EFFICACY OF EXOGENOUS ENZYME SUPPLEMENTATION IN RELEASING METABOLISABLE ENERGY IN BROILER FEEDS

by

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I declare that this thesis that I hereby submit for the degree M.Sc. (Agric) at the University of Pretoria is my own original work and has not previously been submitted by me for degree purposes at any other university.

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ABSTRACT

This study was conducted with the aim to investigate the efficacy of exogenous enzyme supplementation in releasing metabolisable energy in broiler feeds. Two performance trials were conducted during this study. Effects were measured in terms of the body weight gain, feed intake, feed conversion ratio, mortality and production efficiency factor in broilers. Correct interpretation and practical application of the positive effects of exogenous enzyme supplementation to commercial broiler feeds can aid nutritionists to develop nutritionally balanced broiler feeds at lower costs. The negative effects of anti-nutritional factors in broilers feeds can be greatly reduced with the strategic use of exogenous enzyme addition to the feed.

In the first performance trial, four treatment feeds were fed to broilers. The Positive control feed was a balanced diet, formulated according to standard nutrient specifications used by Daybreak Farms, with only the metabolisable energy marginally lower than the standard to ensure that energy was the first limiting nutrient. Nutrient specification met or exceeded recommendations by the NRC (1994). A Negative control feed, similar to the Positive control, was formulated with 0.35 MJ ME / kg feed less than the Positive control. An Avizyme treatment and a Hemicell treatment were formulated similar to the Negative control, with the addition of 0.05% Avizyme and 0.0125% Hemicell to the respective treatments. The addition of Avizyme and Hemicell to the respective treatments was hypothesised to release an additional 0.35 MJ ME / kg feed. Four thousand three hundred and twenty day-old Ross 788 chicks were randomly divided into four treatment groups, each with eight replicates and 135 birds per replicate for the first seven days. After seven days birds were reduced to 126 birds per replicate.

In the second performance trial, five treatment feeds were fed to broilers. The Positive control feed was a balanced diet, formulated according to standard nutrient specifications used by Daybreak Farms, with only the metabolisable energy marginally lower than the standard to ensure that energy was the first limiting nutrient. Nutrient specification met or exceeded recommendations by the NRC (1994). Results of the first performance trial indicated that more than the hypothesised 0.35 MJ ME / kg feed was being released from the feed with exogenous enzyme addition. Subsequently, the difference in metabolisable energy between the Negative control and Positive control treatments were increased for the second trial. A Negative control feed, similar to the Positive control, was formulated with 0.45 MJ ME / kg feed less than the Positive control. An Avizyme treatment and a Hemicell treatment were formulated similar to the Negative control, with the addition of 0.05% Avizyme and 0.0125% Hemicell to the respective treatments.

A Combination treatment was formulated similar to the Negative control, with the addition of both 0.05% Avizyme and 0.0125% Hemicell to the feed. Seven thousand five hundred and sixty day-old Ross 788 chicks were randomly divided into five treatment groups, each with 12 replicates and 126 birds per replicate.

For both trials, birds were housed in environmentally controlled houses with a similar lighting schedule and *ad libitum* access to feed and water. Body weights, feed intake and mortality were recorded weekly for the duration of the five week performance trials.

The data was statistically analysed, using the general linear model function in SAS (Statistical Analysis Systems, 1989; Statistical Analysis Systems, 1994). Fischer's protected test was used for the post hoc multiple comparison test. Repeated tests were included in the model. The confidence interval was set at 95%. Initial body weight was tested as a covariate in all the analyses.

Incorrect dosing of the trial feeds during the first performance trial prevented the evaluation of the treatments for the entire 35 day period. The difference between the Positive control and the Negative control treatments were not large enough to enable the exact determination of the amount of metabolisable energy that the Avizyme released in the feed. It could, however, be concluded that Avizyme addition to a broiler feed increased broiler production efficacy. The Avizyme treatment was contributed at least 0.35 MJ ME / kg feed during the trial released more than 0.35 MJ ME / kg feed during the extended starter phase of the trial. The above mentioned conclusions served as a motivation to increase the metabolisable energy difference between the Positive control and the Negative control treatments for the next performance trial. Addition of Hemicell to the feed contributed 0.35 MJ ME / kg feed over a five week growing period.

With the second performance trial, the Avizyme and Hemicell treatments released an additional 0.45 MJ ME / kg feed, supported by broiler production variables similar to the Positive treatment. The Combination treatment was shown to release more than 0.45 MJ ME / kg feed and significantly increased broiler performance. It was concluded that the combination of Hemicell and Avizyme in a broiler ration had a positive synergistic effect on each other in the young broiler, indicating that exogenous enzymes could be more effective in younger broilers.

Although exogenous enzymes resulted in the release of a significant amount of additional metabolisable energy in the feed, the addition of these enzymes should undergo economical evaluation to ensure that the addition of these enzymes is viable under commercial circumstances. Addition of Avizyme with a calculated energy contribution of 0.35 MJ ME / kg and 0.45 MJ ME / kg to the feed realised an income over feed cost (IOFC) of 25 c / kg live weight and 4 c / kg live weight, respectively, during the five week period. The Hemicell treatment showed a negative IOFC (suggesting that Hemicell inclusion will decrease profit) of 24 c / kg live weight during the first four weeks of the first production trial and an IOFC of 2 c / kg live weight during the five week period of the second trial. The combination of both enzymes in the feed returned an IOFC of 16 c / kg live weight. In general the income over feed cost was the highest during the starter phase because of a higher efficacy of exogenous enzyme addition in younger broilers.

LIST OF ABBREVIATIONS

AGP	:	Antibiotic growth promoter
°C	:	Degrees Celsius
c	:	cents (South Africa)
cm	:	Centimeter
CP	:	Crude protein
FCR	:	Feed conversion ratio
g	:	Gram
GLM	:	General linear model
IOFC	:	Income over feed cost
IU	:	International unit
kg	:	Kilogram
L	:	Litre
LSM	:	Least square mean
m	:	Meter
m^2	:	Square meter
MJ	:	Mega joules

ME	:	Metabolisable energy
PEF	:	Production efficacy factor
rH	:	Relative humidity
R	:	Rand (South African)
RS_1	:	Resistant starch fraction 1
RS_2	:	Resistant starch fraction 2
RS_3	:	Resistant starch fraction 3
SEM	:	Standard error of the mean

Chapter 1

Introduction

Global economic pressures and recent escalations in the prices of livestock feed, land, electricity and fuel caused the commercial broiler industry to become a very marginal operation. Successful broiler farming must be based on scientific principals where even the simplest aspect of the business needs to be thoroughly researched. Broilers have to grow to a uniform, predetermined weight as soon as possible, while consuming the least amount of feed possible, resulting in an efficient feed conversion ratio (FCR). At the same time this objective has to be achieved with a minimum amount of nitrogen wastage (in the form of excreta) while growth variables have to be monitored and controlled to limit the occurrence of metabolic disorders which result from too rapid growth.

There are numerous studies indicating that the addition of different combinations of exogenous enzymes to a maize-soybean based feed improve broiler performance. Amongst other, researchers have found that bird performance (Zanella *et al*., 1999; Yu and Chung, 2004; Cowieson and Adeola, 2005), apparent metabolisable energy (Meng and Slominski, 2005; Saleh *et al*., 2005), ileal protein digestibility (Zanella *et al*., 1999; Cowieson and Adeola, 2005; Meng and Slominski, 2005; Saleh *et al*., 2005) and ileal amino acid digestibility of some amino acids (Zanella *et al*., 1999) improved when adding enzymes to the feed. Similar studies have also been conducted where there has been no significant improvement in apparent metabolisable energy (Scheideler *et al*., 2005), ileal digestibility of energy and nitrogen (Cowiesan and Adeola, 2005), or protein, starch and fat digestibility (Zanella *et al*., 1999; Meng and Slominski, 2005). This suggests that the combination and concentration of enzyme addition as well as the quality of maize and soya may have a significant influence on enzyme efficacy. Maize is the main source of energy in most broiler feeds. Soybeans are a good source of protein with a well-balanced amino acid composition, providing a generous amount of the essential amino acids required by the broiler chicken. There is a multitude of enzymes available on the market to add to feed, all claiming to improve the feed efficiency to some extent. The effective mode of action of enzymes is either to increase the energy availability by deactivating the anti-nutritional characteristics of the feed or by increasing the amino acid digestibility through proteolitic activity. Addition of commercial enzyme preparations is especially of value with younger animals that are not yet producing endogenous enzymes at optimal levels (Classen, 1996).

Chesson (1993) hypothesised that addition of commercial enzyme preparations that increase the solubility of the feed will result in more bacterial fermentation in the gastro-intestinal tract, which, in turn, will lead to a higher metabolisable energy value for the feed. A higher metabolisable energy value will then most likely result in improved growth performance, as long as energy is the most limiting nutrient in the feed. In cases where proteolytic enzymes do not seem to make any advantageous contribution to the performance of broilers, it is possible that the amino acid requirements of the birds are already being met and an increase in amino acid digestibility is of little value (Marsman *et al.*, 1997). Likewise, where energy releasing enzymes do not seem to make any advantageous contribution to the performance of the broilers, the energy requirement of the birds could already be met by the feed without any enzymes, or the feed might be deficient in other nutrients.

The purpose of this study was to determine the efficacy of two commercial feed enzymes, Avizyme 1502 and Hemicell, alone and in combination in releasing metabolisable energy in broiler feeds and the effect of these feed enzyme inclusions on broiler performance. The zero hypothesis is that addition of feed enzymes to the feed will not result in a significantly higher energy availability from the feed and similar broiler performance, compared to a control diet with no enzyme added. The alternative hypothesis is that addition of exogenous enzymes to the feed will result in a higher energy availability from the feed and better broiler performance, compared to a control diet with no enzyme added.

Chapter 2

Literature review

The application of different commercial enzyme preparations to broiler feeds

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Abstract

This review provides an overview on existing literature on the effects of different commercial enzymatic preparations on the production of modern broilers. Special focus was placed upon the efficacy of commercial enzymes in counteracting and neutralising the anti-nutritional effects of certain compounds, especially those found in soybean meals. These anti-nutritional factors are compounds mostly found in the hemicellulose in the cell wall of raw materials. Some of the raw materials in which these compounds are found supply either large amounts of energy or are excellent sources of essential amino acids in a well-balanced ration. It is thus clear that these raw materials cannot be left out of the ration because of their anti-nutritional characteristics, but should rather be enzymatically enhanced in order to decrease the viscosity of the raw material for improved nutrient uptake, feed conversion ratio and weight gain in the broiler.

2.1. Introduction

The aim of this review is to investigate the effect of different combinations of enzymes in broiler feeds on broiler production. The specific enzymes under investigation are β-mannanase, protease, amylase and xylanase. Commercial enzyme preparations offer these enzymes in a range of different combinations. In this review, special attention has been paid to Hemicell (ChemGen Corp., Gathersburg, USA; with β-mannanase as the active enzyme) and Avizyme (Danisco Animal Nutrition, Wiltshire, UK; with protease, amylase and xylanase as the active enzymes), as these were the enzyme preparations that were used in the subsequent research trial.

2.2. Soybean digestibility in broilers

Second only to maize, soybeans and soybean byproducts are the most common raw materials included in South African broiler rations. Tahir *et al*. (2006) claimed that a maize-soybean diet is the most common broiler ration worldwide. The maize component of the ration supplies most of the energy of the diet, while the soybean component of the ration supplies most of the protein (McEllhiney, 1994). Soybean meal contains several anti-nutritional factors, including trypsin inhibitors, ureases, goitrogens, antivitamins, phytates, saponins, estrogens and non-starch polysaccharides (Odetallah *et al*., 2002).

Most of the anti-nutritional factors in soybean meal, but especially trypsin inhibitor are neutralised by proper heat processing prior to feeding. There are, however, some of the anti-nutritional factors that are heat resistant. Different strategies have to be implemented to eliminate these anti-nutritional factors. Exogenous enzyme application is one such strategy that has proven successful.

The presence of non-starch polysaccharides in the cell wall of products such as soybean decreases the digestibility of both energy and protein. Non-starch polysaccharides in soybean meal decrease the metabolisable energy considerably (Pierson *et al*., 1980). Non-starch polysaccharides increase the digesta viscosity, causing decreased digestibility of starch, protein and fat in the diet (Choct and Annison, 1990). A number of studies have revealed that addition of exogenous enzymes to broiler diets known to be high in non-starch polysaccharides, have improved the nutritive value of the diet to the broilers (Cowiesan, 2005; Juanpere *et al*., 2005; Meng *et al*., 2005). Zanella *et al*. (1999), Meng and Slominski (2005) and Saleh *et al*. (2005) found a slight improvement in non-starch polysaccharide digestibility with exogenous enzyme addition. The improved nutritive value of the diets were due to the reducing action of the exogenous enzymes on the anti-nutritional effect of the non-starch polysaccharides (Preston *et al*., 2001; Choct *et al*., 2004).

Although soybean meal is included in broiler diets as the main protein source, it still contains up to 40% total carbohydrates and contains 15 to 22% polysaccharides (Odetallah *et al*., 2002). The polysaccharide portion of the soybean meal is commonly divided into eight to ten percent acidic polysaccharides, five percent arabinogalactans, one to two percent cellulosic materials (MacMasters *et al*., 1941; Honing and Rackis, 1979) and one to two percent heat resistant anti-nutritional mannans (Dierick, 1989). β-Mannans are a group of closely related compounds in soybeans that are extremely heat resistant. The consequence is that β-mannans retain their anti-nutritional characteristics in broiler feeds, even after heat processing of the soybean meal (Dale, 1997). The β-mannan and β-galactomannan content of soybean meal is 1.3 – 1.7% and 1.83 – 2.22%, respectively (Dierick, 1989).

The highly viscous properties of mannans, that are mainly associated with the hull and fibre fractions of the soybean meal, make them an extreme anti-nutritional factor (Reid, 1985; Odetallah *et al*., 2002). The protein percentage of soybean meal can serve as an indicator of its quality. Odetallah *et al*. (2002) stated that the two most common commercial soybean meals contain 44% and 48% protein, respectively. A soybean meal with a lower protein percentage (lower quality) is usually more fibrous. A more fibrous soybean meal will result in a relatively higher mannan content, because most of the mannans are located in the hull and fibre fractions of the soybean meal. Odetallah *et al*. (2002) based his hypothesis that exogenous supplementation of a β-mannanase enzyme will show more dramatic results when a poorer quality soybean meal is used, on the above.

The high viscosity of β-mannans creates a partial blockage of the receptor sites on intestinal surface, decreasing the utilisation of carbohydrates (Dale, 1997). The reduced utilisation of carbohydrates causes a poorer feed conversion ratio. Jackson *et al*. (1999) has demonstrated what a strong anti-nutritional factor mannans are to monogastric animals by using guar gum (contains galactomannan). Including guar meal at two to four percent in a feed resulted in reduced growth and poorer feed conversion ratios in broilers (Couch *et al*., 1967; Ray *et al*., 1982; Verma and McNab, 1982). Leeds *et al*. (1980) found β-galactomannan to interfere with glucose metabolism and insulin secretion rates in pigs. A possible strategy for neutralising the anti-nutritional effect of β-mannans, is to add an exogenous β-D-mannanase enzyme.

β-Mannan is a linear structure of repeating β-1,4-mannose, β-1,6-galactose and glucose units attached to a mannan backbone (Odetallah *et al*., 2002). Chanzy and Voung (1985) have found that mannan heteropolysaccharides like glucomannan, galactomannan and galactoglucomannan commonly form associated structures with cellulose and cellulose-like polymers.

Chesson (2001) determined that approximately ten percent of the protein in soybean is trapped in the cell wall matrix and is unavailable to the broiler digestive system. These entrapped proteins can be made available to the broiler's digestive system by exogenous enzyme addition to the diet (Mandels, 1985). The crude protein and energy digestibility have been improved significantly by the addition of carbohydrases to a maize-soybean based ration (Brenes *et al*., 1993; Frigård *et al*., 1994; McKnight, 1997;

Bedford and Schulze, 1998; Oloffs *et al*., 1999; Mathlouthi *et al*., 2003; Saki *et al*., 2005; Tahir *et al*., 2006). Addition of multi-enzyme preparations that included hemicellulase to poultry feed, showed an overall improvement in poultry performance (Steenfeldt *et al*., 1998; Kocher *et al*., 2000; Malathi and Devegowda, 2001). In further studies, Kocher *et al*. (2002) revealed that the improvement in broiler performance, found with multi-enzyme additions, is most likely due to an increased crude protein and energy digestibility of the soybean meal component of the diet.

2.3. Starch composition and digestibility in broilers

South African broiler diets contain maize as the main ingredient and as the major energy source. Maize comprises mostly of starch. Since maize is the main ingredient in modern broiler diets, starch provides more than half of the energy requirements of the modern broiler chicken and the typical broiler diet consists of 36% starch (Weurding, 2002). Starch is built up entirely of glucose molecules, linked by α-bonds. Starch granules contain two different glucose polymers, namely amylose and amylopectin.

Cereal grains are divided into a pericarp, the germ and the endosperm. The pericarp is found on the outside and helps to protect the kernel. The pericarp and germ contain almost no starch and form the minority of the kernel (Kotarski *et al*., 1992). The endosperm makes up the majority of the kernel (approximately 80% of the total weight) and most of the starch is found in this area. The cell walls of the endosperm cells surround the starch granules that are embedded in a protein matrix (McAllister and Cheng, 1996). The endosperm of maize can be differentiated into two regions, namely, a floury and a horny endosperm. Starch is loosely associated with protein in the floury endosperm, while starch is tightly embedded in the protein matrix in the horny endosperm region (Hoseney, 1986). Weurding (2002) reported that the negative effect of the protein matrix on starch digestibility was highlighted when Michalet-Doreau and Champion (1995) found that the more loosely packed starch in the floury endosperm maize varieties was more digestible than the more protected starch in the horny endosperm maize varieties.

Zobel (1988) illustrated that amylose has a linear structure, consisting of glucose units linked by α-1,4 bonds. The length of amylose chains varies from four to one hundred glucose units. On average, cereal starches contain 25% amylose (Weurding, 2002). Amylose is considered to be less digestible than amylopectin. The linear structure of amylose in comparison to the branched structure of amylopectin creates a smaller surface area per molecule for enzymes to attach to the molecule and for digestion.

Amylose also contains hydrogen bonds between glucose chains (Weurding, 2002), which make this structure even less susceptible to enzymatic hydrolyses. Sievert and Pomeranz (1989) have indicated that the resistant starch content (as a percentage of dry matter) increases with the amylose content. Increasing the broiler's capacity to digest amylose by the supplementation of exogenous amylase in the feed could possibly overcome the negative effects of a high amylose content in the starch.

Amylopectin has a branched structure of short α-linked glucose chains (similar to amylose structure) which are bound together by α-1,6 bonds. The average amylopectin structure consists of 20 glucose units, with the α-1-6 bonds making up approximately five percent of the total glycosidic bonds (Gallant *et al.*, 1992). Amylopectin is responsible for the crystalline structure of the starch granule (Imberty *et al*., 1991). Amylopectin can be divided into three different types, each with a unique susceptibility to enzymatic hydrolysis (Weurding, 2002). The ratio of amylose and amylopectin vary in different cultivars of maize, giving rise to terms such as waxy maize (high amylopectin content) and amylomaize (high amylose content).

Maize also contains cellulose. Weurding (2002) pointed out that cellulose is entirely built up of glucose molecules, but these molecules are linked by β-bonds, which cannot be hydrolysed by the broiler's enzymes. The cellulose content in maize is, however, not high enough to have a great influence on starch digestion. Non-starch polysaccharides in cereals, like β-glucans and arabinoxylans can affect the starch digestion in broilers and other monogastric animals. These polysaccharides serve as physical barriers that inhibit enzyme accessibility to starch granules and increase the viscosity of the digesta, resulting in reduced diffusion rate of enzymes, an increased feed passage time and decreased digestibility of the starch and feed (Classen, 1996; Refstie *et al*., 1999). Although these anti-nutritional factors are present in varying amounts in grains (Classen, 1996), the presence of these molecules can be treated with the supplementation of exogenous enzymes like xylanase.

In broilers, starch can be digested in two different ways. Firstly, starch can be digested by amylolytic enzymes in the small intestine. Starch that escapes digestion in the small intestine can be microbially fermented in the caeca to volatile fatty acids, methane, hydrogen and carbon dioxide. Volatile fatty acids make up 90% of the fermentation products (Weurding, 2002). The aim should be to ensure that all the starch is digested and absorbed in the small intestine, because of two reasons. Firstly, energy is lost as heat during volatile fatty acid production and in products like methane, hydrogen and carbon dioxide. Secondly, the efficiency of utilisation of glucose is higher than the efficiency of utilisation of volatile fatty acids. Dierick *et al.* (1989) supported the above mentioned statements by claiming that hind gut fermentation of starch results in less net energy than digestion of starch in the small intestine. The digestibility of starch is affected by the starch structure and composition (Oates, 1997) and the associations between the starch granules and protein and cell wall structures in the feed (Eastwood, 1992). Weurding (2002) noted that the protein matrix around the starch granules as well as the non-starch polysaccharide fraction reduce the accessibility of enzymes to the starch granules. An exogenous protease enzyme supplementation could assist in digesting the protein matrix and making the starch granules more accessible to the amylolytic enzymes.

Weurding (2002) explained the process of starch digestion in poultry and stated that no enzymatic hydrolysis of starch occurs prior to the stomach. Poultry do not produce salivary α-amylase. Feed is passed from the mouth to the crop and proventriculus. From the proventriculus feed is moved into the gizzard where it is ground before passing into the small intestine. The pancreas secrete α-amylase into the lumen of the small intestine. The optimal activity of pancreatic α-amylase in the jejunum occurs at a pH of 6.9 (Rogel *et al*., 1987). Starch digestion can, however, only commence once α-amylase reaches the unprotected starch molecules. The protein matrix and non-starch polysaccharides will delay or even inhibit complete starch digestion in the small intestine. The broiler does not have enzymes, such as arabinoxylans and β-glucanase to digest non-starch polysaccharides (Weurding, 2002). After the α-amylase attaches to the amylose, amylose is degraded to maltose and maltotriose. Amylopectin is degraded to maltose, maltotriose and α-dextrins. Moran (1982) described the detailed digestion of amylopectin to glucose, where it is finally absorbed through the intestinal wall as an energy source for body tissues. When not utilised immediately, it will be stored as glycogen in muscle and the liver or as fat in adipose tissue.

As previously mentioned, some of the starch might be protected from α-amylase degradation by protein matrixes and non-starch polysaccharides. This starch will pass through the small intestine and is termed resistant starch (Englyst *et al*., 1982). The European Resistant Starch research group defined resistant starch as "… the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). Resistant starch can result from a number of factors, including heat processing, an interaction with other nutrients (Bedford, 1996) and the starch granule structure (Tester *et al*., 2004). Resistant starch can decrease the apparent metabolisable energy of a feed significantly (Rutherfurd *et al*., 2007). Resistant starch can be microbially fermented in the caeca and colon to volatile fatty acids (particularly acetate, propionate and butyrate), methane, hydrogen and carbon dioxide. Volatile fatty acid production and absorption in the hind gut have a beneficial effect on health in the large intestine by inhibiting pathogen growth, increasing fluid and electrolyte absorption and reducing the intestinal pH (Weurding, 2002). The production of volatile fatty acids from the fermentation of starch is, however, less energy efficient than complete digestion of starch in the small intestine (Dierick *et al.* 1989). Englyst *et al*. (1992) defined three different resistant starch fractions (RS_1, RS_2 and RS_3, respectively). RS_1 is the physically inaccessibly starch. In RS_1, plant cell walls and proteins protect the starch from hydrolysis. This fraction is larger in coarsely ground maize. RS_2 is the resistant starch granules, found in unheated feed or feed that has been heated under low moisture conditions. RS_3 is retrograded starch (mainly retrograded amylose), found in feed that has been heated under high moisture and high temperature conditions and / or feed that has been subjected to more than one heat treatment. RS_1 can increase the resistance of starch to enzymatic hydrolysis (Annison and Topping, 1994).

2.4. β-mannanase and Hemicellulase

Soybeans contain a number of anti-nutritional factors, which result in a lower digestibility and reduced performance in the broiler. One of these anti-nutritional factors, namely mannan, is incorporated in the soybean cell wall as a component of hemicellulose. One of the main reasons that mannan is considered to be such a nutritional constraint is because of its extremely high viscosity in solution (Centeno *et al.*, 2006). Bedford and Classen (1992) showed that non-starch polysaccharides will result in an increased FCR and reduce the efficiency of nutrient utilisation by affecting the rate of diffusion of substrates, nutrients and digestive enzymes. The backbone structure of mannan comprises β-1,4-linked mannose residues (Nelson and Fodge, 1996) that can be broken down by adding β-mannanase to the feed.

β-mannanase is the primary active ingredient in Hemicell (ChemGen Corp., Gathersburg, USA). Hemicell is an endohydrolase enzyme (mannan endo-1,4-β-mannosidase), extracted as a fermentation product from *Bacillus lentus* (Odetallah *et al*., 2002). This enzyme degrades β-mannans, neutralising the anti-nutritional effect that β-mannans have in monogastric feeds. Hemicell randomly cleaves within the 1,4-β-D-mannan main chain of galactomannan, galactogluco-mannan and mannan (McCleary, 1988)

The positive effects of dietary β-mannanase supplementation in maize-soya based diets have already been widely studied. Positive effects include improved feed conversion ratios in pigs (Hahn *et al*., 1995; Chen *et al*., 1998) and broilers (Nelson and Fodge, 1996), increased egg weight and increased total egg production in layers (Jackson *et al*., 1999) and improved growth rates and feed conversion ratios in turkeys (Odetallah *et al*., 2002).

In an experiment conducted by McNaughten *et al*. (1998), broilers were fed a treatment feed that had an energy content of 12.57, 12.90 and 13.22 MJ / kg for the starter, grower and finisher, respectively. β-Mannanase was added to all phases of the treatment feed. Broilers in the control group were fed a similar feed, except that the energy content was 13.17, 13.50 and 13.82 MJ / kg for the starter, grower and finisher, respectively. Broilers in the treatment group performed slightly better than the broilers in the control group. This proved that the addition of β-mannanase to a diet can increase the energy availability by up to 0.6 MJ / kg.

The commercial enzyme preparation Ronozyme® VP shows a very high mannanase activity (Centeno *et al.*, 2006) and therefore has the potential to produce results similar to results where Hemicell is added to feed. In an experiment conducted by Centeno *et al.* (2006), 160 day-old Ross 308 broiler chicks were fed a diet containing 299.9 g / kg soybean meal for 28 days with different enzyme preparations added. It was found that chicks fed the basal diet with 2 g / kg Ronozyme® VP (RON) had significantly higher final body weights after 28 days and a significantly shorter caecum (relative to body weight), compared to broilers fed only the basal diet without the addition of any commercial enzymes (CTRL). The average final body weight after 28 days was 1161 g and 1214 g for the CTRL and RON broilers, respectively. The relative length of the caeca of the broilers after 28 days was 1.448 cm / 100 g body weight and 1.206 cm / 100 g body weight for the CTRL and RON broilers, respectively. The FCR of the broilers over the 28 day period was 2.29 g feed / g weight gain and 2.11 g feed / g weight gain for the CTRL and RON broilers, respectively, although these results were not significantly different.

The higher body weight was attributed to the β-mannanase activity that hydrolysed the mannan in the hemicelluloses of the cell wall, resulting in a lower viscosity of the feed, and therefore a better nutrient utilisation and ultimate production efficiency. The shortened caecum was proposed to be, firstly, due to less roughage passing through the lower digestive tract, which lowers bacterial fermentation activity in the caeca. Secondly, less roughage moving through the lower digestive tract resulted in less muscular development in the surrounding location in the body.

Saleh *et al.* (2003) conducted a range of *in vitro* digestibility trails and found that hemicellulase had no effect on the crude protein and dry matter digestibility of maize. It was further also found that hemicellulase had no significant effect on the dry matter digestibility of maize. When evaluating the viscosity of maize after the addition of hemicellulase, no significant alteration was recorded.

In a similar trial, Saleh *et al.* (2003) conducted a range of *in vitro* digestibility trials on a number of individual enzymes instead of the conventional testing of commercial enzymatic combination preparations on soybean meal. This was done in an effort to determine the optimum concentration of enzyme that should be added to a feed. The hemicellulase enzyme was purified from *Aspergillus niger* and it was found that the optimum concentration of hemicellulase was 1 IU. A 0.1 g sample of ground soybean meal was subjected to a typical *in vitro* two phase (gastric and peptic phases) gastro-intestinal digestibility simulation. Hemicellulase was found to significantly improve the crude protein digestibility of the soybean meal. However, the supplementation of hemicellulase did not affect the dry matter digestibility of soybean meal. Viscosity was significantly improved in both digestive phases with the addition of hemicellulase.

2.5. Protease

Karimi *et al.* (2007) formulated a feed containing 645.1 g / kg wheat and 274.1 g / kg soybean meal as a base diet and fed 120 broiler chicks for 19 days. The base diet was fed without any commercial enzyme added as a control feed. A second feed comprised the base feed with the addition of 1 g / kg Avizyme 1300. Weight records for day 15 and day 19 showed that the addition of the enzymatic preparation to the ration had a significant effect on the live weight of the chicks. A significant difference in weight gain between days 10 to 15 was also found between the control and the treatment groups. Feed conversion ratios were significantly improved for days 15 – 19. No significant differences were found in the total length of the small intestine, nor in the weight of the small intestine and gizzard relative to total body weight.

Saleh *et al.* (2003) found that protease had no effect on the *in vitro* digestibility of crude protein and dry matter digestibility in maize. Also, the viscosity of maize did not change after the addition of protease.

The *in vitro* digestibility trials using different individual enzymes was referred to earlier (see β-mannanase). In this trial the protease enzyme was purified from *Aspergillus saitoi* and it was found that the optimum concentration of protease was 0.9 IU. Protein digestibility of the protease fortified soybean meal was evaluated using the method prescribed by Saunders *et al.* (1973). Protease was not found to significantly improve the crude protein digestibility of the soybean meal. Hessing *et al.* (1996) showed that the apparent ileal nitrogen digestibility could be significantly improved in chicks with the addition of acid protease to the ration. Saleh e*t al.* (2003) pointed out that these results are contradictory to the results of Sakomura *et al.* (1998), who concluded that addition of the commercial enzyme preparation, Avizyme® 1500 to a maize and soybean ration, with protease as one of the active enzymes, did not improve the ileal protein, energy, starch and fat digestibility of the feed. The supplementation of protease, however, did not affect the dry matter digestibility of soybean meal. Viscosity was significantly improved in the peptic phase of digestibility, but only slightly in the pancreatic phase with the addition of protease. Saleh *et al.* (2003) also found that protease did not significantly increase the dry matter digestibility of soybean meal.

Mahagna *et al.* (1995) showed that the addition of protease to a sorghum-soybean diet did not have a significant effect on the digestibility of fat, starch or protein. The addition of protease also did not seem to significantly change the energy values of the feed.

Marsman *et al.* (1997) conducted an experiment to determine the effect of two commercial enzyme preparations in soybean meals on the productivity of broilers. Five hundred and twenty day-old female Ross broiler chicks were fed a basal feed of 382.5 g / kg soybean meal and performance variables were measured from seven days to 25 days of age. One hundred and thirty chicks received the basal feed without any commercial enzymes added and 130 chicks received the basal diet with the addition of Neutrase® (Novozymes A/S, Krogshoejvej, 2880 Bagsvaerd, Denmark), a commercial protease enzyme preparation. The Neutrase (NEU) chicks did not show significantly different body weight gain, feed intake or FCR. Body weight gain, feed intake and FCR were 1321 g, 2104 g and 1.59 g / g, respectively, for the control group and 1316 g, 2106 g and 1.60 g / g, respectively, for the NEU chicks. Apparent ileal crude protein and ileal non-starch polysaccharide digestibility were, however, significantly increased in the NEU chicks and were 83.7% and 14.5%, respectively, for the control group and 85.6% and 18.3%, respectively, for the NEU chicks . Enzymatic treatment did not have a significant effect on the chyme viscosity or on the concentration of soluble non-starch polysaccharides in the chyme. The chyme viscosity was 3.53 cP and 3.35 cP for the control group and NEU group, respectively, and the concentration of soluble non-starch polysaccharides was 12.6% and 10.5% for the control and the NEU groups, respectively.

Marsman *et al.* (1997) commented that failure of dietary enzymes to enhance broiler productive performance could be an indication that the diet already fulfills in the maximum crude protein requirement of the broiler.

Café *et al.* (2002) fed 6240 day-old male Cobb 500 broiler chicks a maize-soybean meal based feed for 49 days. Three thousand one hundred and twenty chicks received the ration without the addition of any commercial enzyme preparation and the other 3120 chicks received the feed with the addition of 1 g / kg Avizyme 1500, with protease as one of the active ingredients (AVI). Production variables were measured at 16, 35, 42 and 49 days, with 480 chicks selected randomly from the pens and slaughtered at days 35, 42 and 49 to determine dressing percentage and yield percentages from the different body parts (Table 1). The mean body weight of the AVI treatment group was significantly improved for days 16, 35 and 49. Unexpected results were obtained from the FCR measurements, with the AVI chicks showing a significantly poorer FCR for days 16 and 42. These results are contradictory to the findings of Wyatt *et al.* (1997), who claimed that FCR is significantly improved when adding Avizyme to a sorghum-soybean basis meal. With the addition of Avizyme to a maize-soybean meal based diet, Zanella *et al.* (1999) found significant improvement in FCR and body weight gain in one trial and no significant difference for the same variables in another trial. This is an indication that the quality of raw material could have a marked influence on the efficacy of commercial enzymatic treatments. Interestingly, the AVI treatment group in the trial of Café *et al*. (2002) had significantly higher proportions of abdominal fat at days 42 and 49, showing that more energy was potentially available in the AVI rations. Mortality was significantly lower for the AVI group. The dressing percentage was only significantly higher for the AVI treatment group on day 42. Proportional dressing percentages for breast yield, leg quarters and wings did not differ significantly between the two groups at any of the slaughter ages.

Zanella *et al.* (1999) found the addition of 1 g / kg Avizyme to a maize-soybean based meal to significantly improve the crude protein digestibility by 2.9%, the starch digestibility by 1.8% and the fat digestibility by 2.5%. The addition of protease to the maize portion of the feed did not have a significant effect on the viscosity. It was, however, found that the improvement in crude protein digestibility did not correspond to an improvement in amino acid digestibility. Digestibility of lysine, methionine and arginine did not increase, although valine and threonine digestibility increased significantly. The percentage of abdominal fat, breast weight relative to total body weight and the dressing percentage did not differ significantly between treatments.

Table 1 Production parameters of broilers at day 16, 35, 42 and 49 of production for a ration supplemented with Avizyme 1500 compared to a control feed [adapted from Café *et al.* (2002)]

	Control	Avizyme	Improvement
Day 16			
Body weight (g)	461[a]	475[b]	+ 3%
FCR (g / g)	1.230[a]	1.248[b]	- 1%
Mortality rate (%)	1.02[b]	0.32[a]	+ 1%
Day 35			
Body weight (g)	1 758[a]	1 787[b]	+ 2%
FCR (g / g)	1.598	1.599	0%
Mortality rate (%)	1.90[b]	1.06[a]	+ 1%
Dressing percentage (%)	69.84	69.82	0
Breast yield (%)	22.17	22.12	0%
Leg quarters (%)	34.77	34.97	0%
Wings (%)	12.19	12.18	0%
Abdominal fat (%)	2.43	2.61	0%
Day 42			
Body weight (g)	2 224	2 213	+ 0%
FCR (g / g)	1.874[a]	1.906[b]	- 2%
Mortality rate (%)	1.98[b]	1.25[a]	+ 1%
Dressing percentage (%)	70.20	71.07	+ 1%
Breast yield (%)	21.68	21.38	- 1%
Leg quarters (%)	33.15	33.10	0%
Wings (%)	12.02	12.07	0%
Abdominal fat (%)	2.28[a]	2.42[b]	- 0.1%
Day 49			
Body weight (g)	2 705[a]	2 748[b]	+ 2%
FCR (g / g)	2.122	2.134	- 1%
Mortality rate (%)	2.59	2.08	+ 1%
Dressing percentage (%)	68.70	69.04	0%
Breast yield (%)	21.73	21.72	0%
Leg quarters (%)	32.93	33.17	+ 1%
Wings (%)	11.79	11.83	0%
Abdominal fat (%)	2.19[a]	2.48[b]	- 0.3%

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Douglas *et al.* (2000) added 1 g / kg Avizyme to a soybean meal-based broiler feed and found no significant improvement in body weight gain or in FCR. Ileal digestible energy was increased by the addition of Avizyme. The increase in ileal digestible energy was significantly greater when a soybean meal with a lower ileal digestible energy was used in the basal feed. This indicates, once again, that the quality of the raw materials used in the ration will determine the efficacy of the commercial enzyme preparation.

The net protein ratio of solvent extracted soybean meal was increased significantly in broiler feeds by the addition of an enzymatic combination including protease (Wiryawan *et al.*, 1997). Wiryawan and Dingle (1999) hypothesised that the net protein ratio was increased because protease, amongst other enzymatic reactions, was responsible for digesting the protein that was exposed after the cell wall break down by xylanase. The starch digestibility will also be increased if protease releases starch from starch-protein matrices that are found in the feed.

Zanella *et al.* (1999) fed 1440 day-old Hubbard males six different rations, i.e. a soybean meal based feed containing no commercial enzymes (SBM –), a soybean meal based feed containing 1 g / kg Avizyme® 1500 (SBM +), an extruded full fat soybean based feed containing no commercial enzymes (FSE –), an extruded full fat soybean based diet containing 1 g / kg Avizyme® 1500 (FSE +), a roasted full fat soybean based diet containing no commercial enzymes (FSR –) and a roasted full fat soybean based diet containing 1 g / kg Avizyme® 1500 (FSR +). The SBM, FSE and FSR treatments contained 45, 38 and 37% crude protein, respectively, and all the feeds contained 12.6 MJ / kg. Avizyme® 1500, at an inclusion rate of 1g / kg contained 800 μ / g xylanase (extracted from *Trichoderma longibrachiatum*), 6 000 μ / g protease (extracted from *Bacillus subtilis*) and 2 000 μ / g amylase (extracted from *Bacillus amyloliquifaciens*). In a second trial, the same feeds and enzymatic treatments were fed, but with a lower energy level to account for the positive results obtained from enzymatic addition to the ration. In the first trial it was found that the addition of enzymes to the feeds significantly increased the 45 day body weight of the chicks and significantly improved the FCR, but the mortality, viscosity of the intestinal contents, carcass weight, abdominal fat, breast weight and pancreas weight were not significantly affected by the addition of commercial enzyme to the feed (Table 2). None of the production variables were significantly different between the enzyme treatments and the control treatments in the second trial (Table 3), although the enzyme treatments had lower energy levels than the control treatments. This indicates that energy levels can be reduced to lower levels when enzymes are added to the diet. The above mentioned statement indicates that enzymatic addition has a certain energy value due to increased energy availability from the ration.

Table 2 Production parameters for broilers at 45 days of age on a soybean meal (SBM), an extruded full fat soybean based (FSE), a roasted full fat soybean based (FSR) ration and the mean of all the rations (Σ) supplemented with Avizyme® 1500 (+) and without any enzymes added (–). Energy levels were similar for all diets [adapted from Zanella *et al*. (1999)]

	Body weight gain (kg)	FCR (g / g)	Mortality (%)	Viscosity (cps)	Abdominal fat (% of carcass weight)	Breast weight (% of carcass)	Dressing %	Pancreas weight (g)
SBM –	2.62	1.87	6.05	2.3	3.33	27.8	72.2	4.61
SBM +	2.69	1.82	4.03	2.5	3.26	27.6	73.0	4.27
FSE –	2.68	1.82	3.63	2.7	3.57	27.7	72.3	4.83
FSE +	2.73	1.80	6.45	2.8	3.09	27.3	72.5	4.71
FSR –	2.64	1.88	6.86	2.5	3.21	27.0	72.5	4.58
FSR +	2.69	1.84	4.61	2.4	3.05	27.3	72.6	4.13
Σ –	2.65[a]	1.86[a]	5.51	2.5	3.37	27.5	72.3	4.67
Σ +	2.70[b]	1.82[b]	4.99	2.6	3.13	27.4	72.7	4.37
Improvement	+ 2%	+ 2%	+ 1%	- 4%	0%	0%	0%	+ 6%

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05)

Table 3 Production parameters for broilers at 45 days of age on a soybean meal (SBM), an extruded full fat soybean based (FSE), a roasted full fat soybean based (FSR) rations and the mean of all the rations (Σ) supplemented with Avizyme® 1500 (+) and without any enzymes added (–). Energy levels for the enzymatically treated feeds were reduced [adapted from Zanella *et al*. (1999)]

	Body weight gain (kg)	FCR (g / g)	Mortality (%)	Abdominal fat (% of carcass weight)	Breast weight (% of carcass weight)	Dressing %	Pancreas weight (g)
SBM –	2.61	1.76	4.30	2.83	29.0	72.3	3.94
SBM +	2.61	1.77	2.74	3.14	29.3	72.1	3.75
FSE –	2.65	1.76	2.35	3.32	27.8	72.1	3.94
FSE +	2.66	1.76	2.74	3.20	28.6	71.9	4.14
FSR –	2.56	1.81	3.91	3.25	28.6	72.0	4.04
FSR +	2.61	1.81	3.52	2.77	28.3	72.9	3.67
Σ –	2.60	1.78	3.52	3.13	28.5	72.1	3.97
Σ +	2.62	1.78	3.00	3.04	28.7	72.3	3.85
Improvement	+ 1%	0%	+ 1%	0%	0%	0%	+ 3%

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

2.6. Amylase

Karimi *et al.* (2007) formulated a feed containing 645.1 g / kg wheat and 274.1 g / kg soybean meal (44% crude protein on a dry matter basis) as a base diet and fed 120 broiler chicks for 19 days. The base diet was fed without any commercial enzyme added as a control feed. A second feed comprised the base feed with the addition of 1 g / kg Avizyme 1300. Weights and feed intake were recorded on days 1, 5, 10, 15 and 19, respectively. Weight records for day 15 and day 19 showed that the addition of the enzymatic preparation to the ration had a significant effect on the live weight of the chicks. A significant difference in weight gain between days 10 to 15 was also found between the control and the treatment groups. Feed conversion ratios were also significantly improved for days 15 – 19. No significant differences were found in the total length of the small intestine, or in the weight of the small intestine and gizzard relative to total body weight.

Saleh *et al.* (2003) reported that Sakomura *et al.* (1998) mixed the commercial enzyme preparation, Avizyme® 1500, with amylase as one of the active enzymes, into a maize and soybean ration and found that the ileal protein, energy, starch and fat digestibility of the feed is improved.

Mahagna *et al.* (1995) showed that the addition of amylase to a sorghum soybean feed does not have a significant effect on the digestibility of fat, starch or protein.

Café *et al.* (2002) fed Cobb 500 broiler chicks a maize-soybean meal based feed for 49 days. Half of the chicks received the ration without the addition of any commercial enzyme preparation and the other half received the feed with the addition of 1 g / kg Avizyme 1500, with amylase as one of the active ingredients (AVI). Production variables were measured at 16, 35, 42 and 49 days (Table 1). The mean body weight of the AVI treatment group was significantly improved for days 16, 35 and 49. Unexpected results were obtained from the FCR measurements, with the AVI chicks showing a significantly poorer FCR for days 16 and 42. These results are contradictory to the findings of Wyatt *et al.* (1997), who claimed that FCR is significantly improved when adding Avizyme to a sorghum-soybean basis meal. With the addition of Avizyme to a maize-soybean meal basis diet, Zanella *et al.* (1999) found significant improvement in FCR and body weight gain in one trial and no significant difference for the same variables in another trial. This is an indication that the quality of raw material could have a marked influence on the efficacy of commercial enzymatic treatments. Interestingly, the AVI treatment group in the trial of Café *et al*. (2002) had significantly higher proportions of abdominal fat at days 42 and 49, showing that more energy was potentially available in the AVI rations. Mortality was significantly lower for the AVI group. The dressing percentage was only significantly higher for the AVI treatment group on day 42. Proportional dressing percentages for breast yield, leg quarters and wings did not differ significantly between the two groups at any of the slaughter ages.

Zanella *et al.* (1999) found the addition of 1 g / kg Avizyme to a maize-soybean based meal to significantly improve the crude protein digestibility by 2.9%, the starch digestibility by 1.8% and the fat digestibility by 2.5%. The addition of amylase to the maize portion of the feed did not have a significant effect on the viscosity. It was, however, found that the improvement in crude protein digestibility did not correspond to an improvement in amino acid digestibility. There was no increased efficiency in the digestibility of lysine, methionine or arginine, although valine and threonine were digested significantly more efficiently. The percentage of abdominal fat, breast weight relative to total body weight and the dressing percentage did not differ significantly between treatments.

Douglas *et al.* (2000) added 1 g / kg Avizyme to a soybean meal-based broiler feed and found no significant improvement in body weight gain or in FCR. Ileal digestible energy was increased by the addition of Avizyme. The increase in ileal digestible energy was significantly greater when a soybean meal with a lower ileal digestible energy was used in the basal feed. This indicates, once again, that the quality of the raw materials used in the ration will determine the efficiency of the commercial enzyme preparation.

Zanella *et al.* (1999) fed Hubbard males three different rations with and without the addition of Avizyme® 1500 (six treatments). All six treatments had similar energy levels. Avizyme® 1500, at an inclusion rate of 1g / kg contained 800 μ / g xylanase (extracted from *Trichoderma longibrachiatum*), 6 000 μ / g protease (extracted from *Bacillus subtilis*) and 2 000 μ / g amylase (extracted from *Bacillus amyloliquifaciens*). In a second trial, the same feeds and enzymatic treatments were fed, but at lower energy availability to account for the positive results obtained from enzymatic addition to the ration. In the first trial it was found that the addition of enzymes to the feeds significantly increased the 45 day body weight of the chicks and significantly improved the FCR, but the mortality, viscosity of the intestinal contents, carcass weight, abdominal fat, breast weight and pancreas weight were not significantly affected by the addition of commercial enzyme to the feed (Table 2). None of the production variables were significantly different between the enzyme treatments and the control treatments in the second trial (Table 3), although the enzyme treatments had lower energy levels than the control treatments. This indicates that energy levels can be reduced to lower levels when enzymes are added to the diet. The above mentioned statement indicates that enzymatic addition has a certain energy value due to increased energy availability from the ration.

2.7. Xylanase

Xylose residue is the part of the non-starch polysaccharides in the cell wall of wheat products that acts as an anti-nutritional factor (Karimi *et al.*, 2007). It is present at 50 – 80 g / kg in wheat (Annison, 1993) and can be hydrolysed by the xylanase enzyme to lower the viscosity and improve the nutrient intake in the feed.

Another possible method of counteracting some of the anti-nutritional factors in soybeans is to add xylanase enzyme to the feed ration in the form of commercial enzymatic preparations high in xylanase activity, such as Avizyme and Roxazyme® G200 (Centeno *et al.*, 2006). Cone *et al.* (1994) stated that the Xylanase X-250 commercial enzyme preparation increases the nitrogen solubility in soybean meal. These results would be expected to deliver increased productive performance in broilers.

In an experiment conducted by Centeno e*t al.* (2006), 160 day-old Ross 308 broiler chicks were fed a 299.9 g / kg soybean meal (44% crude protein on a dry matter basis) for 28 days with the addition of different enzyme preparations. Chicks were evaluated for weekly body weight, relative caecum length and FCR, but no significant difference was found between chicks fed the basal ration without any enzymatic addition and the chicks fed the same ration with the addition of 1 g / kg Roxazyme® G200. Although the caecum's length relative to the body weight was shorter in the ROX broilers, compared to the CTRL broilers, the results did not differ significantly.

Karimi *et al.* (2007) formulated a feed containing 645.1 g / kg wheat and 274.1 g / kg soybean meal (44% crude protein on a dry matter basis) as a base diet and fed 120 broiler chicks for 19 days. The base diet was fed without any commercial enzyme added as a control feed. A second feed comprised the base feed with the addition of 1 g / kg Avizyme 1300. Weights and feed intake were recorded on days 1, 5, 10, 15 and 19, respectively. Weight records for day 15 and day 19 showed that the addition of the enzymatic preparation to the ration had a significant effect on the live weight of the chicks. A significant difference in weight gain between days 10 to 15 was also found between the control and the treatment groups. Feed conversion ratios were also significantly lower (improved) for days 15 – 19. No significant differences were found in the total length of the small intestine, or in the weight of the small intestine and gizzard relative to total body weight.

Saleh *et al.* (2003) conducted a range of *in vitro* digestibility trails and found that xylanase had no effect on the crude protein digestibility of maize. It was further also found that xylanase had no significant effect on the dry matter digestibility of maize. When evaluating the viscosity of maize after the addition of xylanase, no significant alteration was recorded.

In a similar trial Saleh *et al.* (2003) conducted a range of *in vitro* digestibility trails on a number of individual enzymes instead of the conventional testing of commercial enzymatic combination preparations on soybean meal. This was done in an effort to determine the optimum concentration of enzyme that should be added to a diet. The xylanase enzyme was purified from *Trichoderma viride* and it was found that the optimum concentration of xylanase was 15 U. A 0.1 g sample of ground soybean meal was subjected to a typical *in vitro* two phase (gastric and peptic phases) gastro-intestinal digestibility simulation. Xylanase was found to improve the crude protein digestibility of the soybean meal, but not enough to bring about a significant effect. The supplementation of xylanase only brought about a marginal improvement to the dry matter digestibility of soybean meal. Viscosity was significantly improved in both digestive phases with the addition of xylanase.

Saleh *et al.* (2003) reported that Sakomura *et al.* (1998) added the commercial enzyme preparation Avizyme® 1500 to a maize and soybean ration, with xylanase as one of the active enzymes, and found that the ileal protein, energy, starch and fat digestibility of the diet was improved.

Café *et al.* (2002) fed Cobb 500 broiler chicks a maize-soybean meal based feed for 49 days. Half of the chicks received the ration without the addition of any commercial enzyme preparation and the other half received the feed with the addition of 1 g / kg Avizyme 1500, with amylase as one of the active ingredients (AVI). Production variables were measured at 16, 35, 42 and 49 days (Table 1). The mean body weight of the AVI treatment group was significantly improved for days 16, 35 and 49. Unexpected results were obtained from the FCR measurements, with the AVI chicks showing a significantly poorer FCR for days 16 and 42. These results are contradictory to the findings of Wyatt *et al.* (1997), who claimed that FCR is significantly improved when adding Avizyme to a sorghum-soybean basis meal. With the addition of Avizyme to a maize-soybean meal basis diet, Zanella *et al.* (1999) found significant improvement in FCR and body weight gain in one trial and no significant difference for the same variables in another trial. This is an indication that the quality of raw material could have a marked influence on the efficacy of commercial enzymatic treatments. Interestingly, the AVI treatment group in the trial of Café *et al*. (2002) had significantly higher proportions of abdominal fat at days 42 and 49, showing that more energy was potentially available in the AVI rations. Mortality was significantly lower for the AVI group. The dressing percentage was only significantly higher for the AVI treatment group on day 42. Proportional dressing percentages for breast yield, leg quarters and wings did not differ significantly between the two groups at any of the slaughter ages.

Zanella *et al.* (1999) found the addition of 1 g / kg Avizyme to a maize-soybean based meal to significantly improve the crude protein digestibility by 2.9%, the starch digestibility by 1.8% and the fat digestibility by 2.5%. The addition of xylanase to the maize portion of the feed did not have a significant effect on the viscosity. It was, however, found that the improvement in crude protein digestibility did not correspond to an improvement in amino acid digestibility. There was no increased efficiency in the digestibility of lysine, methionine or arginine, although valine and threonine were digested significantly more efficiently. The percentage of abdominal fat, breast weight relative to total body weight and the dressing percentage did not differ significantly between treatments.

Douglas *et al.* (2000) added 1 g / kg Avizyme to a soybean meal-based broiler feed and found no significant improvement in body weight gain or in FCR. Ileal digestible energy was increased by the addition of Avizyme. The increase in ileal digestible energy was significantly greater when a soybean meal with a lower ileal digestible energy was used in the basal feed. This indicates, once again, that the quality of the raw materials used in the ration will determine the efficiency of the commercial enzyme preparation.

Grindazym GP 5000, a commercial enzyme preparations containing mostly xylanase, was added to 0.6 g samples of barley and rye, respectively at concentrations of 5 g / kg, 10 g / kg and 20 g / kg by Castañón *et al.* (1997). The samples were then subjected to typical *in vitro* monogastric digestion simulations. Adding the commercial enzyme preparation to the barley sample at 5 g / kg concentration significantly decreased the insoluble non-starch polysaccharides and increased the amount of soluble non-starch polysaccharides. At higher concentrations of enzyme, both the amount of soluble and insoluble non-starch polysaccharides decreased, indicating that the hydrolysis of non-starch polysaccharides is concentration dependent. Adding the three different concentrations of Grindazym GP 5000 to the rye samples did not have any significant reduction in the amount of insoluble or soluble non-starch polysaccharides.

Malathi and Devegowda (2001) mixed three different enzyme preparations at two different concentrations with 0.1 g samples of sunflower meal, soybean meal, deoiled rice bran and a typical broiler starter ration (550 g / kg maize, 315 g / kg soybean meal and 100 g / kg sunflower meal), respectively. Malathi and Devegowda (2001) added enzymes at different concentrations and consulted the work of John and Schmidt (1988) to determine the xylanase activity of the enzymes at different concentrations. Xylanase was active at 900 U / kg feed and 1800 U / kg feed in Nutrizyme-B™ at concentrations of 1 g / kg and 2 g / kg, respectively, 408 U / kg feed and 816 U / kg feed in Biofeed plus-CT™ at concentrations of 0.6 g / kg and 1.2 g / kg, respectively, 540 U / kg feed and 1080 U / kg feed in Energex-CT™ at concentrations of 1.2 g / kg and 2.4 g / kg, respectively. Samples were then subjected to two phase *in vitro* digestion assay (Bedford and Classen, 1993) and analysed for relative viscosity and total sugars released, as shown in Tables 4 and 5. Control samples also underwent the same treatment, excluding the addition of commercial enzyme preparations. For the sunflower meal, both concentration levels of Xylanase and the higher concentrations of Nutrizyme-B™ and Biofeed plus-CT™ significantly reduced the relative viscosity. The total sugars released were significantly increased for both concentration levels of Xylanase and Nutrizyme-B™ and for the higher concentration level of Biofeed plus-CT™. For the soybean meal and the deoiled rice bran, both concentration levels of all the enzymes significantly reduced the relative viscosity and significantly increased the total sugars released. For the broiler starter diet, both concentration levels of Xylanase and the higher concentrations of Nutrizyme-B™ significantly reduced the relative viscosity. The total sugars released were significantly increased for both concentration levels of Xylanase and Nutrizyme-B™.

Table 4 Effect of enzyme supplementation on the relative viscosity and total sugars released on sunflower meal and soybean meal [adapted from Bedford and Classen (1993)]

	Relative viscosity [Improvement]	Total sugars released (g / L) [Improvement]
Sunflower meal		
Control	1.59^{a}	4.24^{d}
1 g / kg Nutrizyme-B	1.33^{cd} [+ 16%]	6.46^{b} [+ 52%]
2 g / kg Nutrizyme-B	1.25^{d} [+ 21%]	7.26^{a} [+ 71%]
0.6 g / kg Biofeed plus-CT	1.50^{ab} [+ 6%]	6.25^{b} [+ 47%]
1.2 g / kg Biofed plus-CT	1.33^{cd} [+ 16%]	6.79^{ab} [+ 60%]
1.2 g / kg Energex-CT	1.50^{ab} [+ 6%]	4.69^{cd} [+ 11%]
2.4 g / kg Energex-CT	1.41^{bc} [+ 16%]	5.28^{c} [+ 25%]
Soybean meal		
Control	1.53	3.39^{e}
1 g / kg Nutrizyme-B	1.42^{a} [+ 7%]	4.01^{d} [+ 18%]
2 g / kg Nutrizyme-B	1.29^{b} [+ 16%]	4.65^{ab} [+ 37%]
0.6 g / kg Biofeed plus-CT	1.33^{c} [+ 13%]	4.11^{cd} [+ 21%]
1.2 g / kg Biofed plus-CT	1.26^{c} [+ 18%]	4.56^{abc} [+ 35%]
1.2 g / kg Energex-CT	1.27^{c} [+ 17%]	4.32^{bcd} [+ 27%]
2.4 g / kg Energex-CT	1.17^{d} [+ 24%]	4.82^{a} [+ 42%]

[abcd] Column means within the same raw material with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

Table 5 Effect of enzyme supplementation on the relative viscosity and total sugars released on deoiled rice bran and a typical broiler starter diet [adapted from Bedford and Classen (1993)]

	Relative viscosity [Improvement]	Total sugars released (g / L) [Improvement]
Deoiled rice bran		
Control	1.97[a]	4.69[d]
1 g / kg Nutrizyme-B	1.56[d] [+ 21%]	6.27[ab] [+ 34%]
2 g / kg Nutrizyme-B	1.50[d] [+ 24%]	6.67[a] [+ 42%]
0.6 g / kg Biofeed plus-CT	1.70[bc] [+ 14%]	5.95[b] [+ 27%]
1.2 g / kg Biofed plus-CT	1.62[cd] [+ 18%]	6.30[ab] [+ 34%]
1.2 g / kg Energex-CT	1.81[b] [+ 8%]	5.24[c] [+ 12%]
2.4 g / kg Energex-CT	1.72[bc] [+ 13%]	5.89[b] [+ 26%]
Broiler starter diet		
Control	1.50[a]	5.16[e]
1 g / kg Nutrizyme-B	1.33[d] [+ 11%]	6.34[bc] [+ 23%]
2 g / kg Nutrizyme-B	1.27[d] [+ 15%]	7.20[a] [+ 40%]
0.6 g / kg Biofeed plus-CT	1.41[abc] [+ 6%]	5.98[cd] [+ 16%]
1.2 g / kg Biofed plus-CT	1.35[bcd] [+ 10%]	6.75[ab] [+ 31%]
1.2 g / kg Energex-CT	1.50[a] [0%]	5.32[e] [+ 3%]
2.4 g / kg Energex-CT	1.44[abc] [+ 4%]	5.66[de] [+ 10%]

[abcde] Column means within the same raw material with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

Wiryawan *et al.* (1995) stated that the addition of 1 g / kg xylanase to a solvent-extracted soybean meal increase the true metabolisable energy for broilers. The net protein ratio of solvent extracted soybean meal was also found to be increased significantly in broiler diets with the addition of an enzymatic combination including xylanase (Wiryawan *et al.*, 1997). Wiryawan and Dingle (1999) hypothesised that the increased net protein ratio was increased because xylanase, amongst other enzymatic reactions was responsible for breaking down the cell wall.

Zanella *et al.* (1999) fed Hubbard males three different rations with and without the addition of Avizyme® 1500 (six treatments). All six treatments had similar energy levels. Avizyme® 1500, at an inclusion rate of 1g / kg contained 800 U / g xylanase (extracted from *Trichoderma longibrachiatum*), 6 000 U / g protease (extracted from *Bacillus subtilis*) and 2 000 U / g amylase (extracted from *Bacillus amyloliquifaciens*). In a second trial, the same feeds and enzymatic treatments were fed, but at lower energy availability to account for the positive results obtained from enzymatic addition to the ration. In the first trial it was found that the addition of enzymes to the feeds significantly increased the 45 day body weight of the chicks and significantly improved the FCR, but the mortality, viscosity of the intestinal contents, carcass weight, abdominal fat, breast weight and pancreas weight were not significantly affected by the addition of commercial enzyme to the feed (Table 2). None of the production variables were significantly different between the enzyme treatments and the control treatments in the second trial (Table 3), although the enzyme treatments had lower energy levels than the control treatments. This indicates that energy levels can be reduced to lower levels when enzymes are added to the diet. The above mentioned statement indicates that enzymatic addition has a certain energy value due to increased energy availability from the ration.

2.8. Conclusion

Most published studies indicated profitable advantages to adding commercial enzyme preparations to broiler rations, especially when making use of high roughage raw materials and raw materials with anti-nutritional factors, such as soybean meal. There are, however, still many experiments that found the contrary where the addition of commercial enzyme preparations did not result in any significant improvements in broiler production variables. Although there were cases where enzymes did not show any advantageous contribution to the productive performance of broilers, it is possible that the diets without enzymes already fulfilled in the nutrient requirements of the birds for maximum production. Where enzymes do not seem to increase production significantly, further research is required using rations with a lower protein and energy concentration. Further research might prove that these controversial commercial enzyme preparations can be efficient and profitable when added to cheaper, lower quality rations.

Chapter 3

Performance Trial 1: Efficacy of Avizyme 1502® and Hemicell® to increase the availibility of metabolisable energy in broiler feeds

Abstract

The aim of this trial was to investigate whether the feed enzymes, Avizyme 1502 (containing amylase, xylanase and protease) and Hemicell (containing β-mannanase), would contribute an additional 0.35 MJ ME / kg of broiler feed. The hypothesis is that the addition of exogenous enzymes to the feed will reduce the anti-nutritional effects in maize and soya, leading to an increased metabolisable energy availability to the broiler. A Negative control feed was formulated to have 0.35 MJ ME / kg less energy than a Positive control feed. Two treatment feeds were formulated to contain similar metabolisable energy values than the Negative control. The commercial enzyme preparations, Avizyme and Hemicell, were added to these two diets, respectively. It was expected that these enzyme preparations would increase the metabolisable energy content of the treatment diets with 0.35 MJ ME / kg. Ross 788 broilers were fed different treatment feeds from day of hatch for 35 days. Weekly body weights, weekly body weight gains, weekly and cumulative feed intake, weekly and cumulative feed conversion ratios, cumulative mortality and production efficiency factors were recorded and calculated as performance variables to compare the respective feeds. This trial revealed that the addition of Avizyme to an extended starter diet contributed at least 0.35 MJ ME / kg feed and resulted in significant improvements in body weight gain, FCR and PEF over a five week growing period ($P < 0.05$). The addition of Hemicell to an extended starter and grower phase of a feed contributed at least 0.35 MJ ME / kg feed and resulted in a significant improvements in byweight gain, fewd intake, FCR and PEF over a five week growing period ($P < 0.05$).

3.1. Introduction

β-Mannans are a group of closely related compounds in soybeans mainly associated with the hull and fibre fractions. The β-mannans are highly viscous (Reid, 1985; Jackson *et al.*, 1999; Odetallah *et al.*, 2002) which causes a partial blockage of receptor sites on intestinal surfaces, and thereby decreasing the utilisation of carbohydrates (Dale, 1997), resulting in poor feed conversion ratio. The β-mannan and β-galactomannan content of soybean meal is 1.3 – 1.7% and 1.83 – 2.22%, respectively, (Dierick, 1989) and are extremely heat resistant, retaining their anti-nutritional characteristics in broiler feeds even after processing of the soybean meal (Dale, 1997). Including raw materials that contained β-mannans at two to four percent in a feed resulted in reduced growth and worse feed conversion ratios in broilers (Couch *et al*., 1967; Ray *et al*., 1982; Verma and McNab, 1982). A possible strategy for reducing the anti-nutritional effect of β-mannans is to add an exogenous β-D-mannanase enzyme to the feed.

Chesson (2001) determined that approximately ten percent of the protein in soybean is trapped in the cell wall matrix and is unavailable to the broiler digestive system. These entrapped proteins can be exposed to the broiler's digestive system by adding exogenous enzymes to the diet (Mandels, 1985). The crude protein and energy digestibility have been improved significantly by the addition of carbohydrases to a maize-soybean based ration (Brenes *et al*., 1993; Frigård *et al*., 1994; McKnight, 1997; Bedford and Schulze, 1998; Oloffs *et al*., 1999; Mathlouthi *et al*., 2003; Saki *et al*., 2005; Tahir *et al*., 2006). Addition of multi-enzyme preparations that included hemicellulase to poultry feed showed an overall improvement in poultry performance (Steenfeldt *et al*., 1998; Kocher *et al*., 2000; Malathi and Devegowda, 2001). In further studies, Kocher *et al*. (2002) revealed that the improvement in broiler performance, found with multi-enzyme additions, is most likely due to an increased crude protein and energy digestibility of the soybean meal component of the diet.

The ratio of amylose and amylopectin vary between different cultivars of maize, giving rise to terms such as waxy maize (high amylopectin content) and amylomaize (high amylose content). On average, cereal starches contain 25% amylose (Weurding, 2002). Amylose is considered to be less digestible than amylopectin. The linear structure of amylose in comparison to the branched structure of amylopectin creates a smaller surface area per molecule for enzymes to attach to the molecule for digestion. Amylose also contain hydrogen bonds between glucose chains (Weurding, 2002), which make this structure less susceptible to enzymatic hydrolyses. Sievert and Pomeranz (1989) have indicated that the resistant starch content (as a percentage of dry matter) increases with the amylose content. Increasing the broiler's capacity to digest amylose by the supplementation of exogenous amylase in the feed could possibly overcome the negative effects of a high amylose content in the starch.

Weurding (2002) pointed out that cellulose is composed entirely of glucose molecules, but these molecules are linked by β-bonds, which cannot be hydrolysed by the broiler's enzymes. The cellulose content in maize is, however, not high enough to have a great influence on starch digestion.

Non-starch polysaccharides in cereals, like β-glucans and arabinoxylans can affect the starch digestion in broilers and other monogastric animals. These polysaccharides serve as physical barriers that inhibit enzyme accessibility to starch granules and increase the viscosity of the digesta, resulting in a reduced diffusion rate of enzymes, an increased feed passage time and decreased digestibility of the starch and feed (Classen, 1996; Refstie *et al*., 1999). Although these anti-nutritional factors are present in varying amounts in grains (Classen, 1996), the negative effects that these molecules have on digestibility of the grains can be reduced with the supplementation of exogenous enzymes like xylanase.

The digestibility of starch is affected by the starch structure and composition (Oates, 1997) and the associations between the starch granules and protein and cell wall structures in the feed (Eastwood, 1992). Weurding (2002) noted that the protein matrix around the starch granules as well as the non-starch polysaccharide fraction reduce the accessibility of enzymes to the starch granules. An exogenous protease enzyme supplementation could assist in digesting the protein matrix and making the starch granules more accessible to the amylolytic enzymes. The protein matrix and non-starch polysaccharides, will delay or even inhibit complete starch digestion in the small intestine. The broiler does not have enzymes to digest non-starch polysaccharides like arabinoxylans and β-glucanase (Weurding, 2002).

As previously mentioned, some of the starch might be protected from α-amylase degradation by protein matrixes and non-starch polysaccharides. This starch will pass through the small intestine and is termed resistant starch (Englyst *et al*., 1982). Resistant starch can decrease the apparent metabolisable energy of a feed significantly (Rutherfurd *et al*., 2007). Resistant starch can be microbially fermented in the caeca and colon to volatile fatty acids (particularly acetate, propionate and butyrate), methane, hydrogen and carbon dioxide. The production of volatile fatty acids from the fermentation of starch is, however, less energy efficient than complete digestion of starch in the small intestine (Dierick *et al.* 1989).

Avizyme 1502 is a commercially available product (Danisco Animal Nutrition, Wiltshire, UK) that combines the enzymes amylase, xylanase and protease. The manufacturer claims that the product increases the apparent metabolisable energy value of maize by up to five percent. At an average energy value for maize of 13.46 MJ / kg, it means that Avizyme might increase the energy of maize with 0.67 MJ

/ kg maize. Therefore, in a typical broiler feed which contains around 60% maize, the additional energy availability would be 0.4 MJ / kg feed with the inclusion of Avizyme.

Hemicell (ChemGen Corp., Gathersburg, USA) is an enzyme product containing β-mannanase, which, according to the manufacturers, would increase the apparent metabolisable energy value of the feed with 0.5 MJ / kg. McNaughten *et al*. (1998) found that the addition of β-mannanase to a diet increased the energy availability by up to 0.6 MJ / kg feed.

The aim of the study was to verify that the two products, Avizyme 1502 and Hemicell, would increase the apparent metabolisable energy content of a maize-soya based broiler feed with at least 0.35 MJ ME / kg feed.

3.2. Materials and methods

3.2.1. Experimental design

A randomised block design was used in this trial. The trial was conducted at the Daybreak experimental farm in Sundra (Mpumalanga, South Africa) in an environmentally controlled broiler house. The house was divided into four blocks to minimise the variation within a block and maximise the variation between blocks. Dividing the house into blocks assisted in minimising the effect of unintentional temperature fluctuations and different noise levels within the house. There were four treatments with eight replicates per treatment. Thus, each treatment was replicated twice within each of the four blocks.

3.2.2. Housing

The broiler house used for this study was divided into 32 pens. Each pen had a surface area of six square meters (2 m × 3 m). The house had a solid concrete floor and pens were covered with a layer of wood shavings. A boiler was used to control the temperature in the house. Hot air was blown through a hot air tunnel that ran through the whole house. Heated air was distributed evenly throughout the house. Six thermometers and three temperature and humidity loggers were installed throughout the house and monitored daily to ensure that a uniform temperature and humidity was maintained throughout the whole house. Curtains on the sides of the house were used to control ventilation. For the first five days chicks were fed from a combination of pan feeders and tube feeders. Water was supplied by a combination of nipple drinkers and fountain drinkers. After five days the pan feeders and fountain drinkers were removed. Feeders were kept full at all times to ensure that feed intake was not affected by low feed levels.

The temperature profile that was followed from day 1 – 35 is shown in Table 6. Minimum and maximum temperatures, and the boiler temperature reading were monitored on a daily basis to ensure that temperature stayed within the range. The lighting program for the 35 day period is shown in Table 7.

Table 6 Temperature profile for Performance Trial 1

Days	Temperature (°C, 50% rH)		
	Lower temperature	Target temperature	Upper temperature
1 – 2	31.5	33.0	34.5
3 – 5	30.5	32.0	33.5
6 – 8	29.5	31.0	32.5
9 – 11	28.2	29.7	31.2
12 – 14	25.7	27.2	28.7
15 – 17	24.7	26.2	27.2
18 – 20	23.5	25.0	26.5
21 – 23	22.5	24.0	25.5
24 – 35	21.5	23.0	24.5

Table 7 Lighting programme for Performance Trial 1

Days	Day light (hours)	Darkness (hours)
1 – 6	23	1
7 – 15	14	10
16 – 22	16	8
23 – 28	18	6
29 – 35	20	4

3.2.3. Birds

Four thousand three hundred and twenty (4320) day-old chicks (Ross 788) were obtained from Midway Hatcheries. One hundred and thirty five (135) chicks were randomly allocated to each pen on day 0. Birds per pen were reduced to 126 birds on day seven (any mortalities during the first week were first taken into account, where after poorer quality birds were removed from the pen, as necessary). Stocking density at day seven was 21 chicks / m^2.

Chicks were graded by the hatchery to ensure placement of good quality chicks.

3.2.4. Dietary treatments

All feed was manufactured at the Afgri Animal Feeds commercial feed factory in Isando, Gauteng. Lines were cleaned properly before trial feed manufacturing commenced and lines were also cleaned thoroughly between treatments to avoid contamination between respective treatments. The Negative control and Positive control treatments were manufactured first to prevent contamination of these diets with traces of Avizyme or Hemicell. The feed was bagged off into 50 kg bags and bags for various treatments were clearly marked for easy identification.

All treatment feeds were formulated to contain a minimum of 18% total soya products. The following feed treatments were tested in this trial:

Positive control: This feed was formulated according to standard nutrient specifications used by Daybreak Farms to meet or exceed the nutritional requirements of broilers, as recommended by the NRC (1994). The apparent metabolisable energy was slightly lower than the standard to ensure that energy is the first limiting nutrient (Table 8). No exogenous enzyme was added to this treatment.

Avizyme treatment: This feed was formulated to have 0.35 MJ less metabolisable energy / kg than the Positive control, assuming an additional 0.35 MJ ME / kg feed to be released by the added 0.05% of Avizyme in the feed.

Table 8 Energy specifications of treatment diets (MJ ME / kg)

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Starter	11.70	11.35	11.35	11.35
Grower	12.20	11.85	11.85	11.85
Finisher	12.46	12.11	12.11	12.11

[1]Included Avizyme 1502 at 0.5 g / kg.
[2]Included Hemicell at 0.125 g / kg.

Hemicell treatment: This feed was formulated to contain 0.35 MJ less metabolisable energy / kg than the Positive control, expecting that the addition of 0.0125% Hemicell to the feed will increase the energy concentration with 0.35 MJ ME / kg feed.

Negative control: This feed was formulated to have 0.35 MJ ME / kg feed less than the Positive control. No exogenous enzymes were added.

These treatments applied to all three feeding phases used in this trial, i.e. an extended starter, grower and finisher phase (Table 8). Birds were fed according to days on feed (18, 10 and 7 days, respectively). With every change to the next phase, the left-over feed from the previous phase was weighed back and discarded. Birds were fed *ad libitum*.

Tables 9 to 14 show the raw material inclusion and calculated nutrient specifications for each feed used in the trial.

Representative samples of the different feeds were collected during feeding, before the birds had access to the feed. Each sample was ground and analysed for dry matter, ash, crude protein, crude fibre, crude fat, calcium, phosphorous, potassium and sodium at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria). Representative samples of each feed were also analysed for Avizyme activity and Hemicell activity by Danisco Animal Nutrition (Wiltshire, UK) and ChemGen Corporation (Gathersburg, USA), respectively. Moisture determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 934.01). Dry matter determination and ashing was done according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 942.05). Crude fibre determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 962.09). The Dosi fibre system was used to determine the crude fibre percentage. Crude fat determination was done according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 920.39). Crude protein determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 988.05). The Leco FP – 428 (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396) was used to determine the nitrogen content of a feed. Samples were prepared for calcium analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 935.13).

Table 9 Raw material inclusion (% as fed basis) for the starter phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Maize	59.08	59.53	59.53	59.53
Full fat soya (35.5% CP)	10.00	10.00	10.00	10.00
Soybean oilcake (46% CP)	17.75	18.43	18.43	18.43
Sunflower oilcake (36% CP)	5.58	6.98	6.98	6.98
Gluten (60% CP)	1.95	0.55	0.55	0.55
L threonine	0.085	0.084	0.084	0.084
DL methionine	0.135	0.143	0.143	0.143
Lysine HCl	0.415	0.390	0.390	0.390
Vegetable oil	1.04	0.00	0.00	0.00
Salt	0.408	0.408	0.408	0.408
Sodium bicarbonate	0.13	0.14	0.14	0.14
Monocalcium phosphate	1.92	1.89	1.89	1.89
Limestone	1.58	1.58	1.58	1.58
Vitamin & mineral premix	0.12	0.12	0.12	0.12
Betafin[3]	0.05	0.05	0.05	0.05
Coccidiostat and AGP[4]	0.09	0.09	0.09	0.09

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Betaine methyl donor.

[4] Stafac 4% and Salinomycin 12%.

Table 10 Raw material inclusion (% as fed basis) for the grower phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Maize	64.91	64.06	64.06	64.06
Full fat soya (35.5% CP)	10.01	7.33	7.33	7.33
Soybean oilcake (46% CP)	13.40	13.00	13.00	13.00
Sunflower oilcake (36% CP)	3.99	8.01	8.01	8.01
Poultry byproduct meal	2.85	2.50	2.50	2.50
L threonine	0.036	0.037	0.037	0.037
DL methionine	0.160	0.146	0.146	0.146
Lysine HCl	0.310	0.346	0.346	0.346
Vegetable oil	1.12	0.98	0.98	0.98
Salt	0.396	0.394	0.394	0.394
Sodium bicarbonate	0.03	0.03	0.03	0.03
Monocalcium phosphate	1.27	1.25	1.25	1.25
Limestone	1.33	1.73	1.73	1.73
Vitamin & mineral premix	0.05	0.05	0.05	0.05
Betafin[3]	0.05	0.05	0.05	0.05
Coccidiostat and AGP[4]	0.09	0.09	0.09	0.09

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Betaine methyl donor.

[4] Stafac 4% and Salinomycin 12%.

Table 11 Raw material inclusion (% as fed basis) for the finisher phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Maize	64.67	64.94	64.94	64.94
Full fat soya (35.5% CP)	10.00	10.00	10.00	10.00
Soybean oilcake (46% CP)	13.05	11.13	11.13	11.13
Sunflower oilcake (36% CP)	4.00	7.00	7.00	7.00
Poultry byproduct meal	2.86	2.59	2.59	2.59
L threonine	0.036	0.036	0.036	0.036
DL methionine	0.157	0.144	0.144	0.144
Lysine HCl	0.310	0.337	0.337	0.337
Vegetable oil	2.09	0.98	0.98	0.98
Salt	0.388	0.390	0.390	0.390
Monocalcium phosphate	1.05	1.03	1.03	1.03
Limestone	1.18	1.18	1.18	1.18
Vitamin & mineral premix	0.10	0.10	0.10	0.10
Betafin[3]	0.05	0.05	0.05	0.05
Coccidiostat and AGP[4]	0.09	0.09	0.09	0.09

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Betaine methyl donor.

[4] Stafac 4% and Salinomycin 12%.

Table 12 Calculated nutrient specifications (% on as fed basis) for the starter phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Dry matter	90.06	90.05	90.05	90.05
Protein	20.61	20.57	20.57	20.57
AME for chicks (MJ / kg)	11.70	11.35	11.35	11.35
Fibre	3.62	3.94	3.94	3.94
Fat	5.35	4.28	4.28	4.28
Lysine[3]	1.13	1.13	1.13	1.13
Methionine[3]	0.47	0.47	0.47	0.47
Total sulphur amino acids[3]	0.73	0.74	0.74	0.74
Threonine[3]	0.70	0.70	0.70	0.70
Tryptophan[3]	0.19	0.19	0.19	0.19
Arginine[3]	1.17	1.21	1.21	1.21
Isoleucine[3]	0.73	0.74	0.74	0.74
Valine[3]	0.81	0.81	0.81	0.81
Leucine[3]	1.62	1.54	1.54	1.54
Glycine and Serine[3]	1.54	1.54	1.54	1.54
C 18:2	2.42	2.10	2.10	2.10
Calcium	0.93	0.93	0.93	0.93
Potassium	0.80	0.83	0.83	0.83
Chloride	0.30	0.30	0.30	0.30
Total Phosphorous	0.79	0.80	0.80	0.80
Retainable Phosphorous	0.46	0.46	0.46	0.46
Sodium	0.20	0.20	0.20	0.20

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Apparent digestible.

Table 13 Calculated nutrient specifications (% on as fed basis) for the grower phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Dry matter	89.59	89.69	89.69	89.69
Protein	18.39	18.48	18.48	18.48
AME for chicks (MJ / kg)	12.20	11.85	11.85	11.85
Fibre	3.31	4.00	4.00	4.00
Fat	6.31	5.60	5.60	5.60
Lysine[3]	0.95	0.95	0.95	0.95
Methionine[3]	0.44	0.44	0.44	0.44
Total sulphur amino acids[3]	0.69	0.69	0.69	0.69
Threonine[3]	0.59	0.59	0.59	0.59
Tryptophan[3]	0.16	0.16	0.16	0.16
Arginine[3]	1.05	1.07	1.07	1.07
Isoleucine[3]	0.65	0.65	0.65	0.65
Valine[3]	0.73	0.74	0.74	0.74
Leucine[3]	1.39	1.37	1.37	1.37
Glycine and Serine[3]	1.45	1.46	1.46	1.46
C 18:2	2.51	2.22	2.22	2.22
Calcium	0.75	0.88	0.88	0.88
Potassium	0.71	0.71	0.71	0.71
Chloride	0.30	0.30	0.30	0.30
Total Phosphorous	0.62	0.64	0.64	0.64
Retainable Phosphorous	0.34	0.34	0.34	0.34
Sodium	0.17	0.17	0.17	0.17

[1] Avizyme was added at 0.5 g / kg.
[2] Hemicell was added at 0.125 g / kg.
[3] Apparent digestible.

Table 14 Calculated nutrient specifications (% on as fed basis) for the finisher phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Negative control
Dry matter	89.65	89.64	89.64	89.64
Protein	18.20	18.32	18.32	18.32
AME for chicks (MJ / kg)	12.46	12.11	12.11	12.11
Fibre	3.30	3.88	3.88	3.88
Fat	7.26	6.09	6.09	6.09
Lysine[3]	0.94	0.94	0.94	0.94
Methionine[3]	0.44	0.43	0.43	0.43
Total sulphur amino acids[3]	0.69	0.69	0.69	0.69
Threonine[3]	0.58	0.58	0.58	0.58
Tryptophan[3]	0.16	0.16	0.16	0.16
Arginine[3]	1.04	1.06	1.06	1.06
Isoleucine[3]	0.64	0.64	0.64	0.64
Valine[3]	0.73	0.73	0.73	0.73
Leucine[3]	1.37	1.37	1.37	1.37
Glycine and Serine[3]	1.44	1.44	1.44	1.44
C 18:2	2.79	2.47	2.47	2.47
Calcium	0.66	0.66	0.66	0.66
Potassium	0.71	0.70	0.70	0.70
Chloride	0.29	0.30	0.30	0.30
Total Phosphorous	0.57	0.59	0.59	0.59
Retainable Phosphorous	0.30	0.30	0.30	0.30
Sodium	0.16	0.16	0.16	0.16

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Apparent digestible.

3.2.5. Measurements

The feeders in all pens were filled with feed and water lines were functional before chicks were placed in the pens. Chicks were placed in their respective pens immediately upon delivery. Chicks were counted once weekly to ensure that migration between pens or unrecorded mortalities did not occur. Pens were checked for mortalities on a daily basis. Individual pen records were kept of all mortalities, day of mortality and weight of the dead chick(s).

Total body weight and the feed remaining in each pen were measured weekly (days 7, 14, 21, 28 and 35). Average body weight (g / bird), weekly body weight gain (g / bird / day), cumulative feed intake (g / bird), weekly feed intake (g / bird / day), cumulative and weekly feed conversion ratio (g feed intake / g body weight gain), cumulative mortality and production efficiency factor were calculated for each pen, by making use of total body weight, feed remaining and mortality records.

3.2.6. Statistical analysis

The treatments in this trial were not structured, so simple analysis of treatment, using the generalised linear model (GLM) function in SAS (Statistical Analysis Systems, 1989; Statistical Analysis Systems, 1994) was used in preference to the balanced ANOVA so that *post hoc* multiple comparison tests could be run on the treatment means, in cases where the GLM found significant differences in performance between treatments. The *post hoc* multiple comparison test used was Fischer's protected test. Repeated tests were included in the model. The confidence level was set at 95%.

The model used in SAS was $y = T_i + B_j + e_{ij}$

Where T_i was i^{th} observation for the treatment

B_j was block as a fixed effect

e_{ij} was the random error effect

The variation due to block effects was accounted for by including Block as fixed effect in the model. Initial body weight was tested as a covariant in all the analyses in this trial.

The variables that were analysed are body weight, weekly body weight gains, weekly feed intake, cumulative feed intake, weekly feed conversion ratio, cumulative feed conversion ratio, performance efficiency factor and cumulative mortality. These could be calculated, respectively, from the following measurements: bird counts, initial body weight, weekly body weights, feed weighed in and feed weighed out (weekly and at 18 days), and mortality records.

3.3. Results

3.3.1. Chemical analysis

Tables 15 to 17 show the results for the chemical analyses of the trial feeds. In order to test if Avizyme and Hemicell were correctly added to the respective treatments and to ascertain that no Avizyme and / or Hemicell was added to the Negative and Positive Control feeds, the enzyme activity was tested in the feed samples by Danisco Animal Nutrition (Wiltshire, UK) and ChemGen Corporation (Gathersburg, USA) for Hemicell and Avizyme activity, respectively. An amylase activity of at least 400 MM units / ton feed and a xylanase activity of at least 600 MM units / ton feed were used as an indication that Avizyme was added to the feed at optimal levels. A mannanase activity of at least 90 MM units / ton feed was an indicator that Hemicell was added to the feed at optimal levels.

Table 15 Chemical analyses of the starter feeds on a dry matter basis

	Positive control[4]	Avizyme treatment[5]	Hemicell treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100
Ash (%)	6.1	6.1	6.2	5.1
Crude protein (%)	21.9	21.8	21.9	22.0
Crude fibre (%)	5.0	4.6	4.5	4.4
Crude fat (%)	6.4	6.5	7.1	5.7
Calcium (%)	1.07	1.04	1.04	0.70
Phosphorous (%)	0.81	0.81	0.83	0.83
Potassium (%)	0.96	1.02	1.00	0.99
Sodium (%)	0.28	0.26	0.29	0.12
Avizyme activity (MM amylase / ton)[1]	<100	1566	<100	<100
Avizyme activity (MM xylanase / ton)[2]	175	638	200	207
Hemicell activity (MM units / ton)[3]	8.6	28.7	298.4	9.9

[1]Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2]Avizyme treated feeds should have a xylanase activity of at least 600 MM units / ton.

[3]Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[4] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[5] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[6] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[7] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

Table 16 Chemical analyses of the grower feeds on a dry matter basis

	Positive control[4]	Avizyme treatment[5]	Hemicell treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100
Ash (%)	5.0	4.9	5.8	5.8
Crude protein (%)	20.2	20.2	19.9	19.8
Crude fibre (%)	4.5	5.0	6.4	4.9
Crude fat (%)	7.9	7.9	7.2	8.0
Calcium (%)	0.78	0.74	1.06	1.10
Phosphorous (%)	0.56	0.53	0.62	0.67
Potassium (%)	0.86	0.84	0.88	0.84
Sodium (%)	0.25	0.27	0.26	0.24
Avizyme activity (MM amylase / ton)[1]	<100	151	<100	<100
Avizyme activity (MM xylanase / ton)[2]	266	217	227	306
Hemicell activity (MM units / ton)[3]	4.7	17.2	218.5	13.7

[1]Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2]Avizyme treated feeds should have a xylanase activity of at least 600 MM units / ton.

[3]Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[4] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[5] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[6] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[7] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

Table 17 Chemical analyses of the finisher feeds on a dry matter basis

	Positive control[4]	Avizyme treatment[5]	Hemicell treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100
Ash (%)	5.0	5.3	5.8	6.7
Crude protein (%)	19.5	19.8	19.1	19.8
Crude fibre (%)	4.2	4.8	6.1	5.3
Crude fat (%)	8.0	7.4	7.3	7.0
Calcium (%)	0.87	0.87	1.04	1.22
Phosphorous (%)	0.63	0.67	0.65	0.80
Potassium (%)	0.88	0.84	0.83	0.84
Sodium (%)	0.22	0.24	0.21	0.39
Avizyme activity (MM amylase / ton)[1]	<100	1336	1971	<100
Avizyme activity (MM xylanase / ton)[2]	304	814	822	239
Hemicell activity (MM units / ton)[3]	8.1	15.0	156.8	7.7

[1]Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2]Avizyme treated feeds should have a xylanase activity of at least 600 MM units / ton.

[3]Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[4] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[5] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[6] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[7] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.2. Body weight

As shown in Table 18 and illustrated in Figure 1, the mean body weight of the different treatments did not differ significantly at the start of the trial.

The body weight of the Negative control was significantly lower than all the other treatments from day seven until termination of the trial on day 35.

Table 18 Least square means (± standard error of the mean) of the average weekly body weights (g / bird) for the different treatments from day of hatch (day 0) until day 35

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Positive[1]	40.9 [± 0.23]	152.1[a] [± 1.84]	362.5[a] [± 3.60]	728.7[a] [± 6.20]	1271[a] [± 9.2]	1723[a] [± 12.0]
Avizyme[2]	41.3 [± 0.23]	158.7[b] [± 1.81]	379.8[b] [± 3.53]	764.0[b] [± 6.08]	1308[b] [± 9.1]	1759[b] [± 11.8]
Hemicell[3]	41.4 [± 0.23]	153.8[ab] [± 1.81]	365.0[a] [± 3.54]	737.8[a] [± 6.09]	1242[c] [± 9.1]	1721[a] [± 11.8]
Negative[4]	40.9 [± 0.24]	133.6[c] [± 1.87]	292.9[c] [± 3.66]	613.8[c] [± 6.30]	1125[d] [± 9.4]	1535[c] [± 12.2]
F-probability						
Treatment	0.229	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Block	0.111	0.274	0.275	0.049	0.401	0.278
R^2	0.283	0.811	0.934	0.934	0.902	0.907

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05)

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

The Avizyme treatment's body weight was significantly higher than all the other treatments' body weights from day 14 until day 35 and significantly higher than that of the Positive and Negative control treatments on day seven.

The body weight of the Hemicell treatment was significantly higher than the Negative control treatment for the period day seven until day 35. The Hemicell treatment's body weight was significantly lower than the Avizyme treatment from day seven until day 35 and lower than the Positive control on day 28.

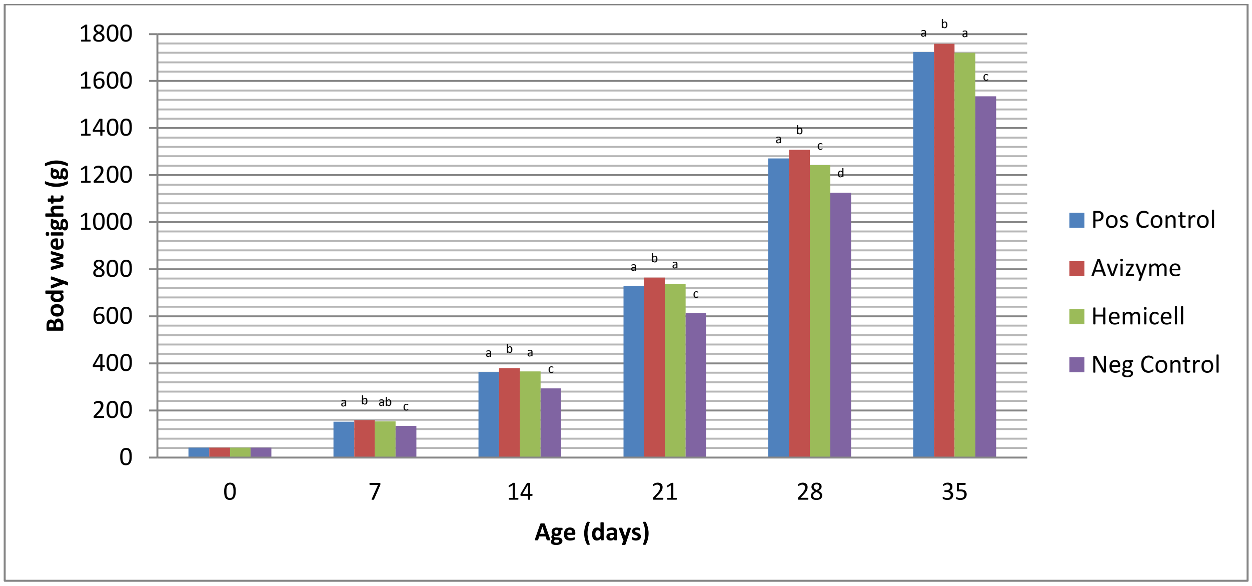

Figure 1 Average weekly body weights of the different treatments from day of hatch (day 0) until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05)

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.3. Body weight gain

As shown in Table 19 and illustrated in Figure 2, the mean body weight gain of the Negative control was significantly lower than all the other treatments for days seven, 14, 21 and 35 and lower than the Positive control and Avizyme treatment on day 28. The total body weight gain of the Negative control treatment on day 35 was significantly lower than all the other treatments.

The Avizyme treatment's body weight gain was significantly higher than all the other treatments' body weight gains from day seven until day 21, and significantly higher than that of the Negative control treatment from days 28 and 35. The body weight gain of the Avizyme treatment was also significantly higher than the Hemicell treatment until day 28, but lower than the Hemicell treatment for day 35. The total body weight gain of the Avizyme treatment was significantly higher than the Hemicell and Negative treatments and also significantly higher than the Positive control treatment until day 21.

Table 19 Least square means (± standard error of the mean) of the average weekly body weight gains (g / bird / day) for the different treatments from day 0 until day 35 and total body weight gain

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35	Day 0 – 35
Positive[1]	13.9^a [± 0.21]	30.1^a [± 0.30]	52.3^a [± 0.47]	77.4^a [± 0.68]	64.6^a [± 1.01]	1682^a [± 11.9]
Avizyme[2]	14.7^b [± 0.21]	31.6^b [± 0.29]	54.9^b [± 0.46]	77.8^a [± 0.66]	64.3^a [± 0.99]	1717^a [± 11.7]
Hemicell[3]	14.1^a [± 0.21]	30.2^a [± 0.29]	53.3^a [± 0.46]	72.1^b [± 0.66]	68.4^b [± 0.99]	1680^b [± 11.7]
Negative[4]	11.6^c [± 0.21]	22.8^c [± 0.30]	45.8^c [± 0.48]	73.0^b [± 0.69]	58.7^c [± 1.02]	1495^c [± 12.2]
F-probability						
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Block	0.301	0.233	0.007	0.554	< 0.001	0.281
R^2	0.831	0.957	0.899	0.722	0.789	0.908

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

The body weight gain of the Hemicell treatment was significantly higher than the Negative control treatment for days seven, 14, 21 and 35 and higher than the Positive and Avizyme treatments for day 35. The Hemicell treatment's body weight gain was only significantly lower than the Avizyme treatment from day seven until day 28. It was only significantly lower than the Positive control treatment on day 28. The total body weight gain of the Hemicell treatment was significantly higher than the Negative control treatment, but lower than the Positive and Avizyme treatments on day 35.

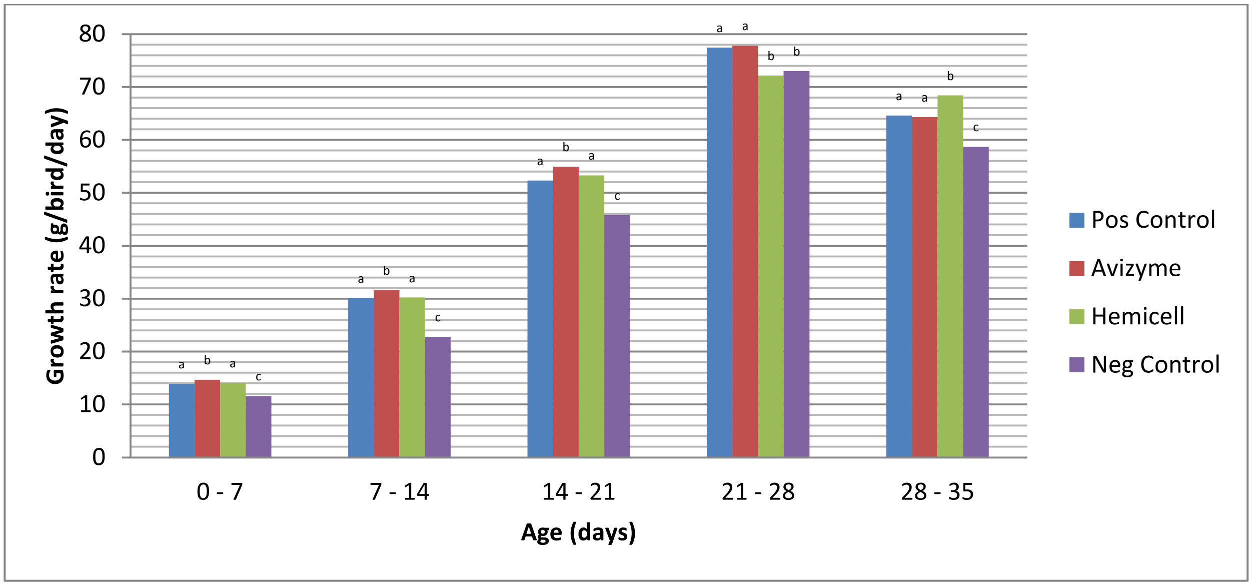

Figure 2 Average weekly body weight gain of the different treatments from day of hatch (day 0) until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.4. Weekly feed intake

As shown in Table 20 and illustrated in Figure 3, the weekly feed intake of the Negative control treatment was significantly lower than all the other treatments for days 14 and 21, but significantly higher than the Positive control treatment treatment's weekly feed intake.

The Avizyme treatment's weekly feed intake was significantly higher than all the other treatments on day 14 and higher than the Negative control treatment on day 21. The weekly feed intake of the Avizyme treatment was significantly lower than the Hemicell treatment on days 28 and 35.

The weekly feed intake of the Hemicell treatment was significantly higher than the Negative control treatment on days 14 and 35 and higher than the Positive control treatment from day 21 until day 35. The Hemicell treatment was also significantly higher than the Avizyme treatment from day 28 until day 35, but lower than the Avizyme treatment on day 14.

Table 20 Least square means (± standard error of the mean) of the average weekly feed intake (g / bird / day) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35
Positive[1]	16.8 [± 0.23]	39.3[a] [± 0.45]	79.7[a] [± 1.20]	129.3[a] [± 1.86]	138.3[a] [± 1.65]
Avizyme[2]	17.2 [± 0.23]	40.9[b] [± 0.44]	81.4[ab] [± 1.18]	128.3[a] [± 1.82]	140.9[ab] [± 1.62]
Hemicell[3]	17.3 [± 0.23]	39.5[a] [± 0.44]	83.6[b] [± 1.18]	134.6[b] [± 1.82]	146.0[c] [± 1.62]
Negative[4]	17.2 [± 0.24]	35.8[c] [± 0.46]	73.0[c] [± 1.22]	129.4[ab] [± 1.89]	144.0[bc] [± 1.68]
F-probability					
Treatment	0.481	< 0.001	< 0.001	0.093	0.013
Block	0.128	0.161	0.308	0.277	0.067
R^2	0.319	0.728	0.627	0.317	0.433

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

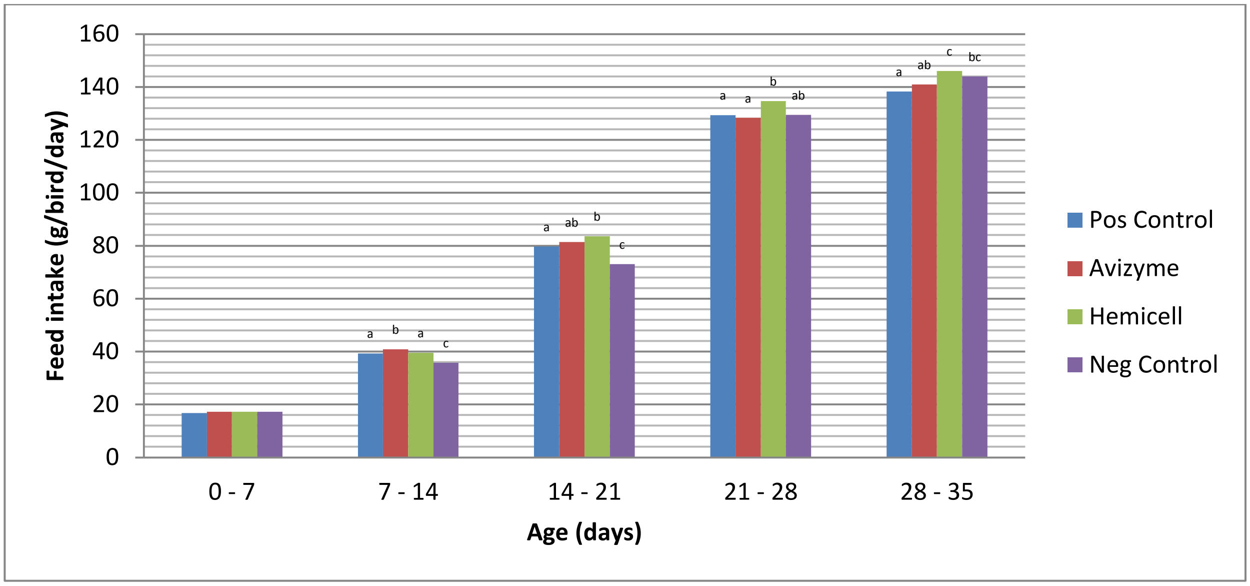

Figure 3 Average weekly feed intake of the different treatments from day 0 until day 35

abc Column means with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.5. Cumulative feed intake

As shown in Table 21 and illustrated in Figure 4, the cumulative feed intake of the Negative control treatment was significantly lower than all the other treatments from day 14 until day 28 and significantly lower than the Hemicell treatment on day 35.

The Avizyme treatment’s cumulative feed intake was significantly higher than the Negative control treatment from day 14 until day 28 and higher than the cumulative feed intake of the Positive control treatment on day 14. The cumulative feed intake of the Avizyme treatment was lower than the Hemicell treatment on day 35.

The cumulative feed intake of the Hemicell treatment was significantly higher than the Negative control treatment for the period day 14 to day 35 and higher than the Positive control treatment from day 21 until day 35. The Hemicell treatment’s cumulative feed intake was significantly higher than all the other treatments on day 35.

Table 21 Least square means (± standard error of the mean) of the average cumulative feed intake (g / bird) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	134.5 [± 1.87]	409.3[a] [± 4.64]	967.4[a] [± 11.19]	1873[a] [± 19.3]	2841[a] [± 24.9]
Avizyme[2]	137.3 [± 1.84]	423.4[b] [± 4.55]	993.4[ab] [± 10.98]	1892[ab] [± 19.0]	2878[a] [± 24.5]
Hemicell[3]	138.5 [± 1.84]	414.6[ab] [± 4.55]	999.7[b] [± 10.99]	1942[b] [± 19.0]	2964[b] [± 24.5]
Negative[4]	137.3 [± 1.91]	388.1[c] [± 4.72]	898.9[c] [± 11.38]	1805[c] [± 19.7]	2813[a] [± 25.4]
F-probability					
Treatment	0.484	< 0.001	< 0.001	< 0.001	0.001
Block	0.129	0.150	0.139	0.326	0.768
R^2	0.319	0.567	0.665	0.520	0.468

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

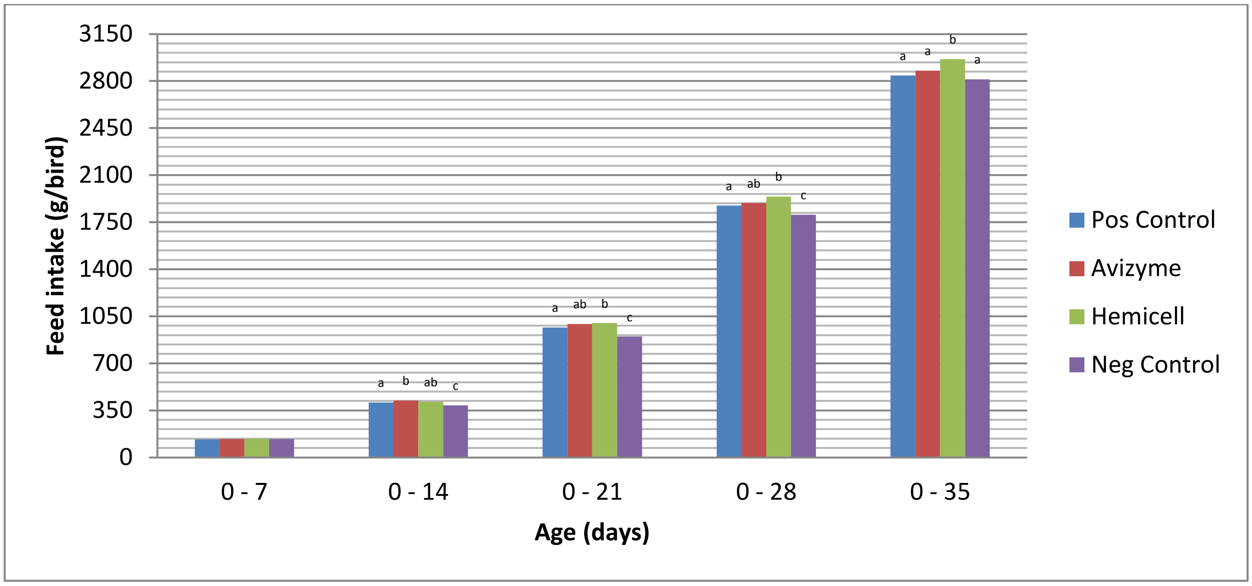

Figure 4 Average cumulative feed intake of the different treatments from day 0 until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.6. Weekly feed conversion ratio

As shown in Table 22 and illustrated in Figure 5, the weekly feed conversion ratio of the Negative control treatment was significantly poorer than all the other treatments for days 7, 14 and 35 and significantly poorer than the Avizyme treatment on days 21 and 28. The Negative control treatment's weekly feed conversion ratio was also poorer than the Positive control treatment on day 28, but better than the Hemicell treatment on day 28.

The Avizyme treatment's weekly feed conversion ratio was significantly better than the Negative control treatment from day seven until day 35 and better than the Positive control treatment for day 7. The weekly feed conversion ration of the Avizyme treatment was also significantly better than the Hemicell treatment for days 7, 21 and 28.

The weekly feed conversion ratio of the Hemicell treatment was significantly better than the Negative control treatment for the period days seven, 14 and 35, but significantly poorer than the weekly feed conversion ratio of the Negative control treatment on day 28. The Hemicell treatment's feed conversion ratio was significantly poorer than the Avizyme treatment on days seven, 21 and 28 and poorer than the Positive control treatment on day 28.

Table 22 Least square means (± standard error of the mean) of the average weekly feed conversion ratios (g feed intake / g body weight gain) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35
LSM [± SEM]					
Positive	1.21[a] [± 0.010]	1.31[a] [± 0.012]	1.52[ab] [± 0.023]	1.67[a] [± 0.026]	2.15[a] [± 0.043]
Avizyme	1.17[b] [± 0.010]	1.30[a] [± 0.012]	1.48[a] [± 0.023]	1.65[a] [± 0.025]	2.20[a] [± 0.042]
Hemicell	1.23[a] [± 0.010]	1.31[a] [± 0.012]	1.57[b] [± 0.023]	1.87[b] [± 0.025]	2.13[a] [± 0.042]
Negative	1.48[c] [± 0.011]	1.57[b] [± 0.012]	1.59[b] [± 0.024]	1.77[c] [± 0.026]	2.47[b] [± 0.044]
F-probability					
Treatment	< 0.001	< 0.001	0.010	< 0.001	< 0.001
Block	0.603	0.653	0.342	0.577	0.076
R^2	0.958	0.943	0.418	0.675	0.715

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

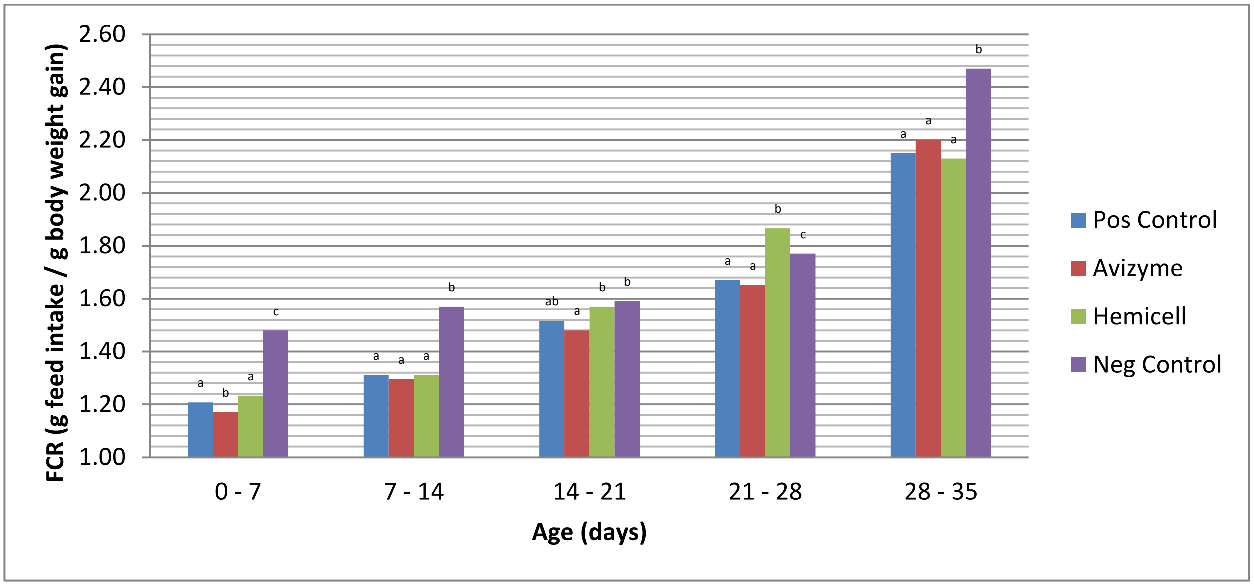

Figure 5 Average weekly FCR of the different treatments from day 0 until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.7. Cumulative feed conversion ratio

As shown in Table 23 and illustrated in Figure 6, the cumulative feed conversion ratio of the Negative control treatment was significantly poorer than all the other treatments on days 14, 21 and 35 and significantly poorer than the Positive and Avizyme treatments on day 28.

The Avizyme treatment's cumulative feed conversion ratio was significantly better than the Negative control and Hemicell treatments from day 14 until day 35. There were no significant differences between the Avizyme treatment and the Positive control treatments.

The Hemicell treatment's cumulative feed conversion ratio was significantly better than the Negative control treatment on days 14, 21 and 35. The cumulative feed conversion ratio for the Hemicell treatment was significantly poorer than the Avizyme treatment from day 14 until day 35 and poorer than the Positive control treatment on days 28 and 35.

Table 23 Least square means (± standard error of the mean) of the average cumulative feed conversion ratio (g feed intake / g body weight gain) for the different treatments from day 0 until day 35

	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	1.27[ab] [± 0.009]	1.41[ab] [± 0.015]	1.52[a] [± 0.017]	1.69[a] [± 0.020]
Avizyme[2]	1.25[a] [± 0.009]	1.37[a] [± 0.015]	1.49[a] [± 0.016]	1.68[a] [± 0.019]
Hemicell[3]	1.28[b] [± 0.009]	1.44[b] [± 0.015]	1.62[b] [± 0.016]	1.76[b] [± 0.019]
Negative[4]	1.54[c] [± 0.009]	1.57[c] [± 0.015]	1.67[b] [± 0.017]	1.88[c] [± 0.020]
F-probability				
Treatment	< 0.001	< 0.001	< 0.001	< 0.001
Block	0.792	0.409	0.368	0.349
R^2	0.969	0.817	0.766	0.776

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

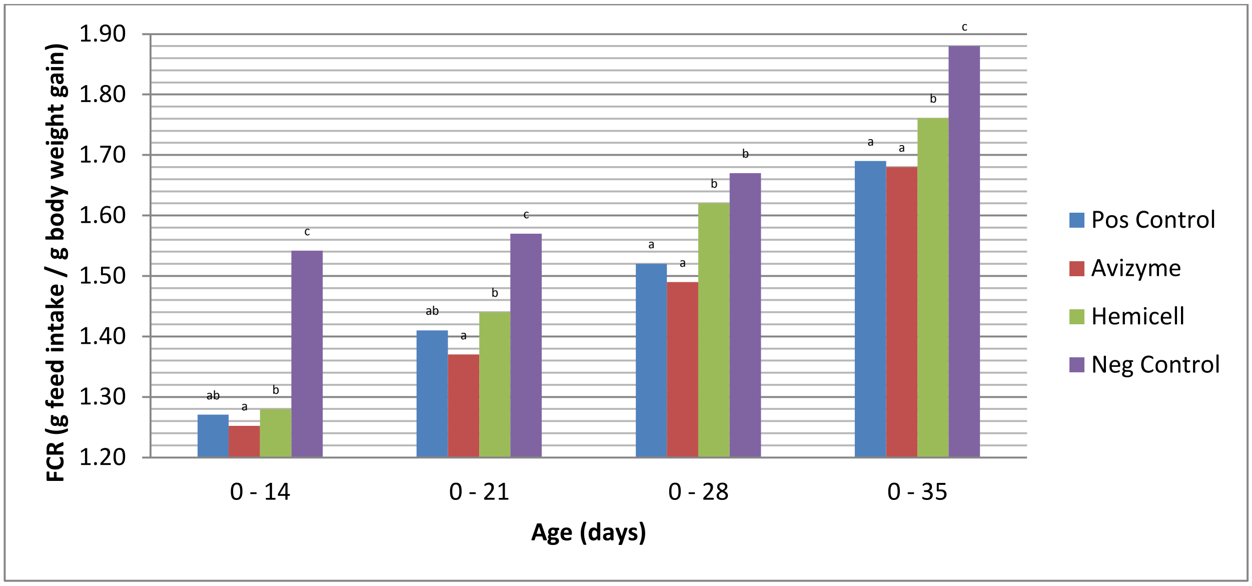

Figure 6 Average cumulative feed conversion ratio of the different treatments from day 0 until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.8. Cumulative mortality

Cumulative mortality was calculated as a percentage of birds placed originally on day 0. As shown in Table 24 and illustrated in Figure 7, the cumulative mortality of the Negative control treatment was not significantly different from any of the other treatments. Likewise, there was no significant difference between the Avizyme treatment and the other treatments.

The cumulative mortality of the Hemicell treatment was significantly lower than the Positive control treatment on day seven.

Table 24 Least square means (± standard error of the mean) of the average cumulative mortality (as a percentage of birds placed) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	1.13[a] [± 0.245]	1.31 [± 0.303]	1.39 [± 0.313]	1.97 [± 0.500]	2.84 [± 0.754]
Avizyme[2]	0.44[ab] [± 0.240]	0.58 [± 0.297]	0.67 [± 0.307]	1.05 [± 0.491]	2.95 [± 0.740]
Hemicell[3]	0.36[b] [± 0.240]	0.59 [± 0.298]	0.66 [± 0.307]	0.96 [± 0.491]	2.60 [± 0.740]
Negative[4]	0.56[ab] [± 0.249]	0.79 [± 0.308]	1.15 [± 0.318]	1.78 [± 0.509]	3.71 [± 0.767]
F-probability					
Treatment	0.130	0.297	0.259	0.375	0.770
Block	0.314	0.350	0.599	0.831	0.644
R^2	0.327	0.266	0.228	0.149	0.102

[ab] Column means with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

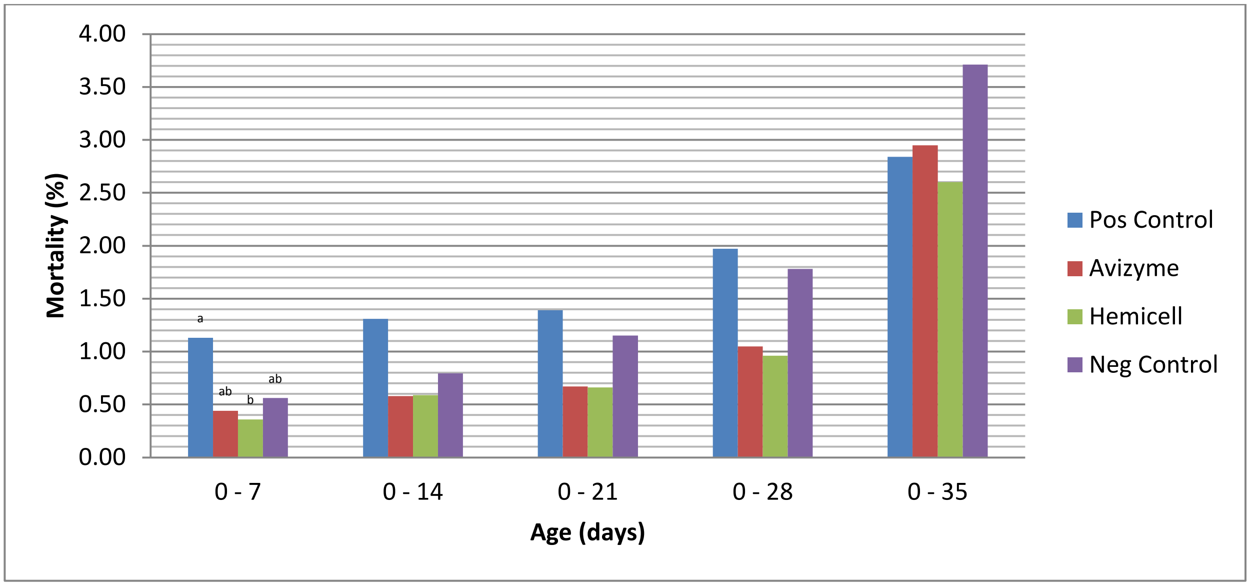

Figure 7 Average cumulative mortality (as a percentage of birds placed) of the different treatments from day 0 until day 35

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.9. Production efficiency factor

The production efficiency factor (PEF) was calculated using the following equation:

$$\text{PEF} = \frac{(100-\text{Cumulative mortality})\times\text{Body weight}}{\text{Cumulative feed conversion ratio}\times\text{Age (days)}} \times 10$$

As shown in Table 25 and illustrated in Figure 8, the production efficiency factor of the Negative control treatment was significantly lower than all the other treatments from day seven until 35. The Avizyme treatment's production efficiency factor was significantly higher than all the other treatments from day seven until day 28 and higher than the Hemicell and Negative groups on day 35.

The production efficiency factor of the Hemicell treatment was significantly higher than the Negative control treatment, but significantly lower than the Avizyme treatment for the period day seven until day 35. There was no significant difference between the Hemicell treatment and the Positive control treatment.

Table 25 Least square means (± standard error of the mean) of the average production efficiency factor for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	177.6[a] [± 2.94]	200.8[a] [± 2.55]	243.4[a] [± 3.26]	292.4[a] [± 4.41]	283.6[ab] [± 4.89]
Avizyme[2]	193.1[b] [± 2.89]	215.7[b] [± 2.51]	263.0[b] [± 3.20]	309.8[b] [± 4.32]	291.1[a] [± 4.80]
Hemicell[3]	177.9[a] [± 2.89]	202.2[a] [± 2.51]	243.2[a] [± 3.20]	271.7[c] [± 4.33]	271.4[b] [± 4.80]
Negative[4]	128.2[c] [± 2.99]	135.0[c] [± 2.60]	184.5[c] [± 3.32]	237.4[d] [± 4.48]	225.4[c] [± 4.97]
F-probability					
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Block	0.521	0.640	0.181	0.663	0.526
R^2	0.919	0.962	0.932	0.866	0.837

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

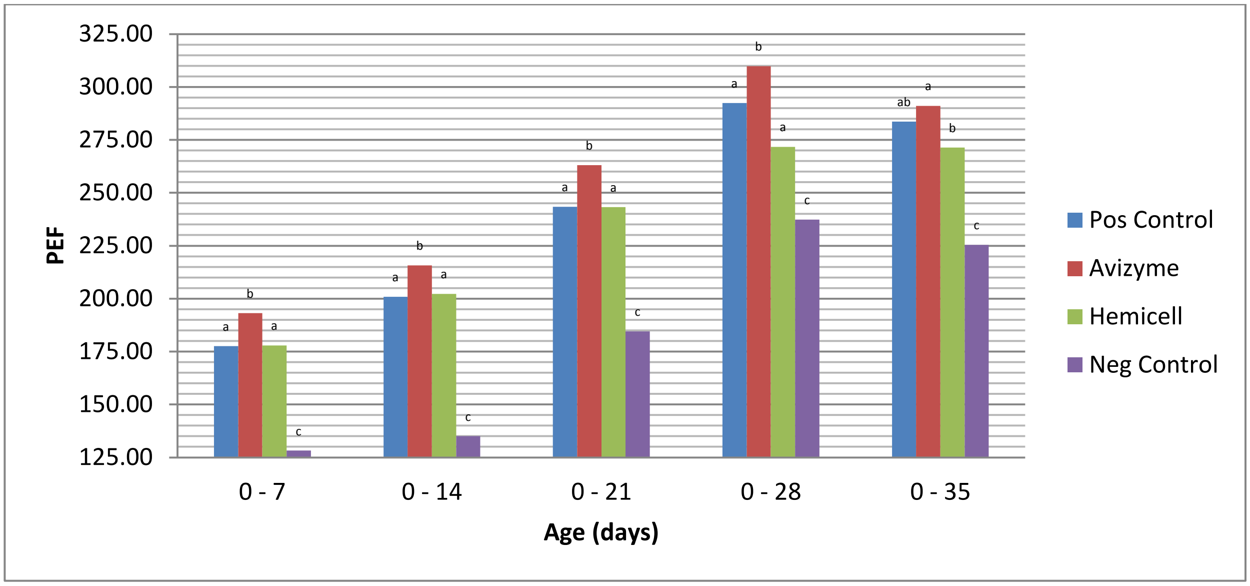

Figure 8 Average production efficiency factor of the different treatments from day 0 until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).

Pos Control: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.35 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.35 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Neg Control: Formulated to have 0.35 MJ less energy than the Positive control. No exogenous enzyme was included.

3.3.10. Economic evaluation

In order to do an economic evaluation of the enzymes added to the feed, both the saving on the feed, as well as the saving on the broilers as final product had to be calculated. Avizyme costed R 63-85 / kg and was included at 500 g / ton. This means that Avizyme was included at a cost of R 31-93 / ton feed. Hemicell cost R 115-00 / kg and was included at 125 g / ton, and Hemicell was therefore included at a cost of R 14-38 / ton feed. A summary of the economic impact of enzyme addition to the broiler diets is shown in Table 26.

The Positive, Avizyme and Hemicell starter feeds were produced at a cost of R 2538-94 / ton, R 2457-98 / ton and R 2441-35 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 80-96 / ton and R 97-59 / ton for Avizyme and Hemicell, respectively. The Positive control group consumed an average of 0.728 kg starter / bird at a cost of R 1-85 per bird for the starter phase. The Avizyme treatment group consumed an average of 0.749 kg starter / bird at a cost of R 1-84 per bird for the starter phase. The Hemicell treatment group consumed an average of 0.749 kg starter / bird at a cost of R 1-83 per bird for the starter phase. The cost saving as a result of the inclusion of enzymes in the starter phase was 1 c / bird and 2 c / bird for Avizyme and Hemicell, respectively. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.531 kg / bird during the starter phase, at a possible income of R 5-09 / bird for the starter phase. The Avizyme treatment group gained an average of 0.558 kg / bird during the starter phase, at a possible income of R 5-35 / bird for the starter phase. The Hemicell treatment group gained an average of 0.537 kg / bird during the starter phase, at a possible income of R 5-14 / bird for the starter phase. The increased income as a result of the inclusion of enzymes in the starter phase was 26 c / bird and 5 c / bird for Avizyme and Hemicell, respectively. The total increased income over feed cost due to the inclusion of enzymes was 27 c / bird and 7 c / bird for Avizyme and Hemicell, respectively.

The Positive, Avizyme and Hemicell grower feeds were produced at a cost of R 2596-35 / ton, R 2534-52 / ton and R 2517-91 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 61-83 / ton and R 78-44 / ton for Avizyme and Hemicell, respectively. The Positive control group consumed an average of 1.145 kg grower / bird at a cost of R 2-97 per bird for the grower phase. The Avizyme treatment group consumed an average of 1.143 kg grower / bird at a cost of R 2-90 per bird for the grower phase. The Hemicell treatment group consumed an average of 1.193 kg grower / bird at a cost of R 3-00 per bird for the grower phase. The cost saving as a result of the inclusion of Avizyme in the grower phase was 7 c / bird. The Hemicell treatment group had a significantly higher feed intake than the Positive control group, which resulted in the Hemicell treatment group's feed costing 3 c / bird more during the grower phase. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.699 kg / bird during the grower phase, at a possible income of R 6-70 / bird for the grower phase. The Avizyme treatment group gained an average of 0.709 kg / bird during the grower phase, at a possible income of R 6-79 / bird for the grower phase. The Hemicell treatment group gained an average of 0.664 kg / bird during the grower phase, at a possible income of R 6-36 / bird for the grower phase. The increased income as a result of the inclusion of Avizyme in the grower phase was 9 c / bird. The Hemicell treatment group had a lower body weight gain than the Positive control group, which resulted in the Hemicell treatment group's birds realising a lower possible value of 34 c / bird less than the Positive control group's birds during the grower phase. The total increased income over feed cost due to the inclusion of Avizyme was 16 c / bird during the grower phase. Inclusion of Hemicell in the grower phase resulted in a decreased income over feed cost of 37 c / bird during the grower phase.

Table 26 Calculation of increased income over feed cost (IOFC) for the Avizyme and Hemicell treatments, compared to the IOFC of the Positive control

	Starter	Grower	Finisher
Positive control			
Live weight gain (kg)	0.531	0.699	0.452
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.728	1.145	0.968
Feed cost (c / kg)	254	260	251
IOFC (c)[1]	324	372	190
Avizyme treatment			
Live weight gain (kg)	0.558	0.709	0.451
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.749	1.143	0.986
Feed cost (c / kg)	246	253	244
IOFC (c)[1]	350	390	192
Increased IOFC (c)[2]	27	17	2
Hemicell treatment			
Live weight gain (kg)	0.537	0.664	0.479
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.749	1.193	1.022
Feed cost (c / kg)	244	252	242
IOFC (c)[1]	332	336	211
Increased IOFC (c)[3]	8	- 37[4]	21

[1] IOFC = (Live weight gain × Live weight price) – (Feed intake × Feed cost).

[2] Avizyme treatment increased IOFC = Avizyme treatment IOFC – Positive control IOFC.

[3] Hemicell treatment increased IOFC = Hemicell treatment IOFC – Positive control IOFC.

[4] Negative value indicates a decreased IOFC.

The Positive, Avizyme and Hemicell grower feeds were produced at a cost of R 2596-35 / ton, R 2534-52 / ton and R 2517-91 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 61-83 / ton and R 78-44 / ton for Avizyme and Hemicell, respectively. The Positive control group consumed an average of 1.145 kg grower / bird at a cost of R 2-97 per bird for the grower phase. The

Avizyme treatment group consumed an average of 1.143 kg grower / bird at a cost of R 2-90 per bird for the grower phase. The Hemicell treatment group consumed an average of 1.193 kg grower / bird at a cost of R 3-00 per bird for the grower phase. The cost saving as a result of the inclusion of Avizyme in the grower phase was 7 c / bird. The Hemicell treatment group had a significantly higher feed intake than the Positive control group, which resulted in the Hemicell treatment group's feed costing 3 c / bird more during the grower phase. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.699 kg / bird during the grower phase, at a possible income of R 6-70 / bird for the grower phase. The Avizyme treatment group gained an average of 0.709 kg / bird during the grower phase, at a possible income of R 6-79 / bird for the grower phase. The Hemicell treatment group gained an average of 0.664 kg / bird during the grower phase, at a possible income of R 6-36 / bird for the grower phase. The increased income as a result of the inclusion of Avizyme in the grower phase was 9 c / bird. The Hemicell treatment group had a lower body weight gain than the Positive control group, which resulted in the Hemicell treatment group's birds realising a lower possible value of 34 c / bird less than the Positive control group's birds during the grower phase. The total increased income over feed cost due to the inclusion of Avizyme was 16 c / bird during the grower phase. Inclusion of Hemicell in the grower phase resulted in a decreased income over feed cost of 37 c / bird during the grower phase.

The Positive and Avizyme finisher feeds were produced at a cost of R 2511-14 / ton and R 2439-24 / ton, respectively. The saving on the feed with the inclusion of Avizyme was R 71-90 / ton. The Positive control group consumed an average of 0.968 kg finisher / bird at a cost of R 2-43 per bird for the finisher phase. The Avizyme treatment group consumed an average of 0.986 kg finisher / bird at a cost of R 2-41 per bird for the finisher phase. The cost saving as a result of the inclusion of Avizyme in the finisher phase was 2 c / bird. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.452 kg / bird during the finisher phase, at a possible income of R 4-33 / bird for the finisher phase. The Avizyme treatment group gained an average of 0.451 kg / bird during the finisher phase, at a possible income of R 4-32 / bird for the finisher phase. The Avizyme treatment group had a lower body weight gain than the Positive control group, which resulted in the Avizyme treatment group's birds realising a lower possible value of 1 c / bird less than the Positive control group's birds during the finisher phase. The total increased income over feed cost due to the inclusion of Avizyme was 1 c / bird.

Inclusion of Avizyme in the feed, realised a total cost saving of 10 c / bird, a total increased income from the birds of 34 c / bird and 19 c / kg live weight. The total increased income over feed cost that could be realised with the inclusion of Avizyme was 44 c / bird and 25 c / kg live weight over a five week period. Due to higher feed intakes with the inclusion of Hemicell in the feed, the inclusion of Hemicell in the feed, increased the feed cost by 1 c / bird during the first four weeks. During the first four weeks, a total decreased income from the birds of 29 c / bird and 23 c / kg live weight was realised with the inclusion of Hemicell in the feed. The total decreased income over feed cost that could be realised with the inclusion of Hemicell was 30 c / bird and 24 c / kg live weight over a four week period.

3.4. Discussion

Several problems were encountered with the dosing of the trial feeds with Avizyme at the feed mill. Dosing of the starter diets was done correctly. Avizyme activity in the grower feed of the Avizyme treatment was lower than the intentional level. Although the amylase was at suboptimal activity in the Avizyme treatment's grower feed, the Avizyme treatment still had the highest amylase activity, indicating that dosing did take place. The dosing of Avizyme in the grower phase may, however, have been at suboptimal levels. For the finisher phase, the Avizyme treatment diet was correctly dosed. The feed of the Hemicell treatment was correctly dosed with Hemicell for all three phases. The finisher feed of the Hemicell treatment was, however, incorrectly dosed with Avizyme as well.

As the Avizyme treatment for the grower phase contained suboptimal activity levels for the enzymes, only the results for the starter phase could be interpreted with confidence to evaluate the efficacy of Avizyme on broiler production. Since the finisher diet also contained the correct levels of Avizyme, and the broilers therefore received the correct enzyme levels for more than 70% of the trial period, results for the entire 35 day period were also discussed. Exogenous enzyme addition to the feed has been shown to be more effective in younger broilers (Classen, 1996), since younger broilers have less endogenous enzymes available. The final results regarding Avizyme efficacy could therefore have been compromised by the low Avizyme levels during the ten day grower phase.

The dosing of Avizyme together with Hemicell in the finisher feed of the Hemicell treatment that was fed from day 29, could have significantly affected the Hemicell treatment's results in the final week of the trial. The complete 35 day period of the trial could therefore not be analysed and interpreted for the effect of Hemicell on broiler production variables. Therefore, the effect of Hemicell on broiler production was only evaluated for the first 28 days of the trial period.

The Negative and Positive control groups were correctly produced and fed throughout the duration of the trial. If the Avizyme and / or the Hemicell products did not supply the level of energy claimed, the treatment feeds would have resulted in performance significantly lower than the Positive control.

The following discussion is relevant to the efficacy of Avizyme for the entire 35 day period of the trial:

Birds that received the Positive control consistently performed better than the Negative control, showing that the Negative control had indeed a lower metabolisable energy content than the Positive control and that energy was the first limiting nutrient.

The Avizyme treatment group consistently performed better than the Negative control group. This indicated that Avizyme application to a feed of relative low energy density had a beneficial effect on broiler performance. The Avizyme treatment group performed at least similar, and in some cases superior to the Positive control group. This indicated that Avizyme made a minimum of 0.35 MJ ME / kg feed available. The results suggested that Avizyme could also have resulted in more additional available energy than the expected 0.35 MJ ME / kg feed.

The following discussion is only relevant for the efficacy of Avizyme during the first 14 days of the trial period:

The Avizyme treatment group consistently performed better than the Negative control group. This indicated that Avizyme application to a feed equal in energy specification of the Negative control during the starter phase had a beneficial effect on broiler performance during the first 14 days of production.

The Avizyme treatment group's performance was for most of the parameters significantly better than the Positive control group during the first 14 days. This indicated that Avizyme application in the starter phase made an additional minimum of 0.35 MJ ME / kg feed available, but results suggested that Avizyme made more than the expected 0.35 MJ ME / kg available during the first 14 days of production.

During the first 14 days of the trial, the Avizyme treatment group's performance was in general superior to the performance of the Hemicell treatment group. This indicated that Avizyme application during the starter phase might deliver better results than Hemicell during the starter phase.

The following discussion is relevant for the efficacy of Hemicell during the first 28 days of the trial period:

The Hemicell treatment group consistently performed better than the Negative control group. This indicated that Hemicell application to a starter and grower feed with a similar energy concentration than the Negative control had a beneficial effect on broiler performance during the first 28 days.

The Hemicell treatment group's performance was consistently equal to the performance of the Positive control group. This indicated that Hemicell application during the starter and grower phases made an additional 0.35 MJ ME / kg feed available during the first 28 days, as was hypothesised.

Trials evaluating the efficacy of exogenous enzymes on broiler performance have shown contradictory results, ranging from no improvement in measured parameters like digestibility and viscosity (Mahagna *et al*., 1995; Zanella *et al*., 1999; Saleh *et al*., 2003) to significant improvement in broiler performance (Centeno *et al*., 2006; Karimi *et al*., 2007).

An exogenous enzyme increases the availability of a specific nutrient in a diet. When evaluating an exogenous enzyme it is critical that this specific nutrient is the first limiting nutrient in the diet. In order to ensure that metabolisable energy was the first limiting nutrient in this trial, balanced diets were formulated, with the exception of energy, which was undersupplied in the diets.

The improved broiler performance in research trials done by Centeno *et al*. (2006) and Karimi *et al*. (2007) are similar to the results found in this performance trial. Although it is beyond the scope of this trial, the increased broiler performance support numerous statements regarding increased ileal digestibility of feed, improvement in viscosity of the diet and increased apparent and true metabolisable energy due to the addition of exogenous enzymes.

Broilers in the Hemicell treatment group performed significantly better than the Negative control group. In the trial conducted by McNaughten *et al*. (1998), broilers in the treatment group only performed slightly better than the broilers in the control group. The difference between these two trials is because the Negative control group in this trial had a metabolisable energy reduction of 0.35 MJ / kg, while the McNaughten *et al*. (1998) trial had an energy reduction of 0.6 MJ / kg.

3.5. Conclusion

Due to incorrect dosing of trial feeds, neither Avizyme nor Hemicell could have been evaluated as feed additives for the entire 35 day production period. It was, however, found that testing feed samples for enzyme activity was critical to evaluate the accuracy of feed mixing. Intense supervision during feed manufacturing and enzyme dosing is as critical as accurate broiler performance recording. The addition of Avizyme to an extended starter phase of a feed contributed more than 0.35 MJ ME / kg feed and resulted in significant improvement in broiler production over a five week growing period. The difference in energy value between the feed of the Positive and Negative control was not significantly high enough as the Avizyme treatment outperformed the Positive control in some instances. This prohibited the determination of an exact metabolisable energy contribution of Avizyme to a broiler starter diet. It could only be concluded that addition of Avizyme to a broiler starter ration will increase the metabolisable energy availability by at least 0.35 MJ ME / kg and will result in a positive influence on broiler production. A repetition of this trial, including a correctly formulated Positive control with a higher energy value, might reveal the exact energy contribution of Avizyme 1502 to a broiler feed. The addition of Hemicell to feeds of an extended starter and grower phase contributed 0.35 MJ ME / kg feed and resulted in a significant improvement in broiler production over a five week growing period.

Chapter 4

Performance trial 2: Efficacy of Avizyme 1502® and Hemicell® as feed additives, alone and in combination, in increasing the availability of metabolisable energy in broiler feeds

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Abstract

The aim of this trial was to investigate whether the feed enzymes, Avizyme 1502 (containing amylase, xylanase and protease) and Hemicell (containing β-mannanase), would contribute an additional 0.45 MJ ME / kg of broiler feed. The hypothesis is that the addition of exogenous enzymes to the feed will reduce the effects of anti-nutritional factors present in maize and soya, leading to an increased metabolisable energy availability to the broiler. A combination of Avizyme 1502 and Hemicell was also added to a feed to determine if a positive synergistic effect exists when adding more than one enzyme product to feed. A Negative control feed was formulated to have 0.45 MJ ME / kg feed less energy than a Positive control feed. Three treatment feeds were formulated to contain similar metabolisable energy values than the Negative control. The commercial enzyme preparations, Avizyme and Hemicell, were added to two of these treatment diets, respectively, and a combination of Avizyme and Hemicell was added to the third treatment diet. It was expected that these enzyme preparations would increase the metabolisable energy content of the treatment diets with 0.45 MJ ME / kg. Ross 788 broilers were fed different treatment feeds from day of hatch for 35 days. Weekly body weights, weekly body weight gains, weekly and cumulative feed intake, weekly and cumulative feed conversion ratios, cumulative mortality and production efficiency factors were recorded and calculated as performance variables to compare the respective feeds. This trial revealed that the addition of Avizyme to a feed increased the energy availability by at least 0.45 MJ ME / kg feed and resulted in significant improvements in bodyweight gain, feed intake, FCR and PEF over a five week growing period ($P < 0.05$). The addition of Hemicell to a feed delivered similar results to the Positive control, indicating that Hemicell might increase the metabolisable energy content of a maize-soya based feed with 0.45 MJ / kg feed. Broilers that received both Avizyme and Hemicell had significantly better bodyweight gain, feed intake, FCR and PEF than the Positive control ($P < 0.05$), indicating a slight positive synergystic effect for the two enzyme products.

4.1. Introduction

The aim of this trial was to investigate whether the feed enzymes, Avizyme 1502 (containing amylase, xylanase and protease) and Hemicell (containing β-mannanase), would release an additional 0.45 MJ ME / kg on a maize-soya based broiler feed. A combination of Avizyme 1502 and Hemicell was also added to a feed to determine if the combination of these two enzymes provided an additive effect, i.e. increased broiler performance more than with any of the enzyme products alone.

4.2. Materials and methods

4.2.1. Experimental design

A randomised block design was used in this trial. The trial was conducted at the Daybreak experimental farm in Sundra (Mpumalanga, South Africa) in two environmentally controlled broiler houses. Each of the houses were divided into three blocks to minimise the variation within a block and maximise the variation between blocks. Dividing the houses into blocks assisted in minimising the effect of unintentional temperature fluctuations and different noise levels within the house. There were five treatments with 12 replicates per treatment, six in House A and six in House B. Thus, each treatment was replicated twice within each of the three blocks per house.

4.2.2. Housing

Each of the two broiler houses used in this trial was divided into 30 pens (60 pens in total over the two houses). Each pen had a surface area of six square meters (2 m × 3 m). Both houses had solid concrete floors and pens were covered with a layer of wood shavings. Each house had a boiler to control the temperature in the house. Hot air was blown through a hot air tunnel that ran through the whole house. Heated air was distributed evenly throughout the house. Six thermometers and three temperature and humidity loggers were installed throughout the house and monitored daily to ensure that a uniform temperature and humidity was maintained throughout the whole house. Houses had curtains on the sides to control ventilation. For the first five days chicks were fed from a combination of pan feeders and fountain feeders. Water was supplied by a combination of nipple drinkers and fountain drinkers. After five days the pan feeders and fountain drinkers were removed. Feeders were kept full at all times to ensure that feed intake was not affected by low feed levels.

The temperature profile that was followed from day 1 – 35 is shown in Table 27. Minimum and maximum temperatures, and the boiler temperature reading were monitored on a daily basis to ensure that temperature stayed within the range. The lighting program for the 35 day period is shown in Table 28.

Table 27 Temperature profile for Performance Trial 2

Days	Temperature (°C, 50% rH)		
	Lower temperature	Target temperature	Upper temperature
1 – 2	31.5	33.0	34.5
3 – 5	30.5	32.0	33.5
6 – 8	29.5	31.0	32.5
9 – 11	28.2	29.7	31.2
12 – 14	25.7	27.2	28.7
15 – 17	24.7	26.2	27.2
18 – 20	23.5	25.0	26.5
21 – 23	22.5	24.0	25.5
24 – 35	21.5	23.0	24.5

Table 28 Lighting programme for Performance Trial 2

Days	Day light (hours)	Darkness (hours)
1 – 6	23	1
7 – 15	14	10
16 – 22	16	8
23 – 28	18	6
29 – 35	20	4

4.2.3. Birds

Chicks were placed in two houses (House A and House B) on two consecutive days. Three thousand seven hundred and eighty (3780) day-old chicks (Ross 788) were obtained from Midway Hatcheries for each of the two houses. One hundred and twenty six (126) chicks were randomly allocated to each pen on day 0. Stocking density at day 0 was 21 chicks / m^2.

Chicks were graded by the hatchery to ensure placement of good quality chicks.

4.2.4. Dietary treatments

All feed was manufactured at the Afgri Animal Feeds commercial feed factory in Isando, Gauteng. Lines were cleaned properly before trial feed manufacturing commenced and lines were also cleaned thoroughly between treatments to avoid contamination between respective treatments. The Negative control and Positive control treatments were manufactured first to ensure that these diets did not contain any traces of Avizyme or Hemicell. The feed was bagged off into 50 kg bags and bags for various treatments were clearly marked for easy identification.

All treatment feeds were formulated to contain a minimum of 18% total soya products. The following feed treatments were tested in this trial:

Positive control: This feed was formulated according to standard nutrient specifications used by Daybreak Farms to meet or exceed the nutritional requirements of broilers, as recommended by the NRC (1994). The apparent metabolisable energy was slightly lower than the standard to ensure that energy is the first limiting nutrient (Table 29). No exogenous enzyme was added to this treatment.

Avizyme treatment: This feed was formulated to have 0.45 MJ less metabolisable energy / kg than the Positive control. Avizyme was included at 0.05%.

Hemicell treatment: This feed was formulated to contain 0.45 MJ less metabolisable energy / kg than the Positive control. Hemicell was included in the feed at 0.0125%.

Table 29 Energy specifications of treatment diets (MJ ME / kg)

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Starter	11.80	11.35	11.35	11.35	11.35
Grower	12.40	11.95	11.95	11.95	11.95
Finisher	12.66	12.21	12.21	12.21	12.21

[1]Included Avizyme 1502 at 0.5 g / kg.
[2]Included Hemicell at 0.125 g / kg.

Combination treatment: This feed was also formulated to have 0.45 MJ ME / kg less than the Positive control. Avizyme was added to this treatment at 0.05% and Hemicell was added at 0.0125%.

Negative control: This feed was formulated to have 0.45 MJ ME / kg feed less than the Positive control. No exogenous enzymes were added.

These treatments applied to three feeding phases used in this trial, i.e. an extended starter, grower and finisher phase (Table 29). Birds were fed according to days on feed (18, 10 and 7 days, respectively). With every change to the next phase, the left-over feed from the previous phase was weighed back and discarded. Birds were fed *ad libitum*.

Tables 30 to 35 show the raw material inclusion and calculated nutrient specifications for each feed used in the trial.

Representative samples of the different feeds were collected during feeding, before the birds had access to the feed. Each sample was ground and analysed for dry matter, ash, crude protein, crude fibre, crude fat, calcium, phosphorous, potassium and sodium at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria). Representative samples of each feed were also analysed for Avizyme activity and Hemicell activity by Danisco Animal Nutrition (Wiltshire, UK) and ChemGen Corporation (Gathersburg, USA), respectively. Moisture determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 934.01). Dry matter determination and ashing was done according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 942.05). Crude fibre determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 962.09). The Dosi fibre system was used to determine the crude fibre percentage. Crude fat determination was done according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 920.39). Crude protein determination was done following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 988.05). The Leco FP – 428 (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396) was used to determine the nitrogen content of a feed. Samples were prepared for calcium analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 935.13).

Table 30 Raw material inclusion (% as fed basis) for the starter phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Maize	58.15	58.75	58.75	58.75	58.75
Full fat soya (35.5% CP)	7.00	7.00	7.00	7.00	7.00
Soybean oilcake (46% CP)	22.73	21.00	21.00	21.00	21.00
Sunflower oilcake (36% CP)	4.00	7.00	7.00	7.00	7.00
Fish meal (65% CP)	1.80	1.38	1.38	1.38	1.38
L threonine	0.042	0.045	0.045	0.045	0.045
DL methionine	0.271	0.128	0.128	0.128	0.128
Lysine HCl	0.235	0.277	0.277	0.277	0.277
Vegetable oil	2.16	0.70	0.70	0.70	0.70
Salt	0.365	0.370	0.370	0.370	0.370
Sodium bicarbonate	0.06	0.06	0.06	0.06	0.06
Monocalcium phosphate	1.42	1.43	1.43	1.43	1.43
Limestone	1.50	1.58	1.58	1.58	1.58
Vitamin & mineral premix	0.12	0.12	0.12	0.12	0.12
Coccidiostat and AGP[3]	0.16	0.16	0.16	0.16	0.16

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Stafac 4% and Salinomycin 12%.

Table 31 Raw material inclusion (% as fed basis) for the grower phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Maize	61.73	63.35	63.35	63.35	63.35
Full fat soya (35.5% CP)	10.00	10.00	10.00	10.00	10.00
Soybean oilcake (46% CP)	16.68	14.90	14.90	14.90	14.90
Sunflower oilcake (36% CP)	4.00	6.05	6.05	6.05	6.05
Fish meal (65% CP)	1.00	1.00	1.00	1.00	1.00
L threonine	0.041	0.043	0.043	0.043	0.043
DL methionine	0.207	0.199	0.199	0.199	0.199
Lysine HCl	0.249	0.279	0.279	0.279	0.279
Vegetable Oil	2.92	0.98	0.98	0.98	0.98
Salt	0.385	0.380	0.380	0.380	0.380
Sodium bicarbonate	0.02	0.03	0.03	0.03	0.03
Monocalcium phosphate	1.19	1.17	1.17	1.17	1.17
Limestone	1.30	1.30	1.30	1.30	1.30
Vitamin & mineral premix	0.11	0.11	0.11	0.11	0.11
Coccidiostat and AGP[3]	0.16	0.16	0.16	0.16	0.16

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Stafac 4% and Salinomycin 12%.

Table 32 Raw material inclusion (% as fed basis) for the finisher phase of the different treatment feeds

Ingredient	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Maize	63.55	66.13	66.13	66.13	66.13
Full fat soya (35.5% CP)	10.00	10.00	10.00	10.00	10.00
Soybean oilcake (46% CP)	14.73	14.50	14.50	14.50	14.50
Sunflower oilcake (36% CP)	4.00	4.00	4.00	4.00	4.00
Fish meal (65% CP)	1.08	1.00	1.00	1.00	1.00
L threonine	0.041	0.039	0.039	0.039	0.039
DL methionine	0.194	0.189	0.189	0.189	0.189
Lysine HCl	0.255	0.260	0.260	0.260	0.260
Vegetable oil	3.43	1.09	1.09	1.09	1.09
Salt	0.373	0.373	0.373	0.373	0.373
Sodium bicarbonate	0.00	0.00	0.00	0.00	0.00
Monocalcium phosphate	0.97	0.97	0.97	0.97	0.97
Limestone	1.15	1.15	1.15	1.15	1.15
Vitamin & mineral premix	0.10	0.10	0.10	0.10	0.10
Coccidiostat and AGP[3]	0.16	0.16	0.16	0.16	0.16

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Stafac 4% and Salinomycin 12%.

Table 33 Calculated nutrient specifications (% on as fed basis) for the starter phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Dry matter	89.58	89.54	89.54	89.54	89.54
Protein	20.96	20.86	20.86	20.86	20.86
AME for chicks (MJ / kg)	11.80	11.35	11.35	11.35	11.35
Fibre	3.24	3.85	3.85	3.85	3.85
Fat	6.05	4.58	4.58	4.58	4.58
Lysine[3]	1.10	1.10	1.10	1.10	1.10
Methionine[3]	0.57	0.43	0.43	0.43	0.43
Total sulphur amino acids[3]	0.83	0.70	0.70	0.70	0.70
Threonine[3]	0.68	0.68	0.68	0.68	0.68
Tryptophan[3]	0.20	0.20	0.20	0.20	0.20
Arginine[3]	1.23	1.24	1.24	1.24	1.24
Isoleucine[3]	0.76	0.75	0.75	0.75	0.75
Valine[3]	0.84	0.84	0.84	0.84	0.84
Leucine[3]	1.55	1.54	1.54	1.54	1.54
Glycine and Serine[3]	1.59	1.59	1.59	1.59	1.59
C 18:2	2.42	2.02	2.02	2.02	2.02
Calcium	0.88	0.90	0.90	0.90	0.90
Potassium	0.84	0.84	0.84	0.84	0.84
Chloride	0.30	0.30	0.30	0.30	0.30
Total Phosphorous	0.71	0.73	0.73	0.73	0.73
Retainable Phosphorous	0.40	0.40	0.40	0.40	0.40
Sodium	0.18	0.18	0.18	0.18	0.18

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Apparent digestible.

Table 34 Calculated nutrient specifications (% on as fed basis) for the grower phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Dry matter	89.72	89.58	89.58	89.58	89.58
Protein	18.81	18.84	18.84	18.84	18.84
AME for chicks (MJ / kg)	12.40	11.95	11.95	11.95	11.95
Fibre	3.07	3.49	3.49	3.49	3.49
Fat	7.44	5.55	5.55	5.55	5.55
Lysine[3]	0.98	0.98	0.98	0.98	0.98
Methionine[3]	0.47	0.47	0.47	0.47	0.47
Total sulphur amino acids[3]	0.71	0.72	0.72	0.72	0.72
Threonine[3]	0.61	0.61	0.61	0.61	0.61
Tryptophan[3]	0.18	0.18	0.18	0.18	0.18
Arginine[3]	1.09	1.10	1.10	1.10	1.10
Isoleucine[3]	0.67	0.67	0.67	0.67	0.67
Valine[3]	0.74	0.75	0.75	0.75	0.75
Leucine[3]	1.41	1.41	1.41	1.41	1.41
Glycine and Serine[3]	1.42	1.42	1.42	1.42	1.42
C 18:2	3.01	2.47	2.47	2.47	2.47
Calcium	0.75	0.75	0.75	0.75	0.75
Potassium	0.77	0.76	0.76	0.76	0.76
Chloride	0.30	0.30	0.30	0.30	0.30
Total Phosphorous	0.63	0.64	0.64	0.64	0.64
Retainable Phosphorous	0.34	0.34	0.34	0.34	0.34
Sodium	0.17	0.17	0.17	0.17	0.17

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Apparent digestible.

Table 35 Calculated nutrient specifications (% on as fed basis) for the finisher phase of the different treatment feeds

	Positive control	Avizyme treatment[1]	Hemicell treatment[2]	Combination treatment[1, 2]	Negative control
Dry matter	89.75	89.52	89.52	89.52	89.52
Protein	18.08	18.12	18.12	18.12	18.12
AME for chicks (MJ / kg)	12.66	12.21	12.21	12.21	12.21
Fibre	3.04	3.08	3.08	3.08	3.08
Fat	7.97	5.71	5.71	5.71	5.71
Lysine[3]	0.94	0.94	0.94	0.94	0.94
Methionine[3]	0.45	0.45	0.45	0.45	0.45
Total sulphur amino acids[3]	0.69	0.69	0.69	0.69	0.69
Threonine[3]	0.58	0.58	0.58	0.58	0.58
Tryptophan[3]	0.17	0.17	0.17	0.17	0.17
Arginine[3]	1.04	1.04	1.04	1.04	1.04
Isoleucine[3]	0.64	0.64	0.64	0.64	0.64
Valine[3]	0.71	0.72	0.72	0.72	0.72
Leucine[3]	1.37	1.38	1.38	1.38	1.38
Glycine and Serine[3]	1.36	1.36	1.36	1.36	1.36
C 18:2	3.17	2.53	2.53	2.53	2.53
Calcium	0.66	0.66	0.66	0.66	0.66
Potassium	0.74	0.74	0.74	0.74	0.74
Chloride	0.29	0.29	0.29	0.29	0.29
Total Phosphorous	0.57	0.58	0.58	0.58	0.58
Retainable Phosphorous	0.30	0.30	0.30	0.30	0.30
Sodium	0.16	0.16	0.16	0.16	0.16

[1] Avizyme was added at 0.5 g / kg.

[2] Hemicell was added at 0.125 g / kg.

[3] Apparent digestible.

4.2.5. Measurements

The feeders in all pens were filled with feed and water lines were functional before chicks were placed in the pens. Chicks were placed in their respective pens immediately upon delivery. Chicks were counted once weekly to ensure that migration between pens or unrecorded mortalities did not occur. Pens were checked for mortalities on a daily basis. Individual pen records were kept of all mortalities, day of mortality and weight of the dead chick(s).

Total body weight and the feed remaining in each pen were measured weekly (days 7, 14, 21, 28 and 35). Average body weight (g / bird), weekly body weight gain (g / bird / day), cumulative feed intake (g / bird), weekly feed intake (g / bird / day), cumulative and weekly feed conversion ratio (g feed intake / g body weight gain), cumulative mortality and production efficiency factor were calculated for each pen, by making use of total body weight, feed remaining and mortality records.

4.2.6. Statistical analysis

The treatments in this trial were not structured, so simple analysis of treatment means, using the generalised linear model (GLM) function in SAS (Statistical Analysis Systems, 1989; Statistical Analysis Systems, 1994) was used in preference to the balanced ANOVA so that *post hoc* multiple comparison tests could be run on the treatment means, in cases where the GLM found significant differences in performance between treatments. Fischer's protected test was used for the *post hoc* multiple comparison test. Repeated tests were included in the model. The confidence level was set at 95%.

The model used in SAS was $y = T_i + H_j + B(H)_k + T{*}H_{ij} + e_{ijk}$

Where T_i was i^{th} observation for the treatment

H_j was house as a fixed effect

$B(H)_k$ was block nested in house as a fixed effect

$T{*}H_{ij}$ was the interaction between treatment and house

e_{ijk} was the random error effect

The variation due to block and house effects were accounted for by including both House, and Block nested in House as fixed effects in the model. Initial body weight was tested as a covariant in all the analyses in this trial.

The variables that were analysed are body weight, weekly body weight gains, weekly feed intake, cumulative feed intake, weekly feed conversion ratio, cumulative feed conversion ratio, performance efficiency factor and cumulative mortality. These could be calculated, respectively, from the following measurements: bird counts, initial body weight, weekly body weights, feed weighed in and feed weighed out (weekly and at 18 days [end of starter phase]), and mortality records.

4.3. Results

4.3.1. Chemical analysis

Tables 36 to 38 show the results for the chemical analyses of the trial feeds. In order to test if Avizyme and Hemicell were correctly added to the respective treatments and to ascertain that no Avizyme and / or Hemicell was added to the Negative and Positive Control feeds, the enzyme activity was tested in the feed samples by Danisco Animal Nutrition (Wiltshire, UK) and ChemGen Corporation (Gathersburg, USA) for Hemicell and Avizyme activity, respectively. An amylase activity of at least 400 MM units / ton feed and a xylanase activity of at least 600 MM units / ton feed were used as an indication that Avizyme was added to the feed at optimal levels. A mannanase activity of at least 90 MM units / ton feed was an indicator that Hemicell was added to the feed at optimal levels.

Table 36 Chemical analysis of the starter feeds on a dry matter basis

	Positive control[3]	Avizyme treatment[4]	Hemicell treatment[5]	Combination treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100	100
Ash (%)	6.0	4.6	6.0	5.8	5.7
Crude protein (%)	21.7	22.1	21.4	21.9	21.5
Crude fibre (%)	4.2	5.0	5.6	4.9	5.1
Crude fat (%)	6.3	5.7	5.4	5.0	6.0
Calcium (%)	1.01	0.62	1.09	1.01	1.02
Phosphorous (%)	0.69	0.78	0.77	0.76	0.75
Potassium (%)	0.98	1.03	0.98	1.01	0.99
Sodium (%)	0.20	0.07	0.27	0.27	0.21
Avizyme activity (MM amylase / ton)[1]	0	1179	< 100	1619	< 100
Hemicell activity (MM units / ton)[2]	10.9	10.9	146.5	174.1	6.4

[1] Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2] Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[3] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[5] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[6] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[7] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

Table 37 Chemical analysis of the grower feeds on a dry matter basis

	Positive control[3]	Avizyme treatment[4]	Hemicell treatment[5]	Combination treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100	100
Ash (%)	5.1	5.0	4.9	5.1	5.1
Crude protein (%)	18.8	18.7	19.1	19.0	19.6
Crude fibre (%)	4.6	4.7	4.6	5.3	5.1
Crude fat (%)	7.4	6.1	5.3	6.8	5.2
Calcium (%)	0.90	0.92	0.91	0.88	0.91
Phosphorous (%)	0.69	0.68	0.66	0.67	0.67
Potassium (%)	0.90	0.90	0.88	0.88	0.84
Sodium (%)	0.19	0.19	0.21	0.17	0.20
Avizyme activity (MM amylase / ton)[1]	0	1075	< 100	1558	< 100
Hemicell activity (MM units / ton)[2]	7.2	2.8	208.1	236.9	7.2

[1] Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2] Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[3] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%..

[5] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[6] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[7] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

Table 38 Chemical analysis of the finisher feeds on a dry matter basis

	Positive control[3]	Avizyme treatment[4]	Hemicell treatment[5]	Combination treatment[6]	Negative control[7]
Dry matter (%)	100	100	100	100	100
Ash (%)	4.6	4.8	4.7	5.0	4.5
Crude protein (%)	18.7	19.1	18.8	18.6	18.2
Crude fibre (%)	3.7	3.7	3.9	4.5	4.6
Crude fat (%)	8.9	6.5	8.3	6.5	6.1
Calcium (%)	0.79	0.78	0.75	0.87	0.84
Phosphorous (%)	0.64	0.64	0.62	0.64	0.67
Potassium (%)	0.86	0.87	0.88	0.90	0.82
Sodium (%)	0.19	0.20	0.19	0.20	0.22
Avizyme activity (MM amylase / ton)[1]	< 100	1339	369	1737	0
Hemicell activity (MM units / ton)[2]	4.2	22.9	157.8	201.3	6.6

[1] Avizyme treated feeds should have an amylase activity of at least 400 MM units / ton.

[2] Hemicell treated feeds should have a Hemicell activity of at least 90 MM units / ton. Untreated feeds should have a Hemicell activity of less than 15 MM units / ton.

[3] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[5] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[6] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[7] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

4.3.2. Body weight

As shown in Table 39 and illustrated in Figure 9, the mean body weight of broilers in both the Avizyme and the Hemicell treatment groups were significantly higher than that of the Positive control group and Combination group at the start of the trial. Although this difference was small (≤ 0.6 g), initial body weight was therefore included as a co-variant during statistical analysis of the data.

Table 39 Least square means (± standard error of the mean) of the average weekly body weights (g / bird) for the different treatments from day of hatch (day 0) until day 35

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Positive[1]	42.8[a] [± 0.15]	156.5[a] [± 1.37]	371.9[a] [± 2.40]	754.7[a] [± 4.78]	1292[a] [± 9.3]	1876[ab] [± 9.6]
Avizyme[2]	43.3[b] [± 0.15]	160.0[ab] [± 1.37]	372.9[a] [± 2.40]	754.2[a] [± 4.78]	1282[a] [± 9.3]	1858[a] [± 9.6]
Hemicell[3]	43.4[b] [± 0.15]	163.7[bc] [± 1.37]	377.0[a] [± 2.40]	756.9[a] [± 4.78]	1290[a] [± 9.3]	1855[a] [± 9.6]
Combination[4]	42.9[a] [± 0.15]	165.1[c] [± 1.37]	388.2[b] [± 2.40]	773.2[b] [± 4.78]	1322[b] [± 9.3]	1893[b] [± 9.6]
Negative[5]	43.0[ab] [± 0.15]	119.8[d] [± 1.37]	226.5[c] [± 2.40]	434.1[c] [± 4.78]	887[c] [± 9.3]	1431[c] [± 9.6]
House A	42.7[x] [± 0.10]	156.5[x] [± 0.87]	353.0[x] [± 1.52]	699.8[x] [± 3.02]	1221 [± 5.9]	1787 [± 6.1]
House B	43.5[y] [± 0.10]	149.5[y] [± 0.87]	341.6[y] [± 1.52]	689.4[y] [± 3.02]	1208 [± 5.9]	1778 [± 6.1]
F-probability						
Treatment	0.024	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
House	< 0.001	< 0.001	< 0.001	0.019	0.120	0.300
Block (House)	0.249	0.397	0.077	0.219	0.346	0.610
House × Treatment	0.617	0.226	0.088	0.152	0.160	0.514
R^2	0.534	0.946	0.986	0.988	0.972	0.973

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The body weight of the Negative control was significantly lower than all the other treatments from day seven until termination of the trial on day 35.

The Avizyme treatment's body weight was significantly higher than the Negative control treatment's body weight, but significantly lower than that of the Combination treatment from day seven until day 35. There were no significant differences between the Avizyme group and both the Positive control and the Hemicell treatment.

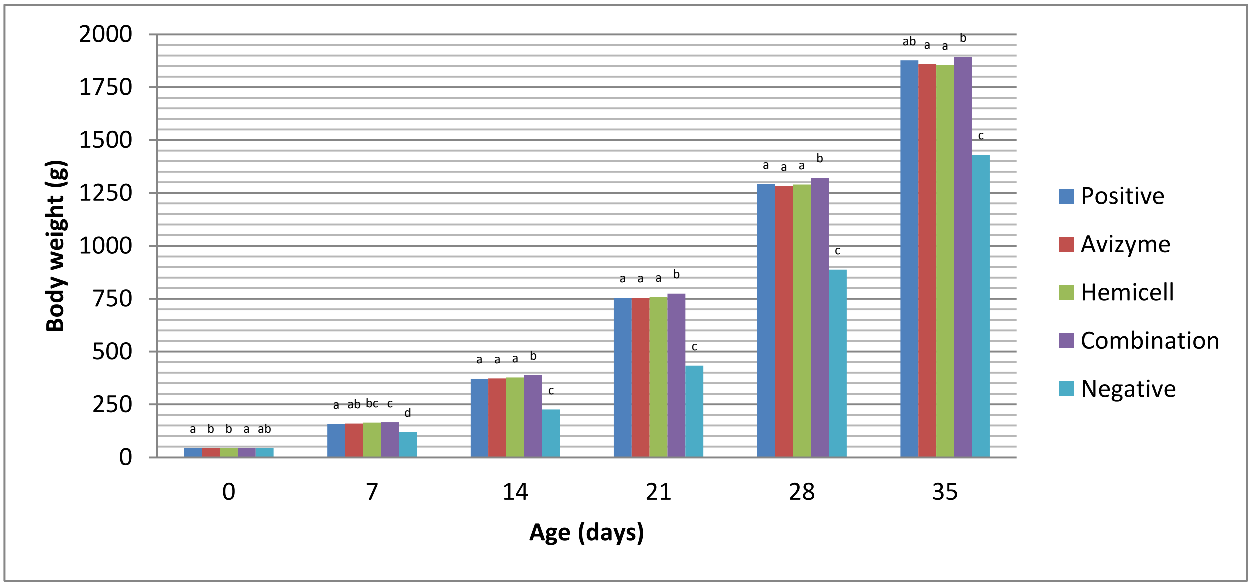

Figure 9 Average weekly body weights of the different treatments from day of hatch (day 0) until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

Likewise, the body weight of the Hemicell treatment was significantly higher than the Negative control treatment for the period day seven until day 35 and significantly lower than the Combination treatment from day 14 onwards. It was only significantly higher than the Positive control treatment on day seven.

The mean body weight of the Combination treatment was significantly higher than the body weights of all the other treatments on all the measuring days, except on day seven, where there was no difference between the Combination treatment and the Hemicell treatment and on day 35, where the body weight of the Combination treatment was not significantly different from the Positive control.

A significant difference in mean body weight was observed between houses from placement up to day 21, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.3. Body weight gain

As shown in Table 40 and illustrated in Figure 10, the mean body weight gain of the Negative control treatment was significantly lower than all the other treatments from day seven until day 28. For the last week of the trial, the body weight gain of the Negative control treatment was still lower than all the other treatments, but it was only significantly lower than the Positive control treatment and the Avizyme treatment. The total body weight gain of the Negative control treatment on day 35 was significantly lower than all the other treatments.

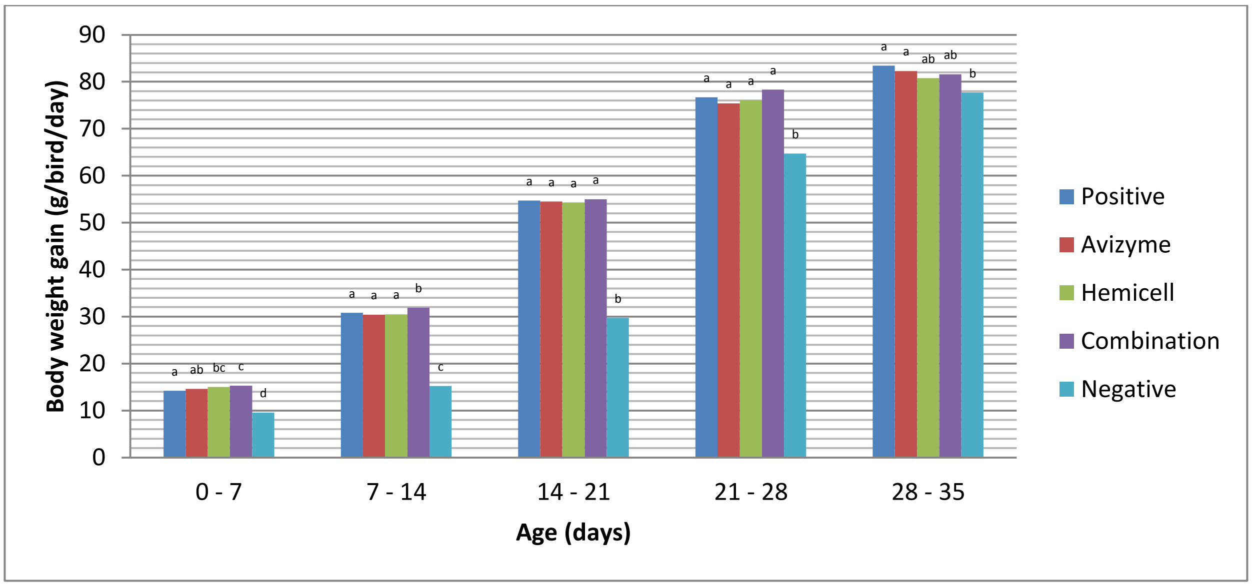

Figure 10 Average weekly body weight gain of the different treatments from day of hatch (day 0) until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The Avizyme treatment's body weight gain was significantly higher than the Negative control treatment's body weight gain, but significantly lower than that of the Combination treatment from day seven until day 14. There were no significant differences between the Avizyme treatment and both the Positive control and the Hemicell treatment. The total body weight gain of the Avizyme treatment was significantly higher than the Negative control treatment, but lower than the Combination treatment on day 35.

Table 40 Least square means of the average weekly body weight gains (g / bird / day) for the different treatments from day 0 until day 35 and total body weight gain

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35	Day 0 – 35
Positive[1]	14.2[a] [± 0.17]	30.8[a] [± 0.21]	54.7[a] [± 0.52]	76.7[a] [± 1.15]	83.4[a] [± 1.28]	1833[ab] [± 9.6]
Avizyme[2]	14.6[ab] [± 0.17]	30.4[a] [± 0.21]	54.5[a] [± 0.52]	75.4[a] [± 1.15]	82.3[a] [± 1.28]	1815[a] [± 9.6]
Hemicell[3]	15.0[bc] [± 0.17]	30.5[a] [± 0.21]	54.3[a] [± 0.52]	76.1[a] [± 1.15]	80.8[ab] [± 1.28]	1812[a] [± 9.6]
Combination[4]	15.3[c] [± 0.17]	31.9[b] [± 0.21]	55.0[a] [± 0.52]	78.3[a] [± 1.15]	81.6[ab] [± 1.28]	1850[b] [± 9.6]
Negative[5]	9.6[d] [± 0.17]	15.2[c] [± 0.21]	29.7[b] [± 0.52]	64.7[b] [± 1.15]	77.7[b] [± 1.28]	1388[c] [± 9.6]
House A	14.2[x] [± 0.11]	28.1[x] [± 0.14]	49.5 [± 0.33]	74.5 [± 0.73]	80.9 [± 0.81]	1744 [± 6.1]
House B	13.3[y] [± 0.11]	27.4[y] [± 0.14]	49.7 [± 0.33]	74.1 [± 0.73]	81.5 [± 0.81]	1735 [± 6.1]
F-probability						
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	0.032	< 0.001
House	< 0.001	0.002	0.758	0.701	0.610	0.261
Block (House)	0.381	0.038	0.750	0.384	0.649	0.606
House × Treatment	0.228	0.095	0.314	0.332	0.410	0.519
R^2	0.946	0.990	0.976	0.684	0.286	0.973

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The body weight gain of the Hemicell treatment was significantly higher than the Negative control treatment for the period day seven until day 28. The Hemicell treatment's body weight gain was only significantly lower than the Combination treatment on day 14. It was only significantly higher than the Positive treatment group on day seven. Similar to the Avizyme treatment, the total body weight gain of the Hemicell treatment was significantly higher than the Negative control treatment, but lower than the Combination treatment on day 35.

The mean body weight of the Combination treatment was only significantly higher than the body weights of all the other treatments on day 14. The combination treatment's body weight gain was higher than the Negative control treatment on all weighing days and was significantly higher than the Positive control and the Avizyme treatment' body weight gain on day seven. The total body weight gain of the Combination treatment on day 35 was higher than all the other treatments, although there was no significant difference between the Combination treatment and the Positive control treatment.

A significant difference in weekly body weight gain was observed between houses from day seven up to day 14, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.4. Weekly feed intake

As shown in Table 41 and illustrated in Figure 11, the weekly feed intake of the Negative control treatment was significantly lower than all the other groups for days 14, 21 and 35. The Negative control treatment's weekly feed intake was only lower than the Avizyme treatment on day seven and the Hemicell treatment on day 28.

The weekly feed intake of the Hemicell treatment was significantly higher than the Negative control treatment for the period day 14 to day 35 and higher than the Positive control treatment on day 28. There were no significant differences between the Hemicell treatment and both the Avizyme and Combination treatments.

The weekly feed intake of the Combination treatment was significantly higher than the Negative treatment group for days 14, 21 and 35. The weekly feed intake of the Combination treatment was also significantly higher than the Positive control treatment on days 14 and 28 and higher than the Avizyme treatment on day 14.

Table 41 Least square means of the average weekly feed intake (g / bird / day) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35
Positive[1]	18.9[a] [± 0.42]	42.1[a] [± 0.95]	79.4[a] [± 1.04]	124.4[a] [± 0.87]	153.2[a] [± 1.29]
Avizyme[2]	20.4[b] [± 0.42]	41.8[a] [± 0.95]	82.0[a] [± 1.04]	126.7[ab] [± 0.87]	153.0[a] [± 1.29]
Hemicell[3]	19.8[ab] [± 0.42]	44.2[ab] [± 0.95]	81.8[a] [± 1.04]	128.5[b] [± 0.87]	152.3[a] [± 1.29]
Combination[4]	19.3[ab] [± 0.42]	46.3[b] [± 0.95]	80.7[a] [± 1.04]	128.2[bc] [± 0.87]	154.2[a] [± 1.29]
Negative[5]	18.9[a] [± 0.42]	34.1[c] [± 0.95]	44.8[b] [± 1.04]	126.0[ac] [± 0.87]	136.5[b] [± 1.29]
House A	19.7 [± 0.27]	41.5 [± 0.60]	73.6 [± 0.66]	132.0[x] [± 0.55]	148.8 [± 0.82]
House B	19.3 [± 0.27]	41.9 [± 0.60]	73.9 [± 0.66]	121.5[y] [± 0.55]	150.9 [± 0.82]
F-probability					
Treatment	0.057	< 0.001	< 0.001	0.011	< 0.001
House	0.302	0.684	0.671	< 0.001	0.079
Block (House)	0.264	0.157	0.858	0.580	0.572
House × Treatment	0.515	0.613	0.529	< 0.001	0.900
R^2	0.300	0.695	0.955	0.960	0.755

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

A significant difference in weekly feed intake was observed between houses on day 28, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

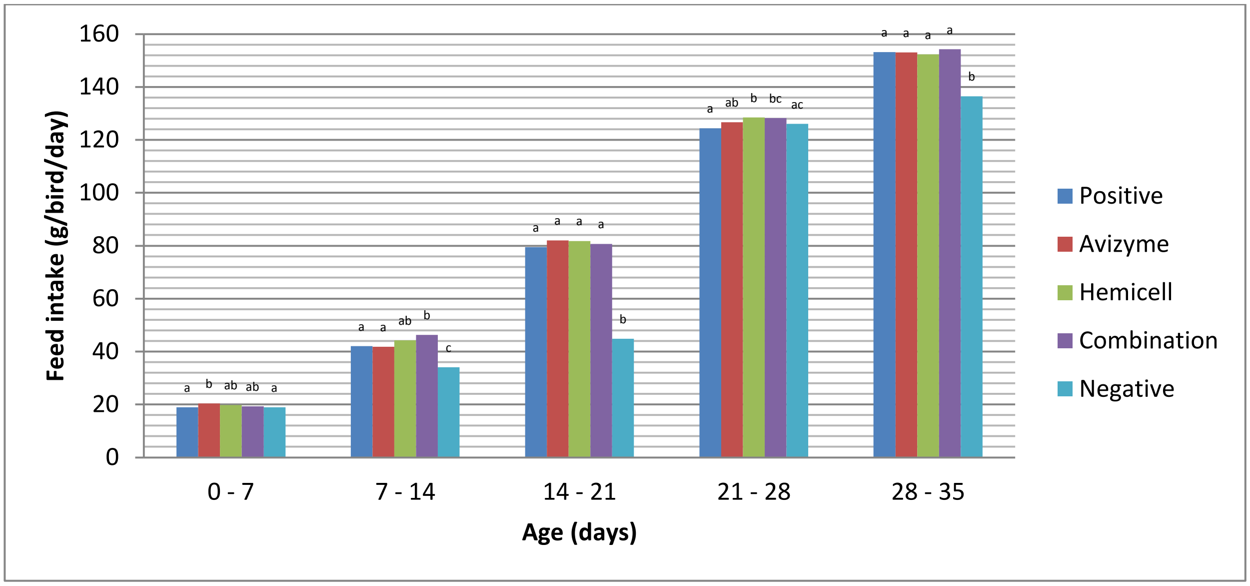

Figure 11 Average weekly feed intake of the different treatments from day 0 until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

4.3.5. Cumulative feed intake

As shown in Table 42 and illustrated in Figure 12, the cumulative feed intake of the Negative control treatment was only significantly lower than the Avizyme treatment on day seven, but lower than all the other treatments from day 14 until day 35.

The Avizyme treatment's cumulative feed intake was significantly higher than the Negative control treatment from day seven until day 35 and higher than the cumulative feed intake of the Positive control treatment on days seven, 21 and 28. The cumulative feed intake of the Avizyme treatment was lower than the Combination treatment on day 14. There were no significant differences between the Avizyme treatment and the Hemicell treatment.

Table 42 Least square means of the average cumulative feed intake (g / bird) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	151.1^{a} [± 3.36]	445.7^{a} [± 6.50]	1001.8^{a} [± 6.81]	1872^{a} [± 10.4]	2945^{a} [± 17.7]
Avizyme[2]	163.5^{b} [± 3.36]	456.2^{ab} [± 6.50]	1030.0^{b} [± 6.81]	1917^{b} [± 10.4]	2988^{ab} [± 17.7]
Hemicell[3]	158.6^{ab} [± 3.36]	467.9^{bc} [± 6.50]	1040.3^{b} [± 6.81]	1940^{b} [± 10.4]	3006^{b} [± 17.7]
Combination[4]	154.4^{ab} [± 3.36]	478.8^{c} [± 6.50]	1043.8^{b} [± 6.81]	1941^{b} [± 10.4]	3020^{b} [± 17.7]
Negative[5]	151.2^{a} [± 3.36]	390.2^{d} [± 6.50]	704.0^{c} [± 6.81]	1586^{c} [± 10.4]	2542^{c} [± 17.7]
House A	157.3 [± 2.13]	448.1 [± 4.11]	963.0 [± 4.31]	1887^{x} [± 6.6]	2929^{x} [± 11.2]
House B	154.2 [± 2.13]	447.4 [± 4.11]	965.0 [± 4.31]	1816^{y} [± 6.6]	2872^{y} [± 11.2]
F-probability					
Treatment	0.056	< 0.001	< 0.001	< 0.001	< 0.001
House	0.302	0.904	0.735	< 0.001	< 0.001
Block (House)	0.265	0.071	0.070	0.201	0.309
House × Treatment	0.516	0.412	0.404	< 0.001	< 0.001
R^2	0.300	0.733	0.976	0.963	0.933

^{abcd} Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

^{xy} Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The cumulative feed intake of the Hemicell treatment was significantly higher than the Positive and Negative control treatments for the period day 14 to day 35. There were no significant differences between the Hemicell treatment and both the Avizyme and Combination treatments.

The cumulative feed intake of the Combination treatment was significantly higher than the Positive and Negative control treatments for days 14 to 35. The cumulative feed intake of the Combination treatment was also significantly higher than the Avizyme treatment on day 14.

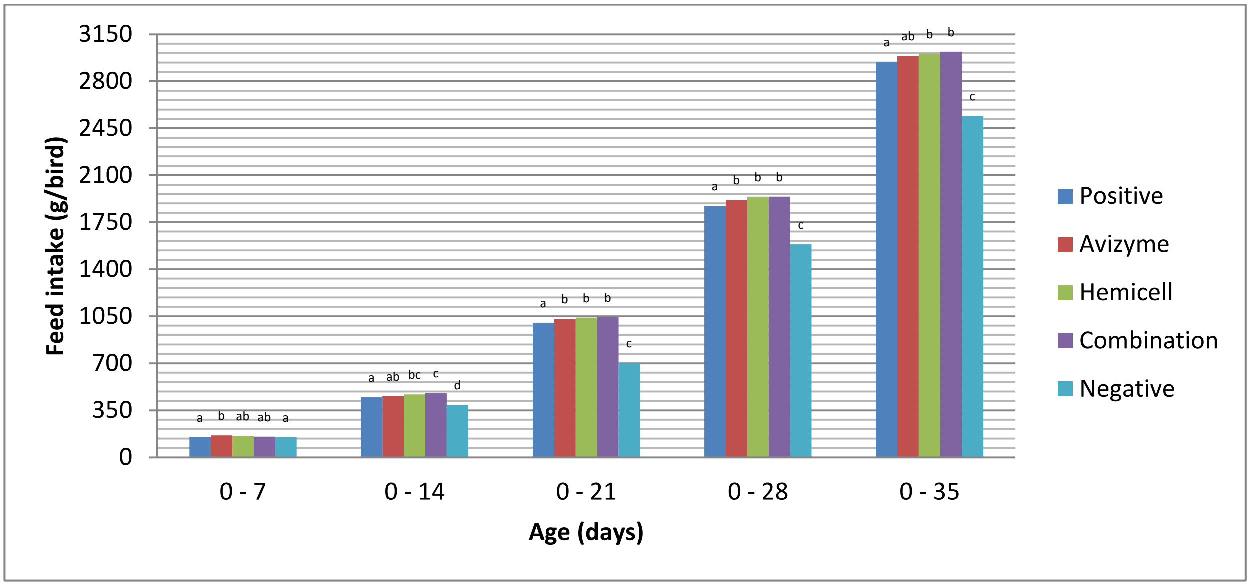

Figure 12 Average cumulative feed intake of the different treatments from day 0 until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

A significant difference in cumulative feed intake was observed between houses from day 28 up to day 35, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.6. Weekly feed conversion ratio

As shown in Table 43 and illustrated in Figure 13, the weekly feed conversion ratio of the Negative control treatment was significantly poorer than all the other groups for days 7, 14 and 28. The Negative control treatment's weekly feed conversion ratio was only poorer than the Positive control treatment on day 21 and better than the Hemicell and Combination treatments on day 35.

The Avizyme treatment's weekly feed conversion ratio was significantly better than the Negative control treatment for days seven, 14 and 28, but poorer than the Combination treatment for day 7. There were no significant differences between the Avizyme treatment and both the Hemicell and Positive control treatments.

Table 43 Least square means of the average weekly feed conversion ratios (g feed intake / g body weight gain) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – 35
Positive[1]	1.33[ab] [± 0.030]	1.37[a] [± 0.029]	1.45[a] [± 0.019]	1.62[a] [± 0.024]	1.84[ab] [± 0.034]
Avizyme[2]	1.40[a] [± 0.030]	1.37[ab] [± 0.029]	1.51[ab] [± 0.019]	1.68[a] [± 0.024]	1.86[ab] [± 0.034]
Hemicell[3]	1.32[ab] [± 0.030]	1.45[ab] [± 0.029]	1.51[b] [± 0.019]	1.69[a] [± 0.024]	1.89[a] [± 0.034]
Combination[4]	1.27[b] [± 0.030]	1.45[b] [± 0.029]	1.47[ab] [± 0.019]	1.64[a] [± 0.024]	1.89[a] [± 0.034]
Negative[5]	1.97[c] [± 0.030]	2.24[c] [± 0.029]	1.51[b] [± 0.019]	1.98[b] [± 0.024]	1.78[b] [± 0.034]
House A	1.42[x] [± 0.019]	1.55 [± 0.019]	1.49 [± 0.012]	1.80[x] [± 0.015]	1.84 [± 0.021]
House B	1.49[y] [± 0.019]	1.60 [± 0.019]	1.49 [± 0.012]	1.64[y] [± 0.015]	1.86 [± 0.021]
F-probability					
Treatment	< 0.001	< 0.001	0.108	< 0.001	0.150
House	0.010	0.089	0.844	< 0.001	0.513
Block (House)	0.861	0.121	0.882	0.560	0.537
House × Treatment	0.337	0.869	0.751	< 0.001	0.316
R^2	0.893	0.935	0.195	0.924	0.253

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The weekly feed conversion ratio of the Hemicell treatment was significantly better than the Negative control treatment for the period days seven, 14 and 28. The Hemicell treatment's feed conversion ratio was significantly poorer than the Positive control treatment on day 21 and poorer than the

Negative control on day 35. There were no significant differences between the Hemicell treatment and both the Avizyme and Combination treatments.

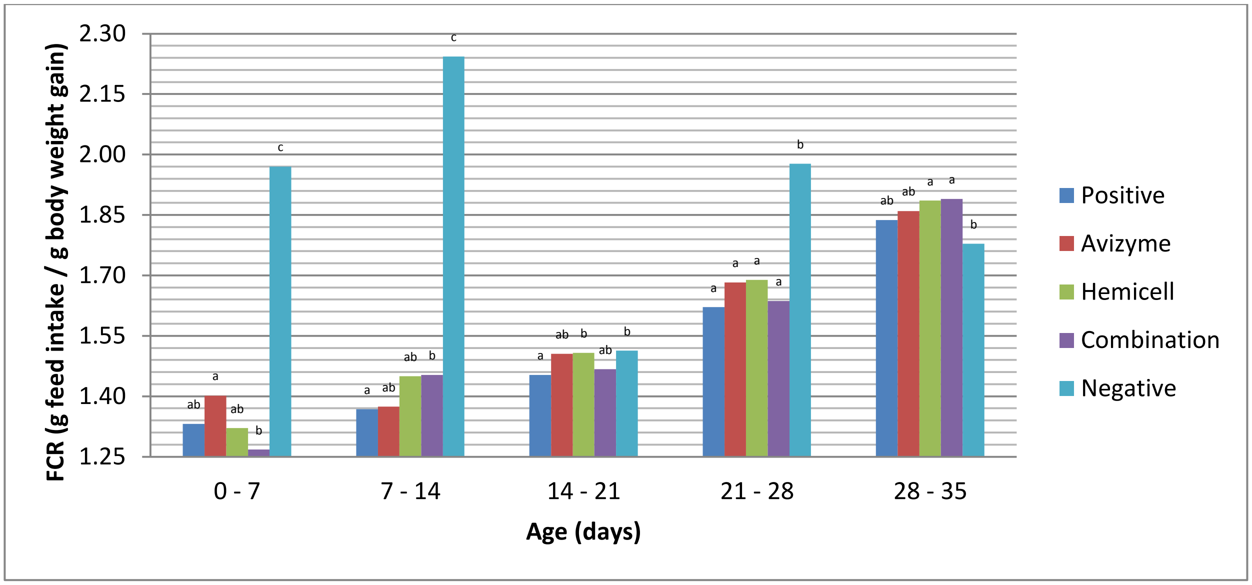

Figure 13 Average weekly FCR of the different treatments from day 0 until day 35

[abc] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The weekly feed conversion ratio of the Combination treatment was significantly better than the Negative control treatment for days seven, 14 and 28. The weekly feed conversion ratio of the Combination treatment was also significantly better than the Avizyme control treatment on day seven, but significantly poorer than the Positive control and Negative control treatments on days 14 and 35, respectively.

A significant difference in weekly conversion ratio was observed between houses on days 7 and 28, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.7. Cumulative feed conversion ratio

As shown in Table 44 and illustrated in Figure 14, the cumulative feed conversion ratio of the Negative control treatment was significantly poorer than all the other groups from day 14 until day 35.

Table 44 Least square means of the average cumulative feed conversion ratio (g feed intake / g body weight gain) for the different treatments from day 0 until day 35

	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	1.36[a] [± 0.020]	1.41[a] [± 0.011]	1.50[a] [± 0.016]	1.61[a] [± 0.009]
Avizyme[2]	1.38[a] [± 0.020]	1.45[b] [± 0.011]	1.55[b] [± 0.016]	1.65[bc] [± 0.009]
Hemicell[3]	1.40[a] [± 0.020]	1.46[b] [± 0.011]	1.56[b] [± 0.016]	1.66[b] [± 0.009]
Combination[4]	1.39[a] [± 0.020]	1.43[ab] [± 0.011]	1.52[ab] [± 0.016]	1.63[c] [± 0.009]
Negative[5]	2.13[b] [± 0.020]	1.80[c] [± 0.011]	1.89[c] [± 0.016]	1.83[d] [± 0.009]
House A	1.50[x] [± 0.012]	1.49[x] [± 0.007]	1.64[x] [± 0.010]	1.69[x] [± 0.006]
House B	1.56[y] [± 0.012]	1.52[y] [± 0.007]	1.57[y] [± 0.010]	1.66[y] [± 0.006]
F-probability				
Treatment	< 0.001	< 0.001	< 0.001	< 0.001
House	0.002	0.004	< 0.001	< 0.001
Block (House)	0.152	0.164	0.591	0.445
House × Treatment	0.514	0.586	< 0.001	< 0.001
R^2	0.963	0.953	0.938	0.929

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05)

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The Avizyme treatment's cumulative feed conversion ratio was significantly better than the Negative control treatment from day 14 until day 35, but poorer than the Positive control treatment from day 21 until day 35. There were no significant differences between the Avizyme treatment and both the Hemicell and the Combination treatments.

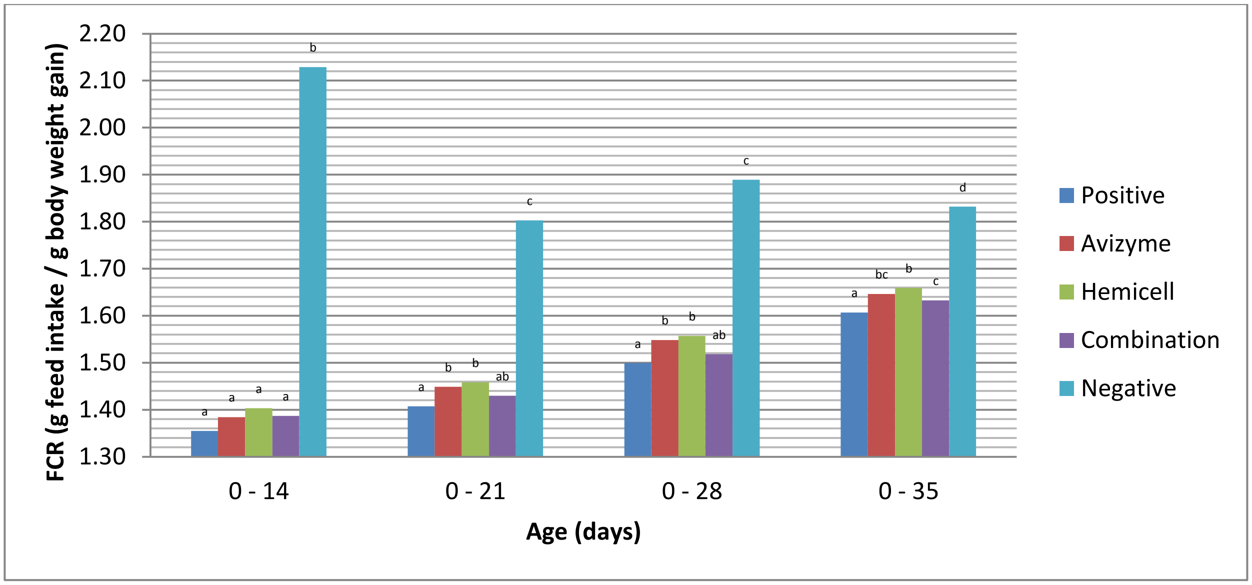

Figure 14 Average cumulative feed conversion ratio of the different treatments from day 0 until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The Hemicell treatment treatment's cumulative feed conversion ratio was significantly better than the Negative control treatment from day 14 until day 35. The cumulative feed conversion ratio for the Hemicell treatment was significantly poorer than the Positive control treatment from day 21 until day 35 and poorer than the Combination treatment for day 35.

The cumulative feed conversion ratio of the Combination treatment was significantly better than the Negative control treatment for days 14 to 35 and better than the Hemicell treatment on day 35. The cumulative feed conversion ratio of the Combination treatment was significantly poorer than the Positive control treatment on day 35.

A significant difference in cumulative feed conversion ratio was observed between houses from day 14 up to day 35, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.8. Cumulative mortality

Cumulative mortality was calculated as a percentage of birds placed. As shown in Table 45 and illustrated in Figure 15, the cumulative mortality of the Negative control treatment was not significantly different from any of the other treatments. The Avizyme treatment's cumulative mortality was only significantly lower than the Positive control treatment on day 7. There was no significant difference between the Avizyme treatment and the Hemicell, Combination and the Negative treatments. There was no significant difference in mortality between the Hemicell treatment and any of the other treatments. Likewise, there was also no significant difference between the Combination treatment and any of the other treatments.

A significant difference in cumulative mortality was observed between houses for days seven and 35, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

Table 45 Least square means of the average cumulative mortality (as a percentage of birds placed) for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	0.86[a] [± 0.171]	1.06 [± 0.208]	1.19 [± 0.248]	1.59 [± 0.355]	2.65 [± 0.456]
Avizyme[2]	0.26[b] [± 0.171]	0.53 [± 0.208]	0.60 [± 0.248]	1.19 [± 0.355]	2.31 [± 0.456]
Hemicell[3]	0.40[ab] [± 0.171]	0.66 [± 0.208]	0.79 [± 0.248]	1.72 [± 0.355]	3.11 [± 0.456]
Combination[4]	0.73[ab] [± 0.171]	0.99 [± 0.208]	0.99 [± 0.248]	1.65 [± 0.355]	3.31 [± 0.456]
Negative[5]	0.40[ab] [± 0.171]	0.86 [± 0.208]	1.52 [± 0.248]	1.32 [± 0.355]	2.25 [± 0.456]
House A	0.32[x] [± 0.108]	0.69 [± 0.132]	0.82 [± 0.157]	1.30 [± 0.225]	2.01[x] [± 0.288]
House B	0.74[y] [± 0.108]	0.95 [± 0.132]	1.11 [± 0.157]	1.69 [± 0.225]	3.44[y] [± 0.288]
F-probability					
Treatment	0.088	0.349	0.312	0.803	0.383
House	0.008	0.162	0.196	0.219	0.001
Block (House)	0.257	0.388	0.539	0.389	0.807
House × Treatment	0.595	0.438	0.226	0.413	0.102
R^2	0.349	0.242	0.254	0.199	0.364

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

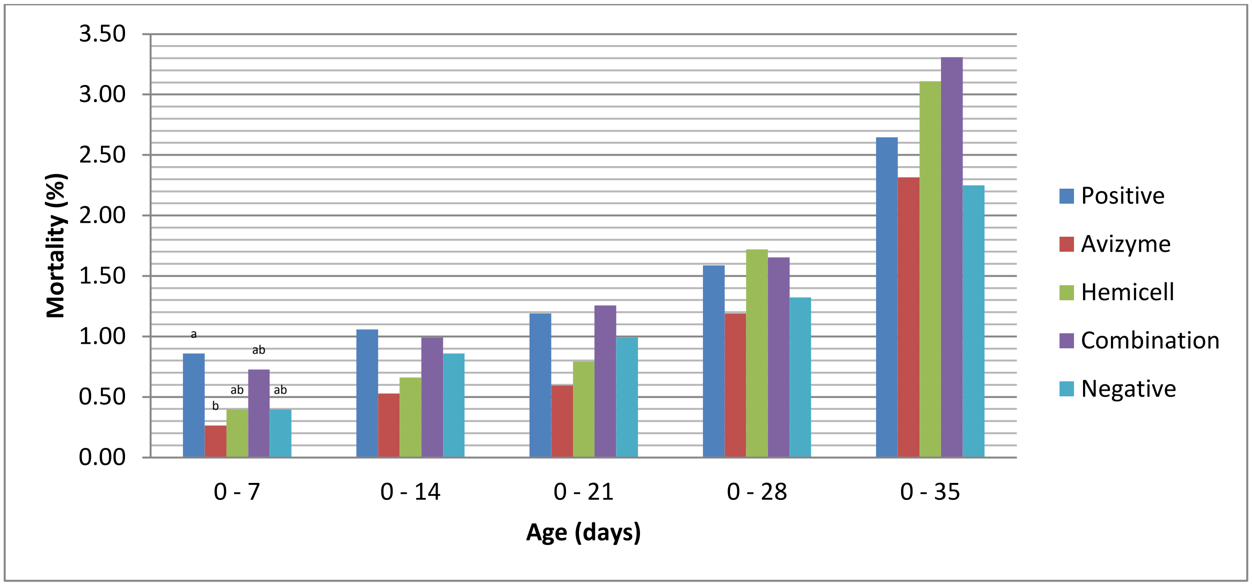

Figure 15 Average cumulative mortality (as a percentage of birds placed) of the different treatments from day 0 until day 35

[ab] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

4.3.9. Production efficiency factor

The production efficiency factor (PEF) was calculated using the following equation:

$$\text{PEF} = \frac{(100 - \text{Cumulative mortality}) \times \text{Body weight}}{\text{Cumulative feed conversion ratio} \times \text{Age (days)}} \times 10$$

As shown in Table 46 and illustrated in Figure 16, the production efficiency factor of the Negative control treatment was significantly lower than all the other treatments from day seven until 35.

The Avizyme treatment's production efficiency factor was significantly higher than the Negative control treatment from day seven until day 35. The production efficiency factor of the Avizyme treatment was lower than the Combination treatment from day seven until day 28 and lower than the Hemicell and Positive treatments on days 7 and 35, respectively.

Table 46 Least square means of the average production efficiency factor for the different treatments from day 0 until day 35

	Day 0 – 7	Day 0 – 14	Day 0 – 21	Day 0 – 28	Day 0 – 35
Positive[1]	167.0[ab] [± 3.66]	194.1[ab] [± 2.38]	252.4[ac] [± 2.15]	303.0[ac] [± 3.89]	324.8[a] [± 2.44]
Avizyme[2]	164.8[a] [± 3.66]	191.5[a] [± 2.38]	246.5[ab] [± 2.15]	292.4[ab] [± 3.89]	315.1[bc] [± 2.44]
Hemicell[3]	176.8[bc] [± 3.66]	190.8[a] [± 2.38]	245.3[b] [± 2.15]	290.9[b] [± 3.89]	309.7[b] [± 2.44]
Combination[4]	185.8[c] [± 3.66]	199.3[b] [± 2.38]	254.4[c] [± 2.15]	305.9[c] [± 3.89]	320.4[ac] [± 2.44]
Negative[5]	86.9[d] [± 3.66]	75.6[c] [± 2.38]	113.9[d] [± 2.15]	170.2[d] [± 3.89]	219.6[d] [± 2.44]
House A	164.5[x] [± 2.31]	176.3[x] [± 1.51]	226.7[x] [± 1.36]	272.7 [± 2.46]	299.6 [± 1.54]
House B	148.0[y] [± 2.31]	164.2[y] [± 1.51]	218.3[y] [± 1.36]	272.2 [± 2.46]	296.2 [± 1.54]
F-probability					
Treatment	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
House	< 0.001	< 0.001	< 0.001	0.893	0.121
Block (House)	0.995	0.263	0.233	0.655	0.479
House × Treatment	0.124	0.135	0.057	< 0.001	< 0.001
R^2	0.916	0.978	0.986	0.953	0.968

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

[xy] Column means with the same superscript did not differ significantly for the house least square means (P > 0.05).

[1] Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

[2] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

[3] Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

[4] Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

[5] Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The production efficiency factor of the Hemicell treatment was significantly higher than the Negative control treatment for the period day seven until day 35 and higher than the Avizyme treatment on day 7. The Hemicell treatment’s production efficiency factor was significantly lower than the Combination treatment from day 14 until day 35 and lower than the Positive control treatment from day 21 until day 35.

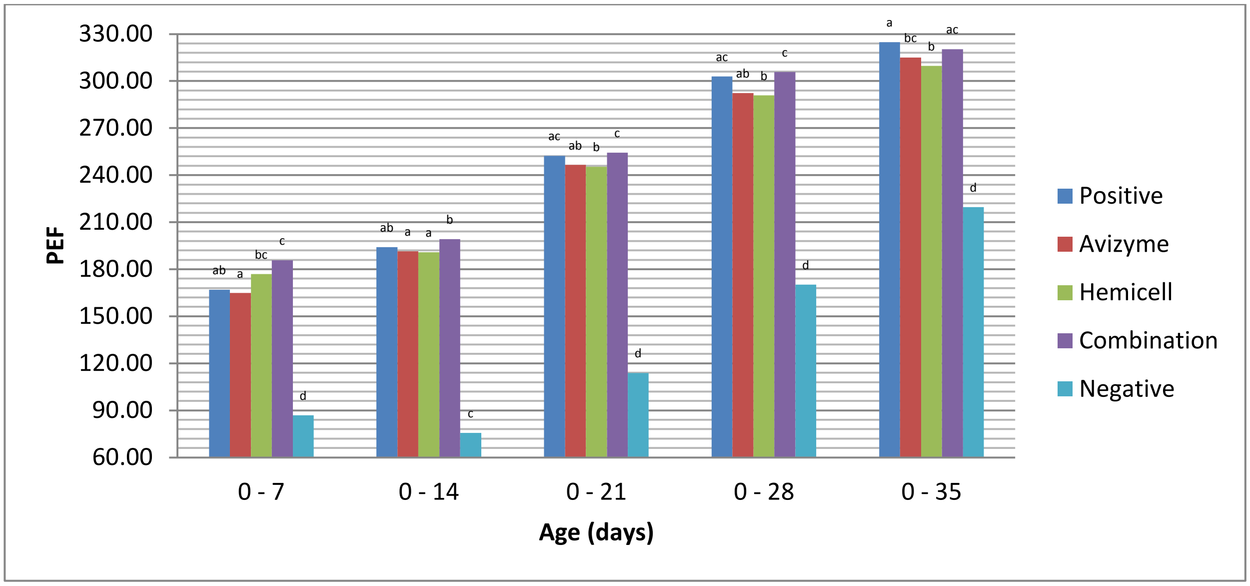

Figure 16 Average production efficiency factor of the different treatments from day 0 until day 35

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (P > 0.05).

Positive: Nutrient specifications were formulated to meet or exceed the recommendations of the NRC (1994), except for a slightly lower apparent metabolisable energy than the standard.

Avizyme: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme included at 0.05%.

Hemicell: Formulated to have 0.45 MJ less energy than the Positive control. Hemicell included at 0.0125%.

Combination: Formulated to have 0.45 MJ less energy than the Positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Negative: Formulated to have 0.45 MJ less energy than the Positive control. No exogenous enzyme was included.

The production efficiency factor of the Combination treatment was significantly higher than the Negative control treatment from day seven until day 35 and higher than the Positive control treatment for day 7. The production efficiency factor of the Combination treatment was also significantly higher than the Avizyme treatment from day seven until day 28 and higher than the Hemicell treatment from day 14 until day 35.

A significant difference in production efficiency factor was observed between houses from day 7 until day 21, but as House was included in the model used for statistical analysis as a fixed effect, the data would have been corrected for house-effect.

4.3.10. Economic evaluation

In order to do an economic evaluation of the enzymes added to the feed, both the saving on the feed, as well as the saving on the broilers as final product had to be calculated. Avizyme costed R 63-85 /

kg and was included at 500 g / ton. This means that Avizyme was included at a cost R 31-93 / ton feed. Hemicell costed R 115-00 / kg and was included at 125 g / ton, and Hemicell was therefore included at a cost of R 14-38 / ton feed. A summary of the economic impact of enzyme addition to the broiler diets is shown in Table 47.

The Positive, Avizyme, Hemicell and Combination starter feeds were produced at a cost of R 2573-00 / ton, R 2457-98 / ton, R 2441-35 / ton and R 2472-05 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 115-02 / ton, R 131-65 / ton and R 100-95 / ton for Avizyme, Hemicell and the combination of both enzymes, respectively. The Positive control group consumed an average of 0.763 kg starter / bird at a cost of R 1-96 per bird for the starter phase. The Avizyme treatment group consumed an average of 0.784 kg starter / bird at a cost of R 1-93 per bird for the starter phase. The Hemicell treatment group consumed an average of 0.795 kg starter / bird at a cost of R 1-94 per bird for the starter phase. The Combination treatment group consumed an average of 0.802 kg starter / bird at a cost of R 1-98 per bird for the starter phase. The cost saving as a result of the inclusion of enzymes in the starter phase was 3 c / bird and 2 c / bird for Avizyme and Hemicell, respectively. The Combination treatment group had a higher feed intake than the Positive control group, which resulted in the Combination treatment group’s feed costing 2 c / bird more during the starter phase. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.548 kg / bird during the starter phase, at a possible income of R 5.25 / bird for the starter phase. The Avizyme treatment group gained an average of 0.548 kg / bird during the starter phase, at a possible income of R 5.25 / bird for the starter phase. The Hemicell treatment group gained an average of 0.551 kg / bird during the starter phase, at a possible income of R 5.28 / bird for the starter phase. The Combination treatment group gained an average of 0.565 kg / bird during the starter phase, at a possible income of R 5.41 / bird for the starter phase. The increased income as a result of the inclusion of enzymes in the starter phase was 3 c / bird and 16 c / bird for Hemicell and the combination of Avizyme and Hemicell, respectively. Inclusion of Avizyme resulted in body weight gains similar to the Positive control group. The total increased income over feed cost due to the inclusion of enzymes was 3 c / bird, 5 c / bird and 14 c / bird for Avizyme, Hemicell and the combination of Avizyme and Hemicell, respectively.

The Positive, Avizyme, Hemicell and Combination grower feeds were produced at a cost of R 2657-95 / ton, R 2560-74 / ton, R 2544-15 / ton and R 2574-80 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 97-20 / ton, R 113-80 / ton and R 83-14 for Avizyme, Hemicell and the combination of Avizyme and Hemicell, respectively. The Positive control group consumed an average of 1.108 kg grower / bird at a cost of R 2-95 per bird for the grower phase. The Avizyme treatment group consumed an average of 1.133 kg grower / bird at a cost of R 2-90 per bird for the grower phase. The Hemicell treatment group consumed an average of 1.145 kg grower / bird at a cost of R 2-91 per bird for the grower phase. The Combination treatment group consumed an average of 1.139 kg grower / bird at a cost of R 2-93 per bird for the grower phase. The cost saving as a result of the inclusion of enzymes in the grower phase was 5 c / bird, 4 c / bird and 2 c / bird for Avizyme, Hemicell and the combination of Avizyme and Hemicell, respectively. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.701 kg / bird during the grower phase, at a possible income of R 6-72 / bird for the grower phase. The Avizyme treatment group gained an average of 0.691 kg / bird during the grower phase, at a possible income of R 6-62 / bird for the grower phase. The Hemicell treatment group gained an average of 0.696 kg / bird during the grower phase, at a possible income of R 6-67 / bird for the grower phase. The Combination treatment group gained an average of 0.714 kg / bird during the grower phase, at a possible income of R 6-84 / bird for the grower phase. The increased income as a result of the inclusion of the combination of Avizyme and Hemicell in the grower phase was 12 c / bird. The Avizyme and Hemicell treatment groups had a lower body weight gain than the Positive control group, which resulted in 10 c / bird and 5 c / bird lower possible value for the Avizyme and Hemicell treatment groups, respectively during the grower phase. The total increased income over feed cost due to the inclusion of the combination of Avizyme and Hemicell was 14 c / bird during the grower phase. Inclusion of Avizyme and Hemicell in the grower phase resulted in a decreased income over feed cost of 5 c / bird and 1 c / bird, respectively during the grower phase.

The Positive, Avizyme, Hemicell and Combination finisher feeds were produced at a cost of R 2578-00 / ton, R 2436-64 / ton, R 2437-55 / ton and R 2436-33 / ton, respectively. The saving on the feed with the inclusion of enzymes was R 109-44 / ton, R 126-07 and R 95-37 / ton for Avizyme, Hemicell and the combination of Avizyme and Hemicell, respectively. The Positive control group consumed an average of 1.073 kg finisher / bird at a cost of R 2-77 per bird for the finisher phase. The Avizyme treatment group consumed an average of 1.071 kg finisher / bird at a cost of R 2-61 per bird for the finisher phase. The Hemicell treatment group consumed an average of 1.066 kg finisher / bird at a cost of R 2-60 per bird for the finisher phase. The Combination treatment group consumed an average of 1.079 kg finisher / bird at a cost of R 2-63 per bird for the finisher phase. The cost saving as a result of the inclusion of Avizyme, Hemicell and the combination of Avizyme and Hemicell in the finisher phase was 16 c / bird, 17 c / bird and 14 c / bird, respectively. The price of broilers at the time of this evaluation was R 9-58 / kg live weight. The Positive control group gained an average of 0.584 kg / bird during the finisher phase, at a possible income of R 5-59 / bird for the finisher phase. The Avizyme treatment group gained an average of 0.576 kg / bird during the finisher phase, at a possible income of R 5-52 / bird for the finisher phase. The Hemicell treatment group gained an average of 0.565 kg / bird during the finisher phase, at a possible income of R 5-41 / bird for the finisher phase. The Combination treatment group gained an average of 0.571 kg / bird during the finisher phase, at a possible income of R 5-47 / bird for the finisher phase. The Avizyme, Hemicell and Combination treatment groups had lower body weight gains than the Positive control group, which resulted in a lower possible value of 7 c / bird, 18 c / bird and 12 c / bird less than the Positive control group's birds during the finisher phase for the Avizyme, Hemicell and Combination treatment groups, respectively. The total increased income over feed cost due to the inclusion of enzymes was 9 c / bird and 2 c / bird for Avizyme and the combination of Avizyme and Hemicell, respectively. The inclusion of Hemicell in the finisher phase resulted in a decreased income over feed cost of 1 c / bird.

Inclusion of Avizyme in the feed, realised a total cost saving of 24 c / kg feed, a total decreased income from the birds of 17 c / bird and 9 c / kg live weight. The total increased income over feed cost that could be realised with the inclusion of Avizyme was 7 c / bird and 4 c / kg live weight over a five week period. Inclusion of Hemicell in the feed, realised a total cost saving of 23 c / kg feed, a total decreased income from the birds of 20 c / bird and 11 c / kg live weight. The total increased income over feed cost that could be realised with the inclusion of Hemicell was 3 c / bird and 2 c / kg live weight over a five week period. Inclusion of the combination of Avizyme and Hemicell in the feed, realised a total cost saving of 14 c / kg feed, a total decreased income from the birds of 1 c / bird and 1 c / kg live weight. The total increased income over feed cost that could be realised with the inclusion of the combination of Avizyme and Hemicell was 30 c / bird and 16 c / kg live weight over a five week period.

Table 47 Calculation of increased income over feed cost (IOFC) for the Avizyme, Hemicell and Combination treatments, compared to the IOFC of the Positive control

	Starter	Grower	Finisher
Positive control			
Live weight gain (kg)	0.548	0.701	0.584
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.763	1.108	1.073
Feed cost (c / kg)	257	266	258
IOFC (c)[1]	328	377	283
Avizyme treatment			
Live weight gain (kg)	0.548	0.691	0.576
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.784	1.133	1.071
Feed cost (c / kg)	246	256	244
IOFC (c)[1]	332	372	291
Increased IOFC (c)[2]	3	- 5[5]	8
Hemicell treatment			
Live weight gain (kg)	0.551	0.696	0.565
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.795	1.145	1.066
Feed cost (c / kg)	244	254	244
IOFC (c)[1]	334	375	281
Increased IOFC (c)[3]	5	- 2[5]	- 1[5]
Combination treatment			
Live weight gain (kg)	0.565	0.714	0.571
Live weight price (c / kg)	958	958	958
Feed intake (kg)	0.802	1.139	1.079
Feed cost (c / kg)	247	257	244
IOFC (c)[1]	343	390	284
Increased IOFC (c)[4]	15	13	1

[1] IOFC = (Live weight gain × Live weight price) – (Feed intake × Feed cost).

[2] Avizyme treatment increased IOFC = Avizyme treatment IOFC – Positive control IOFC.

[3] Hemicell treatment increased IOFC = Hemicell treatment IOFC – Positive control IOFC.

[4] Combination treatment increased IOFC = Combination treatment IOFC – Positive control IOFC.

[5] Negative value indicates a decreased IOFC.

4.4. Discussion

If there was no significant difference in performance between the Negative control feed and the Positive control feed, metabolisable energy was not first limiting in the feeds and no response in production could have been expected to provision of additional metabolisable energy. For this reason the feed energy concentration used as base in the control feeds was lower than values used commercially (see Table 29). A comparison between the Negative control and the Positive control treatments showed that the Positive control consistently performed better than the Negative control, as was expected. This indicated that the difference in energy specification between the two control treatments was large enough to induce differences in performance variables and that energy was the most limiting nutrient.

The Avizyme treatment group performed significantly better than the Negative control group ($P < 0.05$). This indicated that the addition of Avizyme to a maize-soya based broiler feed with a relatively low metabolisable energy level improved broiler performance. The Avizyme treatment group's total body weight, body weight gain, feed intake, weekly FCR and mortality parameters were not significantly different from the Positive control group's parameters. If the exogenous enzymes addition did not supply the level of energy claimed, the treatment feeds would have resulted in performance significantly lower than the Positive control. This indicated that addition of Avizyme to the feed made an additional 0.45 MJ ME / kg feed available to the birds.

Performances of the broilers that received the feed with either Avizyme or Hemicell were similar. The Hemicell treatment group consistently performed better than the Negative control group. These results indicated that the addition of Hemicell to a feed made a positive contribution to broiler performance. The Hemicell treatment group performed similar to both the Positive control group and the Avizyme treatment group. Therefore, the addition of Hemicell to the feed also made an additional 0.45 MJ ME / kg feed available, similar to the addition of Avizyme to the feed.

If the Combination treatment (combination of Avizyme and Hemicell) resulted in performance better than both the Avizyme and the Hemicell treatments, it is likely that the combination of these two enzymes had an additive effect. If the Combination treatment resulted in performance better than the summation of both the Avizyme and the Hemicell treatments, it would have been proven that the combination of these two enzymes had a positive synergistic effect on production. In this trial, the Combination treatment group consistently performed better than the Negative control group, indicating that the combination of Avizyme and Hemicell added to the feed resulted in an improvement in broiler production. The Combination treatment group's performance was statistically similar to the Positive control, Avizyme treatment and Hemicell treatment groups for most parameters. There were, however, some parameters for the Combination treatment group that performed better than the other groups. The Combination treatment's body weight was significantly superior to all the other treatments on days 14 to 28 and the growth rate was significantly higher for days 7 to 14. This indicated that broilers that received a combination of Avizyme and Hemicell added to feed performed better than when these two enzymes added to the feed individually. As previously mentioned, the Positive control contained 0.45 MJ more metabolisable energy per kg than the Negative control feed and Hemicell and Avizyme contributed an additional 0.45 MJ ME / kg to the low energy diets. The Combination treatment results were significantly better than the Positive treatment at earlier stages of the trial (broilers were younger), but the difference between the Combination treatment and the Positive treatment became insignificant at a later stage in the trial when broilers were older. The results obtained in this trial is supported by a study done by Classen (1996), who stated that exogenous enzyme addition to the feed is more effective in younger broilers, since younger broilers have less endogenous enzymes available.

Studies relating to the effect of enzyme activity on broiler performance have been contradictory. Some studies have found that the addition of exogenous enzymes to maize-soya based broiler diets have had no effect on parameters like digestibility and viscosity (Mahagna *et al.*, 1995; Zanella *et al*., 1999; Saleh *et al.*, 2003).

The improved broiler performance that was measured in this performance trial are similar to the results found by other research trials (Centeno *et al.*, 2006; Karimi *et al.*, 2007). The increased broiler performance support numerous statements about the effect of exogenous enzyme addition, including increased ileal digestibility of feed, improvement in viscosity of the diet and increased apparent and true metabolisable energy.

Broilers in the Hemicell treatment group performed significantly better than the Negative control group. In a trial conducted by McNaughten *et al*. (1998), broilers in the treatment group only performed slightly better than the broilers in the control group. A difference between these two trials that might explain this difference in findings is the smaller variation in ME between the Negative control and treatment group. The Negative control group in this trial had a metabolisable energy reduction of 0.45 MJ / kg, while in the study of McNaughten *et al*. (1998) an energy reduction of 0.6 MJ / kg was used. The current trial indicates that an allocation of 0.6 MJ ME / kg feed for Hemicell as used by McNaughten *et al*. (1998) was possibly too high.

4.5. Conclusion

The Avizyme treatment revealed that the addition of Avizyme to an energy deficient diet will result in a significant improvement in broiler production variables, by increasing the metabolisable energy content of the diet by 0.45 MJ ME / kg feed. The addition of Hemicell to an energy deficient diet was also found to make an additional 0.45 MJ ME / kg feed available, leading in a significant improvement in broiler production variables. The Combination treatment revealed that the addition of a combination of Avizyme and Hemicell to an energy deficient diet resulted in a significant improvement in broiler production. The combination of Avizyme and Hemicell was found to release more than 0.45 MJ ME / kg feed and lead to superior production, in comparison with the enzyme added individually to the test feeds. The combination of Hemicell and Avizyme in a broiler ration revealed that these two enzymes have a positive synergistic effect on each other in the younger broiler, due to exogenous enzyme addition being more effective in younger broilers.

Chapter 5

General conclusions and recommendations

Addition of exogenous enzymes to a broiler feed significantly improved broiler production variables in this study. It could be concluded from this study that addition of Avizyme or Hemicell, or a combination of both Avizyme and Hemicell to a broiler feed released additional metabolisable energy from the feed, resulting in improved broiler performance when compared to a Negative control treatment, without any exogenous enzymes added.

Due to incorrect dosing of trial feeds during the first performance trial, neither Avizyme, nor Hemicell could have been evaluated as feed additives for the entire 35 day production period. Testing feed samples for enzyme activity is critical to evaluate the accuracy of feed mixing. Intense supervision during feed manufacturing and enzyme dosing is as critical as accurate broiler performance recording.

In the first trial, the addition of Avizyme to an extended starter phase of a feed contributed more than 0.35 MJ ME / kg feed and resulted in significant improvement in broiler production over a five week growing period. There was no significant difference in performance between the broilers of the Positive control and Avizyme treatment. This prohibited the determination of an exact metabolisable energy contribution of Avizyme to a broiler starter diet. It could only be concluded that addition of Avizyme to a broiler starter ration will increase the metabolisable energy availability by at least 0.35 MJ ME / kg and will result in a positive influence on broiler production. The addition of Hemicell to an extended starter and grower phase of a feed contributed 0.35 MJ ME / kg feed and resulted in a significant improvement in broiler production over a five week growing period.

The data obtained from the first performance trial was considered when planning and implementing the second performance trial. The problems of incorrect enzyme activity in the respective treatments that were experienced during the first performance trial were addressed with the second trial. All treatments were tested for correct enzyme activity before commencement of the trial. For the second performance trial, the difference in metabolisable energy between the Positive control and the Negative control treatments was increased in order to determine the amount of additional metabolisable energy that Avizyme released in a broiler feed.

In the second performance trial, it was found that the addition of Avizyme to an energy deficient diet will result in a significant improvement in broiler production variables, because of an additional 0.45 MJ ME / kg feed being made available. The addition of Hemicell to an energy deficient diet was also found to contribute an additional 0.45 MJ ME / kg feed, leading in a significant improvement in broiler production variables. The addition of a combination of Avizyme and Hemicell to an energy deficient diet resulted in a significant improvement in broiler production. The combination of Avizyme and Hemicell was found to release more than 0.45 MJ ME / kg feed and lead to superior production, in comparison with the enzymes added individually to the test feeds. The combination of Hemicell and Avizyme in a broiler ration showed a positive synergistic effect on each other in the younger broiler, due to exogenous enzyme addition being more effective in younger broilers.

The addition of Hemicell to the feed made at least an additional 0.35 MJ ME / kg available in the first performance trial, and 0.45 MJ ME / kg in the second trial. The increment of 0.1 MJ ME / kg from the first to the second trial was not large enough to be able to determine the value of the enzyme more accurately. Therefore, it was concluded that addition of Hemicell to the feed made an additional 0.35 – 0.45 MJ ME / kg available. The efficacy of enzymes in feed is also dependent on the quality of the feed. Lower quality raw materials, with a lower digestibility will yield more dramatic results when exogenous enzymes are added. The soybean products that were used in the second performance trial could have been inferior in quality, when compared to the soybean products used in the first performance trial.

Although the addition of exogenous enzymes resulted in the release of additional metabolisable energy in the feed, the addition of these enzymes should undergo economical evaluation to ensure it is viable under commercial circumstances. Addition of Avizyme with a calculated energy contribution of 0.35 MJ ME / kg and 0.45 MJ ME / kg to the feed realised an income over feed cost (IOFC) of 25 c / kg live weight and 4 c / kg live weight, respectively, during the five week period. The Hemicell treatment showed a negative IOFC (including the enzyme decreased the profit) of 24 c / kg live weight during the first four weeks of the first production trial and an IOFC of 2 c / kg live weight during the five week period of the second trial. The combination of both enzymes in the feed returned an IOFC of 16 c / kg live weight.

When evaluating the IOFC during the three different phases (extended starter, grower and finisher), it becomes clear that the IOFC is highest during the starter phase and lowest during the finisher phase. Exogenous enzyme addition is conclusively more effective during the earlier phases of feed in the broiler.

The Avizyme treatment also yielded a predominantly higher IOFC in comparison to the Hemicell treatment. It can be concluded that, although the Aviyzme and Hemicell treatments both appeared to have a similar efficacy as exogenous enzyme applications, Avizyme addition realised a higher profit. The combination of both enzymes added to the feed, however, had a superior IOFC and would possibly be the best feeding strategy to follow.

It is important that trial results be placed in perspective to commercial circumstances. Trial results were obtained under ideal circumstances, while commercial production is subject to larger variations. Although addition of these enzymes have been shown to release an additional 0.45 MJ ME / kg feed, it is recommended that commercial broiler diets must be formulated with a substantially lower energy contribution allocated to the enzymes. Both the feed manufacturer, as well as the poultry farmer, will benefit from a lower energy contribution allocated to the enzyme. The feed manufacturer uses the lower energy value as an insurance against suboptimal enzyme activity, as the minimum energy specification might still be achieved. The poultry farmer benefits from increased poultry production, due to a feed with a higher energy value. When a combination of two or more enzymes are used in commercial feed, the approach can be more aggressive. The diet can be formulated with a maximal energy contribution allocated to one of the enzymes, while the other enzyme(s) are added with no additional energy contribution allocated to those enzymes. Again, both the feed manufacturer, as well as the poultry producer will benefit from this strategy, as previously explained. Another strategy that can be recommended where there is vertical integration between the feed manufacturer and the poultry producer, is to “let the enzyme pay for itself.” With this strategy, the enzyme is included in the feed with only a portion of its potential energy contribution being allocated. The fractional energy that the enzyme contributes to the feed is equal to the cost of the enzyme’s addition in energy. As an example, if the addition of an enzyme costs R 40 / ton feed and a 12 MJ ME / kg feed cost R 2 400, the cost of 1 MJ ME / kg would be R200, or R40 / 0.2 MJ ME. The enzyme would then be allocated an energy contribution of 0.2 MJ ME / kg feed.

From the conclusions made in this study, addition of exogenous enzymes to commercial broiler feeds would most certainly be recommended for improved broiler production and decreased raw material costs in feed production. Correct interpretation and practical application of the positive effects of exogenous enzyme application to commercial broiler feeds, can aid nutritionists to develop nutritionally balanced broiler feeds at lower costs. The negative effects of anti-nutritional factors in broilers feeds can be greatly reduced with the strategic use of exogenous enzyme addition to the feed.

Chapter 6

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