viscosity was reduced from 2.9 to 1.7 mPas, jejuna viscosity from 4.6 to 2.3 mPas and ileal viscosity from 14 to 3.9 mPas (Choct, Hughes & Bedford, 1999). The researchers found that starch digestibility increased due to the reduction in gut viscosity. Marron *et al.* (2001) also used xylanase for supplementing a wheat based broiler diet and they also found that enzyme supplementation increased AME and starch digestibility.

Silva and Smithard (2002) tested the effect of xylanase supplementation (30 g/kg of rye) in rye based diets for poultry on intestinal crypt cell proliferation rate (CCPR) of birds. According to Silva and Smithard (2002) studies has shown that soluble and fermentable polysaccharides with high viscosity stimulates the CCPR in the proximal and distal small bowel while the increase in CCPR can lead to large bowel cancer. The rate of crypt cell proliferation was measured as the amount of cells proliferated in two hours.

Their results revealed that enzyme supplementation significantly reduced crypt cell proliferation, crypt cell proliferation was reduced from 45 cells/2h to 29 cells/2h (Silva & Smithard, 2002). This finding of Silva and Smithard (2002) is more related to breeding birds than broilers, the reason being that broilers are slaughtered at the age of 35 days and that cancer would not have an effect on the broilers. Their findings however show that the use of xylanase decreases digesta viscosity. Yasar and

Forbes (2000) found the same result when they supplemented a wheat based diet with xylanase. According to them, xylanase supplementation increased the height of villi, the thickness of the crypts and muscle layers in the intestinal segments and it reduced CCPR. The increased villi height and crypt thickness leads to an increase in the absorptive area for nutrients, thus increasing nutrient absorption. They also stated that the decrease in CCPR can lead to the potential saving of nutrients for maintenance (Yasar & Forbes 2000).

The main objective of the use of NSP-degrading enzymes in wheat and barley based diets is to increase the apparent metabolisable energy of the wheat or barley. As mentioned before, the variability of the apparent metabolisable energy content of wheat is related to the anti-nutritional effects of the NSPs present in the wheat and barley (Choct *et al.,* 1995; 1996; 1999). A summary of the findings of some researchers on the use of NSP-degrading enzymes in wheat, rye and or barley based diets for broilers can be seen in Table 10.

The use of these enzymes in maize-soya based diets has only recently been investigated and some positive results have been found and reported by various researchers (McNab & Boorman, 2002). NSP-degrading enzymes commonly used in trials regarding maize-soya based diets consist of xylanase, β-glucanase, pectinase, cellulase, mannanase, and galactanase (Meng & Slominski, 2005).

Marsman *et al.* (1997) conducted a study where a cell wall degrading enzyme preparation (Energex^{TM}) was added to a maize-SBM based diet for broilers. The carbohydrase preparation consisted of cellulase, hemicellulase and pectinase. Their objective was to look at the effect of enzyme supplementation on broiler performance and apparent ileal nutrient digestibility. Their results indicated that body weight gain (BWG), feed intake (FI) and FCR was not affected by the enzyme supplementation. Enzyme supplementation significantly *(*$P<0.05$) increased apparent ileal crude protein (CP) digestibility from 83.2 % to 85.2 % (Marsman *et al.* 1997). According to Marsman *et al.* (1997) the carbohydrase preparation used in their study exhibited proteolytic activity and therefore it was able to solubilise a considerable amount of protein. Apparent ileal digestibility of NSP was also significantly ($P<0.05$) increased by 9.1 %.

Using the same enzyme preparation as Marsman *et al.* (1997), Saleh *et al*. (2005) studied the effect of enzyme supplementation in a maize-SBM based diet for broilers on abdominal fat content.

Apparent ileal CP digestibility, metabolisable energy (ME) and ash digestibility was increased by enzyme supplementation (Saleh *et al*., 2005). Their study also revealed that the enzyme preparation used had no effect on the abdominal fat content of broilers. Saleh *et al*. (2005) repeated the study, using a different enzyme preparation. The enzyme preparation used for this study was a pure cellulase (1,4-(1,3:1,4)-b-D-Glucan 4- glucano-hydrolase) obtained from the fungus *Trichoderma viride*. The addition of the pure cellulose to the maize-SBM based diet had no effect on BWG and FCR, nor did it have any effect on apparent ileal digestibilities. Interestingly, cellulase addition to the diet had an effect on the abdominal fat content of broilers (Saleh *et al*., 2005). The addition of cellulase led to a significant decrease in the abdominal fat pad weight of the broilers. Saleh *et al*.

(2005) suggested that the effect of cellulase on the abdominal fat content can be explained by two theories.

The first theory is that it is possible for the cellulase to be absorbed by the bird's intestine due to the fact that the molecular weight of cellulase is 56 000. The second theory is based on the alteration of gut microflora. Degradation of cellulose by cellulase into smaller molecules can lead to a change in the type and population of microflora present in the gut. This change in microflora leads to a change in hindgut fermentation, which can result in an increased lipid metabolism (Saleh *et al.*, 2005).

Table 10 Summary of the effect of NSP-degrading enzymes on the performance of broilers fed on wheat, rye and or barley based diets

Diet	Enzyme used	Improvement over control	Reference
Wheat-rye	Xylanase	FCR 6% BWG 4%	(Cowieson *et al.*, 2005)
Wheat	Xylanase	FCR 6% BWG 10%	(Scott, 2005)
Wheat	Xylanase Glucanase	FCR 5% BWG 5%	(Wang *et al.,* 2005)
Wheat	Xylanase	AME 10%	(Shakouri & Kermanshahi, 2005)
Triticale	Xylanase	AME 3%	(Shakouri & Kermanshahi, 2005)
Wheat-rye	Xylanase Glucanase	BWG increased FCE improved	(Pettersson & Åman, 2007)
Low apparent metabolisable wheat	Xylanase Glucunase Pectinase	AME 24.3% FCE 34.1%	(Choct *et al.,* 1995)
Wheat	Xylanase	BWG increased FCR improved	(Engberg *et al.,* 2004)

FCR – Feed conversion ratio; FCE – Feed conversion efficiency; BWG – Body weight gain; AME – Apparent metabolisable energy

Zanella *et al.* (1999) supplemented a maize-SBM based diet with a commercial enzyme preparation, Avizyme®1500, it was added to the diet at 0.1 %. Avizyme®1500 consists of 800 μ/g xylanase from the fungus *Trichoderma longibrachiatum*, 6,000 μ/g protease from the gram-positive bacterium *Bacillus subtilis*, and 2,000 μ/g amylase from the gram-positive bacterium *Bacillus amyloliquifaciens*. The enzyme supplementation resulted in a 2.9 % increase in ileal CP digestibility. Enzyme

supplementation had no significant effects on the digestibility of the most limiting amino acids, lysine and methionine. Amino acids digestibilities which was significantly ($P<0.05$) increased due to enzyme supplementation was those of threonine, serine, glycine, valine and tyrosine. Enzyme supplementation also led to the significant increase of 1.9 % in BW and improvement of 2.2 % in FCR (Zanella *et al.,* 1999). Zanella *et al.* (1999) also looked at the effect of enzyme supplementation on the abdominal fat content of broilers, no significant results were found.

As mentioned before, theoretically the use of NSP-degrading enzymes should result in an increase in NSP digestibility. Kocher *et al.* (2002) tested this theory by supplementing the same maize-SBM based diet with two types of enzyme preparations in two different experiments. The enzyme preparation used in the first experiment consisted of endo-1,3(4)-β-glucanase, hemicellulase and pectinase. Kocher *et al.* (2002) determined that the main neutral NSP constituents present in their SBM were galactose and arabinose minor amounts of rhamnose, xylose and glucose were also present. The concentration of the neutral NSPs in the digestive tract of the broilers was significantly altered when the diet was supplemented with the enzyme preparation. Analysis done by Kocher *et al.* (2002) on the monosaccharide composition of the NSP fractions in the ileum showed that the insoluble NSP fraction (galactose, arabinose and rhamnose) was reduced, thus leading to an increase in the concentration of galactose, arabinose and rhamnose in the free sugar fraction. The partial depolymerisation of galactan, arabinogalactan and rhamnogalacturonan by the enzyme preparation led to a significant increase in AME content in the ileum of the broilers. An increase in ileal CP digestibility was also observed. Kocher *et al.* (2002) explained that the increase in ileal CP digestibility was due to the proteolytic activity exhibited by the enzyme preparation, which was the same explanation given by Marsman *et al.* (1997).

In the second experiment of Kocher *et al.* (2002) an enzyme preparation consisting of galactanase was used to supplement the diet. The enzyme preparation used in the first experiment had no effect on volatile fatty acid (VFA) concentration in the caeca of broilers, but the supplementation with galactanase had a significant effect on VFA concentrations. The VFA's affected were acetic- and propionic acid. The use of galactanase also resulted in a significant decrease in the concentration of galactose in the soluble and insoluble NSP fractions present in the jejunal and ileal digesta of the broilers. This led to a corresponding increase of galactose and glucose concentration in the free sugar fraction. A summary of studies done on the use of NSP-degrading enzymes in maize-SBM based diets for broilers are shown in Table 11.

Table 11 Summary of the effect of NSP-degrading enzymes on the performance of broilers fed on maize-soya based diets

Enzyme used	Enzyme effect	Reference
Cellulase, Hemicellulase and Pectinase	↑ NSP digestibility ↑ CP digestibility	(Marsman *et al.,* 1997)
Cellulase, Hemicellulase and Pectinase	↑ ME ↑ CP digestibility ↑ Ash digestibility	(Saleh *et al.*, 2005)
Cellulase	↓ Abdominal fat content	(Saleh *et al.*, 2005)
Xylanase, Amylase, Protease	↑ CP digestibility (2.9%) ↑ BW (1.9%) Improved FCR (2.2%)	(Zanella *et al,.* 1999)
Endo-1,3(4)-β-glucanase, Hemicellulase, Pectinase	↑ NSP digestibility ↑ CP digestibility ↑ AME	(Kocher *et al.,* 2002)
Galactanse	↑ VFA production ↑ NSP digestibility	(Kocher *et al.,* 2002)
Glucanase, Hemicellulase, Pectinase	No significant results	(Rebolé *et al.,* 1999)
Xylanase, Amylase, Protease	↑ AME (2%) ↑ BW (6%) Improved FCR ↑ AA digestibility	(Cowieson & Ravindran, 2008)
β-Mannanse	↑ BW (4.66%) Improved FCR (2.76%)	(Jackson, 2004)
α-Galactosidase	↑ BW (2.22%) Improved FCR (0.88%)	(Kidd *et al.,* 2001)
Xylanase, Amylase, Protease	↑ BW (10.3%) Improved FCR (10.5%)	(Iji *et al.,* 2003)
Xylanase, Amylase, Protease	↑ BW (3.6%) Improved FCR (0.78%)	(Pack *et al.*, 1998)

FCR – Feed conversion ratio; FCE – Feed conversion efficiency; BWG – Body weight gain; AME – Apparent metabolisable energy; AA – Amino acid; VFA – Volatile fatty acids; ME – metabolisable energy; NSP – Non-starch polysaccharide

Irish and Balnave (1993) tested the supplementation of an enzyme preparation consisting of galactanase, pectinase, arabinase, glucanase and polygalacturonase on a maize-SBM based diet for broilers. No significant results were found, Irish and Balnave (1993) stated that the failure to produce any results was due to the oligosaccharides present in the SBM. Oligosaccharides cannot be broken down due the absence of α-galactosidase activity in monogastric animals (Nitsan *et al.,* 1991; Irish & Balnave, 1993; Iji *et al.*, 2001). A high concentration of oligosaccharides in the small intestine produces an osmotic effect which leads to an increased feed passage rate, this chain reaction has a negative effect on nutrient absorption in the gut (Irish & Balnave, 1993).

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CHAPTER 3

FIBROLYTIC ENZYMES FOR MAIZE-SOYA DIETS: EFFECTS ON PRODUCTION PERFORMANCE

3.1 Abstract

A growth trial was conducted to compare the digestibility of a maize–soybean meal grower feed containing an experimental enzyme at three levels of inclusion with a negative control, containing no enzyme additions, and a positive control, containing a proven commercial enzyme. The commercial enzyme was a product with a xylanase activity of 38114.29 nkat/g and the second enzyme (ABO374) was a liquid experimental product with a xylanase activity of 1426.86 nkat/ml. Five diets were used i.e. control basal diet without enzyme supplementation (negative control), basal diet supplemented with the commercial enzyme (positive control) and three basal diets supplemented with the test enzyme at various inclusion levels (ABO 50, ABO 100 and ABO 200). Supplementation with the test enzyme (ABO 50) significantly improved BW at 23 days of age with 4.6 % (1107.4 g *vs* 960.96 g) and at 37 days of age with 3.2 % (2311.75 g *vs* 2237.81 g) over the negative control. BWG for the total period of the trial significantly improved by 3.24 % (64.32 g/bird/day *vs* 62.24 g/bird/day) with the test enzyme supplementation (ABO 50) when compared to the negative control. During the starter phase, test enzyme supplementation (ABO 50) led to an improvement of 4.58 % (1.25 *vs* 1.31) in FCR in comparison with the negative control. The FCR for the total trial obtained by the test enzyme supplementation was significantly lower than the FCR obtained by the positive control. The highest EPER obtained for this trail was through test enzyme supplemented diets and this was significantly higher than the EPER obtained by the positive control. It is clear from this growth trial that the test enzyme (ABO374) outperformed the commercial enzyme.

Key words: body weight, body weight gain, feed conversion ratio, European production efficiency ratio, xylanase, maize, soybean meal

3.2 Introduction

Poultry production in recent years has undergone tremendous advances in genetics and nutritional management thereby increasing broiler productivity. The diets currently used for broilers are made up of highly concentrated raw materials which provide the nutrients for efficient digestion and utilisation. Sustaining this increase in production relies on the challenges that need to be overcome to maintain the efficiency of production (Hetland *et al*., 2004). The determining factor for the price of broiler feed in South Africa is the cost of raw materials, with maize and soya being the two major raw materials used in broiler feeds (Gous, 1998; Kleyn, 2005; Esterhuizen, 2010). Broiler producers therefore have to overcome these higher input costs for production to remain profitable (Vauqulin, 2009).

Increase in the cost of raw materials has put pressure on feed manufacturing companies to increase the nutrient utilisation efficiency of their feed (Bedford, 2009). According to Bedford (2009) and SAPA (2008), the demand for bio-fuels and for poultry meat in third world countries will not only lead to an increase in the cost of grains, but will also lead to the limitation in the supply of grains. It is estimated that 36 % of the maize production in the United States (US) will be used for ethanol production in the year 2015 (Vauqulin, 2009). Considering this, the feed manufacturing companies are turning their interests on feed additives like exogenous enzymes to improve the efficiency of nutrient utilisation (Bedford, 2009).

The negative correlation between the NSP content of feed and the nutritive value of the feed has clearly been demonstrated in poultry and this has led to the enormous attention that the fibre component of poultry feed has received during the recent years (Bedford, 1993; Bedford & Schulze, 1998; Bedford, 2000; McNab & Boorman, 2002). The application of exogenous fibrolytic enzymes in the poultry industry have been used to neutralise the effects of NSPs present in wheat, barley, rye and oats and this has proven to be a successful means of improving broiler production performance (Choct *et al*., 1995; Brufau *et al*., 2001; Engberg *et al*., 2004; Cowieson *et al*., 2005; Scott, 2005; Wang *et al*., 2005; Pettersson & Åman, 2007). The role of fibrolytic enzymes in broiler diets based on raw materials with substantially lower concentration of NSPs are yet to be proven, although various studies have concluded that maize and soybean meal based diets are responsive to supplemental fibrolytic enzymes (Cowieson & Ravindran, 2008).

The objective of this trial was to compare production performance of broilers receiving a diet containing three different activity levels of an experimental xylanase product with that of a negative control receiving no exogenous fibrolytic enzymes and a positive control receiving a proven commercial xylanase exogenous enzyme.

3.3 Materials and methods

One thousand one hundred and forty Cobb 500 broilers chicks (as-hatched) were placed in 30 floor pens with 38 birds per pen (0.12 m^2/bird), bedding for the pens was supplied in the form of shavings at a depth of 100mm. The experimental units (pen) were allocated to five treatments with six replicates per treatment (Table 12). The experiment was a completely randomised design with dietary treatment as the main effect. Birds were obtained from a commercial hatchery. The birds were housed in an environmentally controlled broiler house, the temperature and lighting was managed according to the specifications of the primary breeder. The environmental temperature of the house for the first day was 33˚C and was decreased by 2˚C every seventh day to 20˚C at 35 days of age. A minimum of six air changes per minute was provided for the birds. The birds had *ad libitum* access to feed and water.

Table 12 A description of the dietary treatments used throughout the trial

Treatment	Description
Negative control	Control diet
Positive control	Control diet + Commercial enzyme (7622858 nkat/ton of feed)
ABO 50	Control diet + ABO374 (3811429 nkat/ton of feed)
ABO 100	Control diet + ABO374 (7622858 nkat/ton of feed)
ABO 200	Control diet + ABO374 (15245716 nkat/ton of feed)

The trail consisted of three treatments, a positive control and a negative control group. A four phase diet according to the nutrient specifications of the primary breeder was formulated using the feed formulation program *Winfeed* and fed to the birds (Table 13). A pre-starter diet was fed to all birds from day 0 to day 1, starter diets were fed from day 2 to day 15, grower from day 16 to day 22 and finisher from day 23 to day 37. The pre-starter and starter diets were fed in a meal form, while the grower and finisher diets were fed as pellets.

A commercial enzyme product was added to the positive control diet at the manufacturer's recommendations of 200 g/ton. The commercial enzyme product was added to the diet in a granular form. The test product, ABO374, was added to the diets of ABO 50, ABO 100 and ABO 200 at various inclusion levels in liquid form. The inclusion level of ABO374 was 5342 ml/ton as determined by the Department of Microbiology at the Stellenbosch University. The inclusion level of ABO374 was determined so that the xylanase activity of the commercial enzyme and ABO374 was the same in the feed. The xylanase activity of the commercial enzyme and ABO374 determined by Department of Microbiology at the Stellenbosch University were 38114.29 nkat/g and 1426.86 nkat/ml respectively. ABO 50 was supplemented with ABO374 at an inclusion level of 2671 ml/ton, ABO 100 with 5342 ml/ton and ABO 200 with 10684 ml/ton. There was no enzyme supplementation in the negative control diet. Pelleting of feed took place 24 hours after enzyme addition.

Cellulose in the form of powder was added to diets to replace the granular enzyme on a weight for weight basis while water was added to diets to replace liquid enzyme additions on a weight for weight basis. These additions are shown in Table 14.

Table 13 Ingredient and calculated nutrient composition of the broiler diets used in the production trial on an as fed basis (g/kg)

	Prestarter	Starter	Grower	Finisher
Ingredient (g/kg)				
Maize	434.38	498.73	503.75	587.76
Soybean full fat	507.74	420.63	289.91	12.41
Soybean 46	10.22	33.61	164.11	357.94
L-lysine HCl	4.07	3.66	1.16	0.72
DL-methionine	2.59	2.32	1.52	0.97
L-threonine	1.45	1.29		
Limestone	16.23	16.60	16.61	17.09
Salt	1.46	2.13	2.47	2.29
Monocalcium phosphate	16.33	16.41	16.39	16.49
Sodium bicarbonate	3.04	2.11	1.58	1.82
Vitamin + mineral premix *	1.50	1.50	1.50	1.50
Calculated nutrient content				
AMEn (MJ/kg)	13.20	13.05	12.40	11.33
Crude protein (%)	23.63	21.96	22.80	22.07
Lysine (%)	1.67	1.51	1.38	1.27
Methionine (%)	0.60	0.55	0.49	0.43
Cysteine (%)	0.39	0.37	0.39	0.39
Methionine+Cystine (%)	0.99	0.93	0.88	0.82
Threonine (%)	1.05	0.97	0.88	0.85
Tryptophan (%)	0.28	0.25	0.27	0.26
Arginine (%)	1.61	1.48	1.57	1.50
Isoleucine (%)	1.06	0.98	1.04	1.01
Leucine (%)	1.98	1.89	1.98	1.97
Histidine (%)	0.64	0.60	0.63	0.61
Phenylalanine (%)	1.09	1.01	1.07	1.03
Tyrosine (%)	0.83	0.78	0.86	0.88
Phenylalanine+Tyrosine (%)	1.92	1.79	1.92	1.92
Valine (%)	1.15	1.08	1.15	1.13
Glycine+Serine (%)	2.16	2.00	2.11	2.03
Crude fibre (%)	3.80	3.58	3.52	3.15
Crude fat (%)	11.06	9.73	7.52	3.00
Calcium (%)	1.00	1.00	1.00	1.00
Phosphorous (%)	0.83	0.82	0.83	0.82
Available Phosphorous (%)	0.50	0.50	0.50	0.50
Sodium (%)	0.16	0.16	0.16	0.16
Chloride (%)	0.18	0.22	0.22	0.22
Potassium (%)	0.98	0.91	0.97	0.94
Linoleic acid (%)	5.89	5.20	4.02	1.64
Choline (mg/kg)	1750.00	1606.13	1600.00	1400.00

*Vitamin+mineral premix provides (per kg of diet): 8160 Iu Vitamin A, 1700 Iu Vitamin D3, 30.6 Iu Vitamin E, 2.7 mg Vitamin K3, 2.05 mg Vitamin B1, 2.05 mg Vitamin B2, 27.2 mg Niacin, 10.2 mg Calcium Pantothenate, 0.02 mg Vitamin B12, 4.1 mg Vitamin B6, 1.7 mg Folic acid, 0.068 mg Biotin, 0.08 mg Iodine, 0.34 mg Cobalt, 0.2 mg Selenium, 120 mg Ronozyme p500, 350 mg Choline, 70 mg Manganese, 70 mg Zinc, 6 mg Copper and 50 mg Ferrous

The body weights of the birds were measured at placement (day 0) and on days 2, 9, 16, 23, 30 and 37. Each pen was weighed and the individual bird weight was calculated as an average. Feed was removed 3 hours prior to the weighing of the birds. Feed intake was also measured by weighing the initial amount of feed offered to the birds and the amount of feed remaining in the feeders on day 2, 9, 16, 23, 30 and 37. The feed intake was then used to calculate feed conversion ratio. Bird mortality was recorded daily, this was done by weighing the dead bird and the remainder of the birds present in the pen and the feed remaining in the feeders of the pen.

Statistical analysis was done by the *Centre for Statistical Analysis* at the Stellenbosch University. The data were tested for homogeneity and normality. Data were of equal variance and normally distributed, it was then analysed by mixed model repeated measures ANOVA with the statistical program *Statistica version 8.*

The european production efficiency ratio (EPER) was calculated using the equation presented in Equation 1.

Table 14 Amount of added water, cellulose and enzyme to the different treatments

Ingredient	Treatments				
	Negative control	**Positive control**	**ABO 50**	**ABO 100**	**ABO 200**
Water (ml/ton)	10684	10684	8013	5342	0
Cellulose (g/ton)	200	0	200	200	200
Commercial enzyme (g/ton)	0	200	0	0	0
ABO374 (ml/ton)	0	0	2671	5342	10684

$$EPER = \frac{\text{Liveweight } (k_g) \times \text{Liveabillity } (\%)}{\text{Age at depletion } (\text{day}_s) \times \text{Feed conversion ratio}} \times 10_0$$

Equation 1 European production efficiency ratio (EPER)

3.4 Results and discussion

The treatment means for body weight (BW), body weight gain (BWG), feed intake (FI), cumulative feed intake, feed conversion ratio (FCR), European production efficiency ratio (EPER) and average daily gain (ADG) are shown in Tables 15 – 20. Mortality during the trial was 2.37 % and the deaths were not related to any of the dietary treatments. The average liveability for the trial was 97.63 %. At the beginning of the trial no significant (P>*0.05*) differences between the mean body weight (BW) of the birds from the different treatments were noted. Mean BW during the first three periods (2 d, 9 d and 16 d) of the trial was not significantly affected by enzyme supplementation. Significant differences in mean BW were noticed during the last two periods (30 d and 37 d) of the trial. At 30 days of age the mean BW of the birds in the negative control was 1617.54 g, the mean BW of the birds in ABO 50 was 1691.28 g, this was significantly (P<*0.05*) higher than the negative control (4.3 % increase over negative control). At 37 days of age the increase in the mean BW for the birds from ABO 50 (2311.75 g) over the negative control (2237.81 g) was 3.2 %, the mean BW for the birds of ABO 100 (2186.03 g) was significantly (P<*0.05*) lower than that of the negative control.

Table 15 Mean body weight (BW) (g/bird) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme as measured weekly from day 2 to 37

Period		2 d		9 d		16 d		23 d		30 d		37 d	
Treatment	N	BW	SE[1]	BW	SE[1]	BW	SE[1]	BW	SE[1]	BW	SE[1]	BW	SE[1]
Negative control	6	59.4	0.3	189.0	2.7	490.6	6.6	960.9[ab]	14.4	1617.5[a]	14.4	2237.8[a]	16.1
Positive control	6	57.3	0.7	191.9	2.4	490.9	4.6	956.2[a]	9.9	1612.4[a]	19.9	2235.5[a]	16.9
ABO 50	6	59.0	0.3	194.8	2.5	511.3	8.9	1007.4[b]	22.8	1691.3[b]	9.3	2311.8[b]	22.0
ABO 100	6	59.5	0.4	193.4	2.4	499.0	6.9	978.7[ab]	11.9	1638.4[a]	20.5	2186.0[c]	17.7
ABO 200	6	60.1	0.3	195.9	4.5	494.8	11.7	973.8[ab]	21.8	1637.1[a]	38.5	2248.5[a]	55.2

[1] Standard error of the mean; [a – c] Means within a column with common superscripts are not significantly different (P>*0.05*)

The results obtained for mean BW is in agreement with previous studies that have used the same type of enzyme and diet (Pack *et al*., 1998; Zanella *et al*., 1999; Kidd *et al*., 2001; Iji *et al*., 2003; Jackson, 2004; Cowieson & Ravindran, 2008).

The enzyme application of ABO 50 resulted in a significant (P<*0.05*) increase (4.46 %) in mean BWG from 55.65 g/bird/day (negative control) to 58.25 g/bird/day (ABO 50) during the period of 2 – 30 days. The mean BWG of ABO 50 was also significantly (P<*0.05*) higher than all the other treatments. The mean BWG of 64.32 g/bird/day for ABO 50 during the total period of the trial (2 – 37d) was significantly (P<*0.05*) higher than the negative control with a mean BWG of 62.24 g/bird/day (3.24 % higher), it was also significantly (P<*0.05*) higher than the rest of the treatments. ABO 100 had a mean BWG significantly (P<*0.05*) lower than all other treatments.

The increase in BWG observed in the ABO 50 treatment may be an indication that the ABO374 enzyme has the ability to improve the digestibility of the feed when it is added at 2671 ml/ton of feed. Researchers who have observed an increase in BWG due to fibrolytic enzyme supplementation have concluded that the increase was due to an increase in the digestibility of the feed (Zanella *et al,.* 1999; Iji *et al.,* 2003; Jackson, 2004; Cowieson & Ravindran, 2008).

Table 16 Mean body weight gain (BWG) (g/bird/day) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme from day 2 to 37

Period		2 - 9 d		2 - 16 d		2 - 23 d		2 - 30 d		2 - 37 d	
Treatment	N	BWG	SE[1]	BWG	SE[1]	BWG	SE[1]	BWG	SE[1]	BWG	SE[1]
Negative control	6	18.5	2.5	30.8	6.4	42.9	14.1	55.7[a]	14.2	62.2[a]	15.9
Positive control	6	19.2	1.9	30.9	4.3	42.8	9.7	55.5[a]	19.3	62.2[a]	16.6
ABO 50	6	19.4	2.1	32.3	8.6	45.1	23.3	58.3[b]	9.3	64.3[c]	21.8
ABO 100	6	19.1	2.1	31.4	6.7	43.7	11.5	56.4[a]	20.5	60.7[d]	17.7
ABO 200	6	19.3	4.3	30.9	11.6	43.4	21.5	56.3[a]	38.3	62.5[a]	54.8

[1] Standard error of the mean; [a – d] Means within a column with common superscripts are not significantly different (P>*0.05*)

Significant differences (P<*0.05*) between the positive control and ABO 100 regarding mean FI, with a mean FI of 29.08 g/bird/day and 26.06 g/bird/day respectively, was only observed for the total period of the trial (2 - 37 d). When the negative control was compared to all the other treatments, there were no significant differences found. The results obtained in this trial regarding mean BWG (Table 16) and mean FI (Table 17) differ from previous studies (Irish & Balnave, 1993; Meng & Slominski, 2005). Irish & Balnave (1993) found that enzyme application in the form of xylanase in their study resulted in an increase in mean BW, but had no effect on mean BWG or mean FI, this was similar to the results reported by Meng & Slomenski (2005) in their study.

Cumulative feed intake is the total amount of feed consumed by the bird from day 0 until the bird reaches it slaughter mass. The mean cumulative feed intake (Table 18) during the total period of the trial was only affected by the addition of the commercial enzyme (positive control). The positive control had a mean cumulative feed intake of 3146.58 g/bird for the total period of the trial, this was significantly higher (P<*0.05*) than the mean cumulative feed intake of 3037.70 g/bird for the negative control and 3056.44 g/bird for ABO 200.

Table 17 Mean weekly feed intake (FI) (g/bird/day) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme from day 2 to 37

Period		2 - 9 d		10 - 16 d		17 - 23 d		24 - 30 d		31 - 37 d	
Treatment	N	FI	SE[1]	FI	SE[1]	FI	SE[1]	FI	SE[1]	FI	SE[1]
Negative control	6	23.77	2.16	56.79	5.59	109.31	8.53	104.52	66.89	139.57[ab]	35.37
Positive control	6	23.58	2.62	56.16	3.49	110.41	5.56	112.55	27.00	145.39[a]	32.74
ABO 50	6	23.64	1.40	56.87	6.84	109.79	9.67	106.84	24.27	138.65[ab]	37.79
ABO 100	6	23.90	2.87	56.20	5.88	111.08	8.99	112.80	16.13	130.28[b]	38.60
ABO 200	6	24.30	4.03	55.88	9.61	110.81	16.25	104.49	5.73	139.29[ab]	45.65

[1] Standard error of the mean; [a – b] Means within a column with common superscripts are not significantly different (P>*0.05*)

Table 18 Mean cumulative feed intake (FI) (g/bird/period) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme from day 2 to 37

Period		2 - 9 d		2 - 16 d		2 - 23 d		2 - 30 d		2 - 37 d	
Treatment	N	FI	SE[1]	FI	SE[1]	FI	SE[1]	FI	SE[1]	FI	SE[1]
Negative control	6	166.42	2.16	563.94	6.55	1329.10	13.18	2060.73	74.25	3037.70[c]	55.22
Positive control	6	165.09	2.62	559.71	5.80	1335.11	9.13	2128.87	29.12	3146.58[b]	12.80
ABO 50	6	165.47	1.40	563.55	7.97	1339.96	17.68	2094.11	44.17	3064.61[bc]	61.97
ABO 100	6	167.29	2.87	561.42	8.25	1346.69	17.34	2136.30	20.23	3068.82[bc]	25.21
ABO 200	6	170.11	4.03	562.73	12.71	1343.95	31.38	2081.45	35.41	3056.44[c]	70.68

[1] Standard error of the mean; [a – c] Means within a column with common superscripts are not significantly different (P>0.05)

Mean feed conversion ratio was significantly (P<*0.*05) affected by ABO 50 during the starter phase (2 – 16 d). ABO 50 had a mean FCR of 1.25 for the starter phase (2 – 16 d) and the mean FCR for the negative control was 1.31. Enzyme application in ABO 50 thus resulted in a significant (P<0.05) improvement in the mean FCR over the negative control, an improvement of 4.58 %. The mean FCR of ABO 50 was also significantly lower than that of the positive control and ABO 200. No significant differences for mean FCR between treatments were found during the grower (17 – 30 d) and finisher (31 – 37 d) phases. During the total trial period, mean FCR was significantly (P<*0.05*) affected by ABO 50 when compared to the positive control and ABO 100. The mean FCR for ABO 50, the positive control and ABO 100 was respectively 1.36, 1.45 and 1.44. These findings are in agreement with previous studies done with the similar types of enzymes (Pack *et al*., 1998; Zanella *et al*., 1999; Kidd *et al*., 2001; Iji *et al*., 2003; Jackson, 2004; Cowieson & Ravindran, 2008).

Table 19 Mean feed conversion ratio (FCR) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme for all feeding phases

Period		Starter (2 - 16 d)		Grower (17 - 30 d)		Finisher (31 - 37 d)		Total (2 - 37 d)	
Treatment	N	FCR	SE[1]	FCR	SE[1]	FCR	SE[1]	FCR	SE[1]
Negative control	6	1.31[a]	0.01	1.33	0.05	1.57	0.05	1.40[ab]	0.02
Positive control	6	1.29[a]	0.00	1.40	0.03	1.63	0.04	1.45[a]	0.01
ABO 50	6	1.25[b]	0.03	1.30	0.03	1.57	0.08	1.36[b]	0.03
ABO 100	6	1.28[ab]	0.01	1.39	0.04	1.73	0.12	1.44[a]	0.02
ABO 200	6	1.30[a]	0.01	1.33	0.02	1.61	0.11	1.40[ab]	0.03

[1] Standard error of the mean; [a – b] Means within a column with common superscripts are not significantly different (P>0.05)

The European production efficiency ratio (EPER) is a production efficiency factor used for comparing live-bird performance of flocks. This value (Equation 1) incorporates live weight, age, liveability and FCR. Using the EPER facilitates the comparison of bird performance in and between farms, it can also be used to asses environmental and managemental variables. Any effect relating to health, environmental stress or feed quality will be reflected in the EPER, this is due to the incorporation of terminal weight and age in addition to liveability and FCR. The EPER of a flock with acceptable growth and liveability parameters should be between the range of 200 to 225 (Shane, 1999).

ABO 50 had the highest mean EPER off all treatments. The mean EPER of ABO 50 (450.25) was significantly (P<*0.05*) higher than the mean EPER of the negative control (434.15) and the positive control (410.70). No significant differences were observed in the ADG.

Table 20 European production efficiency ratio (EPER) and average daily gain (ADG)(g/bird/day) for broilers receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme for the total period of the trial

Period		EPER		ADG	
Treatment	N	EPER	SE[1]	ADG	SE[1]
Negative control	6	434.15[ab]	5.53	62.24	0.0014
Positive control	6	410.70[ac]	5.37	62.23	0.0014
ABO 50	6	450.25[b]	14.75	64.36	0.0016
ABO 100	6	398.37[c]	7.16	60.76	0.0015
ABO 200	6	421.63[abc]	14.00	62.53	0.0032

Standard error of the mean; [a – c] Means within a column with common superscripts are not significantly different (P>0.05)

As mentioned before, the pre-starter and starter diets were the only diets that were not pelletised. A possible reason why there are only significant differences in the starter diet phase in regards to mean FCR and not in the grower or finisher diet phases is that the enzymes in the starter diet had a longer

time period to exert their effect. Pelletisation of the grower and finisher diets of all treatments took place 24 hours after the enzyme where added and thus after 24 hours the enzymes in the feed were inactivated due to the temperature of pelletisation (Silversides & Bedford, 1999). The enzymes in the starter diet of all treatments was thus still active after 24 hours and thus had a longer period of time to exert their effect (Silversides & Bedford, 1999).

According to Irish & Balnave (1993), oligosaccharides cannot be broken down in the broiler's intestine due to the absence of α-galactosidase activity. When the concentration of oligosaccharides in the bird's small intestine is high, it produces an osmotic effect. This leads to fluid retention and increases the feed passage rate, which in turn leads to a negative effect on nutrient absorption (Irish & Balnave, 1993). This may be another possible reason why enzyme supplementation only improved the mean FCR during the starter phase. The starter feed had far less soybean meal than the grower diet, the finisher diet had the highest soybean meal content. Thus, the oligosaccharide content of the starter feed was less than that of the grower and finisher diets. The breakdown of non-starch polysaccharides to oligosaccharides by the enzymes in the grower and finisher diets could have led to a higher oligosaccharide concentration than that of the oligosaccharide concentration obtained in the small intestine of the birds when the starter feed with enzyme was fed (Irish & Balnave, 1993).

Coon *et al.* (1990) conducted a study where they removed the oligosaccharides from soybean meal by means of ethanol. The ethanol-extracted soybean meal was then fed to broilers to determine the nutritional differences due to the removal of the oligosaccharides. They found a significant ($P<0.05$) increase in fibre digestibility and in the metabolisable energy value, this response was attributed to a decrease in feed passage rate and thus an increase in nutrient absorption (Coon *et al*., 1990).

3.5 Conclusion

The objective of this trial was to determine whether an experimental xylanase enzyme (ABO374) product had the potential to increase broiler performance on a maize based diet with soybean meal being the main protein source. The experimental xylanase enzyme (ABO374) product was also compared to a commercial xylanase enzyme product.

The results obtained during this performance trial gives a clear indication that the use of exogenous fibrolytic enzymes, especially xylanase, in a commercial maize - soybean broiler diet can improve broiler performance. The experimental xylanase enzyme ABO374 outperformed the commercial enzyme when it was added at 2671mL/ton with a xylanase activity of 1426.86nkat/mL. With the starter feed FCR and the overall FCR being significantly ($P<0.05$) improved by the application of ABO374 to the feed, it is possible to reduce the amount of feed being used for production and therefore reducing the production cost per bird. One of the possible reasons stated in the discussion why the FCR for the grower and finisher period did not improve when the diet was supplemented ABO374, is that the enzyme could have inactivated during pelleting, the ABO374 enzyme can thus be improved by making it more heat stable. Another reason could be the effect of the enzyme on the oligosaccharide

concentration in the gut. Interesting research on the use of ABO374 will be the replacement of soybean meal with various levels of sunflower oilcake, reason being that the oligosaccharide concentration of soybean meal is much more than the oligosaccharide concentration found in sunflower oilcake.

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CHAPTER 4

FIBROLYTIC ENZYMES FOR MAIZE-SOYA DIETS: EFFECTS ON APPARENT DIGESTIBILITY OF NUTRIENTS

4.1 Abstract

A digestibility trial was conducted to compare the digestibility of a maize–soybean meal grower feed containing an experimental enzyme at three levels of inclusion with a negative control, containing no enzyme additions, and a positive control, containing a proven commercial enzyme. The commercial enzyme was a product with a xylanase activity of 38114.29 nkat/g and the second enzyme (ABO374) was a liquid experimental product with a xylanase activity of 1426.86 nkat/ml. Five diets were used i.e. control basal diet without enzyme supplementation (negative control), basal diet supplemented with the commercial enzyme (positive control) and three basal diets supplemented with the test enzyme at various inclusion levels (ABO 50, ABO 100 and ABO 200). The basal diet used in this trial was a grower phase diet. Supplementation with the test enzyme had no significant improvements on the apparent digestibility of dry matter, organic material, ash, crude protein, gross energy or crude fibre. No significant improvements in the apparent digestibility of the amino acids (threonine, arginine, valine, lysine, methionine, cysteine and isoleucine) were noticed and thus the digestibility of the grower feed was not influenced by the addition of enzymes due to the supplementation of the test enzyme ABO374.

Key words: apparent digestibility, dry matter, organic material, ash, crude protein, gross energy, crude fibre, xylanase, maize, soybean meal

4.2 Introduction

A significant part of all plant feedstuffs are made up by dietary fibre (Hetland *et al*., 2004). The soluble dietary fibre fraction however has anti-nutritive effects in poultry (Bedford, 1993; Bedford, 1996; Knudsen, 1997; Bedford & Schulze, 1998; Bedford, 2000; Hetland *et al*., 2004; Jørgensen *et al*., 2007). These non-starch polysaccharides (NSP) increase gut viscosity and thereby inhibit digestion and absorption of nutrients (Bedford, 1993). The increase in gut viscosity also reduces feed intake due to a slower feed passage rate, which leads to the proliferation of microbes in the gut (Hetland *et al*., 2004). The negative correlation between the NSP content of feed and the nutritive value of the feed has been clearly demonstrated in poultry and this has led to the enormous attention that the fibre component of poultry feed has received in recent years (Bedford, 1993; Bedford & Schulze, 1998; Bedford, 2000; McNab & Boorman, 2002).

Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed-linked β-glucans and arabinoxylans (Parsippany, 2008). Depolymerisation of these NSPs requires specific enzymes, which are specific to the main and side chain structure of the NSP (Bedford, 1993; Henry & Rothwell, 1995; Castanon *et al*., 1997; Bedford & Schulze, 1998; Bhat, 2000; Andersson *et al*., 2003; Dalibard & Geraert, 2004).

The application of exogenous fibrolytic enzymes in the poultry industry have been used to neutralise the anti-nutritive effects of NSPs present in wheat, barley, rye and oats and this has proven to be a successful means of improving broiler production performance and feed digestibility (Choct *et al*., 1995; Brufau *et al*., 2001; Engberg *et al*., 2004; Cowieson *et al*., 2005; Scott, 2005; Wang *et al*., 2005; Pettersson & Åman, 2007). The use of NSP-degrading enzymes in wheat and barley based diets for broilers are therefore well established and accepted (McNab & Boorman, 2002; Bedford, 2009). The role of fibrolytic enzymes in broiler diets based on raw materials with substantially lower concentration of NSPs are yet to be proven, although various studies have concluded that maize and soybean meal based diets are responsive to supplemental fibrolytic enzymes (Cowieson & Ravindran, 2008).

The objective of this trial was to compare the digestibility of a basal grower diet containing three different activity levels of an experimental xylanase product with that of a negative control receiving no exogenous fibrolytic enzymes and a positive control receiving a proven commercial xylanase exogenous enzyme.

4.3 Materials and methods

Four hundred and eighty Cobb 500 chicks at one day-old (as hatch) were bought from a commercial hatchery and grown to the age of 21 days on the starter diet presented in Table 22. The birds were housed in an environmentally controlled house, the temperature and lighting was managed according to the specifications of the primary breeder. The environmental temperature of the house for the first day was 33˚C and was decreased by 2˚C every seventh day to 20˚C at 35 days of age. A minimum of six air changes per minute was provided for the birds. The birds had *ad libitum* access to feed and water.

At 21 days of age, the chicks were placed in individual metabolic cages. All the metabolic cages were equipped with feeders, automatic water nipples and faeces collection trays. The trial was conducted in an environmentally controlled house, the temperature and lighting was managed according to the specifications of the primary breeder.

Table 21 A description of the dietary treatments used throughout the trial

Treatment	Description
Negative control	Control diet
Positive control	Control diet + Commercial enzyme (7622858 nkat/ton of feed)
ABO 50	Control diet + ABO374 (3811429 nkat/ton of feed)
ABO 100	Control diet + ABO374 (7622858 nkat/ton of feed)
ABO 200	Control diet + ABO374 (15245716 nkat/ton of feed)

The trail consisted of three treatments, a negative control and a positive control group. A grower diet according to the nutrient specifications of the primary breeder was formulated using the feed formulation program *Winfeed* and fed to the birds (Table 22). The grower diet was fed as pellets.

A commercial enzyme product was added to the positive control diet at the manufacturer's recommendations of 200 g/ton. The commercial enzyme product was added to the diet in a granular form. The test product, ABO374, was added to the diets of ABO 50, ABO 100 and ABO 200 at various inclusion levels in liquid form. The inclusion level of ABO374 is 5342 ml/ton as determined by the Department of Microbiology at the Stellenbosch University. The inclusion level of ABO374 was determined so that the xylanase activity of the commercial enzyme and ABO374 are the same in the feed. The xylanse activity of the commercial enzyme and ABO374 determined by Department of Microbiology at the Stellenbosch University were 38114.29 nkat/g and 1426.86 nkat/ml respectively. ABO 50 was supplemented with ABO374 at an inclusion level of 2671 ml/ton, ABO 100 with 5342 ml/ton and ABO 200 with 10684 ml/ton. There was no enzyme supplementation in the negative control diet. Pelleting of feed took place 24 hours after enzyme addition of application. Cellulose in the form of powder was added to diets to replace the granular enzyme on a weight for weight basis while water was added to diets to replace liquid enzyme additions on a weight for weight basis. These additions are shown in Table 23.

The total tract digestibility method and total collection method was used to conduct the digestibility trial. The total tract digestibility method measures the difference between the amounts of each nutrient consumed from the amounts of each nutrient excreted in faeces. The grower negative control, positive control, ABO 50, ABO 100 and ABO 200 diets used in the production trial were also used in the digestibility trial.

Each treatment in this digestibility trial consisted of a total of twenty 21 day old broilers. The birds were subjected to 100% *ad libitum* feed intake. The first four days were used for adaptation. During the adaptation period the treatment feeds, to which the broilers were allocated, were fed *ad lib.* Feed intake was measured for each individual bird during the adaption period to determine the amount of feed taken in per day. The measurements were then used to calculate the amount of feed that each individual bird had to receive during the trial.

The trial started on day 25 and ended on day 31. Feed intake and faeces collection was measured for five days. Faeces collection was done 24 hours after the feed was given. Measurements were recorded daily, which included feed intake, feed refusal and faecal weights. Faeces and samples of all treatment feeds were collected, and stored in a freezer room at -20°C. Care was taken to avoid the contamination of faeces with feathers, scales and debris. After the trial the faeces collection of each group in each treatment was pooled and a sample was taken for analyses.

Both dietary and faecal samples were prepared for analyses according to method 950.02 of the AOAC International (Horwitz, 2003). Duplicate samples of both dietary treatments and faecal samples were analysed for dry matter, ash, crude protein, crude fibre, gross energy and amino acid content. Dry matter, ash, crude protein and crude fibre content of the feeds were analysed according using official methods of analysis of AOAC international. Method 934.01 was used for dry matter content analysis, method 994.12 for ash, method 990.03 for crude protein and method 978.10 for crude fibre (Horwitz, 2003). The energy content of the feed was determined by making use of a bomb calorimeter. Both dietary and faecal samples were sent to the Central Analytical Facility of Stellenbosch University for amino acid analysis. The amino acid content of both types of samples was determined by Central Analytical Facility of Stellenbosch University by making use of the *EZ Faast kit* from *Phenomenex.* Apparent digestibility coefficients were calculated on a DM basis as presented in Equation 2.

$$\text{Apparent nutrient digestibility} = \frac{(\text{Nutrient consumed} - \text{Nutrient excreted})}{(\text{Nutrient consumed})}$$

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

and nutrient excreted = DM excreta output x Nutrient content $_{excreta}$

Equation 2 Calculation for apparent digestibility coefficients

Statistical analysis was done by the *Centre for Statistical Analysis* at the Stellenbosch University. The data was tested for homogeneity and normality. Data was of equal variance and normal distributed. It was then analysed through mixed model repeated measures ANOVA with the statistical program *Statistica version 8.*

Table 22 Ingredient and calculated nutrient composition of the broiler diets used in the digestibility trial on an as fed basis (g/kg)

	Starter	Grower
Ingredient (g/kg)		
Maize	498.73	503.75
Soybean full fat	420.63	289.91
Soybean 46	33.61	164.11
L-lysine HCl	3.66	1.16
DL-methionine	2.32	1.52
L-threonine	1.29	
Limestone	16.60	16.61
Salt	2.13	2.47
Monocalcium phosphate	16.41	16.39
Sodium bicarbonate	2.11	1.58
Vitamin + mineral premix *	1.50	1.50
Calculated nutrient content		
AMEn (MJ/kg)	13.05	12.40
Crude protein (%)	21.96	22.80
Lysine (%)	1.51	1.38
Methionine (%)	0.55	0.49
Cysteine (%)	0.37	0.39
Methionine+Cystine (%)	0.93	0.88
Threonine (%)	0.97	0.88
Tryptophan (%)	0.25	0.27
Arginine (%)	1.48	1.57
Isoleucine (%)	0.98	1.04
Leucine (%)	1.89	1.98
Histidine (%)	0.60	0.63
Phenylalanine (%)	1.01	1.07
Tyrosine (%)	0.78	0.86
Phenylalanine+Tyrosine (%)	1.79	1.92
Valine (%)	1.08	1.15
Glycine+Serine (%)	2.00	2.11
Crude fibre (%)	3.58	3.52
Crude fat (%)	9.73	7.52
Calcium (%)	1.00	1.00
Phosphorous (%)	0.82	0.83
Available Phosphorous (%)	0.50	0.50
Sodium (%)	0.16	0.16
Chloride (%)	0.22	0.22
Potassium (%)	0.91	0.97
Linoleic acid (%)	5.20	4.02
Choline (mg/kg)	1606.13	1600.00

*Vitamin+mineral premix provides (per kg of diet): 8160 Iu Vitamin A, 1700 Iu Vitamin D3, 30.6 Iu Vitamin E, 2.7 mg Vitamin K3, 2.05 mg Vitamin B1, 2.05 mg Vitamin B2, 27.2 mg Niacin, 10.2 mg Calcium Pantothenate, 0.02 mg Vitamin B12, 4.1 mg Vitamin B6, 1.7 mg Folic acid, 0.068 mg Biotin, 0.08 mg Iodine, 0.34 mg Cobalt, 0.2 mg Selenium, 120 mg Ronozyme p500, 350 mg Choline, 70 mg Manganese, 70 mg Zinc, 6 mg Copper and 50 mg Ferrous

Table 23 Amount of added water, cellulose and enzyme to the different treatments

Ingredient	Treatments				
	Negative control	**Positvie control**	**ABO 50**	**ABO 100**	**ABO 200**
Water (ml/ton)	10684	10684	8013	5342	0
Cellulose (g/ton)	200	0	200	200	200
Commercial enzyme (g/ton)	0	200	0	0	0
ABO374 (ml/ton)	0	0	2671	5342	10684

4.4 Results and discussion

The mean apparent digestibility coefficients for the nutrients in the broiler chickens fed by dietary treatments at 100 % *ad libitum* are shown in Table 24. The apparent dry matter digestibility of 80 % for the negative control was significantly (P<*0.05*) higher than those of the ABO 50, ABO 100 and ABO 200 diets, with apparent dry matter digestibilities of 75 %, 75 % and 72 % respectively. Apparent digestibility for dry matter of the positive control (78 %) was also significantly higher (P<0.05) than those of the ABO 50, ABO 100 and ABO 200 diets. Significant differences (P<*0.05*) between the negative control and ABO 100 and ABO 200 apparent ash digestibility was observed. The apparent digestibility of ash for the negative control was 44 %, for ABO 100 it was 31 % and for ABO 200 it was 29 %. Apparent ash digestibility of the positive control (43 %) and ABO 50 (42 %) was also significantly higher (P<*0.05*) than the apparent ash digestibilities of ABO 100 and ABO 200. The apparent digestibility for organic matter of ABO 50 (77 %), ABO 100 (78 %) and ABO 200 (76 %) was significant lower (P<*0.05*) than that of the negative control (82%). ABO 50 and ABO 100 had significant higher (P<*0.05*) apparent digestibility for organic matter than ABO 200. Supplementation with the ABO374 enzyme did not result in any improvement in the apparent metabolisable energy. The apparent gross energy digestibility for the negative control (83 %) was significantly higher (P<*0.05*) than those of the ABO 50 (79 %), ABO 100 (81 %) and ABO 200 (77 %) diets. ABO 100 had a significant higher (P<*0.05*) apparent digestibility than ABO 200.

The apparent crude protein digestibility of the negative control (73 %) was significantly higher than those of the positive control (67 %), ABO 50 (65 %), ABO 100 (64 %) and ABO 200 (59 %) diets. ABO 200 had a significantly lower (P<*0.05*) apparent crude protein digestibility than the positive control, ABO 50 and ABO 100 diets. The positive control and ABO 50 diets had significant lower (P<*0.05*) apparent crude fibre digestibilities than the negative control, ABO 100 and ABO 200 diets. The positive control had an apparent crude fibre digestibility that was significantly higher (P<*0.05*) than that of the ABO 50 diet. The negative control, positive control, ABO 50, ABO 100 and ABO 200 had apparent crude fibre digestibilities of 45 %, 35 %, 21 %, 43 % and 37 % respectively.

Table 24 Mean apparent digestibility coefficients of nutrients in broiler chickens receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme

Treatments	Dry matter	Ash	Organic matter	Gross energy	Crude protein	Crude fibre
Negative control	0.80[a]	0.44[a]	0.82[a]	0.83[a]	0.73[a]	0.45[a]
Positive control	0.78[a]	0.43[a]	0.81[ab]	0.82[ab]	0.67[b]	0.35[c]
ABO 50	0.75[bc]	0.42[a]	0.77[cd]	0.79[cd]	0.65[b]	0.21[b]
ABO 100	0.75[b]	0.31[b]	0.78[bc]	0.81[bd]	0.64[b]	0.43[ad]
ABO 200	0.72[c]	0.29[b]	0.76[d]	0.77[c]	0.59[c]	0.37[ad]

[a – d] Means within a column with common superscripts are not significantly different (P<*0.05*)

The mean apparent digestibility coefficients for the individual amino acids in the broiler chickens fed the dietary treatments at 100 % *ad libitum* are shown in Table 25. Enzyme supplementation with ABO374 did not result in any improvements in the apparent digestibilities of any amino acids. The apparent digestibility for threonine, arginine, valine, lysine, methionine, cysteine and isoleucine for the negative control diet was all significantly higher (P<*0.05*) than the positive control, ABO 50, ABO 100 and ABO 200 diets.

Table 25 Apparent total tract digestibility coefficients for individual amino acids in broiler chickens receiving diets containing no enzyme addition, commercial enzyme or three inclusion levels of test enzyme

Treatments	Threonine	Arginine	Valine	Lysine	Methionine	Cysteine	Isoleucine
Negative control	0.97[a]	0.97[a]	0.93[a]	0.96[a]	0.98[a]	0.94[a]	0.97[a]
Positive control	0.95[b]	0.96[b]	0.88[b]	0.93[b]	0.96[b]	0.83[b]	0.92[b]
ABO 50	0.87[c]	0.95[b]	0.83[c]	0.88[c]	0.92[c]	0.79[c]	0.91[b]
ABO 100	0.87[c]	0.94[c]	0.84[c]	0.91[d]	0.95[d]	0.85[b]	0.88[c]
ABO 200	0.91[d]	0.94[c]	0.73[d]	0.91[d]	0.93[e]	0.80[c]	0.87[d]

[a – e] Means within a column with common superscripts are not significantly different (P>*0.05*)

The findings in this digestibility trial in regard of the individual apparent amino acid digestibility are not in agreement with the results found by other researchers who conducted the same type of trials (Pack *et al*., 1998; Zanella *et al*., 1999; Kidd *et al*., 2001; Iji *et al*., 2003; Jackson, 2004; Cowieson & Ravindran, 2008). According to Irish & Balnave (1993), oligosaccharides cannot be broken down in the broiler's intestine due to the absence α-galactosidase activity.

When the concentration of oligosaccharides in the bird's small intestine is high, it produces an osmotic effect. This leads to fluid retention and increases the feed passage rate, which in turn leads to a negative effect on nutrient absorption (Irish & Balnave, 1993).

A possible reason why enzyme supplementation did not increase nutrient digestibility may be that the breakdown of non-starch polysaccharides by the enzymes led to an increase in the concentration of oligosaccharides in the small intestine of the birds, thus leading to the decrease in nutrient absorption (Irish & Balnave, 1993). Coon *et al.* (1990) conducted a study where they removed the oligosaccharides from soybean meal by means of ethanol. The ethanol-extracted soybean meal was then fed to broilers to determine the nutritional differences due to the removal of the oligosaccharides. They found a significant ($P<0.05$) increase in fibre digestibility and in the metabolisable energy value, this response was attributed to a decrease in feed passage rate and thus an increase in nutrient absorption (Coon *et al*., 1990). The commercial enzyme and ABO374 used in this trial was only analysed for xylanase activity and not for proteolytic activities and therefore we do not know if they have any proteolytic activities. Looking at the results for individual apparent amino acid digestibility, it may be possible that the enzymes used in this trial have no proteolytic activity. This may be another possible reason why no significant improvements in individual apparent amino acid digestibility were found. Researchers who found significant improvements in amino acid digestibility due to enzyme supplementation, have all reported that enzymes used by them had proteolytic activity (Zanella *et al*., 1999; Kocher *et al*., 2002; Meng & Slominski, 2005).

4.5 Conclusion

The objective of this trial was to determine whether an experimental xylanase enzyme (ABO374) product had the potential to increase the digestibility of a broiler feed based on maize and soybean meal as the main protein source. The experimental xylanase enzyme (ABO374) product was also compared to a commercial xylanase enzyme product.

According to the results obtained during this digestibility trial, ABO374 did not improve the apparent digestibility of a commercial maize- soybean meal based grower diet. However, these results cannot explain the improvement found in the production trial due to enzyme addition. The digestibility trial was only conducted on the grower feed that was used in the production trial, no digestibility studies were done on the starter or finisher feeds. The findings in this digestibility trial in regard of the individual apparent amino acid digestibility are not in agreement with the results found by other researchers who conducted the same type of trials (Pack *et al*., 1998; Zanella *et al*., 1999; Kidd *et al*., 2001; Iji *et al*., 2003; Jackson, 2004; Cowieson & Ravindran, 2008). According to Irish & Balnave (1993), oligosaccharides cannot be broken down in the broiler's intestine due to the absence α-galactosidase activity. When the concentration of oligosaccharides in the bird's small intestine is high, it produces an osmotic effect. This leads to fluid retention and increases the feed passage rate, which in turn leads to a negative effect on nutrient absorption (Irish & Balnave, 1993). This may be another possible reason why enzyme supplementation only improved the mean FCR during the starter phase. The starter feed had far less soybean meal than the grower diet, the finisher diet had the highest soybean meal content. Thus, the oligosaccharide content of the starter feed was less than that of the grower and finisher diets. The breakdown of non-starch polysaccharides to oligosaccharides by the

enzymes in the grower and finisher diets could have led to a higher oligosaccharide concentration than that of the oligosaccharide concentration obtained in the small intestine of the birds when the starter feed with enzyme was fed (Irish & Balnave, 1993).

Coon *et al.* (1990) conducted a study where they removed the oligosaccharides from soybean meal by means of ethanol. The ethanol-extracted soybean meal was then fed to broilers to determine the nutritional differences due to the removal of the oligosaccharides. They found a significant ($P<0.05$) increase in fibre digestibility and in the metabolisable energy value, this response was attributed to a decrease in feed passage rate and thus an increase in nutrient absorption (Coon *et al*., 1990).

Further research which could be done on ABO374 would be the replacement soybean meal with various levels of sunflower oilcake. The sole reason being that the oligosaccharide content of soybean meal is much more than the oligosaccharide content of sunflower oilcake. It is also possible that better results can be found by testing it on starter feed used in the production trial, this could show whether the increase in oligosaccharide content had an influence on enzyme action,because the starter feed contained less soybean meal than the grower feed.

4.6 References

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CHAPTER 5

GENERAL CONCLUSION

The primary objective of this thesis was to evaluate whether a test enzyme (ABO374), containing mainly xylanase activity, had the potential to improve the digestibility of a maize-SBM based broiler diet and thus the performance of broilers that were fed these diets. The test enzyme was also compared to a commercially available xylanase enzyme.

In the production trial the test xylanase enzyme ABO374 outperformed the commercial enzyme when it was added at 2671mL/ton with a xylanase activity of 1426.86nkat/mL. With the starter feed FCR and the overall FCR being significantly ($P<0.05$) improved by the application of ABO374 to the feed, it is possible to reduce the amount of feed being used for production and therefore reducing the production cost per bird. The results obtained during the digestibility cannot explain the improvement found in the production trial due to the addition of the test enzyme. The digestibility trial was only conducted on the grower feed that was used in the production trial and this may explain why the results obtained during the starter and finisher phases in the growth trial could not be explained by the results of the digestibility trial. Pelletisation of the grower feed could also have had a negative effect on the enzyme action in the feed. The enzyme could have denatured during pelletisation which would have led to the inactivation of the enzyme.

As mentioned before, the oligosaccharide content of soybean meal is higher than that of sunflower oilcake. Further research on ABO374 test enzyme could include the replacement of soybean meal with various levels of sunflower oilcake. The ABO374 enzyme can be improved by making it heat stable. Feed conversion ratio improvement due to enzyme supplementation was only noticed during the starter phase, indicating that the pelletisation of the feed could have had a negative effect on the enzyme activity in the feed. Future digestibility studies regarding ABO374 should be done on all the feed phase diets. By doing this, it can be determined whether pelletisation has an influence on the enzyme activity in the feed or if an increase in oligosaccharide content had any effect on nutrient absorption.

NSP-degrading enzymes have been on the market for more than 12 years, yet it is only recent that the demand for NSP-degrading enzymes has increased. This increase in demand for these enzymes is linked to the rise in cost of raw materials commonly used in broiler feed. The production of biofuel plays an important role in the cost of maize and the influence thereof will only increase in the future. This will force the producer to turn to alternative raw materials to use in their broiler feed. The problem is that these alternative raw materials are high in fibre. Looking at literature, there is much evidence to support the success of NSP-degrading enzymes to increase the digestibility of high fibre broiler diets. Literature and this thesis also support the successful use of NSP-degrading enzymes in commercial broiler diets (maize-soybean meal based diets) to improve FCR and growth of broilers. Improvement of the FCR leads to less feed being used for broiler production and thus increasing the financial gain

for broiler producers. The ban of antimicrobial growth promoters (AGP) holds a great future for NSP-degrading enzymes. Researchers have shown that NSP-degrading enzymes have beneficial effects on the microflora composition in the gut of broilers and are therefore considered as a replacement for AGP.