EFFECTS OF FEEDING GRADED LEVELS OF PALM KERNEL MEAL (PKM) IN BROILER CHICKEN DIETS SUPPLEMENTED WITH MAXIGRAIN® ENZYME.

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November, 2007

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DECLARATION

I declare that the whole of this work is the result of my investigation (except where reference is made to published literature and where any assistance is acknowledged) and has not been part of any presentation for any other qualification.

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Dr. J.J. OMAGE
Date
Major Supervisor

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CERTIFICATION

This thesis entitled “Evaluation of enzyme (Maxigrain®) application of graded levels of Palm Kernel Meal (PKM) on the performance of broiler chickens” by ESUGA, PHILIP MAJIYEBO meets the regulations governing the award of the degree of Master of Science (M.Sc) in Animal Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

Dr. J. J. Omage **Chairman, Supervisory Committee**	**Date**
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DEDICATION

This work is dedicated to Feed Masters Ltd., management and staff for the sponsorship of this project.

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ABSTRACT

A study was conducted to evaluate the effects of feeding graded levels of PKM in broiler chicken diets supplemented with or without Maxigrain® enzyme on the performance of broiler chickens. The first study is on nutrient retention, where eighty one (81) day old chicks of the Arbor Acres strain were randomly allotted to nine (9) isonitrogeneous dietary treatments with three replicates and three birds each in metallic cages. The PKM was included at 0, 10, 20, 30, and 40% levels while PKM treated with Maxigrain® was included at 10, 20, 30, and 40% levels. Results show that there was significant ($P<0.001$) differences in protein, fat, NFE and metabolizable energy retention in birds fed the control and Maxigrain® treated diets than those on diets without Maxigrain®. The second study was conducted to evaluate the effects of PKM in broiler diets treated with or without Maxigrain®. Four hundred and five (405) day old chicks of the Arbor Acres strain were randomly allotted to nine (9) isonitrogeneous dietary treatments where PKM was treated with Maxigrain® prior to its inclusion in the diets. The PKM was included in the diets as in the first study on nutrient retention. The results indicated that Maxigrain® treatment of PKM improved the utilization of PKM, improved feed: gain ratio and reduced the cost of production. The effects of PKM treated with or without Maxigrain® on broiler performance did not show any superiority over the control diet. The inclusion of PKM even at 40% with or without Maxigrain® attained a final weight above 1.5kg at 8 weeks.

Therefore, PKM inclusion at 10, 20, 30, and 40% levels did not have any deleterious effect on broiler performance and health. The dressed weight (g), abdominal fat, pancreas and spleen were significantly ($P<0.001$) different across the dietary treatments. The percentage neck, breast and the intestines were also significantly ($P<0.05$) different across diets but no clear trends were established.

The third study was conducted to evaluate the effects of PKM in broiler diets supplemented with or without Maxigrain®. Four hundred and five (405) day old chicks of the Arbor Acres broiler strain were randomly allotted to nine isonitrogeneous diets. The Maxigrain® was added to the already formulated diets at 0.01% to four of the nine diets. The PKM was included in the diets at the same levels as in the first study on nutrient retention. The results indicate that supplementation of PKM with or without Maxigrain® on broiler performance shows that the inclusion of PKM up to 20% with Maxigrain® was superior to the control diet and it is therefore recommended for optimum performance of broiler chickens.

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LIST OF ABBREVIATIONS

CF	Crude fibre
CP	Crude protein
DM	Dry matter
EE	Ether extract
HB	Hemoglobin
ME	Metabolizable energy
NFE	nitrogen free extract
PCV	packed cell volume
TP	total protein
WBC	White blood cell

CHAPTER ONE

INTRODUCTION

Research efforts over the years had attempted to increase the use of cheap agro-industrial by-products like wheat offal (WO), rice bran (RB), brewer's dried grain (BDG), palm kernel meal (PKM) and maize Milling by-product (MO) (Onwudike, 1988). There is therefore an economic incentive to investigate the use of palm kernel meal (PKM) in broiler diets in Nigeria due to its cost effectiveness compared to other conventional feedstuffs.

There has been a dramatic increase in global production of PKM, with annual growth rate of 15% over the last two decades (FAO, 2002).

The move towards the use of diets devoid of ingredients of animal origin will exacerbate the effects of anti-nutrients on bird performance and nutrient excretion in the environment. One of the key technologies that can help address this challenge is the use of appropriate enzymes. The enzyme technology has progressed a great deal over the past twenty years with respect to efficacy and matching activities with their target substrates. For example, β-glucanases and xylanases for degradation of the β-glucans and arabinoxylans in barley, oats, wheat, rye and triticale have proven efficacy in enhancing the nutritive value of these grains for poultry (broilers in particular). The inclusion of microbial phytase to improve the utilization of organic phosphorus is another good example. The benefits of using enzymes in monogastric diets include not only enhanced growth performance and feed conversion, but also fewer

environmental problems due to reduced output of excreta. Increased accuracy and flexibility in least-cost feed formulations and improved well being of animals are other possible benefits of using feed enzymes. As more and more knowledge is gathered on the detailed chemical structures and the physiological activities of non starch polysaccharides (NSPs) in various ingredients, highly sophisticated enzymes will be developed to target these polymers in a precise manner.

Therefore the use of NSP as energy sources, a more efficient utilization of non-conventional ingredients, such as copra meal (coconut), palm kernel cake, sunflower meal and some industrial wastes, and the elimination of specific anti-expeller pressed palm kernel, popularly known as palm kernel cake and (b) solvent extracted palm kernel or palm kernel meal. So, palm kernel meal or cake is the residue of oil extraction of palm kernel. With an increase of 300% over the last two decades, palm kernel meal production has been one of the fastest growing processed feedstuff (FAO, 2002). The rising cost of conventional feedstuffs, which are regularly imported into palm kernel meal producing countries, has triggered much pressure on local poultry industries to maximize the use of locally available feedstuffs (Hutagalung, 1980).

The poor response of monogastric animals to the use of agro-industrial by-product could be traced to high fibre and low metabolizable energy content of these by-products (Atteh *et al.,* 1993). To make these cheap, high fibre, low energy wastes acceptable to these animals, one must either augment the by-

products with high energy sources like palm oil (Fadipe, 1996) or break down the fibre to release more energy for the animal.

The effect of dietary enzymes is influenced by the type and concentration of the undesirable carbohydrates present in the feedstuff and the class and age of the livestock and poultry that consume it. Young animals are affected to a greater degree by the anti-nutritional compounds than the older ones. (Annison, 1991 and Schuttle *et al.,*1995). Poultry and swine do not have the endogenous enzymes necessary for breaking down of non-starch- polysaccharides (NSPs).

The use of enzymes has been common in many industries for many years. For instance, enzyme uses in the food processing, brewing and leather-working industries are well documented (Partridge and Wyatt, 1995). An increased understanding of the properties of enzymes and their function has led to their introduction in the animal-feed industry, although the application of enzyme technology is relatively new for the livestock feed industry.

Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer, (Vukic Vranjes and Wenk, 1993) Their use in poultry feeds has predominantly been related to the hydrolysis of fibre or non-starch polysaccharide (NSP) fraction of cereal grains. These NSPs cannot be digested by the endogenous enzymes of poultry and can have anti-nutritive effects. They cause an increase in viscosity of intestinal content and entrap large amounts of

well digestible nutrients like starch and proteins. This leads to an impaired digestion and digestive problems, (Almirall *et al.,* 1995)

Numerous researchers have demonstrated that the soluble NSP fraction and not the total NSP fraction are responsible for anti-nutritive responses. These NSPs can bind to large amounts of water and as a result, the viscosity of fluids in the digestive tract is increased. The increased viscosity causes problems in the small intestines because it reduces nutrient availability (particularly fat) and results in increased amount of sticky droppings (Choct, *et al.,* 1995)

The advent and use of commercial feed enzymes in livestock feeding has opened a new horizon for the use of hitherto waste feedstuff. Results of experiments (Atteh, 2001) showed that with the use of a bacterial xylanase enzyme, it was possible to replace 50% of maize with wheat offal without detrimental effect on broiler performance. Atteh (2001) reported 56% and 26% increase in apparent metabolizable energy (AME) of wheat offal and BDG respectively when diets were supplemented with xylanase enzyme.

Palm kernel meal is one of the abundant agricultural by-products that is cheap and readily available. It is aflatoxin free, palatable and has considerable potential as a carbohydrate and protein source. However, due to its low nutritive value, grittiness and potential for deterioration in unhygienic conditions, large amounts of PKM is often discarded and can create environmental problems in the future (Sundu *et al.*, 2005a).

With the availability of feed enzymes to digest fibre, there is need to evaluate the feeding value of enzyme treated PKM. From the composition of PKM, it

appears that three main enzymes are needed to improve their nutritive value namely: mannanase, α-galactosidase and cellulase. (Balasubramaniam, 1976).

PKM has been reported to contain mannan and galactomannan and these are likely to have anti-nutritive properties. Of the total NSPs in PKM, 78% is mannan, 3% is arabinoxylans, 3% is glucoronoxylans which were found to be water-insoluble and 12% is cellulose (Dusterhorft and Voragen, 1992). These problems are mostly tackled by either a careful formulation of diet for protein and energy balance or the inclusion of enzymes, particularly mannanase, galactosidase and cellulase.

The role of enzymes as feed additive in poultry diets is well established. Hastings (1946) and Allen *et al.*, (1997) all observed that enzyme addition to monogastric animal feed reduced viscosity of ingesta in the intestine and showed a marked improvement on the various morphological effects of feeding fibrous materials to non-ruminant animals. The use of PKM in poultry diets has been practiced for several decades. Its low level of key essential amino acids (lysine and methionione in particular), high dietary fibre (particularly in the form of β-mannan) and grittiness have precluded its inclusion in broiler diets. Contradictory results have been reported on the effect of PKM on performance of broilers (Panigrahi and Powell, 1991; Ezieshi and Olomu, 2004 and Sundu *et al.,* 2005a), which may not be related to its contribution to the overall nutrient in the diet, particularly amino acids and metabolizable energy but due to the wide variation in its nutrient content depending on the oil extraction process, the

species of the palm nut and the amount of shell content remaining in the meal on chemical analysis (O'mara *et al.,* 1999).

The aim of this study is to determine the effects of Maxigrain® (a multi-enzyme compound of β-glucanase, xylanase, phytase, arabinoxylanase and a mixture of yeast and minerals) treated PKM on nutrient retention and performance of broiler chicks fed on diets containing PKM with or without Maxigrain® in broiler diets and the appropriate method of Maxigrain® application.

1.1 OBJECTIVES OF THE STUDY

The main objective of this study includes:

1. To determine the effects of Maxigrain® treated palm kernel meal on metabolizable energy and other nutrients (protein, fat, crude fibre) retention.
2. To determine the effects of the Maxigrain® treated palm kernel meal on the performance of broiler chicks
3. To determine the appropriate method of Maxigrain® application on palm kernel meal based broiler diets.
4. To determine the effects of Maxigrain® treated palm kernel meal on carcass characteristics of broilers.
5. To evaluate the economics of raising broiler chicks on Maxigrain® treated palm kernel meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Feeding value of Palm Kernel Meal

Nutritionally, palm kernel meal contains moderate amounts of protein and carbohydrate. Crude protein of palm kernel meal ranges from 14 to 21%. (Sundu *et al.*, 2005c)

Table 2.1 Show the physical characteristics and nutrient composition of PKM, while Table 2.2 show the amino acid availability, exceeding 85%, except for valine and glycine. Examination of the physical characteristics of palm kernel meal reveals that its bulk density is quite high compared to copra meal (coconut) their respective values are 0.67 and 0.56g/cc. Although Palm Kernel Meal and copra meal have some common properties in carbohydrate composition, their water holding capacity differs greatly. Palm kernel meal water holding capacity is half the water holding capacity of copra meal (Sundu *et al.,* 2005c). These two physical characteristics bulk density and water holding capacity are important as they affect feed intake (Kyriazakis and Emmans, 1995).

2.2 PKM protein and amino acids

Methionine and lysine are the two most important essential amino acids to be considered when using PKM because only about 30% and 50% respectively of these amino acids requirement of young chicks is met by PKM.

Table 2.1: Physical characteristics and nutrient content of palm kernel meal

Fractions	Composition	References
Dry matter (%)	94	(1)
Crude protein (%)	14-21	(1) (2) (3)
Gross Energy (KCal/kg)	4998	(1)
Crude fibre (%)	21-23	(1) (4)
Lipid(%)	8-17	(1) (4)
Ash(%)	3-6	(1) (4)
Bulk density (unmodified) (g/cm^3)	0.67	(1)
Bulk density (0.5mm) (g/cm^3)	0.57	(1)
WHC (1.0mm) (g water/g feed)*	2.82	(1)
WHC (0.5mm) (g water/g feed)	2.93	(1)

WHC: Water holding capacity. (1) Sundu *et al.*, 2005c; (2) Nwokolo *et al.,* 1976; (3) Onwudike, 1986; (4) Sue, 2001.

Table 2.2: Amino acid composition and availability of palm kernel meal (percentages).

Amino Acids	Composition (%)			Availability	0-3 weeks of broiler)
	(A)	(B)	(C)	(%) (B)	Requirements (D)
Arginine*	2.18	2.68	2.40	93.2	1.25
Cystine	0.20	-	-	-	(Cys + Meth) 0.90
Glycine	0.82	0.91	0.84	63.3	(Glycine + Serine) 1.25
Histidine*	0.29	0.41	0.34	90.1	0.35
Isoleucine*	0.62	0.60	0.61	86.1	0.80
Leucine*	1.11	1.23	1.14	88.5	1.20
Lysine*	0.59	0.69	0.61	90.0	1.10
Methionine*	0.30	0.47	0.34	91.0	(Cys + Meth) 0.90
Phenylanine*	0.73	0.82	0.74	90.5	(Phenyl + Tyrosine) 1.34
Threonine*	0.55	0.66	0.60	86.5	0.80
Tyrosine	0.38	0.58	0.47	85.0	(Phenyl + Tyrosine) 1.34
Serine	0.69	0.90	0.77	88.7	(Glycine + Serine) 1.25
Valine*	0.93	0.43	0.80	68.4	0.90
Tryptophan*	0.17	-	0.19	-	0.20

***** Essential amino acids: (A): Yeong, 1983; (B) Nwokolo *et al.,* 1976; (C) Hutagalung, 1980; (D) NRC, 1994

Nwokolo *et al.* (1976) reported that the average availability of amino acid for poultry ranges from 63.3% for glycine to 93.2% for arginine.

The low level of valine and methionine in palm kernel meal coupled with the low availability of valine implies that supplementary valine and methionine from other sources are needed when using palm kernel meal as a basal diet. However, palm kernel meal is regarded as an excellent source of arginine because of its high content (2.68%) and availability (93.2%) (Nwokolo *et al.,* 1976)

It has been reported by Chamruspollert *et al.* (2004) that the nutritional requirements for arginine, methionine and lysine are interrelated. Since the ratio of arginine to lysine is very high (1.03) as a consequence of high arginine and low lysine content, the inclusion of palm kernel meal in diets should be supplemented with either synthetic lysine or a high lysine feedstuff to balance these two amino-acids. Failure to provide the correct balance can impair the performance of poultry. Balnave *et al.* (1999) found that arginine: lysine ratio of 1.03 gave better results for birds kept from three to seven weeks under a temperature of 32^{o}C. The requirement for methionine is increased as levels of arginine increases (Chamruspollert *et al.,* 2004). This justifies the importance of considering methionine and lysine when PKM is used in large quantities.

2.3. Carbohydrate and energy content of PKM

A detailed description of the carbohydrate fraction of PKM has been reported by Knudsen (1997). This author reported that total carbohydrate of palm kernel meal, excluding lignin was 50%, of which only 2.4% was of low molecular weight and

1.1% was starch, while 42% is in the form of non-starch polysaccharides (NSPs). This means that 81% of palm kernel meal carbohydrate is in the form of NSPs. Of the total NSPs present in palm kernel meal the main form is an insoluble non-cellulose polysaccharide, accounting for 33.6% of the dry matter. The main sugars in the soluble non-cellulose polysaccharide were mannose and galactose while the sugars in the insoluble non-cellulose polysaccharide were mannose and glucose (Knudsen, 1997). The high amount of lignin (13.6%) in PKM, due possibly to the contamination with nut shell (Knudsen, 1997), makes this feedstuff feel gritty and fibrous. Fractionation of palm kernel meal based on its proximate analysis reveals that 49% of the dry matter of palm kernel meal is in the form of nitrogen free extract (Sue, 2001and Sundu *et al.*, 2004b).

Of the NSPs present, it has been found that 78% is linear mannan with very low galactose substitution, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans (Dusterhoft and Voragen, 1992). It also contains small amount of galactomannan (Daud and Jarvis, 1992, Dusterhoft and Voragen, 1992 and Knudsen, 1997). This type of mannan is characterized as hard and water insoluble (Warren, 1996). Most palm kernel meal mannan is extremely hard, highly crystalline and water insoluble (Aspinal, 1970). However, Dusterhoft and Voragen, (1992) reported that about 66% of palm kernel meal mannan could be solubilised by sequential extraction with alkali and sodium chloride. The metabolizable energy of palm kernel meal varies widely, from at least 2591 kcal/kg (Chin, 2002) to 3959 kcal/Kg (Sundu *et al.,* 2005c). This may be due to the fact that the oil content of palm kernel meal varies due to differences in oil extraction process. The higher metabolisable energy values may be due to higher oil content remaining in the palm kernel meal after the

product is processed by expeller machinery (O'Mara *et al.,* 1999).

2.4 Evaluation of PKM on poultry performance

Research on using palm kernel meal to establish its feeding value and the maximum inclusion rate in broiler diets has been a major concern in palm kernel meal producing countries. Early recommendations for using palm kernel meal indicated that 20% could be given to broilers without any negative effects on the performance of birds (Yeong, 1980, Hutagalung, 1980). Onwudike (1986) investigated the possible inclusion of Palm Kernel Meal, in broiler starter and finisher diets and reported that 28% and 35% respectively could be fed without any ill effect. Later reports suggested that diets containing 40% palm kernel meal could be fed to broilers when methionine and lysine were added. (Panigrahi and Powell, 1991 and Sundu *et al.,* 2004b) The different recommendations may be due to the source and quality of palm kernel meal as these vary widely as shown in Table 2.2 (Nwokolo *et al.,* 1976, Yeong, 1983). Another more plausible reason is the formulation of the diet. Panigrahi and Powell (1991) and Sundu *et al.* (2004a) formulated balanced diets with the inclusion of methionine and lysine. This may indicate that the problem arising from the studies of Yeong (1980) and Hutagalung (1980) may not be from the palm kernel meal per se, but from the deficient nutrient in the diet. Amino acids and metabolisable energy are two important nutrients in diet formulation, particularly for diets containing a high fibre ingredient.

The feed intake of birds fed a palm kernel meal based diet is usually higher than for a maize-based diet (Onwudike, 1986; Ezieshi and Olomu, 2004 and Sundu *et al.,* 2005a). This is probably due to its faster rate of passage in the digestive tract (Onifade and Babatunde, 1998), high bulk density and its low water holding

capacity. Sundu *et al.* (2005b) compared the bulk densities of many poultry feedstuff and found that the bulk density and water holding capacity of palm kernel meal were 0.57g/cm^3 and 2.93 water/g feed respectively. These values are very close to the values of bulk density and water holding capacity of soybean meal. Low bulk density and high water holding capacity are believed to impair feed intake (Sundu *et al.,* 2005b). This phenomenon indicates that palm kernel meal has potential benefit for poultry provided that the diet consumed can be digested and made available for the birds. Panigrahi and Powell (1991) reported that the inclusion of up to 50% palm kernel meal in a diet was tolerated provided that the birds were kept up to 7 weeks of age. Although body weight and weight gain of birds were not affected, digestibility of the diet was impaired. Onifade and Babatunde (1998) and Sundu *et al.* (2005a) reported that the inclusion of increasing levels of palm kernel meal decreases feed digestibility, apparent nitrogen retention, and apparent calcium retention. This indicated that a high proportion of PKM nitrogen or protein is located inside cell walls. The water intake of birds fed palm kernel meal based diets also increased (Panigrahi and Powell, 1991) and there was increased moisture content of the excreta (Onifade and Babatunde, 1998). The decrease in digestibility may be due to the fact that broiler chickens have a limited ability to digest dietary fibre, such as α-mannan, because of the absence of any mannan degrading enzymes in the digestive tract of birds. Accordingly, two possible ways to cope with this problem is to formulate the diet based on digestible nutrients such as amino acid and metabolisable energy with enzyme application to improve palm kernel meal digestibility and reduce the moisture content of excreta.

Table2.3: Body weight and feed intake differences of birds fed Palm Kernel Meal based diets compared with birds fed a maize based diet (percentages)

PKM Diet	Body weight (g)			Feed intake (%)		
	A	B	C	A	B	C
20-23%	-	-0.3	-1	-	+6	+2
30-35%	+4	+2	+0.5	+7	+1	+2
40-47%	+2	-0.3	-7	+6	+5	-1

(A) Panigrahi and Powell, 1991; (B) Sundu *et al,* 2004b; (C) Onwudike, 1986

Table 2.3 indicated that at 20-23% PKM inclusion, the bird body weight was inferior to maize based diet but increased feed intake was reported by all the authors. At 30-35% inclusion of PKM, the report by all the workers indicated that PKM was superior to maize based diets with higher feed intake. However, at 40-47% the reports were contradictory with Panigrahi and Powell (1991) reporting higher weight gain and feed intake, Sundu *et al*, (2004b) and Onwudike, (1986) reported lower weight gains compared with maize based diets. There was a

Depression of feed intake reported by Onwudike, (1986) at 40-47% inclusion of PKM compared to maize based diet.

The grittiness of palm kernel meal caused by contamination by the nut shell may help chickens grind the diet and it may also increase the size of the gizzard (Onwudike, 1986). A study by Onifade and Babatunde (1998) indicated that passage rate of digesta of a diet containing 20% palm kernel meal was faster than that of a diet containing maize offal or brewers dried grains. The gritty lignified shell of palm kernel meal may contribute to an increased rate of passage of the digesta in the digestive tract. Duke (1986) stated that the contraction of the gizzard,

proventriculus and duodenum are totally coordinated. Hard and fibrous feedstuff may increase the contraction of the gizzard and may speed up the peristaltic movement of ingesta in the duodenum and throughout the small intestine. This could account for the increase rate of passage of digesta and could in turn result in increased feed intake. The lignin content of the nut shells in palm kernel meal is a contributing factor to low digestibility.

2.5. Effect of PKM on poultry health

There has been increased interest in PKM over the last five years due to its efficacy in improving the immune system of broilers. Many current findings suggest that palm kernel meal could replace commercial manno-oligo-saccharide as a probiotic to improve chicken health and immunity (Allen *et al.,* 1997 and Fernandez *et al.,* 2002).

Poultry diets contain a wide variety of readily digestible carbohydrates such as starch, sugars and indigestible oligosaccharides and non-starch polysaccharides (NSPs). Numerous studies on the role of indigestible carbohydrates in the digestive tract of chickens over the last two decades have improved our understanding of the role and fate of these carbohydrates. This is now an era when carbohydrates in poultry diets are not only recognized as a source of energy but also have beneficial effects on chicken health. Study in this aspect is expected to increase in the near future as the uses of antibiotic in the European Union and North America have been banned over concerns of antibiotic resistance. (Philips *et al*., 2004)

Oligosaccharides have been substances of choice to replace antibiotics, due to their capacity to block the colonization of pathogenic bacteria in the intestines of broilers. Among oligosaccharides, fructo-oligosaccharides (Waldroup *et al.*, 1993) and manno-oligosaccharide (Fernandez *et al.*, 2000) have been of great interest. The efficacy of Mannose based carbohydrates, either as manno-oligosaccharide (MOS) (Lyons, 2002) or mannose (Oyofo *et al.,* 1989) to improve the immune system of animals has been well accepted. Commercially, the product "BioMos" which is extracted from yeast, has been marketed with efficacious effects in 92% of all the animal studies (Lyons, 2002).

Allen *et al.* (1997) pioneered research into the use of palm kernel meal as a source of mannose based carbohydrates. Since then, several authors reported the success of using palm kernel meal to improve the immune system of broilers (Fernandez *et al.,* 2000 and 2002). β-mannan in palm kernel meal have been reported to have similar properties to the mannan from yeast to increase immunity. Allen *et al.* (1997) reported that the addition of 25g palm kernel meal/kg diet reduced the degree of salmonella colonization in the intestinal tract of broilers consuming feed which was inoculated with *Salmonella kedeugou,* or *Salmonella enteritidis*. The birds became clear of infection by three weeks of age while birds fed the unsupplemented diet remained infected. The effectiveness of using palm kernel meal in broiler diets to protect against colonization with salmonella increased to the same level as using commercial mannan oligosaccharide (MOS) (Fernandez *et al.,* 2002).

In viral-caused disease, palm kernel meal appeared to work well. Zulkifli *et al,*

(2003) conducted research on Newcastle disease anti-body titre of broiler chickens fed palm kernel meal based diets and found that Newcastle disease antibody titre of birds fed palm kernel meal diets remained constant throughout a week period of heat stress while broilers fed the control diet had a large reduction in antibody titre.

The mechanism of improvement in the immune system of birds due to the consumption of palm kernel meal is still unclear. It may be through several modes of action in which mannose based carbohydrates either β-mannan or MOS, in the palm kernel meal are fermented in the caeca due to indigestibility of this fraction. This has beneficial effects in promoting the growth of desirable bacteria. This mode of action is based on the fact that mannose based carbohydrate in palm kernel meal increased the growth of non-pathogenic bacteria, such as *Bifidiobacterium spp* (Fernandez *et al.*, 2002). One of the products of fermentation in the caeca is an increased concentration of lactic acid (Wang and Gibson, 1993 and Okumura *et al.,* 1994) and this prevents the growth of pathogenic species such as Salmonellae. The ability of palm kernel meal to reduce the population of pathogenic bacteria in the gut has been found in four week old broilers (Fernandez *et al.*, 2002).

Another possible mechanism of action is that dietary MOS from palm kernel meal may attract micro-organism away from the intestinal binding sites. Mannan-oligosaccharides have been reported to have receptor sites for the fimbriae of *E. coli* and *Salmonella spp* which resulted in elimination of these particular bacteria as the digesta flows out (Spring *et al.,* 2000). Accordingly, colonization of the

microbes in that organ decreases and thus the birds are less susceptible to these organisms.

2.6. Enzyme supplementation of poultry diets

The main goals of enzyme supplementation of poultry diets include:

1. To remove or destroy the anti-nutritive factors in cereals

2. To enhance the overall digestibility of the feed to render certain nutrients biologically more available.

3. To reduce enviromental pollution from animal excreta by reducing dry- matter excretion

4. To allow the use of a wide range of ingredients without compromising bird performance and provide great flexibility in least- cost feed formulation.

To achieve these goals, enzymes must be able to survive the processing of feed and resist the acidic conditions and proteolytic enzymes in the proventriculus and gizzard.

2.7. Adding enzymes to practical cereal-based diets

Numerous researchers have demonstrated that the soluble-NSP fraction and not the total NSP fraction, is responsible for anti-nutritive responses. These NSPs can bind to large amounts of water, and as a result, the viscosity of fluids in the digestive tract is increased. The increased viscosity causes problems in the small intestine because it reduces nutrient availability (particularly fat) and results in increased amounts of sticky droppings. (Choct, 1998). To counteract these anti-nutritional effects, enzymes are often added to feeds such as these.

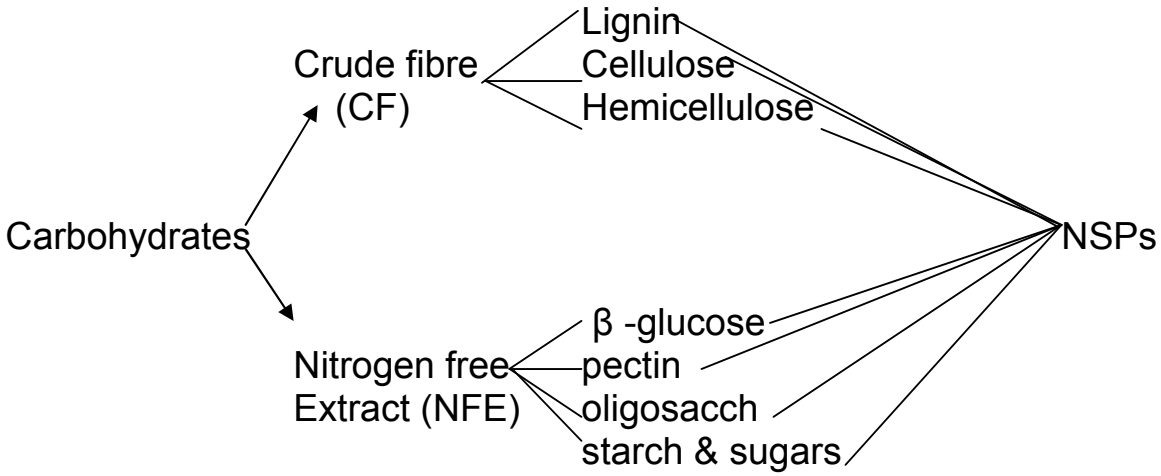

Fig.1 Carbohydrate fractionalization

TABLE 2.4 NSPs present in frequently used raw materials

	Arabinoxylans		Betaglucans	Cellulose	Mannose	Galactose	Uronic Acid	Total
	Sol	Insol.						
Wheat	**1.8**	**6.3**	**0.8**	**2.0**	**T**	**0.3**	**0.2**	**11.4**
Sorghum	**0.1**	**2.0**	**0.2**	**2.2**	**0.1**	**0.15**	**T**	**4.8**
Corn	**0.0**	**5.1**	**T**	**2.0**	**0.2**	**0.6**	**T**	**8.1**
Rice	**T**	**0.2**	**0.1**	**0.3**	**T**	**0.1**	**0.1**	**0.8**
Rice Bran	**0.2**	**8.3**	**T**	**1.2**	**0.4**	**1.2**	**0.4**	**21.8**
Wheat bran	**1.1**	**20.8**	**0.4**	**10.7**	**0.4**	**0.8**	**1.1**	**35.3**

Source: Derived from Choct 1998; T = traces, Sol=Soluble, Insol=Insoluble

The feed industry formulates poultry diets on the basis of least cost. Under these circumstances, the price of cereal and the nutrient content will determine whether a cereal enters the computer formulation. With the introduction of enzymes, nutritionist should be able to release more of the nutrient in a particular cereal, thereby making it more competitive with corn. Supplementation of feeds with enzymes could increase the energy available in wheat by 6-8%.

Phytase use is reported to reduce phosphorus excretion by as much as 40% for broilers. When phytase was added to layer diets, increased egg production and positive effects on egg weight was observed. (Simons and Versteegh, 1991)

The hidden benefits of using glycanases in birds fed viscous cereals include (a) reduction in output of manure containing large amounts of undigested nutrients, and (b) alleviation of problems associated with wet droppings, such as increased percentage of dirty eggs, increased gas production (i.e., ammonia) and increased fly and rodent populations in the shed. However, glycanase supplementation was not the answer to problems associated with phosphorus levels in pig and poultry manure. This led to the development of phytase for use in monogastric animal diets. Phytase increases the digestibility of phytate from around 25% to 50-70% in poultry and its use has been on the increase since banning the use of animal protein sources, such as meat and bone meal, in the European Union. It is also understood that phytase can improve the digestibility of other nutrients as well as energy (Ravindran *et al.,* 1999; 2000 and Kornegay, 2001).

Cereal by-products, such as rice bran, are important feed ingredients but their efficient use in monogastric diets is hindered by the presence of high levels of NSPs and phytate. Supplementing ducks diets with microbial phytase allowed rice bran to be used at high levels (up to 60%) without detrimental effects. (Simons and Versteegh, 1991) Phosphorus excretion was reduced by 9.6%, and sufficient decreases in excretion of manganese, copper and zinc were also noted.

2.8. Enzymes for poultry diets based on non-viscous cereals

Although the insoluble NSP have mainly been regarded as nutrient diluent in the diet, they can also affect digesta transit time and gut motility. Another facet of the role of insoluble NSP in poultry diets that is worthy of reiteration is their ability to act as a physical barrier to digestive enzymes, such as amylase and proteases, thus reducing their efficient digestion of nutrient embedded in the cell wall matrix of grains. Evidence in the literature appears to suggest that enzymes with affinity for insoluble NSP can elicit a positive response in growth performance of broilers (Cowan, 1995 and Choct, 1998). This breakdown of cell wall matrix, especially the insoluble components, may facilitate easier access of digestive enzymes to their substrates within the short feed transit time in birds. Wiseman and McNab (1998) also showed that the rate of starch digestion *in vitro* correlates closely with the AME values of different wheats. It suggests that the accessibility of aminolytic enzymes to starch granules differs depending on the wheat type, and some of the key factors influencing it may relate to the cell wall architecture of the wheat. Bedford (2002) demonstrated that a considerable amount of nutrients such as starch remains encapsulated in the cell walls in the small intestine of chickens and

was removed upon xylanase supplementation. Indeed, D'Alfonso (2003) reported significant variation in the nutritive in value of corn (93 samples examined) for chickens with ileal digestible energy value varying by 2.04MJ/kg DM and starch digestibility ranging from 84% to 90%. This variation was reduced by an enzyme product containing xylanase, protease and amylase. Cowieson (2005) speculated that the ability of enzymes, in particular, glycanases, to enhance the nutritive value of some corn-soy diets is probably mediated through changes in cell wall architecture of the grain, rather than viscosity reduction as it is often the case for viscous grains.

2.9. Vegetable protein sources

Grain legumes are usually included in monogastric diets as protein sources. However, when some grain legumes such as lupins are included in poultry diets as the sole protein source, bird performance suffered due to the high levels of NSP and the oligosaccharides present. Legume NSP are more complex in structure than those in cereals, containing a mixture of colloidal polysaccharides called pectic substances (galactouronans, galactan and arabinans). Neutral polysaccharides such as xyloglucans and galactomannans have also been reported. The Australian sweet lupin, for instance, contains a rhamnogalactouronan with arabinose and galactose side chains, with at least 4-6 different linkages (Cheetham *et al.,* 1993). A detailed analysis of the carbohydrate composition of 6 lupin samples showed that total amount of carbohydrates ranges from 34.1% to 39.3% on dry matter basis, which is composed of 2.31-2.71% rhamnose, 0-0.15% fucose, 0.03-0.21% ribose, 10.94-11.51% arabinose,1.97-

2.44% xylose, 0.89-1.12% mannose, 72-75% galactose and 8.05-10.34% glucose. Starch is present at less than 1%. Undoubtedly, a total depolymerisation of the complex NSP of grain legumes requires extremely complex enzyme activities. Thus, Annison *et al.* (1996) demonstrated that some commercial enzymes had a significant effect on lupin NSP in vivo. If the NSP in lupins are efficiently utilized as an energy source, their nutritive value for poultry will be increased by 50%. Appreciable improvements can be expected for other vegetable protein sources if effective enzymes are developed to degrade the NSP present in these ingredients. However, an effective enzyme to degrade pectic polysaccharides present in commonly used vegetable protein sources for poultry is yet to be developed. Furthermore, under commercial conditions some of the vegetable protein sources, such as lupins, peas and beans are used at small quantities and therefore the characterization of their substrates for the purpose of producing targeted enzymes is probably not on the priority list for enzyme manufacturers for economic reasons.

2.10. Substrate structure and enzyme affinity

Matching an enzyme activity with a substrate does not guarantee the efficacy of the enzyme in degrading the substrate. Currently most of the glycanases used in the poultry industry target the soluble carbohydrates in an endo-active manner, i.e, cleaving the molecules from the middle to reduce the molecular size with little or no monomeric sugars released (Bhat and Hazlewood, 2001). Substrate specificity depends largely on the source of the enzyme. Choct *et al.* (2004) reported three xylanases that are of different substrate affinities. A xylanase derived from *Thermomyces lanuginosus,* significantly increased the soluble NSP content of the

small intestinal digesta, but decreased its viscosity, whereas a xylanase derived from *Humicola insolens* markedly increased both the content of the soluble NSP in the small intestine as well as its digesta viscosity. This clearly demonstrated that the xylanase from *T. lanuginosus* had affinity for both soluble and insoluble arabinoxylans, e.g. whilst releasing soluble NSP from the insoluble cell wall xylans, it also degraded them into smaller polymers, but that from the *H. insolens* had affinity for only the insoluble cell wall xylans. The third xylanase used in this study was from *Aspergillus aculeatus,* which effectively degraded the soluble arabinoxylans, but had no effect on the insoluble fractions. The NSP degradation patterns appear to indicate that enzymes having affinity for both soluble and insoluble NSP will be more efficacious. However, these differences, in general, did not translate into apparent differences in bird performance despite some numerical trends.

Each grain of Maxigrain® contains cellulase-(10,000 i.u.), Beta- glucanase-(200 i.u.), Xylanase-(10,000 i.u.), Phytase-(2500 FTU).

Cellulase breaks down cell-wall for more energy and relocked nutrients. Xylanase and β-glucanase degrades non-starch polysaccharides in feeds. Phytase efficiently releases bound phosphorus from plant phytates and also liberates minerals and amino acids.

Benefits: Converts feed into performance by:

- Optimisation of metabolisable energy and feed conversion efficiency. (FCE)
- Increased use of low cost non- conventional feed ingredients.

- Reduction in requirement of di-calcium phosphate (DCP) or bone meal in feed.
- Significant improvement in better quality and dropping consistency of feces.
- Improvement in weight gain.
- Improvement in egg production and shell quality.

Monogastric animals do not have the endogenous enzymes to hydrolyze non-starch polysaccharide contained in fibrous feed materials.

See table 2.5.

As a result of these, exogeneous enzymes as shown on table 2.6 must be added to improve monogastric digestion.

2.11. Supplementary enzymes in poultry nutrition

Supplementary enzymes have found only limited practical use in animal nutrition until recently. The main reason was an unfavorable relationship between enzyme cost and their benefits. However due to the advances in biotechnology leading to cheaper production costs, economical uses of enzymes in animal nutrition are now possible (Broz and Perrin-Voltz, 1994).

There is increasing experimental evidence that specific enzyme complexes used as feed additives may offer a practical means of enhancing the nutritive quality of various low-energy cereals for poultry. Increasing interest in application of feed enzymes resulted in the development of specific preparations designed for particular feed.

Table2.5: Endogeneous enzymes in poultry

Organ	Enzyme produced	Substrate acted upon	End product
1. Mouth (Saliva)	Alpha-Amylase	Starch	Glucose, Maltose Dextrins
2. Proventiculus	Pepsin	Protein	Peptides
3. Pancreas	Amylase	Starch	Glucose, Maltose Limit Dextrins. Fatty
	Lipase	Fat	Acids, Monoglycerides. Amino acids and small
	Trypsin	Proteins	peptides.
	Chymotrypsin	Peptides	Amino acids
	Elastase		
	Carboxypepti-dase		
4. Intestinal Mucosa	Oligo-1,6 – glucosidase	Dextrin	Glucose
	Maltase	Maltose	Glucose
	Sucrase	Sucrose	Glucose & Fructose
	Amino-peptidase	Peptides	Amino acids
	Dipeptidases	Dipeptides	Amino acids

Source: Card and Neshien (1972)

2.12. Sources of supplementary enzymes

Commercial enzymes are mostly derived from fungal, bacterial and at times other sources such as protozoans which are cultured and the enzymes produced by these microbes extracted from the culture. Some of the available supplementary enzymes and their origins are listed below.

Studies to increase the digestibility of palm kernel meal based diet for poultry have rarely been carried out. The utilization of enzymes has been of interest to the poultry industry for several decades and various enzymes are now available in the market. Studies on enzyme addition in palm kernel meal based diets are now possible. Early reports of the use of enzymes to increase the solubility of palm kernel meal were based on laboratory test (Dowman, 1993 and Dusterhoft, *et al.,* 1993). In feeding trials, the inclusion of enzymes, particularly mannan degrading enzymes, has been studied by Luis (2002) and Sundu *et al.* (2004b). Luis (2002) found that the use of mannan degrading enzymes can support maximum growth of broilers fed a diet having an energy reduction of 1699Kcal/kg. A study of Iyayi and Davis (2005) indicated that the highest growth of broilers was reached when a 30% palm kernel meal diet was supplemented with enzymes, compared to a growth of broilers fed a corn-soy diet.

The digestibility, feed efficiency and AME of the diet were increased when mannan degrading enzymes were added to a 40% PKM diet (Sundu *et al.,* 2004b).

Table 2.6: Sources of supplementary enzymes

Enzyme	Origin
(1) Xylanase	*Bacillus subtiles*
(2) Hostazyme	*Trichoderma longibrachiatum*
(3) Avizyme	*Trichoderma longibrachiatum*
(4) Natugrain	*Trichoderma longibrachiatum*
(5) Allzyme	*Aspergillus niger*
(6) Grindazyme	*Aspergillus niger*
(7) Roxazyme	*Trichoderma viride*
	Trichoderma longibrachiatum
(8) Biofeed	*Humicola insolens*
(9) Maxigrain	*Aspergillus oryzae*

Source: Bio-ingredient Ltd (2000) (Unpublished data)

Protein digestibility and NDF digestibility of the diet containing palm kernel meal increased by about 6-8% and 12-13% respectively when a mannan degrading enzyme was added. The mechanism of how the enzymes increase the digestibility of nutrients in palm kernel meal is unclear.

2.13. Mechanism of action of enzymes

Enzyme action involves the formation of a complex between the enzymes and the substrate to be acted upon. The complex then undergoes breakdown yielding the products and the unchanged enzyme. The complexes are formed between the substrate or substrates and relatively few active centres on the enzyme (McDonald *et al.,* 1983). It may be due partly through the mechanism of decreasing digesta viscosity (Dingle, 1995). Sundu *et al.* (2005a) reported that using mannan degrading enzymes decrease jejunal digesta viscosity of birds fed palm kernel meal diet by 3-4%. When mannan degrading enzymes were used in combination with a mixed enzyme preparation (Maxigrain®) a greater reduction of about 27%, was found in jejunal digesta viscosity.

Since the use of mannan degrading enzymes increased digestibility of dietary fibre (NDF), protein and lipid, this may indicate that protein and lipid are located inside cell walls that contain mannan. So when mannan is broken down by exogenous enzymes, the access of endogenous protease and lipase to attack protein and lipid is enhanced. When a combination of enzymes containing mannan degrading enzymes and a multi-enzyme preparation (Allzyme SSF) was used, dry matter digestibility and NDF digestibility were further increased. The increase in nutrient

digestibilities probably helped to increase the apparent metabolizable energy of a palm kernel meal diet supplemented with enzymes. Importantly, the moisture content of feces was drastically reduced by 16-23% when mannan degrading enzymes were included in palm kernel meal diets (Sundu *et al.,* 2004b). This is beneficial for coping with the problem of wet feces, one of the main issues in the poultry industry today.

2.14. Responses to enzyme supplementation by chickens

2.14.1 Broilers

One of the main reasons for supplementing wheat and barley-based poultry diets with enzymes is to increase the available energy content of the diet and increase energy digestibility (Patridge and Wyatt, 1995 and Ven der Klis *et al.,* 1995). Enzyme supplementation improves carbohydrates digestibility, reduces gut viscosity, and improves fat utilization (Almirall *et al.,* 1995). The improvements from enzyme supplementation are variable because of the variability in the NSP content of wheat. Classen *et al*, (1995), Schutte *et al*, (1995), Ven der klis *et al*, (1995) reported improvements of 5-16%, 3.1-4.5%, and 4.5-12.4%, respectively. The increase in available metabolizable energy with the use of enzymes is difficult to predict, as nutrient ratios, such as energy: protein, and other factors also play an important part in poultry-feed formulations. The importance of energy compensation in feed formulation was demonstrated in a cost-benefit study in Australia in 1991 (unpublished data). A wheat-based diet was formulated with or without enzyme supplementation, a 5% increase in AME was observed in the diets with enzyme supplementation, without compensating for the result of improved

growth and feed efficiency, the cost per kilogram broiler in the enzyme-treated group was 1.3% lower than in the wheat-control group. Compensating for the additional energy further improved production characteristics and also reduce feed cost, giving a reduction in cost per kilogram broiler of 8.8% compared with the wheat control. Partridge and Wyatt (1995) cited similar benefits when allowances were made for the improvements in energy and amino acid digestibility. The problem facing the feed formulator is estimating the correct energy allowance for wheat-based diets. Typically, a conservative 5-6% upgrading of the AME of wheat is recommended for commercial situations. This allowance effectively improves the energy value of wheat to about 13800KJ/kg in a least-cost matrix, bringing the value of a wheat-enzymes combination closer to that for maize and allowing the use of less supplementary energy. Amino acid adjustments may also be made, as enzyme supplementation also improves protein digestibility (Bedford, 1992 and Patridge and Wyatt 1995). Typically, the digestibility of amino acids should be expected to increase by 10% with added enzymes (Bedford, 1992 and Ward 1995). Responses to enzyme supplementation depend on the bird's age, which is apparently related to both the type of gut microflora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal with negative viscosity effects (Allen *et al.,* 1995; Choct *et al.,* 1995 and Vukic Vranjes and Wenk 1993).

The dry matter content of the litter of wheat-or barley-fed broilers is improved (reduced sticky droppings) by adding enzymes to their diets (Wiedmer and Volker, 1989; Jansson *et al.,* 1990 and Mohammed, 1995). The improved litter condition

reduces ammonia build up in sheds and reduces the incidence of hock burns and breast blisters. Also birds fed high-barley or high-wheat diets have been shown to have elevated intestinal weight, which negatively affects the carcass yield. This negative effect is reduced after supplementation with the appropriate enzymes (Francesch *et al*, 1989 and Jeroch and Danicke, 1993).

It has been suggested that supplementation with enzymes could increase the energy available in wheat by 6-8% and would result in growth rates similar to, and feed: gain ratios better than, those obtained with a control wheat diet. To investigate this suggestion, a study was conducted using a wheat-based diet formulated without enzymes, based on National Research Council (NRC, 1994) nutrient requirements. The same diet was reformulated to upgrade metabolizable energy by 6% and the amino acid content by 10%. This diet was fed with and without enzyme supplementation.

2.14.2 Laying hens

The responses of laying hens to enzyme-supplemented feeds are also well documented. Typically, enzymes added to layer feed appear to have little effect on egg mass but improve feed efficiency (Benabdeljelil and Arbaoui, 1994 and Vukic Vranjes and Wenk, 1993), energy utilization (Wyatt and Goodman, 1993; Vukic Vranjes and Wenk, 1993), and laying rate (Poultry International, 1996). Wyatt and Goodman (1993) reported that corn-fed layers exhibited better feed efficiency than those fed enzyme-supplemented barley-based diet.

The results of this study are shown in Table 7.

TABLE 2. 7 : Evaluation of male-broiler performance when nutrient matrix is upgraded in an enzyme-supplemented wheat-based diet

Parameters	Control	Upgraded nutrients[a]	
		Control	Enzyme
	Avg. BW (g)		
21days	750[a]	707[b]	745[a]
42days	2256	2184	2252
Avg. feed consumption (g/bird)			
0-21days	1064	1037	1054
0-42days	4303	4237	4265
Feed-gain ratio			
0-21days	1.518[b]	1.574[a]	1.513[b]
0-42days	1.941	1.973	1.922

Source: Guenter, 1994, University of Manitoba.

Note: *Avg. BW, average body weight.*
[a]Nutrient values of wheat were upgraded by 6% for nitrogen-corrected apparent metabolizable energy and by 10% for amino acids. [b]Enzyme used was Avizyme TX @ 0.2kg/t. a,b, Means within a row not followed by the same letters are significantly different (P<0.05).

The data clearly demonstrated that when the nutrient matrix for wheat is upgraded without enzyme supplementation, the performance of the birds is depressed. However, the same diet supplemented with enzymes yielded performance values equal to those of birds fed the control diet, suggesting that upgrading the nutrient content of the cereal will make the cereal cost-effective enough to enter the least-cost formulation.

Nevertheless, enzyme supplementation improved the utilization of barley diets. Increased energy utilization in laying hens appears to be due to microbial fermentation of solubilized NSPs (Vukic Vranjes and Wenk, 1993) and the subsequently higher absorption of volatile fatty acids (Choct *et al.,* 1995)

Wet litter arising from the use of barley and newly harvested wheat can result in an increased incidence of dirty egg shells and ammonia build up in poultry pens. Adding enzymes to both wheat and barley-based diets has been shown to reduce the moisture content of fecal matter in layers (Marquardt *et al.,* 1994) this means that barley can effectively be used if diets are supplemented with the appropriate enzymes.

Egg-yolk pigmentation should also be considered if maize is excluded from the diets. Wheat and barley contain very low levels of xanthophylls and if these grains are fed to layers, the yolk will be practically colorless and consumers will reject them. The diets therefore need to be supplemented with dietary carotenoids. The quantity required is well documented because of the extensive use of wheat and barley in the layer feed (Bird, 1994). Pigments are fat-soluble compounds and are therefore less efficiently absorbed in the presence of highly viscous compounds such as those found in barley-based diets (Benabdeljelil and Arbaoui, 1994 and Poultry International, 1996). Therefore, if barley and wheat-based diets are being supplemented with enzyme where yolk coloration is desired, appropriate pigments should be added.

2.15. Enzyme supplementation and improved poultry health

Morgan and Bedford (1995) reported that coccidiosis problems could be prevented by using enzymes. Birds fed a wheat-based diet with and without enzyme (glycanase) supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme supplemented group, which also had a much better lesion score.

An increase in digesta passage rate and a reduction in excreta moisture were often noted when enzymes were added to poultry diets, which may be detrimental to the life cycle of the organism. (Ravindran *et al.,* 1999)

Elevated levels of intact soluble NSPs detrimentally increased the activity of fermentative microorganisms in the small intestine. Enzymes (xylanase) supplementation largely eliminated fermentation in the small intestine and improved the performance of the birds. A reduction in the moisture content of poultry excreta is often noted when enzymes (glycanases) are included in the diet. (Kornegay, 2001)

2.16. Enzyme synergy with antibiotics

Researchers have observed a synergistic response to antibiotic and enzyme supplements in broiler feeds containing wheat (Schurz *et al.,* 1993, Broz and Perrin-Voltz*,* 1994, Allen *et al.,* 1995, Choct *et al.,* 1995, Langhout and Schutte, 1995 and Pijsel, 1996) and in those containing barley (Broz and Perrin-Voltz, 1994; Vukic Vranjes and Wenk, 1993).

Typically, weight-gain and feed conversion responses are observed for each supplement, with a degree of non-additive synergism. The importance of

established gut microflora in the digestion of fibre is greater in older birds than in younger birds, with the positive effects of enzymes in layers appearing to require active microflora to degrade the NSP solubilized by enzyme action (Choct *et al.,* 1995). The beneficial effects of enzymes in barley-fed layers can be eliminated by the addition of flavomycin, a compound that reduces the fermentative capability of gut microfloral (Vukic Vranjes and Wenk, 1993). Allen *et al.* (1995) reported that the inclusion of antibiotics in the gut of broilers not only improved production parameters, including weight gain, but also increased the viscosity of digesta. This result is the opposite of that observed when enzymes are added to the diet. These data indicated that both high and low viscosities are associated with improved nutrient utilization. Presumably, enzymes and antibiotics have different modes of action and therefore increase nutrient utilization in different pathways.

2.17. Future prospects

2.17.1 The use of NSP as energy sources

Large amounts of grain by products, such as wheat bran and rice bran, and non-conventional ingredients, such as palm kernel meal, copra meal (coconut) and sunflower meal, are available for use in poultry diets in many parts of the world, but these materials are characterized by their by-products containing large amounts of arabinoxylans and cellulose as the main NSP. The structure of both polymers is well characterized and the enzyme technology is available for a complete breakdown of these substrates. Cellulose is a straight chain 1-4 β-glucan and requires a combination of cellobiohydrolase, endoglucanase and β-glucanases. Rice bran, for instance, contains approximately 20-25% NSP, half of which is

cellulose (Saunders, 1986). Copra meal and palm kernel meal contain high levels of mannans or galactomannans, with a total NSP level reaching 70%. Of particular significance is the increased use of grains for biofuel production, which yields the so-called distillers' grains. This co-product is further classified into distillers' dry grains (DDG), the dried residue of distillers' grains, and distillers' dry grain with solubles (DDGS), the DDG with syrups added. DDG accounts for approximately 30% of dry grains for ethanol production, and it contains 25-28% protein, 8-9% fat, 5% ash and the remainder is believed to be NSP. This figure agrees well with the 42.1% NDF content reported for DDGS (Spiehs *et al*., 2002). It is estimated the world produces as much as 60 million tones of DDG each year. The nature of the NSP is not known, but it may be deduced that they would be composed mainly of cellulose and arabinoxylans. Currently DDG is used predominantly in cattle feed although in some countries it is also used in swine rations. The potential of using enzymes to DDGS for the release of metabolisable energy for monogastric animals is enormous. However, the current enzymes are not designed to degrade NSP to monomeric sugars within the food transit time of the chicken and pre-treatment is necessary in order to yield sophisticated enzymes and fine-tuning of pre-treatment procedures. All these NSP not only represent a large source of potential energy but also prebiotics with specific functions for poultry.

2.17.2 Tailoring enzymes for their secondary effects

It is possible that the digestibility of feed components can be tailored to produce end products with specific effects on the gut microflora and the immune system. Austin *et al.* (1999) reported that a single cloned endo. 1-4 xylanase produced

much the same range of oligosaccharides from different wheats fed to chickens. This means that a specific range of oligomers can be produced from a given NSP source in situ with a particular enzyme. Some of these carbohydrates may be used to stimulate the development of beneficial microflora in the gut. The gut harbours a highly evolved and complex microbial ecosystem containing a vast number of diverse populations. For example, microbes make up approximately 60g/kg of the wet weight of poultry excreta. The proper feeding of poultry should therefore consider the provision of "correct" substrates for the microflora to keep it stable. The consequences of altered rate of nutrient digestion in the gut may be manifested in the number and type of microorganisms present in the gut. Production of xylo-oligosaccharides by the use of xylanases in wheat-based diet could be one way to encourage the development of a healthy gut microflora (Vahjen *et al*., 1998). Indeed the direct benefit of enzyme supplementation for poultry health has been demonstrated. Thus, Sinlae and Choct (2000) demonstrated that broilers fed a wheat-based diet with xylanase had a negligible number of *Clostridium perfringens* compared with the control birds. A more detailed study by Bedford and Apajalahti, (2002) showed that xylanase supplementation of birds fed wheat-based diets markedly reduced the coliforms, lactic acid bacteria, enterococci and the total bacterial count in the small intestine. Another example is the use of specific carbohydrate entities to boost the immune system or reduce the load of pathogens in the gut. Manno-oligosaccharides have been reported to enhance the immune functions in poultry (Spring *et al*., 2000). Producing specific manno-oligomers from copra meal and palm kernel meal in situ is possible.

2.17.3 Deactivation of anti-nutrients in feed.

In addition to NSP, many feed ingredients contain a number of anti-nutritive factors. These include inhibitors of various digestive enzymes (protease inhibitors, trypsin inhibitors), polyphenolics (lignin and tannins), lectins, alkaloids, saponins and glucosinolate. For example, breakdown of glucosinolate in rapeseed using thioglucosidase has been tried (Lawrence *et al.,* 1995 and Huo *et al.*, 1993) demonstrated that trypsin inhibitors in soybean were completely deactivated by a protease within 80minutes in vitro. Another exciting area of development will be the use of specific strains of micro-organisms that produce a high level of enzymes as feed additives. Copper *et al.* (1995) applied such an approach to degrade an anti-nutrient in pasture species, fluoroacetate, which is a significant problem in Africa, Central America and Australia. Under a strictly controlled experimental condition, they used a strain of rumen bacterium *Butyrivbrio fibrisolvens* which was genetically engineered to produce an enzyme to degrade fluoroacetate. The approach was highly effective.

It is envisaged that in the near future enzymes that are more stable and can withstand pelletisation of feed will be developed. Enzymes with longer shelf life are also expected as researchers also look for other non-conventional sources of producing enzymes for industrial use at cheaper cost. Enzymes will play very vital roles in animal feed production and invariable human nutrition.

In Nigeria, both human and animals especially monogastrics compete for the same energy rich foods, but with the use of enzymes, more food will be made available

for human use as the inclusion rate of high fibre diet in monogastric feed is increased.

The importation of animal feed grade wheat in Nigeria has been increasing steadily. To get the best out of animal feed grade wheat there is need to use appropriate enzymes.

The consumption of a high fibre diet is known to increase faecal moisture content (Atteh, 2003). Wet litter leads to increased incidences of bacterial and protozoan diseases. The use of enzymes reduces wet faecal droppings which is beneficial in wet humid tropical environment like Nigeria.

Various low-energy raw material feed ingredients such as brewers dry grain (BDG), palm kernel meal (PKM), rice offal (RO), maize offal (MO) and other offals abound in Nigeria. The use of supplementary enzymes complexes used as feed additives will offer a practical means of enhancing their nutritive quality. It is also envisaged that in future, enzymes that are mostly derived from fungal, bacterial and at times other sources such as protozoans will be cultured and extracted locally in Nigeria, making them cheaper and readily available. Enzymes will play an indispensable role in 21st century animal production.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the study

This study was conducted at a standard poultry unit located at Kakuri Kaduna.Kaduna state.

3.2 Determination of the chemical composition of feed ingredients used.

The palm kernel meal (PKM) used in this study was obtained from Rivoc Oil Mills Port-Harcourt, Nigeria. It was processed by the solvent extraction method. Samples of these materials were taken for proximate analysis by the A.O.A.C. (1990) methods. The analysis was done to know the nutrient composition (crude protein, ether extract, dry matter, crude fibre, ash and nitrogen free extract) of PKM in order to permit its usage for poultry ration formulations.

Protein was determined by Kjedahl procedure, ether extract was determined by subjecting the sample to Petroleum ether extraction at 60-80^{o} C, using the Soxhlet extraction apparatus. Dry matter was determined by oven drying the sample at 100^{o}C over a 12-hour period. Crude fibre was determined by boiling the sample under reflux in weak sulphuric acid (0.255N H_2SO4), then in weak sodium hydroxide (0.312N NaoH) for 1 hour. The residue which consists of cellulose, lignin and mineral matter was dried and weighed. The ash content was determined by igniting a weighed sample in a Muffle furnace at 600^{o}C. The nitrogen free extract (NFE) was obtained by the

difference after the percentages of the other fractions were subtracted from 100%. Gross Energy (GE) was determined by using the Ballistic Bomb Calorimeter.

3.3 EXPERIMENT 1: Determination of the effects of enzyme (Maxigrain®) on nutrient retention/metabolizable energy

Eighty one day old broiler chicks (mix sexes) of Arbor Acres strain

Obtained from Ajanla Farms Limited Ibadan was used for this experiment. The birds were weighed at day old and brooded and fed a control diet shown in Table 3.1 during a two weeks adjustment period.

At two weeks of age, they were randomly assigned to nine groups, housed in metallic cages and fed nine isonitrogeneous experimental diets formulated to contain 23%CP. The treatments were replicated three times with three chicks per replicate.

The experimental diets were fed during a 7-day pre- fecal collection period. This was followed by a 3 day excreta collection period using the total collection procedure. The excreta collected were oven dried at a temperature of 70^{o}C for 48hours, weighed and ground prior to chemical analysis. Feed and water were supplied *ad-libitum* during the trial period which lasted for 24 days.

The diets for experiments 1, 2 and 3 were as follows:

Treatment 1 – Control diet without PKM or Enzyme

Treatment 2 – contain 10% PKM with Enzyme treatment or supplementation.

Treatment 3 – contain 20% PKM with Enzyme treatment or supplementation.

Treatment 4 – contain 30% PKM with Enzyme treatment or supplementation.

Treatment5–contain40%PKM with Enzyme treatment or supplementation

Treatment 6 – contain10% PKM without Enzyme

Treatment 7 – contain 20% PKM without Enzyme

Treatment 8 – contain 30% PKM without Enzyme

Treatment 9 – contain 40% PKM without Enzyme

The PKM was treated with 100gm per metric ton of Maxigrain® and subsequently included at 10%, 20%, 30%, 40% levels of inclusion.

The enzyme (Maxigrain®) was thoroughly mixed by mechanical means.

The compositions of the diets are as shown on Table 3.1

3.3.1 Chemical analysis

Samples of basal diet, test diets and excreta were analyzed for moisture, ether extract and crude fibre, using the method of AOAC (1990).

The apparent metabolisable energy (AME), true metabolisable energy (TME) and nitrogen corrected metabolisable energy (MEn) of each diet were calculated using the following equations.

AME = GE_f x Quantity of feed consumed – GE_{fa} x Quantity of fecal output.

TME = GE_f x Quantity of feed consumed – GE_{fa} x Quantity of excreta of fed bird – GE_{fa} x Quantity of excreta of fasted birds

MEn = GE_f x Quantity of feed consumed – GE_{fa} x Quantity of fecal output X (Nitrogen retained).

Where GE_f = Gross Energy of feed in Kcal/kg

GE_{fa} = Gross Energy of feces in Kcal/kg

Table 3.1: Percentage composition of starter diets

Ingredient	Control 1	10% 2	20% 3	30% 4	40% 5
Maize	55.00	47.50	40.00	33.00	25.00
Soyabean Meal	40.00	37.50	35.00	32.00	30.00
*PKM	0.00	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.60	2.60	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100
Cost/kg diet(₦)	43.00	40.00	38.00	35.00	33.00
Calculated analysis of starter diets					
MetabolizableEnergy(Kcal/kg)	2800	2757	2657	2561	2456
Crude Protein(%)	23.00	23.00	23.00	23.00	23.00
Crude Fibre(%)	4.00	5.00	5.40	6.00	7.00
Ether Extract(%)	3.50	3.80	4.00	4.20	4.50
Lysine (%)	1.35	1.33	1.30	1.30	1.30
Methionine(%)	0.44	0.45	0.46	0.48	0.50
Calcium (%)	1.50	1.50	1.50	1.50	1.50
Available phosphorus(%)	0.70	0.70	0.70	0.70	0.70

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U Vit D_3 2500 I.U Vit E 50mgL Vit K_3 2.5mg; Vit B_1, 3.0mg: Vit B_2 6.0mg; Vit B_6 6.0mg: Niacin 40.0mg; calcium pantothenate 10.0mg; Biotin 0.80mg; Vit B_{12} 0.25mg; Folic acid 1.0mg; Chloline chloride 300mg; manganese 100mg: Iron 50mg; Zinc 45; copper 2.0mg; cobalt 0.25mg; iodine 1.55, selenium 0.1mg.

*The PKM based diets consist of 10, 20, 30 and 40% with Maxigrain® and 10, 20, 30 and 40% without Maxigrain®

Nutrient retention (NR) was determined for crude protein, fat, crude fibre, ash and NFE, using the equation.

$$N.R = \frac{\text{Nutrient Intake} - \text{Nutrient output}}{\text{Nutrient Intake}} \times 100$$

Where: - Nutrient Intake (g) = Dry feed intake x Nutrient in diet

Nutrient Output (g) = Dry faecal output x Nutrient in faeces.

3.4. EXPERIMENT 2: Determination of treated PKM with or without Maxigrain® on broiler performance

3.4.1 Starter phase (0-4Weeks).

Four hundred and five (405) day old chickens (mixed sexes) of Arbor Acres strain obtained from Ajanla Farms Ltd. Ibadan were used for this study. The birds were weighed at day old and randomly assigned to nine isonitrogeneous dietary treatments. The birds were housed on deep litter system and fed dietary levels of PKM with and without Maxigrain® in a completely randomized design (CRD). The treatments were replicated three times with fifteen chicks per replicate. The chicks were brooded conventionally using kerosene stoves and electric bulbs as sources of heat and light, throughout the brooding phase.

The dietary treatments for this experiment were as in experiment 1. The composition of the starter diet is as shown on Table 3.1. The proximate chemical analysis of the formulated starter diet is presented in Table 3.2

Feed and water were supplied *ad-libitum*. The vaccination schedule was strictly adhered to. The first and second gumboro vaccination was given at 9th and 16th

day of age respectively, while Newcastle lasota vaccination was administered on the 23rd day.

Mortality was recorded as it occurred. The birds were weighed on weekly basis while average daily gain, feed intake, water intake, feed: gain ratio and feed cost (₦/kg gain) were calculated.

3.4.2 Finisher phase (5-8weeks).

At the end of the broiler starter phase, the birds were fed on broiler finisher diet at the beginning of the fifth week. There were nine (9) dietary treatments, the compositions of which are shown in Table 3.3. The proximate analysis of the formulated finisher diet is presented in Table 3.4. The diets were isonitrogeneous and formulated to contain 20% crude protein with each treatment replicated three times.

Feed and water were supplied *ad-libitum* during the experimental period. Mortality was recorded as it occurred. The birds were weighed on weekly basis while average daily gain, feed intake, water intake, feed: gain ratio and feed cost (₦/kg gain) were calculated.

3.4.3 Carcass evaluation

At the end of eighth weeks of the feeding trial, two birds from each replicate were randomly selected and weighed. The selected birds were slaughtered by severing the structures of the neck with a sharp knife, bled, defeathered and eviscerated. The breast, neck, thigh, drumstick, wings, back, head, heart, liver, lungs, kidney, gizzard, abdominal fat, pancreas, intestines and spleen were removed and weighed individually and expressed as a percentage of the dressed live weight.

Table 3.3: Percentage composition of finisher diets

Ingredient	Control 1	10% 2	20% 3	30% 4	40% 5
Maize	65.00	55.00	50.00	40.00	32.00
Soyabean Meal	30.00	30.00	25.00	25.00	23.00
*PKM	-	10.00	20.00	30.00	40.00
Bone meal	2.60	2.60	2.60	2.60	2.60
Limestone	1.60	1.60	1.60	1.60	1.60
Salt	0.35	0.35	0.35	0.35	0.35
**Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Cost/kg diet(₦)	40.00	38.5	35.00	33.00	31.00
Calculated analysis					
ME Kcal/kg	3000	2850	2758	2635	2528
Crude Protein (%)	20.00	20.00	20.00	20.00	20.00
Crude Fibre (%)	3.00	4.00	5.00	6.00	7.00
Ether Extract (%)	3.70	3.90	4.10	4.30	4.50
Lysine (%)	1.10	1.14	1.00	1.00	1.00
Methionine (%)	0.39	0.42	0.42	0.40	0.45
Calcium (%)	1.50	1.50	1.50	1.50	1.50
AVailable Phosphorus (%)	0.60	0.60	0.60	0.60	0.60

**Bio-mix premix supplied per kg of diet: Vitamin A 12500 I.U: Vit D_3 2000 I.U: Vit E 30mg; Vit K_3 3.75mg; Vit B_1, 2.5mg: Vit B_2 5.0mg; Vit B_6 3.75mg: Niacin 30mg; Pantothenate acid 12.5mg; Biotin 0.50mg; Vit B_{12} 25mg; Folic acid 0.750mg; Chloline chloride 375mg; Manganese 150mg: Iron
25mg; Zinc 37.5; copper 2.0mg; Iodine 1.0mg; cobalt 0.200mg; selenium 0.100mg; Growth promotant 20mg; Antioxidant 100mg.
*The PKM based diets consist of 10, 20, 30 and 40% with Maxigrain® and 10, 20, 30 and 40% without Maxigrain®

3.4.4 Statistical analysis

All data obtained were subjected to the analysis of variance using the model for a completely randomized design (CRD). The statistical analysis was performed by using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (2001).

Significance of difference between means were determined by applying the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

3.4.5 Hematological analysis

At the end of both the starter and finisher phase, 2ml blood samples were taken from the wing vein of three birds chosen at random from each pen totaling twenty seven birds. The blood was collected in tubes containing EDTA salt for hematological determinations. Hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), and total protein were determined using the procedures outlined by Dacie and Lewis (1977).

3.4 EXPERIMENT 3: Determination of the effects of enzyme (Maxigrain®)supplementation of PKM based diets on broiler performance

This experiment was similar to Experiment 2 in all aspects except in the method of application of the enzyme. Here the compounded diet was supplemented with Maxigrain® at 100gm per metric ton of finished feed irrespective of the PKM levels in the diet. The experimental procedures for both the starter and finisher phase was as described for Experiment 2.

3.5.1 Carcass evaluation

At the end of eighth week of the feeding trial, two birds from each replicate were randomly selected and weighed and the carcass evaluation was carried out as in Experiment 2.

3.5.2 Statistical analysis

All data obtained were subjected to the analysis of variance using the model for a completely randomized design (CRD). The statistical analysis was performed by using the General Linear Model procedure of Statistical Analysis System (SAS) computer software package (2001).

Significant differences between means were determined by applying the Duncan's Multiple Range Test (DMRT) (Duncan, 1955) as in Experiment 2.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. Results

4.2. Proximate composition of palm kernel meal and formulated diets

The proximate composition of PKM is shown in Table 4.1. The average dry matter content is 92.91%, crude protein 16.63%, crude fibre 23%, ether extract 3.5%, total ash 5.23%, nitrogen free extract 51.64%.

The proximate analysis of the PKM and fecal samples were carried out at the Grand Cereals and Oil Mills laboratory Jos, Plateau State Nigeria.

The proximate chemical analysis of the broiler starter diet is shown in Table 3.2. The diets were all isonitrogeneous at 23% as in the calculated analysis.

The proximate chemical analysis of the broiler finisher diet is shown on Table 3.4

The blood sample parameter in the broiler starter and finisher phases is shown in Table 4.2 and 4.3 respectively. The blood parameters show that the different diets did not show deviation from normal blood parameters of healthy birds.

Table 4.1: Proximate analysis of palm kernel meal

Dry matter (%)	92.91
Crude protein (%)	16.63
Crude fibre (%)	23.0
Ether extract (%)	3.5
Total ash (%)	5.23
Calcium (%)	0.25
Available phosphorus (%)	0.16
Nitrogen free extract (NFE)	51.6

4.3 Effects of enzyme (Maxigrain®) treated PKM on nutrient retention / metabolizable energy.

The effects of graded levels of PKM with or without Maxigrain® treatment on nutrient retention is presented in Table 4.4. The protein retention in the birds fed the control, 20, 30 and 40% Maxigrain® treated diets and 10 and 20% diets without Maxigrain® were similar but significantly ($P<0.001$) higher than values for 30, 40% diets without Maxigrain® and 40% with Maxigrain®. Protein retention of those fed the 10% Maxigrain® treated diet show best results compared with those on the control and other treatments with or without Maxigrain®. The superiority of protein retention by birds fed PKM treated with Maxigrain® compared to the PKM without Maxigrain® indicated that Maxigrain® improves the protein availability of PKM.

Fat retention profile was significantly ($P<0.001$) different across the dietary treatments. The values for the birds fed the control, 10 and 20% PKM with Maxigrain® were significantly ($P<0.001$) higher than those on all other diets. Fat retention in the Maxigrain® treated diets were similar in 10, 20 and 30% PKM levels followed by a decline in 40% PKM level. The fat retention values were similar in the birds fed 40% PKM with Maxigrain® and 10, 20, 30 and 40% PKM without Maxigrain®.

The crude fibre retention in the birds fed the control diet was significantly ($P<0.05$) lower than all other diets with or without Maxigrain®. The crude fibre retention increased with increasing levels of PKM inclusion with or without enzyme.

The ash retention was significantly ($P<0.001$) higher in birds fed the 10% PKM with Maxigrain® diets compared to those on all other treatments. The values in the

birds fed the control, 20 and 30% PKM with Maxigrain® and 10 and 20% PKM without Maxigrain® were similar but significantly ($P<0.001$) higher than values for those fed the 40% PKM with Maxigrain® and 30 and 40% PKM without Maxigrain®.

The NFE retention in the birds fed the control, 10, 20 and 30% PKM with Maxigrain® and 10% PKM without Maxigrain® were significantly ($P<0.001$) higher than birds fed on all other treatments. Similarly, the NFE retention values for all levels of PKM with Maxigrain® diets were significantly ($P<0.001$) higher than the values for the corresponding levels of PKM without Maxigrain® diets except at 40% with and without Maxigrain® where the values were similar.

The effects of PKM with or without Maxigrain® treatment on metabolizable energy is presented in Table 4.5. The values for the apparent metabolizable energy and nitrogen corrected metabolizable energy in the birds fed the control and 10% PKM with Maxigrain® diets were significantly ($P<0.001$) higher than those fed on all other diets. Similarly, the apparent metabolizable energy values for all levels of PKM with Maxigrain® were significantly ($P<0.001$) higher than the values for corresponding levels of PKM without Maxigrain® except at 40% with and without Maxigrain® where the values were similar. The true metabolizable energy (True ME) values in the birds fed the control and all those fed on PKM with Maxigrain® diets were significantly ($P<0.001$) higher than those fed on PKM diets without Maxigrain® except at 40% with or without Maxigrain® where the values were similar.

4.4 Effects of Maxigrain® treated PKM on broiler performance

The effect of PKM with and without Maxigrain® on the performance of broiler starter chicks (0-4weeks) is presented in Table 4.6. The final body weight and average daily gain between control and 10, 20% Maxigrain® treated PKM and 10% diet without Maxigrain® were similar but significantly ($P<0.001$) higher than those on other diets without Maxigrain® treated PKM.

The feed intake of birds fed the control, 10 and 20% diets with Maxigrain® were similar to those fed the 10 and 20% diets without Maxigrain®. The feed intake of birds fed on 30% PKM inclusion without Maxigrain® treatment was significantly ($P<0.001$) higher than those fed 30% diet with Maxigrain®. The relative consumption of feed was significantly ($P<0.001$) higher among birds on diets without Maxigrain® treatment except for 40% without Maxigrain®. The feed: gain ratio was similar among control and all diets with or without Maxigrain® except 30 and 40% diets without Maxigrain®.

The feed cost/kg weight gain was similar between control and 10% diets with Maxigrain® and all other diets without Maxigrain® treatment. The least cost and hence the best diets were those fed on 30 and 40% Maxigrain® treatment.

There was no significant ($P>0.05$) difference in mortality in all the treatments with or without Maxigrain®.

The effect of the Maxigrain® treatment of PKM on the performance of broiler finisher chickens (5-8weeks) is presented in Table 4.7.

The initial weight in this phase is the same as the final weights of the starter phase. The final body weight and average daily gain of the birds on the control diet was similar to those on the 10% diets with or without Maxigrain® but significantly ($P<0.001$) superior to birds on all other diets with or without Maxigrain®. The body weights of the birds fed the Maxigrain® treated diets were significantly ($P<0.001$) higher than diets without Maxigrain® treatment at the same level of PKM inclusion except for those fed the 40% diet with or without Maxigrain® which were similar for average daily gain.

The feed intake of the birds fed the control diet was significantly ($P<0.001$) higher than those on all other diets with or without Maxigrain®. The feed intake among birds fed the Maxigrain® treated diets was relatively higher than those on diets without Maxigrain® at all levels of inclusion except at 10% where the intake was lower.

The feed: gain ratio is similar for the control group and those fed the 10% diets with or without Maxigrain®. Feed intake was similar for birds at the same level of PKM inclusion, with or without Maxigrain®. The lowest intake was at 40% with or without Maxigrain® treatment.

Feed cost/kg gain was significantly ($P<0.001$) lower at 10% diets with or without Maxigrain® treatment and the control diet. The most expensive diet was at 40% with Maxigrain® treatment.

4.4.1 Carcass characteristics

The carcass parameter of the birds is shown in Table 4.8.

The dressed weight shows that the birds on the control diet were similar to those fed 10 and 20% diets with Maxigrain® and 10% without Maxigrain® treatment. The % drumstick, wings, back, head, heart, liver, lungs, gizzard and intestinal length were not significantly ($P>0.05$) different. There was significant ($P<0.001$) difference in the % thigh, kidney, abdominal fat, pancreas, and spleen. The % breast, neck and intestines was significantly ($P<0.05$) different in diets with or without Maxigrain® but no definite trends were established.

4.5 Effects of Maxigrain® supplementation of PKM on broiler performance

The effects of Maxigrain® supplementation of PKM on the performance of broiler starter chicks (0-4weeks) is presented in Table 4.9. The final weights and average daily gain of the birds fed the 10 and 20% diets with Maxigrain® were significantly ($P<0.001$) superior to the control while those fed the 20, 30 and 40% diets without Maxigrain® were inferior to the control. The birds fed the Control diet was similar to those on the 30 and 40% diets with Maxigrain® and 10% diet without Maxigrain® in their final weights. All the birds fed the Maxigrain® supplemented diets show relatively higher weights than diets without Maxigrain® at comparative levels.

Feed intake was similar for birds on the control and 10% Maxigrain® supplemented diet but significantly ($P<0.001$) different from all those fed other diets with or without Maxigrain® inclusion. The feed in-take among Maxigrain® supplemented diets was relatively higher than diets without supplementation.

Feed: gain ratio was similar between the birds fed the control diet and all other groups on diets with or without Maxigrain® except 10, and 20% diets with Maxigrain® which were superior to the control diet. The best diet was 10% PKM with Maxigrain® supplementation with a feed: gain ratio value of 1.34. The feed: gain ratio at 30%, were similar with or without Maxigrain®.

Feed cost/kg weight gain was significantly (P<0.05) different between control (with the highest value) and all other diets with or without Maxigrain® supplementation. The feed cost/kg weight at 10, 20 and 30% with Maxigrain® was similar to 10, 20, 30 and 40% diets without Maxigrain®. The best diet in feed cost/kg weight gain was at 40% with Maxigrain® with a value of ₦50.43/kg body weight gain.

Water intake showed that the control was similar to 10% diet with Maxigrain® but significantly (P<0.001) higher than all other diets.

The water intake for birds fed the 10, 20 and 30% PKM Maxigrain® supplemented diets were significantly (P<0.001) higher than in birds fed diets without Maxigrain®. The water intake at 40% PKM inclusion was similar to diets with or without Maxigrain®.

The water: feed ratio was similar in all the birds fed on diets with or without Maxigrain® supplementation.

There was no significant (P>0.05) difference in mortality in all the birds fed the diets with or without Maxigrain® supplementation.

The effects of Maxigrain® supplementation of PKM on the performance of broiler finisher chickens (5-8weeks) is presented in Table 4.10

The initial weight of the birds in the broiler finisher phase is the same as the final weight of the birds in the broiler starter phase of this study.

The birds fed the control diet was significantly (P<0.001) different compared with all other birds fed diets with or without Maxigrain® supplementation in their final weights but inferior to the birds fed 10 and 20% diets with Maxigrain®.

Birds fed Maxigrain® supplemented diets were significantly (P<0.001) higher in their final weights when compared with the birds fed unsupplemented diets at the same levels of PKM inclusion. PKM inclusion at 10% was the best followed by 20% in the supplemented diets as measured by final weight gain of the birds while the control diet was significantly (P<0.001) superior to all the other diets with or without Maxigrain®. The feed intake by the birds on the control was the lowest and significantly (P<0.001) different from all other groups fed diets with or without Maxigrain® supplementation. Feed intake increased with increasing levels of PKM inclusion with or without Maxigrain®. Maxigrain® supplemented diets at 30 and 40% were similar when compared with the same levels without Maxigrain® supplementation.

The feed: gain ratio was similar for birds fed the control, 10 and 20% Maxigrain® supplemented diets. The feed: gain ratio increased with increasing levels of PKM with or without Maxigrain®.

The best diet was 10% with Maxigrain® at a feed per kg gain value of 2.26 when compared with all other diets with or without supplementation.

Feed cost/kg gain was observed to have similar trend as in the feed: gain ratio at all levels. The best diet was 10% PKM with Maxigrain® supplementation at a value of ₦93.46/kg weight gain similar to the control and 20% PKM diet with Maxigrain® supplementation.

There was significant (P<0.001) difference in water intake between the birds fed the control diets and all other treatments.

There was significant (P<0.001) difference at 10% PKM inclusion with Maxigrain® compared with the same level without Maxigrain®. Water intake was similar among birds fed at 20, 30 and 40% diets with Maxigrain® when compared with the same levels without Maxigrain®. There was no significant (P>0.05) difference in mortality in all the treatments with or without Maxigrain®.

Water: feed ratio did not differ significantly (P>0.05) among birds fed diets with increasing levels of PKM with or without Maxigrain® inclusion.

4.5.1 Carcass characteristics

Table 4.11 shows the results of the carcass characteristics of the birds fed graded levels of PKM with or without Maxigrain® supplementation. The results showed that there was significant (P<0.001) difference in the dressed weight of the birds on the control diet compared to all those on other diets with or without Maxigrain® supplementation.

The dressed weights were progressively lower with increasing levels of PKM with or without supplementation except at 10 and 20% with Maxigrain® where dressed weights were higher. There was no significant difference (P>0.05) in the dressing percentage of the birds fed diets with or without Maxigrain® supplementation. There was significant difference (P<0.05) in the %breast, neck, thigh, drumstick, wings, head, lungs, gizzard, pancreas, intestines, spleen and intestinal length (cm) but there was no specific trend established.

The % heart of the birds fed Maxigrain® supplemented diets were significantly ($P<0.001$) different than those fed the unsupplemented diets which were bigger than those on the control and all supplemented diets. The weight of the heart of birds on the control diet were similar to the weight of the heart of all those fed Maxigrain® supplemented diets at all levels of inclusion.

The % liver in the control was similar to 10 and 20% diets with Maxigrain® and 10% without Maxigrain®. At all levels of PKM inclusion, the liver from birds on Maxigrain® supplemented diets were significantly ($P<0.001$) lower than those in the unsupplemented diets where the size of the liver was observed to increase with increasing levels of PKM inclusion with or without Maxigrain®.

The % kidney weight were similar to the control and those fed 10, 20 and 40% PKM diets with Maxigrain® and 10% PKM without Maxigrain® but significantly ($P<0.001$) lower than all other diets.

The size of the kidney was observed to increase with increasing levels of PKM in the unsupplemented diets compared with the Maxigrain® supplemented diets.

The abdominal fat was significantly ($P<0.001$) higher for birds in the control group compared to those on other diets. Similarly, the abdominal fat in the Maxigrain® supplemented diets were significantly ($P<0.001$) lower than the unsupplemented diets at all levels of PKM inclusion.

4.6 Discussion

4.6.1 Proximate composition of palm kernel meal

Fractionation of PKM based on its proximate analysis showed that 51.6% of the dry matter was in the form of NFE. This agrees with Sue (2001) and Sundu *et al.* (2004a) who reported 49% of the dry matter is in the form of NFE. Chin (2001) reported 18% crude protein and classified PKM as a protein source of medium grade. The ether extract was 3.5% which is lower than 7% reported by Aduku (2004). The crude fibre was 23% compared with 11% reported by Aduku (2004). Chemical analysis of palm kernel meal showed that its nutrient content varies widely, depending on the oil extraction process, the species of the palm nut and the amount of shell content remaining in the meal (O'Mara *et al.,* 1999).

4.6.2 Effects of enzyme (Maxigrain®) treated PKM on nutrient retention/metabolizable energy

The effects of Maxigrain® treated PKM on nutrient retention shows that protein retention of diets treated with Maxigrain® was higher compared to diets without Maxigrain®. This could have contributed to the higher body weight gains observed among birds fed Maxigrain® treated diets with optimum at 20% PKM inclusion compared with control and other diets with and without Maxigrain®. This agrees with the findings of Ariff Omer *et al*, (1998) who reported optimum PKM inclusion rate in poultry ration at 20%.

There was significant variation in ether extract retention among all diets, which is higher in the control and Maxigrain® treated diets compared with diets without Maxigrain®. This is in agreement with Marquardt *et al.* (1996) who reported improvement in the body weight and feed conversion efficiency due to an increase

in fat and protein digestibility. Increase fat retention also increases the bioavailability of fat soluble vitamins. The observation of Swain and Johri (1999) that addition of enzyme to feed caused increase in energy but a reduction in fat retention is not in agreement with this study since retention of fat relates to vitamin storage in the body, a reduction may be detrimental to broilers. The results of experiments conducted by Langhout and Schutte, (1995) showed that the effects on chick performance and nutrient digestibility of dietary endo-xylanase in wheat and rye based diets are influenced by the type of fat in the diet.

The crude fibre retention was poorest in the control birds and those fed Maxigrain® treated diets which are thought to be due to the method of application of the enzyme. Retention of crude fibre was lower in 20 and 30% PKM Maxigrain® treated diets compared to the corresponding diets without Maxigrain®. Even at 10 and 40% PKM levels with or without Maxigrain® where crude fibre retention values were not significantly different; the values for the Maxigrain® treated diets were numerically lower. Of the NSPs in PKM, 78% is linear mannan, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans. (Dusterhoft and Voragen, 1992). Since Maxigrain® contains cellulase, β-glucanase, xylanase and phytase, these enzymes must have acted on cellulase, glucoronoxylans and arabinoxylans thereby reducing the crude fibre content and subsequently increasing the energy content retention and NFE. The NFE represent the soluble carbohydrates in the diet. The addition of enzymes to PKM is expected to ameliorate the antinutritive effect of β-mannan and also to degrade large percentage of NSPs and oligo-saccharide components of the diet. (Akpodiete *et al.*, 2006) The higher values of NFE retention in PKM diets with Maxigrain® as compared with PKM diets without Maxigrain® can be attributed to

the degradative effect of Maxigrain® on the NSPs of PKM with subsequent release of soluble carbohydrates. As reported by Akpodiete *et al*. (2006) a higher level of the enzyme may have shown better results. Choct, (1998) reported that the current enzymes are not capable of depolymerizing NSP to their simple constituents during the digesta transit time of poultry. PKM contains 78% linear mannan with very low galactose substitution, 12% cellulose, 3% glucoronoxylans and 3% arabinoxylans but Maxigrain® does not contain mannanase for specific enzymatic cleavage. (Dusterhoft and Voragen, 1992)

The effect of Maxigrain® treatment on ME of broiler chicks show increase in bioavailable energy (AME, TME and MEn) in the PKM diets treated with Maxigrain® to a maximum inclusion of 30% PKM. The TME of all the diets without Maxigrain® were lower than the calculated analysis of the respective diets. This may suggest that Maxigrain® allows the birds to utilize fibre in the diet more efficiently and subsequently extract more energy from the diets.

Atteh (2000) had earlier observed improvement in crude fibre digestion and ME of enzyme supplemented diets where 50% wheat bran replaced 50% of maize in a control diet relative to an unsupplemented wheat bran diet. The AME and MEn of the different diets and the control with or without Maxigrain® show little variation. This was supported by the work of Muztar et al., (1977) who observed a close correlation between corrected and classical ME values and therefore stated that since the amount of tissue protein which is catabolized is small relative to the amount stored by growing birds, or deposited in eggs of laying hens, the imposition of penalty of nitrogen retention is a questionable procedure. They argued that since storage of protein characterize growth, it is difficult to justify the imposition of

penalty for nitrogen retention. They concluded that application of nitrogen correction does not seem to be of major consequence when one considers the amount of time and labor in determining the nitrogen content of feed and excreta samples. Therefore the little variations observed between the AME and MEn of the different diets in this study support the earlier works that believe that the exertion of penalty of nitrogen correction of energy was not necessary.

4.6.3 Effects of Maxigrain® treated PKM on broiler performance

The performance of broiler starter (0-4weeks) chicks fed PKM diets with or without Maxigrain® revealed that at 10% PKM both diets were similar to the control diet as indicated by their final weight and average daily gain. However, there was a downward trend with increasing levels of PKM with or without treatment. This agrees with the reports of (Ariff Omer *et al.,* 1998 and McDonald *et al*., 1995) who observed that lower weights of the birds fed PKM diets could be attributed to the high content of fibre that may have resulted from the diets which increases with increased percentage inclusion of PKM. This may have led to dilution of the energy concentration of the diets and reduced nutrient availability to the birds. In addition, the higher the PKM level the higher the expected concentration of β- mannan which is reported to cause growth depression and reduced feed conversion ratio (Dienick, 1989 unpublished data and Ariff Omer *et al.,* 1998). The feed intake increased with increasing level of PKM with or without Maxigrain® treatment. This was also confirmed by the higher value of feed: gain ratio obtained as the level of PKM increased with or without Maxigrain®. Timbrell (1992) had explained that there could be instances where enzyme treatment or supplementation adds little

value to particular ingredients or even fails to result in improved performance. It may be an indication that the quantity of the enzyme was not enough as was probably the case in this study. The economic analysis show that the cost of the diets decreased with higher level of inclusion of PKM with or without treatment. However, this was more apparent in the Maxigrain® treated diets which agrees with the report of Akpodiete *et al.*, (2006).

The performance of broiler finisher (5-8weeks) chickens fed PKM diets with or without Maxigrain® treatment were similar to the results obtained in the starter phase in their final weight, average daily gain and feed intake trends. The feed: gain ratio, and the feed cost/kg weight gain (₦/kg) were best at 10% PKM diets with or without enzyme treatment which were similar to the control diet. This indicated that the effect of the enzyme treatment on the performance of the birds was minimal as observed also by Timbrell (1992).

The overall mortality for the period was minimal and their occurrence was not attributed to the dietary effects since the losses occurred in both the control and PKM diets with or without Maxigrain® treatment.

The carcass characteristics did not show any particular trends in the control birds or those fed PKM diets with or without Maxigrain® inclusion.

4.6.4 Hematological study

Packed cell volume (PCV), hemoglobin (Hb) content and total protein (Tp) are important blood parameters used to assess the health status of animals. It has been established that reduction in the levels of nutrients in feeds result in decrease in PCV, Hb and Tp of animals (Oladele and Ayo, 1999). The aim of estimating the

hemoglobin content is to determine the oxygen carrying capacity of the birds circulatory system (Mmereole, 1996). Birds with low Hb can be said to have low oxygen carrying capacity and can easily succumb to any form of respiratory disease while birds with high Hb concentration can be regarded as having high oxygen carrying capacity and such birds are likely to withstand some levels of respiratory stress (Salisbury, 1983). The blood parameters did not show any pattern or variation from established ranges and therefore bird health was not affected irrespective of the diets fed with or without Maxigrain®.

4.6.5 Effects of Maxigrain® supplementation of PKM on broiler performance

The performance of broiler starter (0-4weeks) chicks with or without Maxigrain® supplementation showed increase in final weight gain, improved feed: gain ratio in the control and Maxigrain® supplemented diets relative to diets without Maxigrain®. This is expected as Choct, (2006) reported the degradation of β - mannan and 70% NSPs into soluble metabolizable products for monogastrics when enzymes are added to high fibre monogastric diets. Feed and water intake were slightly higher in birds fed up to an optimum of 30% PKM in the Maxigrain® supplemented diets compared to those fed diets without Maxigrain®. Onwudike, (1986), Ezieshi and Olomu (2004) reported higher feed intake of birds fed a PKM based diet compared with a maize – based diet due probably to its faster passage rate in the digestive tract. Feed: gain ratio was better among Maxigrain® supplemented diets compared with the control and all other diets without Maxigrain®. This is similar to the report of Atteh (2000) who observed an

improvement in weight gain and feed conversion efficiency in birds fed enzyme supplemented diets. McDonald *et al.* (1995) and Ariff Omer *et al.* (1998) and observed lower weights of birds fed increasing levels of PKM without enzyme supplementation.

The performance of broiler finisher (5-8weeks) chicks with or without Maxigrain® indicated that the birds in the control and Maxigrain® supplemented diets had a higher final weight gain, improved feed: gain ratio compared to diets without Maxigrain®. Feed and water intake increased with increasing inclusion of PKM probably due to the critical need for energy in finisher chickens but this did not translate into higher live weight or weight gain especially among diets without supplementation. Feed: gain ratio and feed cost/kg weight gain was best at 10% diet with supplementation similar to the control diet. This is supported by the report of Classen *et al*. (1995) and Scott *et al.* (1998) who reported that the responses to the use of enzymes is highest on poor quality raw materials.

Panigrahi and Powell, (1991) found that the inclusion of PKM up to 50% was tolerated provided that the birds were kept above 7 weeks of age.

The water intake of birds fed PKM based diets also increased (Panigrahi and Powell, 1991) and there was increased moisture content of excreta (Onifade and Babatunde, 1998).

The economic analysis revealed a comparative feed cost/kg weight (₦/kg) similar to the control diet in the Maxigrain® supplemented diets up to 20% inclusion of PKM with the best result of ₦93.46 per kg weight gain recorded at 10% PKM inclusion with Maxigrain® supplementation. This was expected as the cost per metric ton of maize was widely higher than PKM at about ₦40.000 to ₦8.000 for

maize and PKM respectively. Akpodiete *et al*. (2006) reported that the cost of a kilogram feed reduced in cost with increasing PKM inclusion with the lowest cost at the highest PKM replacement level of 40%.

The carcass evaluation of broiler chickens fed diets with or without Maxigrain® supplementation showed similar trend as that of the growth performance. The birds fed on Maxigrain® supplemented diets had higher pre-slaughter weight and carcass weight, compared to those on similar diets without Maxigrain®. There was no significant variation in the dressing percentage of the birds with or without Maxigrain®.

There was significant variation in the % breast, neck, thigh, drum stick, wings, head, gizzard, and intestinal length (cm) but no trend was established. The lungs, liver, kidneys, pancreas and intestines were within the normal ranges reported for chickens suggesting the absence of toxic effects by the treatments.

However, a trend was observed in the % heart, liver, kidney and abdominal fat. The heart appears to increase in weight for birds fed diets without Maxigrain® supplementation probably due to increased function. This agrees with Brenes *et al*., (1993), who reported that enzyme supplementation affects the relative size of some organs with relative reductions in the weights of such organs. Similar trend was observed with the liver as reported by Fasina *et al*., (2004) and Odunsi *et al*., (2006). Atteh, (2004) also reported reduced liver weight with enzyme supplementation. The increased fat deposit observed in the control diet and other diets could be attributed to the higher bioavailable ME levels which could be converted to fat in the Control and Maxigrain® supplemented PKM diets relative to the diets without Maxigrain® as reported also by Akpodiete *et al*. (2006)

CHAPTER FIVE

5.0 SUMMARY AND CONCLUSION

The high cost of maize including other factors such as competition between man and animals has contributed immensely to the current high cost of poultry feeds. Feed millers and farmers will appreciate any ingredient that is cheap and yet enhance and sustain production. If Maxigrain® supplementation enhances the utilization of cheap high fibre PKM; there will be remarkable decrease in the cost of poultry feeds to the benefit of Poultry farmers. The increase in price and the concern for continued availability of conventional feedstuff in livestock feeding led to this research to establish the use of palm kernel meal in feeding of broiler chickens.

The first experiment was conducted using palm kernel meal treated with or without Maxigrain® to determine the effect on nutrient retention and metabolizable energy. The trial which lasted 21 days used 81 birds fed nine isonitrogeneous diets in which palm kernel meal was pre-treated prior to its inclusion in the formulation.

The second experiment was conducted using four hundred and five 405) broilers chicks divided into broiler starter (0-4 weeks) and broiler finisher (5-8 weeks). They were fed nine isonitrogeneous diets as in experiment 1.

The third experiment was conducted using four hundred and five (405) broiler chicks similar to Experiment 2 except in the method of enzyme application where the complete diets were supplemented with Maxigrain®.

The following observations were made from the results of these studies:

1. The apparent metabolizable energy (AME), true metabolizable energy(TME), and nitrogen corrected metabolizable energy (MEn) of the Maxigrain® treated PKM diets were higher than PKM diets without Maxigrain®. Maxigrain® (a cocktail of exogenous enzymes)

treatment of PKM improved nutrient retention on a relative bases. However, to maximize the benefit of enzyme supplementation of PKM, a more specific cocktail of enzyme containing mannanase should be considered. As at the time of this study, such an enzyme has not been commercially introduced into the market.

2. The improvement in protein, fat and NFE retention were observed to be more than crude fibre retention due probably to non inclusion of a mannanase degrading enzyme in Maxigrain®

3. The PKM pre-treated diets were not better than the control diet at all levels of inclusion (10, 20, 30, and 40%) in both the broiler starter and finisher phases.

4. When Maxigrain® was applied by the supplementation method, the maximum impact on broiler performance was observed to be between 10 and 20% PKM diets which were both better than the control diet in the Starter and Finisher phases.

5. PKM at all levels (10, 20, 30 and 40%) with or without Maxigrain® and irrespective of the method of application did not result in any deleterious effect on overall broiler performance. Broiler performance attained an acceptable average weight of 1.5kg at 8 weeks of age with or without Maxigrain®.

6. The carcass analysis did not show any increase in the parts of the breast, thigh and drumstick with or without Maxigrain® and irrespective of the method of application.

7. The economic analysis revealed a downward reduction in cost of feed (₦/kg) with increased inclusion level of PKM at the broiler starter phase. The best result was between 10 and 20% PKM diets supplemented with Maxigrain® at 8 weeks of age.

8. The health of the birds was not affected and mortality was not significant throughout the study.

This study should encourage farmers and the poultry industry in palm kernel meal producing countries like Nigeria to use more of this ingredient in commercial rations due to its cost effectiveness and possibility of reducing environmental problems through increased usage and minimize waste disposal.

5.1 RECOMMENDATIONS

The following recommendations are made from these studies:

1. Palm kernel meal is beneficial for inclusion in the diets of broilers. Its inclusion up to 40% with or without enzyme (Maxigrain®) did not have any negative effect on the growth performance of broilers.

   However, palm kernel meal inclusion up to a maximum of 20% showed best results when Maxigrain® was applied by supplementation.
2. Feed digestibility and feed efficiency were improved when Maxigrain® was added to broiler diets resulting in higher weight gains.
3. There is an economic advantage of Maxigrain® supplementation of palm kernel meal in broiler diets with lower feed cost per kg weight gain and therefore; its usage should be encouraged.

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TABLE 4.4 Effects of treated PKM with or without Maxigrain® on nutrient retention of broiler chicks.

Parameters	Control	PKM with Maxigrain® Diets				PKM without Maxigrain® Diets				SEM	Level of Significance
		10+	20+	30+	40+	10-	20-	30-	40-		
Protein%	**54.33^{bc}**	**64.67^{a}**	**58.39^{ab}**	**51.65^{bcd}**	**47.28^{cde}**	**50.94^{bcd}**	**47.00^{cde}**	**44.64^{de}**	**42.65^{e}**	**1.41**	***
Ether extract%	**79.94^{a}**	**78.33^{ab}**	**71.91^{bc}**	**70.80^{cd}**	**62.39^{ef}**	**59.64^{f}**	**63.93^{def}**	**66.33^{cdef}**	**68.67^{cde}**	**1.34**	***
Crude fibre%	**17.16^{a}**	**25.99^{b}**	**28.33^{b}**	**48.00^{c}**	**54.42^{d}**	**28.00^{b}**	**48.67^{c}**	**54.32^{d}**	**56.33^{d}**	**0.90**	*
Ash%	**33.00^{b}**	**42.00^{a}**	**35.33^{b}**	**31.67^{b}**	**26.00^{c}**	**34.33^{b}**	**33.00^{b}**	**26.00^{c}**	**21.67^{d}**	**0.78**	***
N.F.E%	**71.00^{ab}**	**75.67^{a}**	**74.67^{a}**	**66.00^{bc}**	**52.00^{ef}**	**68.67^{b}**	**61.00^{cd}**	**55.33^{de}**	**49.00^{f}**	**1.10**	***

abcdef Means with different superscript within the same row are significantly (P<0.001) different.

*= (P<0.05), *** = (P<0.001)

TABLE 4.5: Effects of treated PKM with or without Maxigrain® on metabolizable energy of broiler chicks

		PKM with Maxigrain® Diets				PKM without Maxigrain® Diets					
Parameters	Control	10+	20+	30+	40+	10-	20-	30-	40-	SEM	Level of Significance
Apparent ME	**3496.70^{a}**	**3407.00^{a}**	**3017.00^{b}**	**2724.70^{bc}**	**1742.00^{de}**	**2839.00^{b}**	**2439.33^{c}**	**2035.00^{d}**	**1641.70^{e}**	**71.63**	***
ME n	**3498.70^{a}**	**3409.00^{a}**	**3019.00^{b}**	**2725.70^{bc}**	**1744.00^{de}**	**2841.00^{b}**	**2441.30^{c}**	**2037.00^{d}**	**1643.70^{e}**	**71.63**	***
True ME	**3402.70^{a}**	**3315.30^{ab}**	**2922.00^{bc}**	**2625.30^{cd}**	**1640.30^{f}**	**2593.70^{cd}**	**2276.00^{de}**	**1867.30^{ef}**	**1525.00^{f}**	**79.83**	***

abcdef Means with different superscript within the same row are significantly (P<0.001) different.

*** = (P<0.001)

Apparent ME = Apparent Metabolizable Energy

MEn = Nitrogen Corrected Metabolizable Energy

True ME = True Metabolizable Energy

TABLE 4.6: Performance of broiler starter (0 - 4weeks) chicks fed treated PKM diets with or without Maxigrain®

		PKM with Maxigrain® diets				PKM without Maxigrain® diets					
Parameters	**Control**	**10+**	**20+**	**30+**	**40+**	**10-**	**20-**	**30-**	**40-**	**SEM**	**Level of significance**
Initial weight(g)	42.13	42.13	42.13	42.13	42.13	42.13	42.13	42.13	42.13		NS
Final weight(g)	1390.66^{a}	1349.33^{a}	1305.00^{abc}	1216.00^{cde}	1174.66^{de}	1327.66^{ab}	1238.33^{bcd}	1123.66^{e}	991.00^{f}	18.03	***
Weight gain(g)	1348.66^{a}	1307.33^{a}	1263.00^{abc}	1174.00^{cde}	1132.66^{de}	1285.66^{ab}	1196.33^{bcd}	1081.66^{e}	949.00^{f}	18.03	***
Average daily gain(g)	38.53^{a}	37.35^{a}	36.08^{abc}	33.54^{cde}	32.35^{de}	36.73^{ab}	34.18^{bcd}	30.90^{e}	27.11^{f}	0.51	***
Feed intake (g)	1740.66^{abc}	1725.66^{abc}	1797.66^{a}	1580.00^{d}	1499.33^{de}	1772.66^{ab}	1693.66^{bc}	1671.00^{c}	1474.00^{e}	15.83	***
Feed: Gain Ratio	1.30^{b}	1.33^{b}	1.40^{b}	1.36^{b}	1.33^{b}	1.36^{b}	1.43^{ab}	1.56^{a}	1.56^{a}	0.03	***
Feed cost/kg wt gain(₦)	55.52^{a}	53.23^{a}	54.89^{a}	48.30^{bc}	45.29^{c}	55.17^{a}	53.97^{a}	54.15^{a}	51.50^{ab}	0.9	***
Mortality	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.09	NS

abcdef Means with different superscript within the same row are significantly (P<0.001) different

*** = (P<0.001)

TABLE 4.7: Performance of broiler finisher (5-8weeks) chickens fed treated PKM diet with or without Maxigrain®

Parameters	Control	PKM with Maxigrain® diets				PKM without Maxigrain® diets				SEM	Levels of Significance
		10+	20+	30+	40+	10-	20-	30-	40-		
Initial weight(g)	1390.66^{a}	1349.33^{a}	1305.00^{abc}	1216.00^{cde}	1174.66^{de}	1327.66^{ab}	1238.33^{bcd}	1123.66^{e}	991.00^{f}	18.03	***
Final weight(g)	3040.00^{a}	2953.33^{a}	2633.33^{b}	2323.33^{c}	2073.33^{d}	2933.33^{a}	2430.00^{c}	2113.33^{d}	1900.00^{e}	24.89	***
Weight gain(g)	1649.33^{a}	1604.00^{a}	1328.33^{b}	1107.33^{c}	898.66^{d}	1605.66^{a}	1191.66^{c}	989.66^{d}	909.00^{d}	22.72	***
Average daily gain(g)	78.54^{a}	76.38^{a}	63.25^{b}	52.73^{c}	42.79^{d}	76.45^{a}	56.74^{c}	47.12^{d}	43.28^{d}	1.08	***
Feed in take (g)	4583.33^{a}	4321.66^{cd}	4487.33^{ab}	4321.00^{cd}	4139.00^{ef}	4397.00^{bc}	4306.00^{cd}	4230.00^{de}	4029.33^{f}	22.54	***
Feed: Gain Ratio	2.76^{e}	2.70^{e}	3.36^{d}	3.93^{bc}	4.63^{a}	2.73^{e}	3.60^{cd}	4.30^{ab}	4.43^{a}	0.08	***
Feed cost/kg wt gain (₦)	111.42^{ef}	104.69^{f}	120.21^{de}	132.48^{bcd}	148.31^{a}	105.47^{f}	126.79^{cd}	141.37^{ab}	137.72^{abc}	2.52	***
Mortality (%)	0.33	0.00	0.00	0.00	0.33	0.00	0.33	0.33	0.33	0.14	NS

abcdef Means with different superscript within the same row are significantly (P<0.001)different

*** = (P<0.001)

TABE 4.8: Carcass characteristics of broiler chickens fed on treated PKM diets with or without Maxigrain® (expressed as percentage of live weight)

Parameters	Control	PKM with Maxigrain® diets				PKM without Maxigrain® diets				SEM	Level of Significance
		10+	20+	30+	40+	10-	20-	30-	40-		
Live Wt (g)	2903^{a}	2621^{b}	2496^{bc}	2335^{cd}	2073^{ef}	2466^{bc}	2211de	2151^{edf}	1948^{f}	31.88	***
Dress Wt (g)	2050^{a}	1991^{a}	1950^{ab}	1633^{c}	1558^{cd}	1941^{ab}	1716^{bc}	1641^{c}	1366^{d}	33.32	***
Dressing (%)	71	76	78	70	75	79	78	76	70		NS
Breast	31^{a}	26^{b}	27^{ab}	28^{ab}	29^{ab}	28^{ab}	27^{ab}	29^{ab}	30^{ab}	12.26	*
Neck	7^{ab}	8^{a}	5^{b}	7^{ab}	6^{ab}	7^{ab}	5^{b}	7^{ab}	6^{ab}	5.18	*
Thigh	30	27	28	29	27	28	27	26	30	11.65	NS
Drum stick	15	14	13	15	14	13	14	13	15	7.33	NS
Wings	11	10	11	12	11	11	11	11	12	5.54	NS
Back	15	17	15	15	16	16	15	15	16	8.67	NS
Head	4	3	3	4	2	3	2	2	4	3.66	NS
Heart	0.47	0.49	0.49	0.51	0.44	0.43	0.57	0.43	0.55	0.23	NS
Liver	2.9	1.9	2.0	1.9	2.1	2.1	2.1	1.4	3.3	3.08	NS
Lungs	0.7	0.6	0.5	0.7	0.5	0.5	0.6	0.4	0.7	0.42	NS
Kidney	0.5^{a}	0.5^{a}	0.4^{abc}	0.4^{ab}	0.3^{abcd}	0.3^{cd}	0.5^{a}	0.3^{bcd}	0.2^{d}	0.43	***
Gizzard	3.0	3.0	2.0	5.0	4.0	4.0	3.0	4.0	4.0	3.19	NS
Abdominal Fat	1.6^{a}	0.8^{cd}	1.0^{bc}	0.6^{d}	0.9^{c}	0.9^{c}	1.3^{bc}	0.7^{cd}	0.5^{d}	0.71	***
Pancreas	0.4^{bc}	0.7^{a}	0.3^{c}	0.3^{c}	0.5^{b}	0.4^{bc}	0.7^{a}	0.4^{bc}	0.3^{c}	0.38	***
Intestines	6^{a}	5^{abc}	4^{bc}	5^{ab}	5^{abc}	4^{abc}	3^{c}	4^{abc}	6^{a}	3.4	*
Spleen	0.17^{bcd}	0.2^{ab}	0.17^{bcd}	0.21^{a}	0.17^{bcd}	0.13^{d}	0.18^{bc}	0.18^{bcd}	0.16^{cd}	0.1	***
Intestinal Length (cm)	301	267	269	281	270	272	260	269	282	4.69	NS

abcdef Means with different superscript within the same row are significantly (P<0.001) different

* = P<0.05 *** = (P<0.001)

TABLE 4.9: Performance of broiler starter (0 - 4weeks) chicks fed on PKM diets supplemented with or without Maxigrain®

Parameters	Control	P K M With Maxigrain® Diets 10+ 20+ 30+ 40+	P K M without Maxigrain® diets 10- 20- 30- 40-	SEM	Level of Significance
Initial weight(g)	40.00	40.00 40.00 40.00 40.00	40.00 40.00 40.00 40.00	-	NS
Final weight(g)	916.33^{c}	1034.67^{a} 978.00^{b} 895.67^{c} 878.33^{c}	894.00^{c} 826.00^{d} 748.33^{e} 745.67^{e}	8.30	***
Weight gain(g)	876.33^{c}	994.67^{a} 938.00^{b} 855.67^{c} 838.33^{c}	854.00^{c} 786.00^{d} 708.33^{e} 705.67^{e}	8.30	***
Ave daily gain(g)	31.30^{c}	35.52^{a} 33.50^{b} 30.56^{c} 29.94^{c}	30.50^{c} 28.07^{d} 25.30^{e} 25.20^{e}	0.29	***
Feed intake (g)	1363.67^{a}	1332.67^{ab} 1311.33^{b} 1243.00^{c} 1181.00^{d}	1248.00^{c} 1198.00^{d} 1171.00^{d} 1168.00^{d}	8.47	***
Feed:Gain Ratio	1.56^{de}	1.34^{a} 1.40^{bc} 1.45^{bcde} 1.41^{bcd}	1.46^{cde} 1.53^{de} 1.66^{e} 1.6^{e}	0.02	***
Feed cost/kg wt (₦)	66.92^{c}	57.35^{b} 57.06^{b} 54.92^{b} 50.43^{a}	58.46^{b} 58.02^{b} 57.94^{b} 54.77^{b}	0.75	*
Water intake (ml)	3206.33^{a}	3209.33^{a} 3086.33^{b} 2952.00^{c} 2787.33^{de}	2834.33^{d} 2828.33^{d} 2756.00^{e} 2756.67^{e}	10.57	***
Water:Feed Ratio	2.36	2.41 2.36 2.38 2.36	2.28 2.36 2.36 2.36		

Mortality	**0.33**	**0.00**	**0.00**	**0.00**	**0.33**	**0.00**	**0.00**	**0.33**	**0.33**	**0.12**	**NS**

abcdef Means with different superscript within the same row are significantly (P<0.001)different
* = (P<0.05) *** = (P<0.001)
NS: No significant difference (P>0.05).

TABLE 4.10: Performance of broiler finisher (5-8weeks) chickens fed on PKM diets supplemented with or without Maxigrain®

Parameters	Control	P K M with Maxigrain® diets 10+	20+	30+	40+	P K M without Maxigrain® diets 10-	20-	30-	40-	SEM	Level of Significance
Initial weight(g)	**916.33^{c}**	**1034.67^{a}**	**978.00^{b}**	**895.67^{c}**	**878.33^{c}**	**894.00^{c}**	**826.00^{d}**	**748.33^{e}**	**745.67^{e}**	**8.30**	***
Final weight(g)	**2107.00^{c}**	**2376.33^{a}**	**2279.00^{b}**	**1870.00^{e}**	**1722.00^{f}**	**1942.33^{d}**	**1654.67^{g}**	**1377.00^{h}**	**1304.67^{i}**	**12.43**	***
Weight gain(g)	**1190.67^{b}**	**1341.67^{a}**	**1301.00^{a}**	**974.33^{d}**	**843.67^{e}**	**1048.33^{c}**	**828.67^{e}**	**628.67^{f}**	**559.00^{g}**	**9.67**	***
Ave daily gain(g)	**42.52^{b}**	**47.92^{a}**	**46.46^{a}**	**34.80 ^{d}**	**30.13^{e}**	**37.44^{c}**	**29.60^{e}**	**22.45^{f}**	**19.96^{g}**	**0.34**	***
Feed intake (g)	**2781.33^{a}**	**3036.33^{c}**	**3259.67^{e}**	**3269.00^{e}**	**3397.97^{f}**	**2923.67^{b}**	**3130.67^{d}**	**3263.00^{e}**	**3388.00^{f}**	**12.16**	***
Feed: Gain Ratio	**2.34^{a}**	**2.26^{a}**	**2.60^{ab}**	**3.36^{c}**	**4.03^{d}**	**2.79^{b}**	**3.78^{d}**	**5.19^{e}**	**6.10^{f}**	**0.07**	***
Feed cost/kg wt(₦)	**93.53^{a}**	**93.46^{a}**	**94.71^{a}**	**121.00^{c}**	**136.13^{d}**	**107.34^{b}**	**132.24^{cd}**	**171.41^{e}**	**189.18^{f}**	**2.32**	***
Water intake (ml)	**6655.67^{a}**	**7056.33^{b}**	**7303.67^{d}**	**7385.67^{e}**	**7459.33^{f}**	**7163.33^{c}**	**7256.00^{d}**	**7389.33^{e}**	**7507.00^{f}**	**10.57**	***
Water : Feed Ratio	**2.40**	**2.33**	**2.24**	**2.26**	**2.20**	**2.45**	**2.32**	**2.27**	**2.22**		
Mortality (%)	**0.66**	**1.00**	**0.33**	**0.66**	**0.66**	**0.33**	**0.33**	**0.66**	**1.00**	**0.30**	**NS**

abcdef Means with different superscript within the same row are significantly (P<0.001)different

*** = (P<0.001)

NS: No significant difference (P>0.05).

TABLE 4.11: Carcass characteristics of broiler chicks fed on PKM diets supplemented with or without Maxigrain® (expressed as percentage of live weight)

Parameters	Control	P K M With Maxigrain® 10+	20+	30+	40+	PKM Without Maxigrain® 10-	20-	30-	40-	SEM	Level of Significance
Live Wt (g)	**2073^{b}**	**2360^{a}**	**2274^{a}**	**1875^{c}**	**1722^{d}**	**1966^{c}**	**1641^{d}**	**1370^{e}**	**1326^{e}**	**27.09**	***
Dressed Wt (g)	**1670^{b}**	**1871^{a}**	**1806^{a}**	**1389^{d}**	**1353^{ed}**	**1575^{c}**	**1273^{e}**	**1080^{f}**	**1046^{f}**	**25.17**	***
Dressing %	**80.54**	**79.48**	**79.42**	**77.08**	**78.57**	**80.06**	**77.55**	**78.80**	**78.80**	**0.93**	**NS**
Breast	**26^{abc}**	**29^{a}**	**25^{bc}**	**24^{bc}**	**25^{abc}**	**23^{c}**	**28^{ab}**	**25^{bc}**	**26^{abc}**	**0.82**	**
Neck	**5.0^{d}**	**7.7^{ab}**	**8.0^{a}**	**7.0^{abc}**	**5.0^{d}**	**7.6^{bcd}**	**5.0^{d}**	**6.0^{cd}**	**6.0^{cd}**	**0.36**	***
Thigh	**27.5b^{c}**	**30.5^{ab}**	**25.8^{c}**	**30.7^{a}**	**25.6^{c}**	**28.5^{abc}**	**27^{c}**	**25.7^{c}**	**26.6^{c}**	**0.82**	**
Drum stick	**14.9^{a}**	**13.5^{a}**	**14^{a}**	**12.9^{ab}**	**13^{a}**	**11^{b}**	**13.5^{a}**	**13^{a}**	**13.5^{a}**	**0.50**	*
Wings	**9.4^{bcd}**	**9.0^{cd}**	**8.8^{d}**	**11^{a}**	**10^{abcd}**	**10.5^{abcd}**	**11^{a}**	**10.9^{abc}**	**11^{ab}**	**0.46**	*
Back	**14.0**	**15.0**	**13.9**	**15.3**	**15.4**	**14.4**	**14.4**	**13.7**	**14.0**	**0.59**	**NS**
Head	**2.5^{b}**	**3.0^{ab}**	**3.0^{ab}**	**3.5^{a}**	**3.0^{a}**	**2.9^{ab}**	**2.9^{ab}**	**3.5^{a}**	**3.0^{a}**	**0.16**	*
Heart	**0.47^{cd}**	**0.47^{d}**	**0.48^{bcd}**	**0.45^{d}**	**0.45^{d}**	**0.5^{abcd}**	**0.56^{ab}**	**0.55^{abc}**	**0.5^{a}**	**0.03**	**
Liver	**1.46^{ef}**	**1.3^{f}**	**1.29^{f}**	**1.86^{cd}**	**1.99^{c}**	**1.6^{de}**	**2.09^{c}**	**2.4^{b}**	**2.98^{a}**	**0.08**	***
Lungs	**0.57^{c}**	**0.58^{c}**	**0.57^{c}**	**0.74^{ab}**	**0.6^{c}**	**0.55^{c}**	**0.6^{c}**	**0.7^{b}**	**0.8^{a}**	**0.02**	***
Kidney	**0.4^{d}**	**0.4^{d}**	**0.44^{cd}**	**0.5^{bc}**	**0.45^{cd}**	**0.4^{d}**	**0.5^{bc}**	**0.57^{ab}**	**0.6^{a}**	**0.06**	***

Gizzard	**2.49^{c}**	**2.75^{bc}**	**2.99^{abc}**	**4.0^{a}**	**3.8^{abc}**	**3.0^{abc}**	**2.66^{bc}**	**3.6^{abc}**	**4.0^{ab}**	**0.37**	*
Abdominal Fat	**1.5 ^{a}**	**0.85^{c}**	**0.7^{cde}**	**0.6^{de}**	**0.5^{e}**	**1.1^{b}**	**1.3^{b}**	**0.8^{cd}**	**0.6^{de}**	**0.05**	***
Pancreas	**0.32^{c}**	**0.34^{c}**	**0.36^{bc}**	**0.44^{a}**	**0.49^{a}**	**0.34^{c}**	**0.40^{ab}**	**0.50^{a}**	**0.40^{ab}**	**0.02**	***
Intestines	**3.0^{c}**	**4.7^{ab}**	**4.4^{ab}**	**4.56^{ab}**	**4.34^{ab}**	**3.99^{bc}**	**4.44^{ab}**	**5.04^{ab}**	**5.0^{a}**	**0.26**	**
Spleen	**0.17^{bcd}**	**0.15^{cd}**	**0.19^{bc}**	**0.19^{bc}**	**0.17^{bcd}**	**0.12^{d}**	**0.15^{cd}**	**0.21^{ab}**	**0.25^{a}**	**0.01**	***
Intestinal Length	**224^{bcd}**	**250^{a}**	**237^{ab}**	**235^{ab}**	**231^{bc}**	**234^{b}**	**214^{d}**	**213^{d}**	**217^{cd}**	**4.15**	***

abcdef Means with different superscript within the same row are significantly (P<0.001) different

NS: No significant difference (P>0.05), * = (P<0.05), ** = (P<0.01), *** = (P<0.001)

Table 4.3 Hematological analysis – broiler finisher chickens fed treated PKM with or without Max

Table 4.2 Hematological analysis - broiler starter chicks fed treated PKM with or without M

	P C V %	TOTAL PROTEIN (g/dl)	HB (g/dl)	Neutrophil (g/dl)	Eosinophil %	Lympho %
Normal Range	30—40		9—13	3—7	0--.05	10—
Control	29	3.00	9.3	1.00	0.40	9.50
10 +	26	3.00	8.3	3.00	0.60	3.00
20 +	34	2.20	11.0	ND	1.40	8.50
30 +	27	3.30	8.5	2.00	2.50	7.30
40 +	26	4.70	8.3	4.00	1.20	8.10
10 -	26	2.70	8.3	ND	1.40	8.60
20 -	31	2.20	10.0	8.00	1.40	7.80
30 -	29	3.20	9.30	ND	1.50	8.40
40 –	25	2.00	8.0	3.00	2.10	7.40

+ with Maxigrain®

_ without Maxigrain®

ND= not determined.

	P C V(%)	TOTAL PROTEIN	HB
Normal Range	30—40	(g/dl)	9—13(g/
Control	35	4.00	11.0
10 +	40	4.00	13.3
20 +	38	4.00	12.6
30 +	39	4.00	13.0
40 +	43	4.00	14.3
10 –	38	5.00	13.3
20 –	34	3.50	11.3
30 –	33	4.50	11.0
40 –	38	5.00	13.3

+with Maxigrain®

_ without Maxigrain®

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Proximate chemical analysis - broiler starter diets

Moisture %	Dry Matter %	Met. Energy kcal/kg	C/Protein %	C/Fat %	Total Ash %	C/Fibre %	N. F. E. %
9.82	92.18	2888	23.57	3.05	9.13	4.50	59.75
9.28	90.72	2861	23.05	2.25	8.46	5.50	60.74
9.09	90.91	2730	23.45	2.45	9.05	6.50	58.55
8.69	91.31	2690	23.09	2.50	8.78	9.50	56.13
8.55	91.45	2510	23.19	2.50	8.89	12.20	53.22
9.28	90.72	2845	23.19	2.15	9.43	5.50	59.73
9.24	90.76	2735	23.19	2.00	8.21	7.00	59.60
8.98	91.02	2693	23.22	1.55	8.13	9.50	57.60
8.06	91.94	2515	23.19	1.50	7.69	11.50	56.12

+ with Maxigrain®

_ without Maxigrain®

Proximate chemical analysis - finisher diets

	Moisture	Dry Matter	Met Energy	C/Protein	C/Fat	Total Ash	C/Fibre	N. F. E
	%	%	kcal/kg	%	%	%	%	%
I	7.49	92.51	2601	23.63	3.31	8.81	3.31	54.61
	7.67	92.33	2643	25.75	4.50	8.81	4.50	51.09
	7.48	92.52	2406	23.73	4.34	7.93	4.34	55.12
	7.10	92.90	2488	24.78	5.43	8.55	5.45	50.62
	7.10	92.90	1799	23.75	6.86	9.48	6.86	50.03
	7.77	92.23	2593	25.25	4.00	9.68	4.01	51.54
	7.14	92.86	2370	23.44	4.70	8.51	4.70	54.62
	7.36	92.64	1711	25.43	6.11	9.20	6.11	49.96
	7.03	92.97	1861	24.45	6.32	8.30	6.32	51.85

+ With Maxigrain®

_ without Maxigrain®