EXOGENOUS ENZYMES AND IRRADIATION OF BARLEY REDUCE THE ANTI–NUTRITIONAL ACTIVITY OF NON-STARCH POLYSACCHARIDES IN BROILERS

by

PAUL DREW

Submitted in partial fulfilment of the requirements for the degree

M.Sc. (Agric)

in the

Department of Animal and Wildlife Sciences, Faculty of Natural and
Agricultural Sciences, University of Pretoria, Pretoria, 0002
South Africa

April, 2008

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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

__________________________ Date: 9 July 2008
Mr PJ Drew

TABLE OF CONTENTS

Page

LIST OF FIGURES i

LIST OF TABLES iii

ACKNOWLEDGEMENTS iv

ABSTRACT v

SUMMARY vii

LIST OF ABBREVIATIONS ix

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 LITERATURE REVIEW 3

2.1) ENERGY IN THE BROILER DIET 3
- a) Gross Energy and Digestible Energy 4
- b) Metabolisable Energy 4
- c) Net Energy 7

2.2) FAT IN THE BROILER DIET 7
- a) Digestion of Fats 8
- b) Factors Affecting Fat Digestibility 9

2.3) THE USE OF BARLEY IN BROILER DIETS 12

2.4) SOLUBLE NON-STARCH POLYSACCHARIDES 13
- a) Effects On Digestive Parameters 17
- b) Effects on Performance Parameters 20
- c) Summary 21

2.5) IRRADIATION OF ANIMAL FEEDSTUFF 22

Page

2.6) INTERACTIVE EFFECTS BETWEEN DIETARY FAT TYPE AND EXOGENOUS CARBOHYDRASE ENZYMES IN BROILER DIETS CONTAINING HIGH CONTENTS OF NON-STARCH POLYSACCHARIDES 28

CHAPTER 3 MATERIALS AND METHODS 33
- a) Experimental Design and Treatments 33
- b) Performance Trial 37
- c) Digestibility Trial 37
- d) Sample Analysis 38
- e) Statistical Analysis 40

CHAPTER 4 RESULTS 41

CHAPTER 5 DISCUSSION 52

CHAPTER 6 CONCLUSION 56

CHAPTER 7 REFERENCES 58

LIST OF FIGURES

Page

Figure 1. Pie Chart comparisons of soya oil and yellow grease's principle fatty acid compositions, the two lipid sources used in this trial 10

Figure 2.1. Illustration of the direct action of radiation on the oxygen bridges in the aqueous state (Siddhuraju *et al.*, 2002) 24

Figure 2.2. A. Alteration of monosaccharide units in the polymer chain by radiation – induced dehydration 25

Figure 2.2. B. Alteration of monosaccharide units in the polymer chain by β–splitting caused by irradiation (Siddhuraju *et al.*, 2002) 25

Figure 3. 35-day liveweight with the calculated standard error of the mean for each treatment 42

Figure 4. Cumulative Feed Conversion Ratio at 35 days of age with the standard error of the mean calculated for each treatment 43

Figure 5. Apparent Metabolisable Energy values of the different diets with the standard error of the mean for each treatment 45

Figure 6. Ileal crude fat digestibility of the different diets and the standard error of the mean 48

Figure 7. Fecal crude fat digestibility of the different diets and the calculated standard error of the mean of each treatment 49

Page

Figure 8. Fecal dry matter digestibility and the standard error of the mean of the different diets 50

Figure 9. Ileal dry matter digestibility of the different diets shown with standard error of the mean per treatment 51

LIST OF TABLES

Page

Table 1. Measures of Carbohydrate composition (% dry matter) (Smits and Annison, 1996) 14

Table 2. Treatment diets used in the performance and digestibility trial 33

Table 3. Fatty acid profiles of yellow grease and soya oil 34

Table 4. Raw material composition and calculated feed specifications of the experimental diets on an as fed basis (g/kg) 35

Table 5. Determined analysis of experimental diets on an as fed basis (g/kg) 36

Table 6. Performance trial results for maize and barley based diets containing either soya oil or yellow grease as the main lipid source (± standard error of the mean) 46

Table 7. Digestibility trial results for maize and barley based diets containing either soya oil or yellow grease as the main lipid source (± standard error of the mean) 47

ACKNOWLEDGEMENTS

Thank you to the University of Pretoria for the financial support for the experiment as well as the use of their facilities and to all those involved in giving a helping hand and making the trial a possibility.

Thank you to DSM Animal Nutrition for the exogenous enzyme used in the project.

Thank you to Mr. R. Coetzee for helping with the statistical analysis of the results of this project.

ABSTRACT

Interactions between non-starch polysaccharide (NSP) level, dietary lipid type, exogenous carbohydrase enzymes and irradiation were investigated. Ten treatment diets were fed to broilers in a performance and digestibility trial. Eight of the diets contained high levels of NSP, achieved by a high barley inclusion of 55% of the diet. Four of the treatments made use of non-irradiated barley, whereas the barley included in the other four diets was irradiated. By adding either 10% soya oil or yellow grease (fat) as the lipid source, sub groups were created which differed in fatty acid profile. Lastly, these treatments were further subdivided by supplementing one of the two diets from each subgroup with a commercially available combination of exogenous carbohydrase enzymes consisting of cellulases, xylanases and β - glucanases (Roxazyme G at 150 g/ton). The two control diets were based on maize (low NSP diets) with either soy oil or yellow grease.

The high NSP diets had significantly lower ($P<0.05$) apparent metabolisable energy (AME) and lipid digestibility values than the low NSP diets. Lipid digestibility and AME values were also significantly lower ($P<0.05$) for diets containing yellow grease compared to soya oil.
The birds that received yellow grease performed worse in terms of growth, feed intake and feed conversion ratio (FCR) than the oil-containing diets. These trends were evident throughout all treatments, although not always significant. The addition of carbohydrase to diets based on barley improved the dietary lipid digestibility and AME values. Significant improvements ($P<0.05$) in bird performance were noted for the barley diets with the yellow grease.

Pre-irradiation of barley significantly increased ($P<0.05$) the AME value of diets, and improved lipid digestibility of the fat-containing treatment.

The simultaneous combination of carbohydrase supplementation and barley irradiation proved to have an additive positive effect on feed quality and bird performance. For all treatments this combination improved the barley based diets to such an extent that it performed equally or significantly better ($P<0.05$) than its maize based counterpart. The irradiated barley-

yellow grease based diets showed a more pronounced benefit with the addition of carbohydrase enzymes to the feed ($P<0.05$).

SUMMARY

A trial was conducted with the purpose to investigate the interactive effects in broiler diets containing high non-starch polysaccharide (NSP) levels, exogenous carbohydrase enzyme addition and irradiation of grain containing high levels of NSP and the effect that lipid type has in such diets. Barley was the main source of carbohydrate in the diets high in NSP, and maize in the diets containing low NSP levels. Effects were measured in terms of performance and diet digestibility in broilers. In understanding these interactions, nutritionists can make informed economical decisions to improve feed quality using various nutritional technologies.

Ten treatment diets were fed to broilers in a performance and digestibility trial. Eight of the diets contained high levels of NSP, achieved by a high barley inclusion of 55% of the diet. Four of the treatments made use of non-irradiated barley, whereas the barley included in the other four diets was irradiated. By adding either 10% soya oil or yellow grease (fat) as the lipid source, sub groups were created which differed in fatty acid profile. Soya oil contained predominantly unsaturated fatty acids, while the yellow grease contained predominantly saturated fatty acids. Lastly, these treatments were further subdivided by supplementing one of the two diets from each subgroup with a commercially available combination of exogenous carbohydrase enzymes consisting of cellulases, xylanases and β - glucanases (Roxazyme G at 150 g/ton). The two control diets were based on maize (low NSP diets) with either soy oil or yellow grease.

600 Ross broiler chickens were randomly divided into 10 treatment groups, each with 3 replicates and 20 birds per replicate. The birds were housed in deep litter pens in an environmentally controlled broiler house, with a constant lighting schedule and *ad libitum* access to feed and water. Body weights, mortality and feed intakes were recorded weekly up to day 35, when the performance trial was terminated. Thereafter, 5 birds per pen, uniform in weight and size, were selected and randomly assigned to metabolism cages for conduction of the digestibility trial.

Crude fat content of the feed and faeces was determined by ether extraction (Büchi Soxhlet) and the gross energy value of the treatment diets and faeces were determined using an adiabatic bomb calorimeter with oxygen ignition. The chromic oxide in the feed and faeces was determined using a Varian Spectrometer (absorbance measured at 425.4nm) after acid extraction of the marker, to determine apparent digestibility coefficients of the crude fat and the metabolisability of the gross energy by standard calculations. Statistical analyses of the data were carried out using Duncan's Multiple Range test and the SAS Software System.

In general, the diets high in NSP showed lower digestibility and performance values compared to those diets low in NSP concentrations. In both the high and low NSP diets the yellow grease (high saturated fatty acid concentration) indicated lower digestibility and performance values than the diets containing soya oil (high unsaturated fatty acid concentration). This effect was more pronounced in the diets containing high NSP levels.

The addition of the carbohydrase enzymes improved digestibility and performance parameters in the high NSP diets, containing either lipid types. The effect was more pronounced in the barley based diets containing the yellow grease, showing an interaction between lipid type and NSP level of the diet. As observed, the more saturated the lipid type combined with a high NSP diet, the more negative the effect on digestibility parameters and performance parameters.

Irradiation showed a generally additive effect, when combined with the enzyme compared to those diets either irradiated or only containing the enzyme. Irradiation showed an improvement of digestibility and performance parameters, due to the effect that irradiation has on anti-nutritional factors in the high NSP contain diets.

LIST OF ABBREVIATIONS

AME : Apparent Metabolisable Energy

CMC : Carboxy-Methyl Cellulose

EEL : Endogenous Energy Losses

FAO : Food and Agricultural Organisation

FCR : Feed Conversion Ratio

FDA : Food and Drug Administration

FFA : Free Fatty Acid

IAEA : International Atomic Energy Agency

JKg^{-1} : Joules per Kilogram

kGy : Kilo Grays

LCSFA : Long Chain Saturated Fatty Acid

ME : Metabolisable Energy

MJ/Kg : Mega Joules per Kilogram

NE : Net Energy

NSP : Non-Starch Polysaccharides

RVA : Real Applied Viscosity

WHO : World Health Organisation

TME : True Metabolisable Energy

CHAPTER 1. INTRODUCTION

Animal production is no longer viable without an efficient production system. Feed, being an input into a production system, is a major expense to the production unit and may realise up to seventy percent or more of the total running costs of the unit. Therefore, it makes the most sense to the producer to maximise the efficiency at which feed is utilised in order to optimise outputs efficiently and maximise profits. Many years of research and knowledge can be used to optimise the efficiency at which feed is utilised. Ongoing research is required to further enhance nutritional knowledge, and allow for improvements to be made in the utilisation of feed in production units. Many nutritional factors interfere with feed utilisation. The more that is understood about these factors, the greater the understanding of how to improve feed utilisation will be.

In birds, soluble non-starch polysaccharides (NSP) such as mixed-linked β-glucans in barley (Alimirall *et al.*, 1995), increase viscosity of the intestinal content causing a disturbance of nutrient, especially fat, absorption (Refstie *et al.*, 1999). Consequently, viscous digesta promotes significant decreases in organic matter digestibility, voluntary feed intake and productive performance (Gasa *et al.*, 2000).

It has been suggested that the addition of carbohydrases to the feed could improve viscosity of digesta through disruption or solubilisation of cell wall polysaccharides, resulting in the reduction or elimination of the encapsulating effects of the cell wall (Dierick and Decuypere, 1994; Yin *et al.*, 2000). Choct *et al.* (1995) noted that the addition of fibre degrading enzymes increased the nutritional value of wheat with a low apparent metabolisable energy (AME) and that the variable AME value of wheat could therefore be attributed to anti-nutritive effects of soluble fibre.

Inclusion of lipids with a high saturated fatty acid content in broiler diets resulted in lower weight gains, increased feed conversion ratio (FCR), increased digesta viscosity, reduced lipid digestibility and decreased metabolisable energy (Danicke *et al.*, 2000).

It was shown that if a carbohydrase enzyme was added to a feed containing high NSP levels, the greatest effect would be seen in the diets with the highest content of saturated fatty acids (Danicke *et al*., 2000).

Currently employed conventional processing methods are relatively ineffective for the reduction or removal of anti-nutritional factors, especially for the hydrolysis of NSP (Siddhuraju *et al*., 2002). Irradiation is one possible alternative and an additional processing technique for reducing both heat stable and heat labile anti-nutrients. Siddhuraju *et al*. (2002) showed that by using ionizing radiation on legume seed, a reduced digesta viscosity was noticed and suggested that this was due to the depolymerization of NSP in the treated seeds.

A joint study group (FAO/IAEA/WHO) on high dose irradiation reviewed data relating to the toxicological, nutritional, radiation chemical and physical aspects of food irradiated to doses above 10 kGy (measured in Grays, 1Gy = 1 Jkg^{-1}) and concluded that foods treated with doses greater than 10 kGy can be considered safe and nutritionally adequate when produced under the established, Good Manufacturing Practice (WHO, 1999).

The purpose of this study was to evaluate the interactive effects of NSP inclusion and dietary fatty acid saturation, with or without exogenous carbohydrases and irradiation of NSP, and the effect of these on the AME value and crude fat digestibility of the feed and overall broiler performance.

CHAPTER 2. LITERATURE REVIEW

2.1. ENERGY IN THE BROILER DIET

Energy represents a major component of the broiler diet and since the feed is a major expense to the production system, it is important to estimate precisely the energy value of feeds. This will enable for least cost formulation to be applied and for adapting the feed supply to the energy requirements of the animals (Noblet *et al*., 1993).

The role of energy in the physiology of respiration has provided the basis for nutrition science and energy intake has also been implicated in the physiology of appetite and satiety and in the control of feed consumption (McNab, 1990). It is important to be able to describe the energy content of feedstuffs for economic reasons. Metabolisable Energy (ME) has become the most universally accepted measure of energy content in poultry diets (McNab, 1990). The establishment of the relationship between the ME content of the diet and its intake and relating the concentrations of other nutrients to the dietary ME value, has improved the precision with which poultry can be fed (McNab, 1990). Although ME is generally considered to be a property of the diet, it is really a characteristic of an animal to which the diet is given (McNab, 1990).

Tabular digestible and metabolisable energy or digestible nutrient contents of feedstuffs have usually been obtained when they were given alone or incorporated in simplified diets. Under practical conditions, most ingredients are used in complex diets and at relatively high feeding levels where digestive interactions are likely to occur (Frape and Tuck, 1977). Therefore, digestible or metabolisable energy values expected from the summation of the ingredient contributions will differ from the measured values (Noblet *et al*., 1993).

Theoretically, the net energy (NE) of a feed is the closest estimate of its true energy value, in comparison with digestible energy (DE) or metabolisable energy (ME). But the NE value is difficult to measure directly and is usually calculated from prediction equations (Noblet *et al*., 1993). Therefore, several NE values can be estimated from a food (Noblet *et al.*, 1993).There are many reasons why several NE values are true for a single feed ingredient.

a) Gross Energy and Digestible Energy

Gross Energy (GE) of a food is determined by Bomb Calorimetery and represents the total energy of the feedstuff. The difference between the energy content of the faeces and the GE of the feed, gives the DE value in simplistic terms. Much research has shown the negative effects that dietary fibre has on the digestibility coefficient of energy (Noblet *et al.*, 1993). The negative effect of fibre on dietary energy digestibility is due not only to its lower degradation but also due to modifications in the apparent digestibility of the other chemical constituents of the diet (Noblet *et al.*, 1993). Noblet *et al.* (1993) showed that other nutrients such as crude protein and crude fat digestibilities decrease with an increase in the NDF content of the diet. In this experiment it is clearly stated that the application of DE values from feeding tables to the calculation of DE content of diets induces an overestimation of their energy content when fibrous and, or high fat ingredients are included at high levels. Degree of fatty acid saturation, fat level in the diet and subsequent rates of passage in the digestive tract and the possible interactions between fat and other chemical constituents (such as fibre) of the diet, explain the effects that fat can have on DE values. DE values of diets and ingredients cannot be considered as constant. It is highly dependant on animal characteristics and feeding level (Noblet *et al.*, 1993).

b) Metabolisable Energy

Apparent Metabolisable Energy (AME) is the difference between the gross energy of the food and the energy lost as faeces, urine and combustible gases when that food is eaten (Harris, 1966). Due to the gaseous losses from poultry being very small, they are almost always ignored (McNab, 1990). The excreta of a food eaten, consists of undigested and unmetabolised dietary residues and a part of the excreta is of endogenous origin (McNab, 1990). This part of the excreta is referred to as Endogenous Energy Losses (McNab, 1990). The energy metabolised, measured through various bioassays, is considered “apparent” as only a part of the energy excreted is due to the dietary residues (McNab, 1990). Part of the Endogenous Energy Losses (EEL) is faecal in origin and is generally reported as consisting of sloughed off gut lining, bile excretions and unabsorbed enzymes. The other part of the EEL is of urinary origin and consists primarily of the excretory products of nitrogen metabolism (McNab, 1990).

Fisher and McNab (1987) identified three general types of experiments to determine ME. Firstly, traditional assays involve preliminary feeding periods to establish a state of equilibrium. Differences in the contents of the digestive tract between the beginning and end of the assay period are controlled by trying to ensure that they are the same. Complete diets may be fed and substitution methods must be used for ingredients.

Secondly, rapid assays, using starvation before and after allowing the birds free access to the diet to control the end effects. Complete diets must be used or substitution methods for ingredients.

Thirdly, rapid assays using tube feeding to place the food directly into the crop of the bird can also be used. Using this method avoids the use of the substitution method for ingredients tested.

The different assays should be judged on their ability to provide the three essential pieces of information; energy balance, food intake and endogenous energy losses (McNab, 1990).

The AME system is widely used, however, there were concerns and problems associated with AME determinations. For example, values for rapeseed meal, dehydrated alfalfa meal, rye and guar meal all tended to decline as their concentrations were increased in the diet (McNab, 1990). Other problems with AME value determinations were differences detected between species, strains, and experimental animals of different ages (McNab, 1990). Reasons for these differences and occurring problems have been the subject of much research, and today many of the problems described above are understood as to why they occur.

However, much more research needs to take place with respect to these problems in understanding them totally and ways to utilise this knowledge in practice to optimise production.

One problem was exposed by Guillaume and Summers (1970) when they showed that AME values derived for diets fed to adult cockerels were profoundly affected by the amount of feed eaten during the assay. The lower the food consumption, the lower the AME value of the diet. This effect was attributed to the contribution made to the excreted energy by the EEL (McNab, 1990).

Sibbald (1976) noticed this effect and determined an assay to directly determine the EEL and said that the status of both diets and feedstuffs should be expressed in terms of their True Metabolisable Energy (TME) contents, where the EEL is subtracted from the AME. Due to major and minor variations introduced to published methods of energy determination, there are now more methods being applied to the derivation of ME values and not just one standard procedure, which leads to different ME systems being derived (McNab, 1990).

For some years it has been common practice to ‘correct’ AME values determined in balance experiments for changes in the nitrogen status of the bird during the experimental period (McNab, 1990). The rationale for this adjustment is that the complete catabolism of protein stored in the body results in the need to dispose of the nitrogen it contained. Nitrogen is excreted as uric acid in poultry which contains energy. For each gram of nitrogen excreted as uric acid, 34.4 kJ of energy is lost from the body and appears in the urine. When a bird is storing protein it is spared the energy cost of excreting nitrogen and less uric acid appears in urinary excretion. Therefore, the same diet or feedstuff, when given to different birds, may have a different ME value because of the differences in the amount of ingested protein the bird has retained. To make ME values independent of the conditions under which they were derived, it has become very common to correct them to what they might have been under standard conditions (McNab, 1990). The most commonly used standard is where the birds are in nitrogen equilibrium (nitrogen retention is zero). The principle of nitrogen retention has been criticised because a diet or feedstuff is penalised when it is promoting the retention of protein, often the objective of animal production. Due to the function of the ME system being to evaluate the energy status of feedstuffs rather than their ability to promote protein synthesis, correction to nitrogen equilibrium can be justified. AME values as well as TME values should be corrected to nitrogen equilibrium (AME_N or TME_N) (McNab, 1990).

c) Net Energy

The efficiency of utilisation of ME (k) was estimated as the NE/ME ratio (multiplied by 100) (Noblet *et al*., 1993). The highest values for 'k' were observed with the lower protein diets and 'k' was higher in diets, which contained additional fat as reported by Noblet *et al*. (1993). Since 'k' is affected by the chemical characteristics of the feed, the hierarchy between feeds with regard to their energy content will depend on the energy system.

Therefore, assuming that NE content is the best energy value estimate, DE was shown to overestimate the feeds that contain high amounts of protein and fibre, according to Noblet *et al*. (1993) and Vermorel and Martin-Rosset (1997). On the other hand it underestimates starch or fat ingredients. The underestimation due to the DE system is the most important for fat sources (Noblet *et al*., 1993). Consequently, more emphasis should be given to studying the effects of digestive interactions occurring with changes in feeding level or digestive capacity of the animal (Noblet *et al*., 1993).

This shows the benefit of adopting the NE system for estimating the energy value of ingredients. It provides an evaluation of the diet energy content, which is closer to the true value.

2.2. FAT IN THE BROILER DIET

In broiler production systems where the main objective of the producer is to grow birds at an optimal rate, energy is an important component of the diet. Animal or plant fat is regularly included in broiler diets to provide the high energy levels required by the bird. Portions of supplemented fat in the diet may range from 3 – 10% of the diet (Danicke *et al.*, 1997b). The proportion of fat in the diet will be dependant on factors such as the price of the fat, availability, effect of fat on pellet quality and any physiological restraints caused by a particular fat.

a) Digestion of Fats

Digestion and absorption of fat mainly occur in the upper intestinal tract. With the passage of the fat into the duodenum, it is mixed with secretions from the pancreas and gall bladder. Pancreatic juices contain lipases and co-lipases, which is essential for the digestion of the fat and the bile acids contain conjugated bile salts which promote fat emulsification (Krogdahl, 1985).

Binding of the co-lipase at the surface of these aggregates is the requirement for the action of the lipases. The lipase enzymes hydrolyse triglycerides at the 1 and 3 positions of the glycerol molecule. Fatty acids, 2-monoglycerides, phospholipids and bile salts then form what is known as micelles (Krogdahl, 1985). These are much smaller than the emulsified fat aggregates. They are able to solubilise non-polar lipid compounds such as fat soluble vitamins or long chain saturated fatty acids, within their lipophyllic core. Under conditions of high fat digestibility, the micelles release their contents to the intestinal membranes in the proximal part of the small intestine. The contents then passively diffuse into the intestinal cells. The majority of the bile salts released by the micelles is absorbed by the distal intestine, but some of the bile salts are used again for micelle formation in the lower part of the intestine (Krogdahl, 1985). The bile salts that are absorbed are transported back to the liver, where they are again used in bile formation. This conserves the metabolic bile salt pool.

The absorbed fatty acid will only enter the intracellular pool if it is bound to the intracellular fatty acid binding protein. The fatty acid binding protein and fatty acid complex moves the fatty acid to other intracellular compartments where it is re-esterified to a triglyceride and further processed (Krogdahl, 1985).

b) Factors Affecting Fat Digestibility

The degree of unsaturation and the polarity of the fatty acid affect fat digestibility (Garret and Young, 1975). Long chain-saturated fatty acids (LCSFA) are more non-polar than long chain-unsaturated fatty acids of the C18 family. The polarity of this family increases as the number of double bonds in the fatty acid increases. Among the saturated fatty acids, polarity increases as chain length increases. The more non-polar a fatty acid is, the more it relies on an adequate presence of bile salts and phospholipids for emulsification. Therefore, medium chain saturated fatty acids and long chain unsaturated fatty acids can be absorbed in large quantities without the presence of bile salts (Garret and Young, 1975).

The differences in the polarity of the fatty acids are shown by the differences in their absorbability. Absorbability increases from C18:0 < C16:0 < C14:0 < C18:1, C18:2, C18:3 (Danicke *et al.*, 1997b). As stated by Danicke *et al.* (2000), fatty acid utilisation from saturated fatty acid sources, such as beef tallow, can be improved by the addition of small amounts of an unsaturated fatty acid, such as plant derived oils. This statement was supported by research done by the like of Leeson and Summers (1976), Wiseman and Lessire (1987), Garret and Young (1975) and Danicke *et al.* (1999a).

The ratio of unsaturated to saturated fatty acids in the diet influences digestibility of fat and ME content of fats or fat blends (Danicke *et al.*, 2000). This relationship was described by non-linear regression models characterised by saturation kinetics. These models considered that at lower ratios of more saturated fatty acids, fat digestibility and ME responded more dramatically to addition of unsaturated fats than at higher ratios. The physiological basis of this synergistic effect between unsaturated and saturated fatty acids is most likely due to the greater emulsification properties of the unsaturated fatty acids and the dependence of long chain saturated fatty acids on such emulsifying agents for effective digestion and absorption (Danicke *et al.*, 2000). As an example, Garret and Young (1975) found that palmitic and stearic acid from soya oil are found to be better absorbable than those originating from beef tallow and the reason being due to the synergistic effects of

Long Chain Unsaturated Fatty Acids, which support micellar solubilisation of Long Chain Saturated Fatty Acids. The order of digestibility of fats in addition to the absolute digestibility value might be modified by other factors such as dietary inclusion level, age of the birds and free fatty acid content (Wiseman *et al*., 1991).

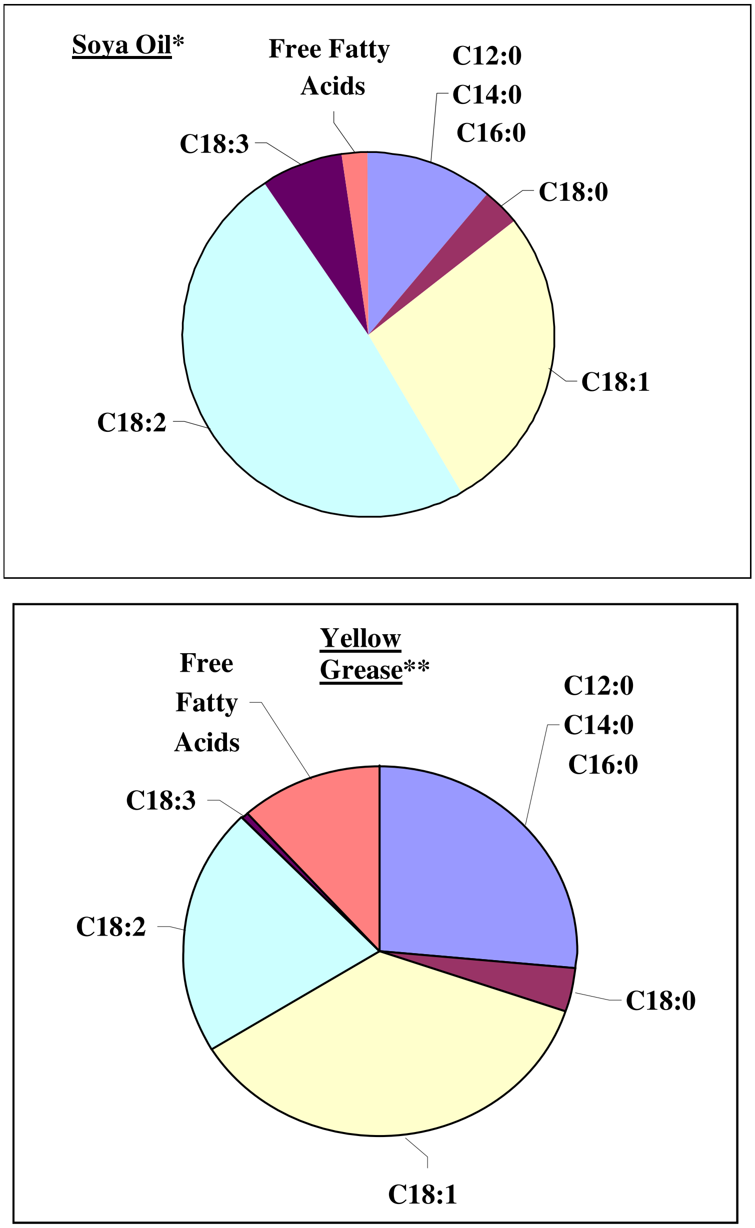


Figure 1. Pie Chart comparisons of Soya Oil and Yellow Grease's principle fatty acid compositions, the two lipid sources used in this trial

*Danicke *et al*. (2000)

**Analysis of yellow grease used in the trial

Bile salt availability affects fat digestibility. In research done where bile salts were added to the broiler diet, especially in young broilers, an improvement in fat digestibility was seen and even more greatly in diets containing high levels of beef tallow (Fedde *et al*, 1960; Polin, 1980).

Mineral soaps are formed with the binding of fat with minerals such as magnesium and calcium (Fedde *et al.*, 1960) and are indigestible. This is of more importance if either fat absorption is low or if high amounts of those minerals are present in the diet.

Lipase availability is another factor affecting fat digestibility, especially in young chicks (Nir *et al.*, 1993). Lipase availability is compromised in young chicks compared to older birds and therefore are less efficient at digesting fats.

The free fatty acid content of a fat can also influence the availability of a fat. The greater the free fatty acid content of a fat, the lower the efficiency of absorption of the products of its digestion, this reduction being more pronounced the more saturated the fat and the younger the bird (Blanch *et al.*, 1995). Digesta viscosity also effects fat digestibility and utilisation as discussed in detail later.

Wiseman *et al.* (1991) proposed an equation based on fatty acid composition, to predict the ME values of fats reasonably well. The equation shows the effects of age, free fatty acids and unsaturated/ saturated fatty acid ratio on the AME of an individual fat.

$$\text{AME of fat (kcal/kg)} = 239 \text{ x } (A + B \text{ x } FFA + C \text{ x } e^{(D \text{ x } U/S)})$$

Where;

	Age <21 days	**Age >21 days**
A	38.112	39.025
B	-0.009	-0.006
C	-15.337	-8.505
D	-0.506	-0.403

FFA = Free-Fatty Acid content (%)

U/S = Unsaturated and Saturated Fatty Acid Ratio.
In using this equation, fatty acids with a chain length of 12 or less are counted as unsaturated.

2.3. THE USE OF BARLEY IN BROILER DIETS

Barley is the preferred grain for cultivation in many areas in the world due to its resistance to drought and ability to mature in climates with a short growing season. Its use for poultry has however been limited by the considerable amounts of fibre contained in the grain. The soluble fibre fraction, which mainly consists of mixed-linked (1-3)(1-4)-β-glucans, is associated with an increase in gut viscosity, which in turn inhibits digestion and absorption of nutrients (Svihus and Gullord, 2002).

Svihus and Gullord (2002), found a positive correlation between energy and fat content of barley to chick performance, measured as weight gain and AME value. It was also found that the specific weight of the barley kernel was negatively correlated to performance, when enzymes were not used, as well as the kernel falling number (the falling number is positively correlated to viscosity). The falling number was determined on a Falling Number 1600 instrument where 7g samples were mixed with 25ml of water and heated in boiling water under agitation for 60 seconds, followed by the measurement of the time needed for a metal stirrer to fall a fixed distance through the hot flour suspension (Svihus and Gullord, 2002).

The crude fibre content of barley was found by Svihus and Gullord (2002) to be positively correlated to chick performance. Transformed *in vitro* digestion viscosity and transformed water extract viscosity were highly negatively correlated to performance in the same trial. Intestinal viscosity was also negatively correlated to performance when enzymes were not used. Between 71% and 89% of the variation in nutritional value could be explained by chemical and physical properties of barley (Svihus and Gullord, 2002).

Svihus *et al*. (1997) measured performance of chicks fed 14 cultivars of barley with or without enzyme supplementation. Findings showed that the different barley samples did not have the same feeding values. This demonstrated that both the cereal cultivar and the growing conditions were important for determining feeding value. Appropriate commercial enzymes were used in the trial to reflect the level of non-starch polysaccharides in the barley grain type, which is strongly correlated to its feeding value. The results suggested that the variation found between barley grains, necessitates the need to routinely test the grains before they are incorporated into diets and before they are supplemented with enzymes. Certain barley cultivar grains showed a low response. This may also indicate that genetic and possibly agronomic intervention could increase the feeding value of barley and possibly negate the need for enzymes to reduce the anti-nutritive effect of NSP (Svihus *et al.*, 1997).

2.4. SOLUBLE NON-STARCH POLYSACCHARIDES

Many of the ingredients utilised in poultry nutrition have anti-nutritional factors. As noted in soyabean ingredients, several soyabean compounds may disturb the digestive process (Rackis, 1974). Among the most apparent, though little investigated, are non-starch polysaccharides (NSP) (Refstie *et al.*, 1999).

NSP refers to those compounds which are polysaccharides but are not starch compounds. The chemical nature of NSP differs from one ingredient to another (Evans *et al.*, 1993). These compounds vary in solubility and viscosity and therefore, individual compounds have varying effects on digestive parameters and productive parameters. NSP is present in several feed ingredients, ranging from cereal grains to legume seeds (Choct and Annison, 1992). Some of the variation in the performance of broiler chickens on different cereal grains and some other ingredients has been ascribed to the nature and concentration of NSP (Smits and Annison, 1996).

Nine of the 100 monosaccharides found in nature are predominant building blocks of NSPs (Smits and Annison 1996). These include pentoses (arabinose and xylose), hexoses (glucose, mannose and galactose), the 6-deoxyhexoses (L-rhamnose and L-fucose) and hexauronic acids (galacturonic acid and glucuronic acid). The sugars are joined by glycosidic bonds between the hemi-acetal group of one sugar and the hydroxyl group of another. The bonds are identified by the carbon atoms of each sugar that are involved in the bond (1 to 6 for hexoses and to 5 for pentoses) and the orientation of the oxygen atom in the hemi-acetal group (primarilyβ). Table 1 shows the fibre fractions of different plant materials.

Table 1. Measures of carbohydrate composition (% dry matter) (Smits and Annison, 1996)

Ingredient	Crude Fibre	Soluble NSPs*	Insoluble NSPs*	Total NSPs*	Main NSP	NSP Conc** (%DM)
Wheat	3.0	2.4	9.0	11.4	β-D-Glucan Arabinoxylan Cellulose	0.5 6.05 2.0
Rye	3.0	4.6	8.6	13.2	β-D-Glucan Arabinoxylan Cellulose	1.2 8.9 1.5
Barley	6.5	4.5	12.2	16.7	β-D-Glucan Arabinoxylan Cellulose	7.6 3.3 3.9
Sorghum	3.0				Arabinoxylan β-D-Glucan	2.8 0.1
Maize	2.5				Arabinoxylan β-D-Glucan	4.2 0.1
Lupin White	16.5	8.0	20.0	28.0	Complex Polymer	
Peas	7.0	2.5	32.2	34.7	Complex Polymer	
Soya Bean Meal	7.0	7.5	16.4	30.3	Complex Polymer	
Rape Meal	13.6	11.3	34.8	46.1	Complex Polymer	

*NSP: Non-Starch Polysaccharide

**Conc.: Concentration

The complexity is further increased by the covalent bonding to non-carbohydrate compounds such as methyl and acetyl groups, proteins, lignins and non-covalent bonding within and between polysaccharides. This diversity in chemical structure, both within and between dietary sources, is reflected in physical structure and physiological activities of various NSP fractions (de Lange, 2000).

NSPs have varying effects on animals when included in the diet. They may affect the production and activity of digestive enzymes, intestinal morphology, the microbial population in various segments of the gut, and secretions of certain hormones including insulin, glucagon, gastric inhibitory polypeptide and possibly secretin and cholecystokinin (Chesson, 1990; Smits, 1996).

Some of these effects may be indirect, rather than direct effects of NSPs. For example, independent of the presence of NSP, microbial activity can affect gut secretions and morphology of the small intestine (Sakata, 1987). These may have implications for gut health, the rate and extent of nutrient digestion and absorption, and the metabolic efficiency of nutrient utilisation after absorption. Anugwa *et al*. (1989) demonstrated that the inclusion of 40% lucern meal in a maize-soyabean meal based diet increased the size of visceral organs in growing pigs. As visceral organs are major contributors to whole body energy expenditure , it is likely that feeding NSPs will increase the basal metabolic rate in monogastric animals (de Lange, 2000).

NSPs, unlike starch and sucrose, cannot be hydrolysed by enzymes produced by mammals or birds themselves (Dierick *et al*., 1989).
The digestibilities of NSPs are lower than those of starch and sugars. Furthermore, the efficiency of utilisation of energy derived from digested NSP is about 30% lower than that of starch and sugars. This has a direct implication for the supply of effective energy to the animal (de Lange, 2000).

Soyabean NSP mainly consists of arabinans, arabinogalactans and acidic polysaccharides (pectic type compounds), and approximately one-third of the NSP are reported to be soluble (Bach-Knudsen, 1997). A fraction of the soyabean NSP, probably an arabinogalactan, is reported to be highly viscous (Thompson *et al.*, 1987).

Many animal feeds contain varying amounts of different NSP. Soluble NSP constituents seem to increase intestinal viscosity and have greater effects than non-soluble NSP. In birds, soluble NSP such as mixed-linked β-glucans in barley (Alimirall *et al.*, 1995), arabinoxylans in rye (Bedford and Classen, 1992) or wheat pentosans (Choct *et al.*, 1996) disturb the absorption of nutrients, especially fat, associated with increased viscosity of the intestinal content (Refstie *et al.*, 1999).

Viscosity is defined by the "Collins Gem English Dictionary" as: Thick and sticky. As described above, different feed constituents affect the viscosity of intestinal digesta, depending on their solubility and own viscosity. This is especially prevalent with the NSP constituents of feed ingredients. The variability in viscosity of intestinal contents mainly depends on the dietary Water Soluble NSP (WSNSP) level and, therefore, on the level of cereals such as barley, rye and wheat (Carre, 1992).

Viscosity can be measured in many different ways. Mostly, viscosity is measured relative to water viscosity. One of these measurements are measured by flow rate in the CANNON-FENX viscosimeter, according to the procedure of Choct and Annison (1992). Real Applied Viscosity (RAV) is the relative viscosity corresponding to the ratio of the viscosity of the diet in acetate buffer (0.2 M, pH 4.5) to that of the buffer. The RAV is the ratio of applied viscosity divided by the grams of diets per millimeter of buffer added to the diet. It can be measured with a Rheomat 115 A (a rheonanalyser) (Carre *et al.*, 1999).

Triticale, barley and rye showed viscosity levels reaching 7, 20 and 27 mL/g, respectively, and some wheat varieties may have considerable viscosity levels reaching 5 mL/g (Carre *et al.*, 1994). Critical viscosity levels of diets are considered to be higher than 6 mL/g and bioavailability of nutrients can be inhibited at this level (Carre *et al.*, 1999).

a) Effects on Digestive Parameters

The broiler chicken's digestive tract is especially sensitive to the presence of soluble NSP (such as β-glucans and arabinoxylans). Soluble NSPs are proven to increase the viscosity and microbial activity of digesta, and reduce its rate of passage through the small intestine (Choct *et al.*, 1995). In particular, viscous digesta have been related to longer retention times in the gastrointestinal tract (Van Der Klis and Van Woorst, 1993). As a consequence, viscous digesta promotes significant decreases in organic matter digestibility, voluntary feed intake and productive performance (Gasa *et al.*, 2000).

Soyabean products have been extensively tested in broiler feed to measure NSP content and effects on dietary viscosity and dietary parameters. Soyabean meal was both the most NSP rich and viscous compared to soya protein concentrates (Refstie *et al.*, 1999). In chickens, the digestibility of dry matter coincided with the content of indigestible soyabean NSP (Refstie *et al.*, 1999). Digestibility of nitrogen, starch, phosphorous and calcium was lower in the soyabean meal diet (high NSP) compared to the Isolated Soya Protein and Soya Protein Concentrate, which contain low NSP levels. Fat digestibility was also lower with ingestion of the soyabean meal. An interesting observation is that the cholesterol blood level was higher in the low NSP diets than the soyabean meal diet (Refstie *et al.*, 1999).

These experiments by Refstie *et al.* (1999), indicated a negative effect of soyabean NSP on the digestive processes in chickens. It was also found by Carre *et al.* (1999) that highly viscous raw materials affected zinc availability in the young chick.

The effect of high NSP levels on blood cholesterol is also related to the effect of viscosity and NSP on fat digestion. A reduced blood cholesterol level has been observed after high intakes of soyabean NSP (Lo and Cole, 1990). This is probably associated with the binding or trapping of bile salts in the diets due to higher viscosities, as seen for galactomannans from the Indian Cluster Bean (guar gum) in rats (Levrat *et al.*, 1996; Favier *et al.*, 1997).

Due to the increased microbial activity in the small intestine with high viscous digesta (Annison, 1993), digestibility of nutrients will also be reduced due to the increased microbial activity.

Fengler and Marquardt (1988) also proved a reduction in digestion and absorption of dietary nutrients in the presence of NSP and increased viscosity.

The negative effects of increased dietary NSP levels on the digestibility and rate of absorption of nutrients from starch, protein and fats involve several mechanisms in reducing the rate and extent of apparent nutrient digestion and absorption (de Lange, 2000):

- The endogenous secretions and losses of enzymes and mucus and the sloughing of mucosal cells are likely increased when intake of NSP is increased.
- Endogenous secretions, such as bile acids, can be bound, particularly by viscous or gelling and lignified NSP thus reducing the effect of recycling.

These effects result in reductions in the apparent nutrient digestion (de Lange, 2000):

- Viscous NSP in particular, will interfere with digesta movement and the mixing of digestive enzymes and nutrients in the lumen.
- NSPs can also increase the resistance of the unstirred water layer at the intestinal surface layer.
- NSPs from cell walls can physically hinder the access of digestive enzymes to nutrients that are enclosed inside cell walls.
- Soluble NSPs stimulate microbial growth and increase the amount of microbial protein and fat at the terminal ileum or faeces. Selected NSPs may also stimulate the growth of toxin producing microbes, which may affect gut health directly and digestive function indirectly.
- Feeding NSPs may alter intestinal morphology and the capacity of the gut to absorb nutrients.

The effect of NSPs on the viscosity of digesta is influenced by factors such as feed processing, the presence of endogenous hydrolytic enzymes in the plant material, exogenous enzymes, as well as microbial fermentation and enzymatic digestion in the intestine (de Lange, 2000). Associated with viscous feed ingredients in chickens is also the problem of wet and sticky droppings, which adhere to the birds and dirty the cages (Svihus *et al.*, 1997). This was shown by Refstie *et al.* (1999) and was demonstrated by dirty cages when feeding diets with high levels of viscous soya products.

Different mechanisms have been used to explain the detrimental effects of dietary NSP on poultry digestion. The simplest explanation is that β-glucans and arabinoxylans from the endosperm cell wall could restrict access of digestive enzymes to nutrients found in the cereal's endosperm (Bedford, 1996).

An alternative explanation that has been more widely accepted, is that soluble NSP promotes increases of digesta viscosity, which could affect intestinal digestion by limiting the diffusion of digestive enzymes and nutrients, and through primary or secondary increases on the microbial activity in the small intestine (Gasa *et al.*, 2000). Molecular weight and degree of branching of the polysaccharide chain mainly determine the so called intrinsic viscosity, i.e. the fractional increase in viscosity per unit concentration polymer (Morris *et al.*, 1981). Bedford and Classen (1992) reported a positive correlation between high molecular weight carbohydrate complexes (molecular weight >500 000) and viscosity in the intestine of chickens fed diets varying in pentosan content. It has been suggested that a viscosity of a polysaccharide solution of approximately 10mPa's indicates a critical polysaccharide concentration above which viscosity responds more steeply to increasing polysaccharide concentrations. This is thought to be the result of the onset of the formation of an entangled network of individual polysaccharide coils (Morris *et al.*, 1981).

It is also interesting to note that there was a reduction in weight gains and nitrogen retention when soyabean hulls were used in the diet instead of starch or sorghum hulls in the experimental diet. This could indicate an anti-nutritional effect of the hull fraction (Wenk & Messikommer, 1991). Thus, the reason for a lack of anti-nutritional effects of NSP in soyabean meal in other research could be due to the use of soyabean meal made from dehulled soya (Refstie *et al*., 1999).

Iji *et al*. (2001) concluded that there are no reports on the direct inhibition of intestinal enzyme synthesis by NSP but the activities of most enzymes may be reduced through the coupling to NSP or physical restriction of enzyme access to the substrate.

It was also noted by Carre (1992) that high viscosities in the small intestine leads to a reduction in enzyme activities, absorption efficiencies, hypertrophy of the pancreas and of the small intestine, alteration in intestinal mucosa and stimulation of intestinal bacterial activity.

b) Effects on Performance Parameters

It has been shown that feeding diets with high NSP levels have a negative effect on animal performance. Broiler chickens fed on maize (low NSP level in diet) and barley-wheat (medium NSP level in diet) showed higher live weights at 22 days than those fed on carboxy-methylcellulose based maize (high viscosity) (Gasa *et al*., 2000).

It was found in an experiment conducted by Iji *et al*. (2001), that over a period of seven days of feeding diets with various viscosities, there were significant decreases in body weight and weight gain in chickens supplemented with guar gum and gum xanthan (high NSP levels) as well as a deterioration in the feed conversion ratio. Although the more viscous diets did not affect feed intake over the first period of exposure, they reduced weight gain and final body weight, suggesting a reduction in nutrient content or utilisation. This is reflected in the high feed conversion ratio of highly viscous diets (Iji *et al*., 2001).

There were also significant differences in voluntary intake and feed conversion ratios observed in the trial by Gasa *et al*. (2000). Monogastric animals attempt to increase feed intake when given diets that contain increasing levels of NSP. This is probably an attempt to maintain the rate of available energy intake. Increases in feed intake coincide with decreases in digesta retention time and thus reduced exposure time of digesta to digestive enzymes. However, feed intake will be limited and digesta retention time may be increased at high NSP contents, presumably because of the bulking effects of NSP. This in turn, may be determined by the digestibility of NSP, the water holding capacity of NSP, or the mass of microbes (Kyriazakis and Emmans, 1995; Smits, 1996; de Lange, 2000).

c) Summary

NSPs have shown to have the following effects on poultry performance according to the respective studies:

- Decrease voluntary feed intake (Kyriazakis and Emmans, 1995)
- Decrease the supply of available energy to the animal, including the digestibility and utilisation of nutrients other than NSPs (Dierick *et al.*, 1989; Annison 1993; Danicke *et al.*, 2000)
- Negatively affect gut and animal health (Smits, 1996)
- Decrease weight gain (Danicke *et al.*, 2000)

High levels of NSP in the diet induce high viscosities and lower animal performance, nutrient digestibility and nutrient availability.

Similar detrimental effects on growth performance and digestibility have been obtained in broiler chickens after consumption of isolated viscous NSP such as carboxy-methylcellulose (CMC) (Smits *et al.*, 1997), pentosans from wheat (Choct *et al.*, 1996), and glucans from barley (White *et al.*, 1981).

The digestible NSP content of a feedstuff should be determined when evaluating its available energy content. Furthermore, diet NSP contents have considerable effects on digestibility of other dietary nutrients, voluntary feed intake, metabolism of nutrients after absorption and gut health. These effects can be attributed to the effects of NSPs on gut microflora, viscosity and water holding capacity of the digesta, and to the mechanical properties of NSP. There are likely to be threshold values below which NSP levels do not effect animal performance. In fact low inclusion levels, selected NSPs or oligosaccharides may stimulate digestive function and improve gut health (de Lange, 2000).

2.5. IRRADIATION OF ANIMAL FEEDSTUFFS

In some feedstuffs the utilisation of available carbohydrates, proteins and other nutrients is much less than that calculated from the chemical composition because of the presence of anti-nutritional or anti-physiological substances such as trypsin inhibitors, chymotrypsin inhibitors, lectins, phytates, goitrogens, glucosinolates, cyanogenic glucosides, oligosaccharides, polyphenols, toxic non-protein amino acids, anti-vitamins, allergens, alkaloids, saponins (Siddhuraju *et al*., 2002).

Conventional processing methods can reduce, but are not able to completely remove, these anti-nutritional factors present in seeds, grains and feed materials and a combination of processing methods is generally more effective than a single method (Siddhuraju *et al*., 2002).

Ionising radiation is commonly used to decontaminate food to increase the shelf life of fresh and dry food materials (Molins, 2001). Food/feed irradiation is a physical process involving an energy input, that does not induce radioactivity in foods. The amount of energy input is called the radiation absorbed dose and is measured in Grays (1Gy = 1 J/kg) (Siddhuraju *et al*., 2002). It is similar in nature to the use of heat via either thermal (infrared) or microwave energies. In contrast to the gross and easily detectable effects that conventional heat treatments have on foods, the radiation dose generates minute and mostly undetectable changes in chemical composition. This is due to the nature of radiation and the selectivity and high efficiency with which it is transferred to the orbiting electrons in the atoms constituting food molecules or contaminants. When the activated orbiting electrons leave the atom, chemical changes occur in the atoms and molecules. This process is called ionisation, that is, the formation of positively charged atoms or molecules known as cations, formed by losing a negatively charged electron. The free electron is rapidly trapped by surrounding atoms, forming negatively charged anions. The ionisation process forms highly reactive atoms and molecules called free radicals. Most of the absorbed radiation energy is used in generating free radicals and in inducing chemical reactions between radicals or between radicals and other molecules. A minimal fraction of the absorbed energy is converted to thermal energy. Without heat or with only minimal heat, the freshness and the typical sensory and nutritional properties of foods are preserved (Siddhuraju *et al.*, 2002).

Free radicals formed in the irradiation process are not unique or different in nature or reactivity from those formed in biological or other cooking processes. Dry heating and conventional cooking result in the production of considerably higher amounts of free radicals than irradiation. Irradiation at 10kGy produces only a 2.4°C temperature increase in 1kg of food with the heat capacity of water (4.184 J/°C). This is about 3% of the energy required to boil 1 litre of water at 100°C. The FDA and international standards are considered safe because no pattern or trends of toxicological significance have been established (WHO, 1981; FDA, 1986).

Irradiation causes chemical changes in foods in amounts directly related to the radiation dose. Several statements about these foods can be made (Siddhuraju *et al.*, 2002):

1) Basic radiation chemistry kinetics and mechanisms allow prediction of the amount of radiolytic products formed,

2) Types of reactions induced by radiation are (none of which are unique to the radiation process):

    a) Oxidation of metals and ions

    b) Oxidation and reduction of carbonyls to and from hydroxyl derivatives

    c) Elimination of double bonds

    d) Decrease of aromaticity in aromatic and heterocyclic compounds

    e) Hydroxylation of aromatic and heterocyclic compounds

3) No unique radiolytic product can be detected with current analytical methods in foods irradiated under established guidelines and/or commercial conditions

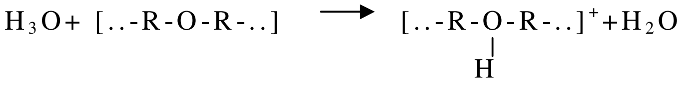
4) All radiolytic products formed as a result of radiation-induced chemistry are already known and are formed in larger amounts than during the application of conventional food/feed processing methods.

Irradiation of high molecular weight carbohydrates in the solid state, as well as in their aqueous solutions, causes the breaking of the external ether bridges. Two mechanisms can be assumed which take place simultaneously (Siddhuraju *et al.*, 2002):

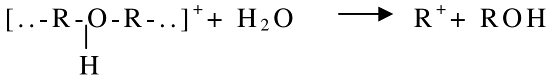
a) Direct action of radiation on the oxygen bridges in the solid state leads to the formation of an -O- radical; then the -O-C- linkage to the next hexose unit is split off producing a positive ion at the carbon atom and a glycosyl radical. In aqueous solutions H_3O^+ ions are formed as the primary species that are sufficiently energetic to hydrolyse the glycosidic bond according to the mechanism of normal acid hydrolysis (Figure 2.1).

$$H_2O \longrightarrow H_2O^+ + e^-$$

$$H_2O^+ + H_2O \longrightarrow H_30^+ + OH^-$$



$$H_3O + [..\text{-}R\text{-}O\text{-}R\text{-}..] \longrightarrow [..\text{-}R\text{-}\underset{\overset{|}{H}}{O}\text{-}R\text{-}..]^+ + H_2O$$



$$[..\text{-}R\text{-}\underset{\overset{|}{H}}{O}\text{-}R\text{-}..]^+ + H_2O \longrightarrow R^+ + ROH$$

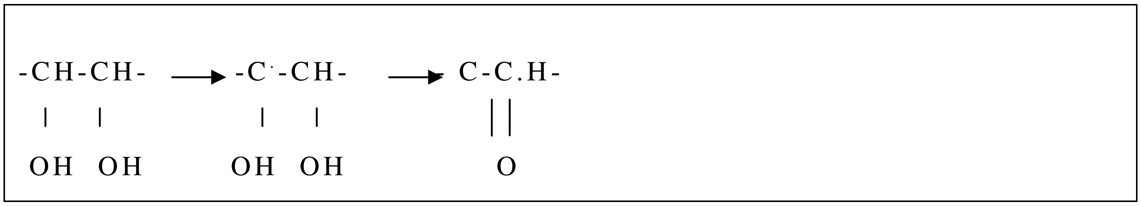
$$R^+ + 2H_2O \longrightarrow ROH + H_3O^+$$

$$H_3O^- + OH^- \longrightarrow 2H_2O$$

Where R is the hexose unit

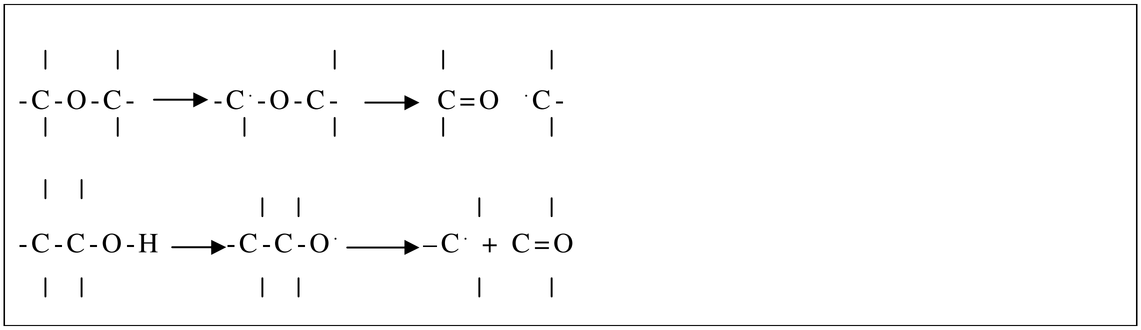
Figure 2.1. Illustration of the direct action of radiation on the oxygen bridges in the aqueous state (Siddhuraju *et al.*, 2002)

b) Irradiation, directly or indirectly, causes alterations of some of the monosaccharide units in the polymer chain by radiation-induced dehydration (Figure 2.2 A) and *β*-splitting (Figure 2.2 B) hereby causing deoxycarbonyl or deoxyacid groups in the chain (Siddhuraju *et al*., 2002).



$$\text{-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-} \rightarrow \text{-}\underset{\text{OH}}{\underset{|}{\text{C}^{\cdot}}}\text{-}\underset{\text{OH}}{\underset{|}{\text{CH}}}\text{-} \rightarrow \text{C-}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{.H-}$$

Figure 2.2 A. Alteration of monosaccharide units in the polymer chain by radiation-induced dehydration (Siddhuraju *et al*., 2002)



$$\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-O-}\overset{|}{\underset{|}{\text{C}}}\text{-} \rightarrow \text{-}\underset{|}{\text{C}^{\cdot}}\text{-O-}\overset{|}{\underset{|}{\text{C}}}\text{-} \rightarrow \overset{|}{\underset{|}{\text{C}}}\text{=O}\quad {}^{\cdot}\overset{|}{\underset{|}{\text{C}}}\text{-}$$

$$\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-O-H} \rightarrow \text{-}\overset{|}{\underset{|}{\text{C}}}\text{-}\overset{|}{\underset{|}{\text{C}}}\text{-O}^{\cdot} \rightarrow \text{–}\overset{|}{\underset{|}{\text{C}^{\cdot}}} + \overset{|}{\underset{|}{\text{C}}}\text{=O}$$

Figure 2.2. B. Alteration of monosaccharide units in the polymer chain by β-splitting caused by irradiation (Siddhuraju *et al*., 2002)

Phytic acid in cereals, chelates mineral cations and proteins, forming insoluble complexes, which leads to a reduced bioavailability of trace minerals and a reduced digestibility of proteins (Reydan and Selvendran, 1993). It was found in one trial that the treatment of soyabeans with irradiation, alone or in combination with soaking, reduces the level of phytate compared to controls (Sattar *et al*., 1990). This reduction is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the irradiation. Another possible mode of phytate loss during irradiation could have been through cleavage of the phytate ring itself (Siddhuraju *et al*., 2002). Nene *et al*. (1975) demonstrated that the irradiation of Red Gam protein increased its *in vitro* digestibility.

The observed enhancement in proteolytic digestion was attributable either to the degradation of protein fragments making them more susceptible to enzymes, or to partial destruction of trypsin inhibitors (Siddhuraju *et al.*, 2002).

Campbell *et al.* (1986) and Campbell *et al.* (1983) showed that irradiation of oat groats and rye respectively, improved the nutritional value of the two cereals. This was attributed to the partial breakage of the polymeric structure of the viscous carbohydrate of the two cereals. This decreased their intestinal viscosity and produced a corresponding reduction in their anti-nutritive effects (Wang *et al.*, 1997). Wang *et al.* (1997) showed no beneficial effects of irradiation of rice bran at 10 or 50 kGy with respect to its nutritive value. This lack of response was probably not caused by a failure to break the rice bran polysaccharides as the dosage was of sufficient energy to bring this about. Irradiation may rather have been ineffective due to the absence of viscous carbohydrates in rice bran. Under such conditions no change in viscosity will occur with irradiation and, therefore the corresponding improvements associated with these changes would not be seen (Wang *et al.*, 1997).

Gamma irradiation has been reported to increase the food value of rye (Campbell *et al.*, 1983) and hull-less barley (Campbell *et al.*, 1985) for chicks. These improvements noted for the rye and hull-less barley are thought to be the results of depolymerisation of soluble pentosans and β-glucans, respectively (Siddhuraju *et al.*, 2002).

These carbohydrate polymers cause the intestinal contents to become viscous and interfere with the nutrient assimilation and the general well-being of the chick and irradiation has been found to improve:

- Apparent absorption of fat, amino acids and starch in rye and hull-less barley (Campbell *et al.*, 1983; Campbell *et al.*, 1985).
- Hull-less oats and hulled oats irradiated showed significant improvements in weight gain to feed ratio of chicks (Campbell *et al.*, 1986).
- Apparent fat retention and tibia ash were higher in chicks fed irradiated hull-less oats than in those fed untreated hull-less oats (Campbell *et al.*, 1986).

Irradiation appears to benefit cereals containing soluble or mucilaginous fibre types, as typified by the β-glucans of barley and oats. These fibres appear prone to irradiation-induced depolymerisation, as indicated by the increased β-glucan solubility and the reduced extract viscosity of irradiated barley and oat samples (Siddhuraju *et al*., 2002).

Campbell *et al*. (1987) evaluated the use of oat groats. Poor performance of 3-week old chicks was noticed with body weight, feed conversion and fat absorption being severely affected when the oat groats were included at 20%, 40% and 60% of the diet. These problems can be rectified by the addition of an enzyme containing β-glucanase activity and by the irradiation of the oat groats before addition to the diet (Campbell *et al*., 1987). Siddhuraju *et al*. (2002) found that the irradiation of three different species of unconventional legume (*Sesbania*) and one species of a common legume of *Vigna*, at a dose of 6 kGy, reduced the viscosities of the different species. It was speculated that this was to be due to the depolymerisation of the non-starch polysaccharides present in the seeds.

Improvements in performance corresponded with radiation induced damage to rye polysaccharides, as indicated by reduced viscosity and increased concentrations of reducing sugars, in diets containing irradiated rye (0-100 kGy) (Campbell *et al*., 1983). Brown (1979) speculated that fibre suspensions in general may impede nutrient diffusion, thereby influencing the digestion and absorption process. Gamma irradiation may alleviate this effect and improve the digestion and retention of most nutrients by depolymerisation and consequent disruption of the gel network (Siddhuraju *et al*., 2002).

A joint FAO/IAEA/WHO study group on high-dose irradiation reviewed data relating to the toxicological, nutritional, radiation chemical and physical aspects of food irradiated to doses above 10 kGy and concluded that foods treated with doses above 10 kGy can be considered safe and nutritionally adequate when produced under established Good Manufacturing Practice (WHO, 1999). Free radicals and other compounds produced by the irradiation of food are identical to those produced during cooking, steaming, roasting, pasteurisation, freezing and other forms of food preparation (Siddhuraju *et al*., 2002). As with pasteurisation, the evidence suggests that food irradiation can only expect to improve the quality of food supply and that the products produced during irradiation pose no unique threat to human beings or animals (Siddhuraju *et al.*, 2002).

2.6. INTERACTIVE EFFECTS BETWEEN DIETARY FAT TYPE AND EXOGENOUS CARBOHYDRASE ENZYMES IN BROILER DIETS CONTAINING HIGH CONTENTS OF NON STARCH POLYSACCHARIDES

Smits and Annison (1996) explains the most potent and probably the most practical interaction between NSPs and lipid digestibility:
'Ingestion of NSPs increases the viscosity of gut contents and increases fermentation and bacterial colonisation. The raised viscosity of the gut content increases retention time of the digesta, increases the thickness of the unstirred water layer adjacent to the mucosa and reduces diffusive and convective transport. Collectively these changes result in reduced solubilisation of fat and possibly, hydrolysis of fat and protein. Nutrient uptake at the mucosal surface is reduced and uptake is also reduced by the effects of the enhanced bacterial growth on the proliferation rate of enterocytes and changes in the morphology of the villi and microvilli. Microbial activity increases the deconjugation and loss of bile acids, impairing the return of bile acids to the liver and subsequent recycling. Finally endogenous nutrient losses may be enhanced by digestion of viscous NSPs.'

Intestinal viscosity also plays a role in fat digestion and often inhibits it (Danicke *et al.*, 2000). Intestinal viscosity was found to interfere with the digestibility of saturated fatty acids more dramatically than with the digestibility of unsaturated fatty acids based on rye (Danicke *et al.*, 1997b) and barley based diets (Danicke *et al.*, 1999c). Furthermore, fatty acid utilisation from saturated fat sources such as beef tallow, can be improved through the addition of small amounts of unsaturated fats such as plant derived oils (Leeson and Summers, 1976; Danicke *et al.*, 2000).

A reduction in gut motility, caused by increased viscosity, decreases the mixing of digesta with pancreatic and biliary secretions, which is of particular importance for emulsification of saturated fatty acids and the formation of micelles. As intestinal viscosity increases and mixing is decreased, the thickness of the Unstirred Water Layer (UWL), an extracellular barrier covering the intestinal microvilli, is proportionally increased (Lund *et al.*, 1989). Westergaard and Dietschy (1976) proposed that the principle role of the micelle is to overcome the resistance of the UWL. Therefore, an increase in the thickness of the UWL would increase this resistance and decrease the transfer rate between intestinal bulk and the absorptive site, which is especially important for saturated fatty acids. Lund *et al.* (1989) showed a significant reduction in cholesterol absorption even at low concentrations of oat gum and continued to fall as the concentration increased, but proportionally less with each increase.

This initial decrease could indicate a sieving effect of dispersed β-glucan polymers on the micelles, which have a relatively high molecular volume (Lund *et al.*, 1989). This decrease coincided with a relatively low *in vitro* viscosity of approximately 5-20 mPa, which is in the range where polysaccharides in solution begin to form an entangled network of individual polysaccharide coils, associated with a steep incremental increase in viscosity (Morris *et al.*, 1981). Higher intestinal lipid concentrations are paralleled by a corresponding increase in the size of the formed micelles (Ockner *et al.*, 1972). This can also explain why tallow feeding is more sensitive to small increases in intestinal viscosity when compared to soya oil feeding. High viscosity has also been shown to affect emulsification negatively, and to reduce lipolysis (Pasquier *et al.*, 1996).

Low bile acid concentration in the digesta of young birds may limit lipid absorption (Inarrea *et al.*, 1989) and make lipid digestibility especially sensitive to the presence of dietary viscous NSP (Danicke *et al.*, 1995). Increases in microbial activity in the small intestine (Annison, 1993) could promote the deconjugation of bile acids and significant decreases in the digestibility of dietary fat (Gasa *et al.*, 2000).

Consumption of carboxy-methylcellulose base maize (maize-CMC), which is highly viscous, also reduced nutrient apparent digestibilities, associated with an increase in digesta viscosity. Fat digestibility was reduced considerably in this experiment (Gasa *et al.*, 2000). A similar pattern was also shown in birds fed wheat pentosans (Choct and Annison, 1992).

Numerous studies with birds have established an influence of the chemical composition of the diet on the utilisation of dietary fat and on its metabolisable energy value, indicating that chain length and degree of saturation of the constituent fatty acids will have an important effect upon the nutritive value of the diet (Blanch *et al*., 1995).

It is possible to increase the utilisation of fats high in saturated fatty acids by increasing the proportion of fats or oils high in unsaturated fatty acids, in the diet (Danicke *et al*., 2000), which influences the metabolisable energy contents of fats or fat blends. This is due to the emulsifying capacity of unsaturated fatty acids and the dependence of long chain saturated fatty acids on such emulsifying agents for more efficient digestion and absorption (Danicke *et al*., 2000).

It was shown that if a carbohydrase enzyme, xylanase, was added to diets high in NSP, the greatest effect would be seen in the diets with the highest tallow concentrations or otherwise fats with a higher content of saturated fatty acids (Danicke *et al*., 2000).

This is specifically so with regard to animal fats and plant oils (Danicke *et al*., 1997a; Langhout *et al*., 1997), confirming that intestinal viscosity does indeed interfere in fat digestion. It was also stated that xylanase's effects depend on the dietary fat type, especially when diets are high in pentosans. Carbohydrase supplementation of diets based on barley, rye or wheat has been shown to accelerate the gastrointestinal time in chickens (Danicke *et al*., 1997a). In turn, an increase in intestinal viscosity has shown to have a negative effect on feed intake and performance. Danicke *et al*. (1997a) showed that xylanase addition to a rye-based diet, resulted in accelerated transit times for broilers fed on soya oil and beef tallow.

Not only do enzymes, but processing methods have ameliorating effects on the actions of NSPs too. It has been shown that the use of irradiation may have positive nullifying effects of diets high in NSPs. Siddhuraju *et al*. (2002) showed that by using ionising radiation on unconventional legume seeds, reduced viscosities were seen in broiler digesta. It was suggested that this was due to the depolymerisation of NSP in the treated seeds. This processing method will therefore have obvious positive influences on the utilisation of feed and bioavailability of nutrients in the broiler chicken. Irradiation also has negating effects on other anti-nutritional factors such as protease inhibitors (especially trypsin inhibitors), alpha-amylase inhibitors, phytohaemagglutins (lectins), oligosaccharides, phytates and tannins.

However, Svihus and Gullord (2002) demonstrated a pronounced negative effect of viscosity on the nutritional value of barley grain, even when enzymes are used.

The effects of dietary unsaturated fatty acids and saturated fatty acids, and their interactions with intestinal viscosity and effectiveness of exogenous enzyme addition, have not been extensively studied with the addition of processing such as irradiation. These interactions and effects have a practical importance, as broiler producers are constantly looking for ways to improve the feed utilisation of their production unit.

Interactions between nutrients, exogenous enzymes, and other dietary components have always been of interest to the animal nutritionist as these interactions and effects influence the utilisation of the feed and therefore, the animal’s performance. The above interactions and effects are important to broiler producers as the interactions and processes effect fat digestibility and Apparent Metabolisability of the feed, a very important component of the diet to a fast growing animal.

Irradiation may also improve the utilisation of feed due to higher nutrient bioavailabilities and by inactivating anti-nutritive compounds in the feed. It may also improve fat digestibility due to the depolymerisation of NSPs in grains. Taking into account these interactions and actions of compounds in the feed, as well as processing methods, a diet that can be utilised to a much greater degree can be produced knowing these necessary facts.

The interactions between lipid types and rations containing high levels of NSP is important to determine, especially in countries using rations high in NSP. The value of additives or processing methods in negating negative interactions or effects of lipid type and rations high in NSP is important for the animal feeding industry, to manufacture diets to produce the best results at the greatest efficiency.

CHAPTER 3. MATERIALS AND METHODS

a) Experimental Design and Treatments

Ten iso-nitrogenous treatment diets were fed to broilers in two parts of the trial, a performance and a digestibility trial. The ten treatments used in the trial are summarised in Table 2. Each diet's raw material composition and calculated feed specification are shown in Table 3, while the analysed feed specification of the diets are shown in Table 4.

Two control diets with low NSP levels were based on maize and included either 10% soya oil or yellow grease (a spent fat) as main lipid source, each with different fatty acid profiles (Table 3).

A high NSP diet containing 55% barley was used as basal diet for the other 8 treatments. Half of these diets contained barley which was pre-irradiated, while the barley of the other half remained unprocessed.

Table 2. Treatment diets used in the performance and digestibility trial

Treatment	**Grain**	**Irradiated**[1]	**Lipid type**[2]	**Enzyme inclusion**[3]
1	Maize	No	Soya oil	No
2	Maize	No	Yellow grease	No
3	Barley	No	Soya oil	No
4	Barley	No	Soya oil	Yes
5	Barley	No	Yellow grease	No
6	Barley	No	Yellow grease	Yes
7	Barley	Yes	Soya oil	No
8	Barley	Yes	Soya oil	Yes
9	Barley	Yes	Yellow Grease	No
10	Barley	Yes	Yellow grease	Yes

[1] Irradiated at 10 kGy

[2] Included as 10% of the diet on an as is basis

[3] Enzyme complex containing glucanase, xylanase and cellulase (Roxazyme G, DSM Animal Nutrition, Kaiseraugst, Switzerland)

Either yellow grease or soya oil was then added at a level of 10% to these diets. The subgroups were again subdivided into treatments receiving either exogenous carbohydrase supplementation (Roxazyme G, DSM Animal Nutrition, Kaiseraugst, Switzerland) or no supplementation.

Table 3. Fatty acid profiles of yellow grease and soya oil

	Soya Oil [*] **%**	**YellowGrease %** [**]
C 12:0	-	0.35
C 14:0	-	0.93
C 16:0	9.49	28.0
C 18:0	3.16	4.20
C 18:1	24.6	40.5
C 18:2	44.0	24.4
C 18:3	6.51	0.66
Free Fatty Acids	2.13	13.2

*Danicke *et al.* (2000)

[**] Analysis of yellow grease used in trial

Roxazyme G contains an enzyme complex, derived from *Trichoderma viride,* with a wide range of enzyme activities and was included into the corresponding treatment diets at 150g/ton. The enzyme for this trial was sponsored by DSM Animal Nutrition, South Africa. The major enzyme activities of this complex are:

- Cellulase (endo-1,4-β-glucanase; EC 3·2·1·4)
- Xylanase (endo-1,4-β-xylanase; EC 3·2·1·8)
- β-glucanase (endo-1,3(4)-β-glucanase; EC 3·2·1·6)

The two control maize-based diets were not supplemented with Roxazyme G as these diets contained very low NSP levels.

The barley was irradiated before the treatment diets were mixed. Irradiation was conducted at Isotron South Africa (Pty) Ltd., Johannesburg. Using a Cobalt 60 Gamma irradiation source, the barley was loaded in bags onto a monorail and rotated around the source. The concrete walls were 3 meters thick that surrounded the radiation source. One rotation submitted the barley to approximately 2 kGy.

Table 4. Raw material composition and calculated feed specifications of the experimental diets on an as fed basis (g/kg)

	Treatment Diets									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Grain	**Maize**	**Maize**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**
Lipid type	**Oil**	**Fat[#]**	**Oil**	**Oil**	**Fat**	**Fat**	**Oil**	**Oil**	**Fat**	**Fat**
Irradiation[1]	-	-	-	-	-	-	+	+	+	+
Carbohydrase inclusion[2]	-	-	-	+	-	+	-	+	-	+
Ingredient										
Maize	550	550	-	-	-	-	-	-	-	-
Barley	-	-	550	550	550	550	-	-	-	-
Irradiated barley	-	-	-	-	-	-	550	550	550	550
Wheat bran	-	-	28	28	28	28	28	28	28	28
Gluten	19.5	19.5	-	-	-	-	-	-	-	-
Soya ocm 44%	170	170	170	170	170	170	170	170	170	170
Fishmeal	129.4	129.4	121	121	121	121	121	121	121	121
Bone meal	17.2	17.2	17.2	17.2	17.2	17.2	17.2	17.2	17.2	17.2
Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Limestone	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Premix	10	10	10	10	10	10	10	10	10	10
Salt	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Yellow Grease	-	100	-	-	100	100	-	-	100	100
Soya Oil	100	-	100	100	-	-	100	100	-	-
Enzymes	-	-	-	0.15	-	0.15	-	0.15	-	0.15

Feed specifications on an as fed basis	**Crude Protein**	**Lysine**	**Methion-ine**	**Ca**	**P**	**Total Dietary NSP***
Maize[*] diets	219.57	12.99	4.91	9.36	4.15	27.5
Barley[*] diets	219.57	13.29	4.48	9.29	4.29	92.0

[1] Irradiation at 10kGy

[2] Complex of cellulases, xylanases and β-glucanases (Roxazyme G)

* Total diet NSP levels calculated as maize or barley contribution to Total NSP using Smits and Annison (1996) cereal's NSP levels

[#] Yellow grease denoted as "fat"

Table 5. Determined analysis of experimental diets on an as fed basis (g/kg)

	Treatment Diets									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Grain	**Maize**	**Maize**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**	**Barley**
Lipid type	**Oil**	**Fat***	**Oil**	**Oil**	**Fat**	**Fat**	**Oil**	**Oil**	**Fat**	**Fat,**
Irradiation[1]	-	-	-	-	-	-	+	+	+	+
Carbohydrase inclusion[2]	-	-	-	+	-	+	-	+	-	+
Crude Protein	213	212	214	213	214	211	207	216	211	212
Crude Fat	103	105	109	103	107	104	104	103	104	105
Crude Fibre	20.0	21.9	35.2	35.5	35.4	33.9	34.6	35.6	33.5	35.4

[1] Irradiation at 10kGy

[2] Complex of cellulases, xylanases and β-glucanases (Roxazyme G)

* Yellow grease denoted as “fat”

The bagged barley was rotated around the radiation source for 5 laps to expose the product to 10 kGy. The length of time to complete one rotation or lap depended on the rate of decay of the Cobalt 60 source and was taken into acount to ensure that the barley was exposed to the correct amount of irradiation. On average, one rotation or lap took approximately 2 hours to complete.

The treatment diets were mixed every 4 days to avoid prolonged periods of storage that could lead to oxidation of the lipid sources. A feed mixer was used on the University of Pretoria's Experimental Farm. Commercially available broiler starter and finisher premixes were obtained from Epol – A division of Rainbow Farms (Pretoria, South Africa) that contained anti-oxidants to prevent rancidity of lipids in animal feed.

b) Performance Trial

600 Ross 788 broiler chickens obtained from a local hatchery, were randomly divided into 10 treatment groups, each with 3 replicates and 20 birds per replicate. The birds were housed on the experimental farm (University of Pretoria, Hatfield) in deep litter pens in an environmentally controlled broiler house, with a constant lighting schedule and *ad libitum* access to feed and water. The chicks were fed a commercially available starter crumble for the first seven days of age to allow for development of the gastrointestinal tract before the commencement of feeding the high fat diets as from day 8. Body weights were recorded weekly, mortality and feed intakes were recorded daily up to 35 days of age, when the performance trial was terminated.

c) Digestibility Trial

After the termination of the performance trial, 5 birds per pen, uniform in weight and size, were selected and randomly assigned to metabolism cages for the conduction of the digestibility trial. Water and treatment diets were available *ad libitum*, and a constant lighting schedule was used.

The use of markers is particularly useful in digestibility trials, especially when animals are fed in a group and when it is impracticable to measure feed intake and faeces output directly. If the concentration of the marker is known in the feed and the concentration is determined in a sample of the feces, the ratio between these concentrations gives a good estimate of digestibility. Chromic oxide is very insoluble and hence indigestible, essential characteristics of a marker.

From day 35, chromic oxide was added at a rate of 5g/kg to all diets as an indigestible marker. A 3-day adaptation period was used. Feed intakes and total faecal collection and weighing were done daily from day 38 to 42. The samples were frozen immediately after collection.

The birds were then sacrificed on day 42 by cervical dislocation. The gastrointestinal tracts were immediately exposed and segmented into the duodenum, jejunum and ileum. The total digesta from each segment was collected and weighed. The duodenum was defined from the pylorus to the entrance of the main biliary and pancreatic ducts, the jejunum from the end of the duodenum to Meckel’s Diverticulum, and the ileum from the end of the jejunum to approximately 1cm from the ileo-caecal-junction. The digesta was then frozen immediately for transport to the laboratory, where it was immediately freeze dried.

d) Sample Analysis

Excreta and digesta samples were freeze dried to minimise nutrient loss and then ground, to prepare for analysis. Feed dry matter was determined by setting 2g samples in an oven set at 105^{o}C, overnight. The samples were then weighed back after 24 hours.

Crude fat content of the feed, ileal digesta and faeces was determined by ether extraction using a Büchi Soxhlet (AOAC, 2000). Petroleum ether was used as a reagent, heated to approximately 50^{o}C. 3g of sample was weighed onto a round piece of filter paper of 12.5 cm diameter, which was then folded and inserted into the extraction thimble. After extraction, the flasks were put into an oven at 70^{o}C for 5 hours, cooled in a dessicator and then weighed.

The gross energy value of the treatment diets and faeces were determined by complete oxidation using an adiabatic bomb calorimeter with oxygen ignition (MC – 1000 Modular Calorimeter).

The chromic (III) oxide in the feed, ileal digesta and faeces was determined using a Varian Spectrometer (absorbance measured at 425.4nm) after acid extraction of the marker, to determine apparent digestibility coefficients of the crude fat and the metabolisability of the gross energy by a standard calculation (Jamroz *et al.*, 2002):

$$\text{Digestibility} = 1 - \left(\frac{Cr_2O_3 \text{ in diet}}{Cr_2O_3 \text{ in digesta/excreta}}\right)\left(\frac{\text{Nutrient or E in digesta or exreta}}{\text{Nutrient or E in diet}}\right)$$

Pyrex glassware was used for the acid extraction of the marker, which was cleaned thoroughly with detergent and deionised water and soaked in diluted nitric acid overnight. The glassware was then rinsed in deionised water and dried before use. 3mL nitric acid was used during the acid digestion of the sample, heated under a ventilated hood. Hydrochloric acid was then added to dissolve any residue. The solution was then diluted with deionised water in preparation for the chromium determination using the mass spectrometer.

Five standards were used for the chromium determination, 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L and 40 mg/L. A calibration standard of 0 mg/L was also used and after every 10 samples, the spectrometer was recalibrated. The calibration Flame stoichiometry was reducing, using acetylene as fuel and air as support, giving a rich yellow flame. A 10 mA lamp current was used for the chromium analysis. The slit width was set to 0.2 nm.

Crude Fibre was determined in feed samples using the Fibertec 2010 system using 0.313M sodium hydroxide and 0.128M sulphuric acid. The sintered glass crucibles were brushed free and rinsed thoroughly with a hydrochloric acid solution and dried before use. 1g samples were weighed into the crucible, which was then placed in the hot extraction unit and locked into position. The reagents and sample were boiled for 30 minutes, before the crucible was vaccumed and rinsed. The samples were then dried overnight at 105°C before being cooled and weighed.

Nitrogen content of the feed, ileal digesta and faeces samples was determined using the LECO system. There are three phases during the analysis cycle – purge, burn and analyze. During the purge stage the encapsulated sample is placed in the loading head, sealed and purged of any atmospheric gases in the container, tank and all gas lines. The sample is then dropped into a hot furnace of approximately 850°C and flushed with oxygen. The gases of combustion are mainly CO_2, NO, H_2O and N_2. The gases are collected in the tank and mixed. The homogenous mixture of gases is the sampled and this sample is passed over hot copper to remove oxygen and convert NO to N_2. It is then passed over Lecosorb and Anhydrone to remove the CO_2 and H_2O. A thermal conductivity cell then measures the N_2 content. The results are displayed as a nitrogen content and proetin content on a desk top computer used to control the LECO equipment (AOAC, 2000).

e) Statistical Analysis

The results were analysed by regression analysis and analysis of variance. Statistical analyses of the data were carried out using the Duncan's Multiple Range test. The linear regression analysis of results using the SAS Software System, was used to determine relationships between results (Statistical Analysis Systems, 1994).

CHAPTER 4. RESULTS

The results of the performance and digestibility trial are presented in Table 6 and Table 7. Graphs for the observed results of various parameters have also been presented in this chapter to show the general trends of the various treatment diets. Yellow grease used in the diets is denoted as “fat” to describe the saturated lipid type used in the treatment diets.

No significant differences in bird performance between maize and barley based diets were observed (Figures 3 and 4) when comparing Treatment 1 (maize and oil without enzyme) with Treatment 4 (Barley and oil without enzyme), and Treatment 2 (maize and fat without enzyme) with Treatment 6 (barley and fat without enzyme). Birds that received the maize-oil diet performed slightly, but not significantly, better than the birds on the maize-fat diet. Body weight and feed intake for the birds on the barley-oil (Treatment 4) diet were significantly higher ($P<0.05$) than for the birds on the barley-fat diet (Treatment 6). This elevation of feed quality was even more remarkable when the enzyme was added to the barley-fat diet, where a significant improvement in bird performance ($P<0.05$) was observed by comparing the difference in results of Treatment 3 (barley and oil with enzyme) and 4 compared to Treatment 5 (barley and fat with enzyme) and 6. No significant effects of barley irradiation, however, could be noted for bird performance without enzyme supplementation in the barley oil diets (Treatments 7, irradiated barley and oil with enzyme, and Treatment 8, irradiated barley and oil without the enzyme). Bird performance for the barley-oil diet (Treatment 4) were, although not significantly, poorer than the maize-oil (Treatment 1) diet.

The combined use of irradiated barley and supplementation of exogenous carbohydrase enzymes to a broiler diet containing high levels of soya oil, had a significant positive effect ($P<0.05$) on broiler performance. Even more notably is the significant superior body weight and feed intake figures ($P<0.05$) for the barley diet compared (Treatment 7) to the control maize diet (Treatment 1) where both irradiation and enzyme supplementation were used in the barley diet. The birds performed significantly better ($P<0.05$) in terms of body weight and feed intake compared to the control maize diet when the barley was irradiated and the treatment diets supplemented with the enzyme (Treatment 1 compared to Treatment 7 and Treatment 2 compared to Treatment 9 (Irradiated Barley and Fat with enzyme).

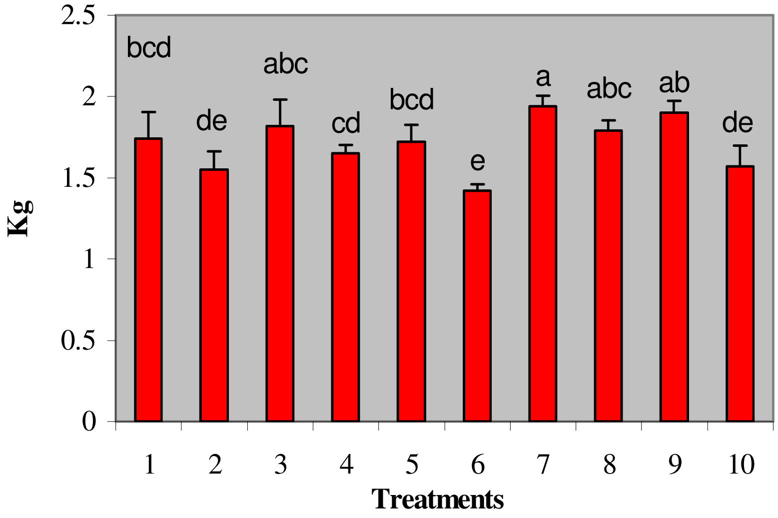


Fig 3. 35-day liveweight with the calculated standard error of the mean for each treatment

abcdef means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

The low NSP (maize based) diets had evidently higher fecal and ileal lipid digestibility coefficients and AME values (P<0.05) when compared with the high NSP (barley based) diets, for both the oil- and fat-containing rations, respectively (Treatment 1 compared to treatment 4 and Treatment 2 compared to Treatment 6). Lipid digestibility and AME were significantly higher ($p<0.05$) for the maize-oil diet than for the maize-fat diet (Treatment 1 and Treatment 2).

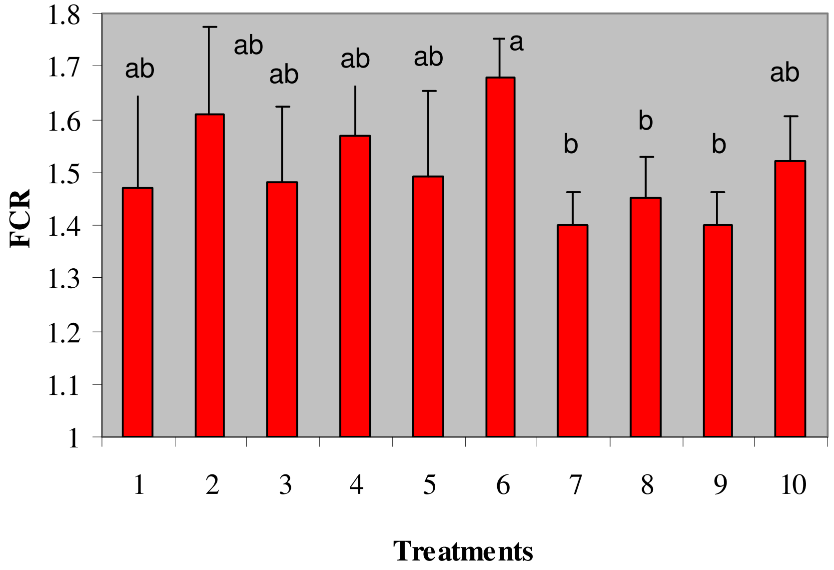


Fig 4. Cumulative Feed Conversion Ratio at 35 days of age with the standard error of the mean calculated for each treatment

^{abcdef} means treatments with the same superscript do not differ significantly ($P>0.05$) as determined by Duncans Multiple Range Test.

Treatments:
1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

The AME value and crude fat digestibility were significantly improved in the barley-oil diet than the barley-fat diet (Treatment 4 compared to Treatment 6). The same trends were noted for all corresponding treatments when comparing diets containing soya oil with diets containing yellow grease.

AME values were significantly higher ($P<0.05$) in the barley-oil based diet containing the enzymes (Treatment 3) compared to the barley-oil based diet without enzymes (Treatment 4) (see Figure 5). This elevation of feed quality was even more remarkable when the enzyme was added to the barley-fat diet (Treatment 5 compared to Treatment 6), where a significant improvement (P<0.05) was also observed in lipid digestibility, shown in Figure 6.

The irradiation of barley had a significant positive effect (P<0.05) on AME for the barley diets containing soya oil (Treatment 4 compared to Treatment 8), and improved both AME and lipid digestibility significantly (P<0.05) when used together with yellow grease (Treatment 6 compared to Treatment 10, which is irradiated barley and fat without the enzyme).

Comparing the results of barley plus irradiation and barley plus enzymes, no significant differences could be noted for either lipid sources, respectively (Treatment 3 compared to Treatment 8 and Treatment 5 compared to Treatment 10) for both AME, fecal and ileal lipid digestibility. However, when combining irradiation and enzyme addition to barley diets, the feed quality (AME and ileal lipid digestibility) was improved compared to the use of only irradiation or only enzyme supplementation.

The combined use of irradiated barley and supplementation of exogenous carbohydrase enzymes (Treatment 7) to a broiler diet containing high levels of soya oil (Treatment 4), had a significant positive effect (P<0.05) on feed quality (see Figure 5 and Figure 6) . The barley-oil diet (Treatment 4) without any additional treatments had a significant lower AME and lipid digestibility coefficient (P<0.05) than the maize-oil diet (Treatment 1) showing the lower feed quality of the non-treated barley based diets compared to the maize based diets. This is evident when AME, ileal and fecal digestibility are taken into account.

Pre-irradiation of the barley, together with enzyme inclusion, improved the AME and lipid digestibility to such an extent that no difference could be observed between the AME and lipid digestibility values of the barley-oil based (Treatment 7) and maize-oil based diets (Treatment 1).

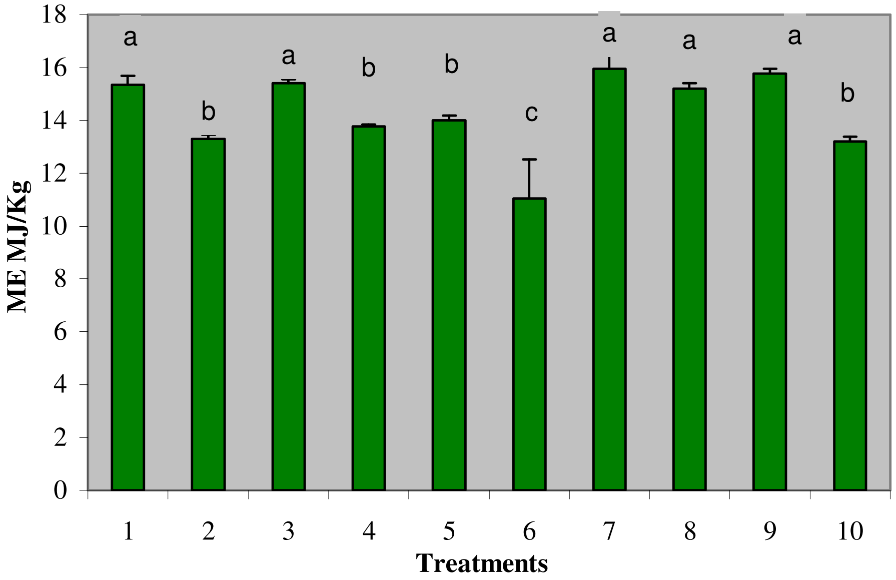


Fig 5. Apparent Metabolisable Energy values of the different diets with the standard error of the mean for each treatment

^{abcdef} means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:
1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

This is similar for the pre-irradiation of barley, together with the enzyme, in the fat based diet (Treatment 9). However, AME improves significantly (P<0.05) in the barley-fat based diet compared to the maize-fat diet (Treatment 2).

Table 6. Performance trial results for maize and barley based diets containing either soya oil or yellow grease as the main lipid source (± standard error of the mean).

Treatment	35 day Body weight (kg)	Total Feed Intake (kg)	FCR	AME (MJ/Kg)
1	1.74 (±0.16)^{bcd}	2.53 (±0.08)^{cd}	1.47 (±0.18)^{ab}	15.3 (±0.34)^{a}
2	1.55 (±0.11)^{de}	2.48 (±0.08)^{de}	1.61 (±0.17)^{ab}	13.3 (±0.15)^{b}
3	1.82 (±0.16)^{abc}	2.69 (±0.05)^{ab}	1.48 (±0.14)^{ab}	15.4 (±0.14)^{a}
4	1.65 (±0.05)^{cd}	2.57 (±0.08)^{bcd}	1.57 (±0.10)^{ab}	13.8 (±0.07)^{b}
5	1.72 (±0.11)^{bcd}	2.56 (±0.12)^{bcd}	1.49 (±0.16)^{ab}	14.0 (±0.17)^{b}
6	1.42 (±0.04)^{e}	2.39 (±0.05)^{e}	1.68 (±0.07)^{a}	11.0 (±1.47)^{c}
7	1.94 (±0.07)^{a}	2.71 (±0.06)^{a}	1.40 (±0.06)^{b}	16.0 (±0.80)^{a}
8	1.79 (±0.06)^{abc}	2.58 (±0.05)^{bcd}	1.45 (±0.08)^{b}	15.2 (±0.22)^{a}
9	1.90 (±0.08)^{ab}	2.66 (±0.04)^{abc}	1.40 (±0.06)^{b}	15.8 (±0.20)^{a}
10	1.57 (±0.13)^{de}	2.38 (±0.05)^{e}	1.52 (±0.09)^{ab}	13.2 (±0.18)^{b}

^{abcdef} Column means with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

Table 7. Digestibility trial results for maize and barley based diets containing either soya oil or yellow grease as the main lipid source (± standard error of the mean).

Treatment	Ileal crude Fat Digest.[*]	Fecal Crude Fat Digest.	Ileal DM[**] digest.	Fecal DM digest.
1	0.90 (±0.03)^{a}	0.91 (±0.03)^{a}	0.82 (±0.04)^{a}	0.85 (±0.04)^{ab}
2	0.67 (±0.03)^{c}	0.71 (±0.02)^{c}	0.74 (±0.06)^{ab}	0.79 (±0.08)^{abcd}
3	0.87 (±0.03)^{ab}	0.87 (±0.03)^{ab}	0.80 (±0.04)^{a}	0.84 (±0.03)^{abc}
4	0.83 (±0.04)^{b}	0.82 (±0.05)^{b}	0.75 (±0.03)^{ab}	0.78 (±0.04)^{abcd}
5	0.64 (±0.03)^{cd}	0.65 (±0.03)^{de}	0.73 (±0.05)^{ab}	0.76(±0.05)^{cd}
6	0.50 (±0.02)^{e}	0.51 (±0.04)^{f}	0.61 (±0.24)^{b}	0.73 (±0.05)^{d}
7	0.91 (±0.03)^{a}	0.91 (±0.04)^{a}	0.83 (±0.03)^{a}	0.86 (±0.02)^{a}
8	0.86 (±0.02)^{ab}	0.86 (±0.04)^{ab}	0.77 (±0.01)^{ab}	0.80 (±0.03)^{abcd}
9	0.67 (±0.06)^{c}	0.69 (±0.02)^{cd}	0.76 (±0.03)^{ab}	0.79 (±0.05)^{abcd}
10	0.58 (±0.04)^{d}	0.60 (±0.03)^{e}	0.73 (±0.02)^{ab}	0.77 (±0.01)^{bcd}

^{abcdef} Column means with the same superscript do not differ significantly ($P>0.05$) as determined by Duncans Multiple Range Test.

[*] Digest.: Digestibility

[**] DM: Dry Matter

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

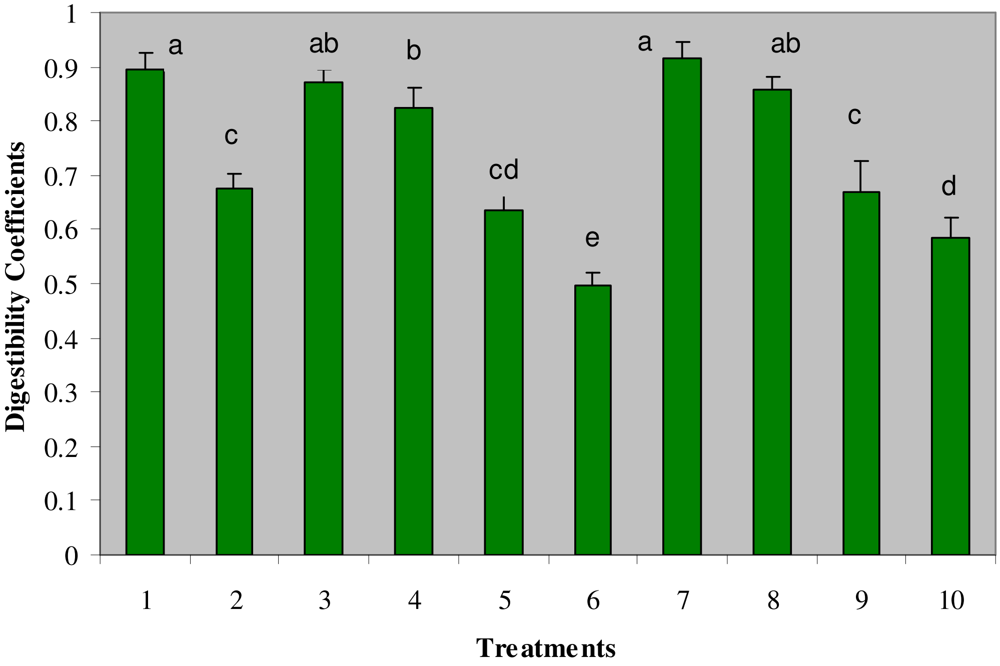


Fig 6. Ileal crude fat digestibility of the different diets and the standard error of the mean

abcdef means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme

2: Maize and Fat without enzyme

3: Barley and oil with enzyme

4: Barley and oil without enzyme

5: Barley and fat with enzyme

6: Barley and fat without enzyme

7: Irradiated Barley and oil with enzyme

8: Irradiated Barley and oil without enzyme

9: Irradiated Barley and fat with enzyme

10: Irradiated Barley and fat without enzyme

Where yellow grease was included in the diets, the AME for the barley diet was 11.0 MJ/kg and for the irradiated barley diet with enzymes (Treatment 9) it was 15.8 MJ/kg, significantly higher than the 13.3 MJ/kg of the maize-fat diet (Treatment 2). The combination of irradiation of barley and enzyme supplementation also increased lipid digestibility up to the same level as that of the maize based diet.

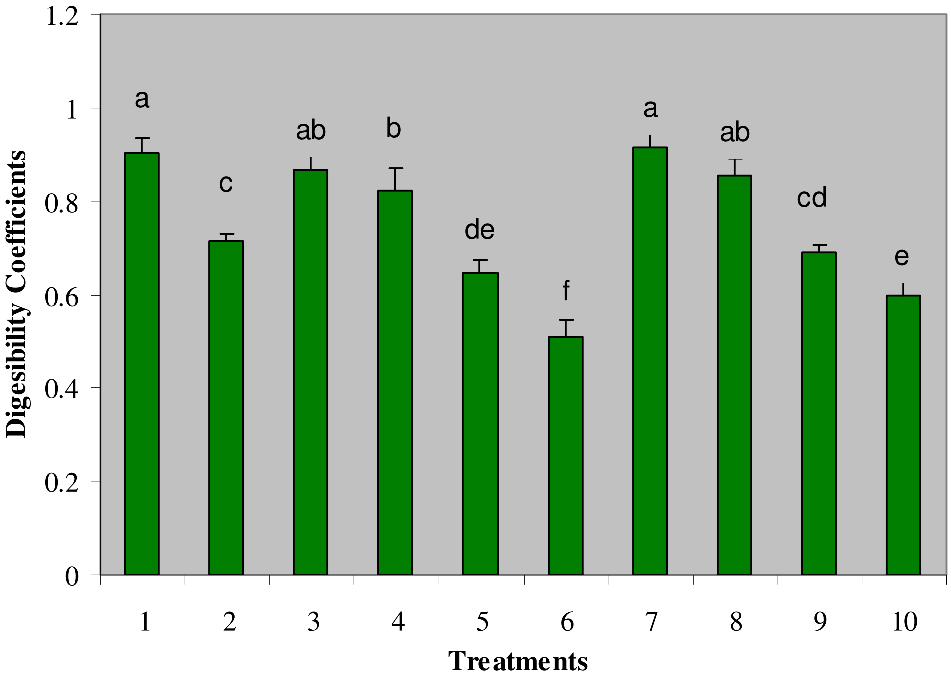


Fig 7. Fecal crude fat digestibility of the different diets and the calculated standard error of the mean of each treatment

^{abcdef} means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

Fecal dry matter digestibility and fecal crude fat digestibility showed the same trends as their ileal digestibility coefficient counterparts (Figure 8). Their similarities can be observed as shown in Table 7.

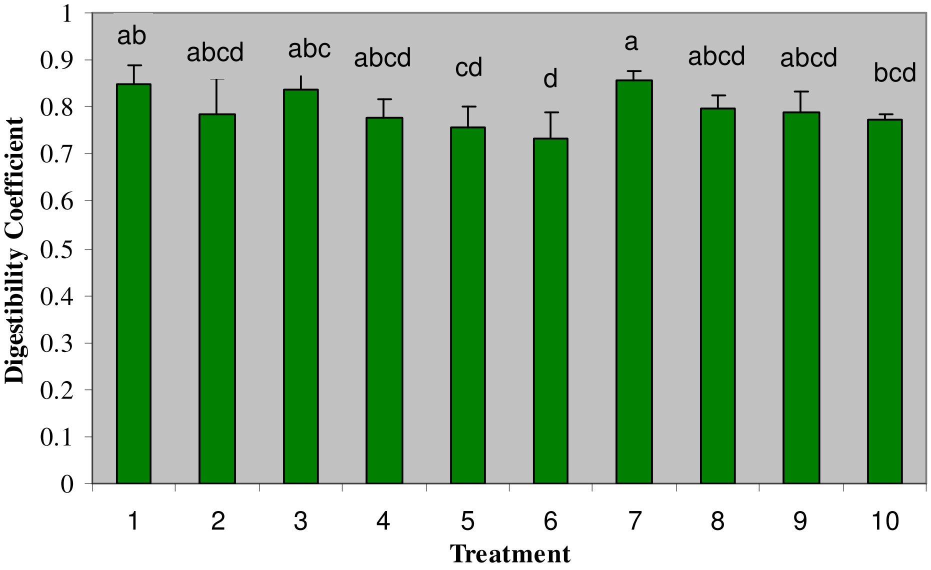


Fig 8. Fecal dry matter digestibility and the standard error of the mean of the different diets

^{abcdef} means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

Ileal dry matter digestibility showed similar trends in the treatment diets when compared to the other digestibility parameters (Figure 9). However, the results were not significantly different from each other when analyzing the digestibility coefficients. The irradiation of barley showed no significant effect on ileal dry matter digestibility when the soya oil was used as the lipid source. Ileal dry matter digestibility showed an improvement when the lipid source of the irradiated barley used was yellow grease, however this improvement was not significant (P>0.05).

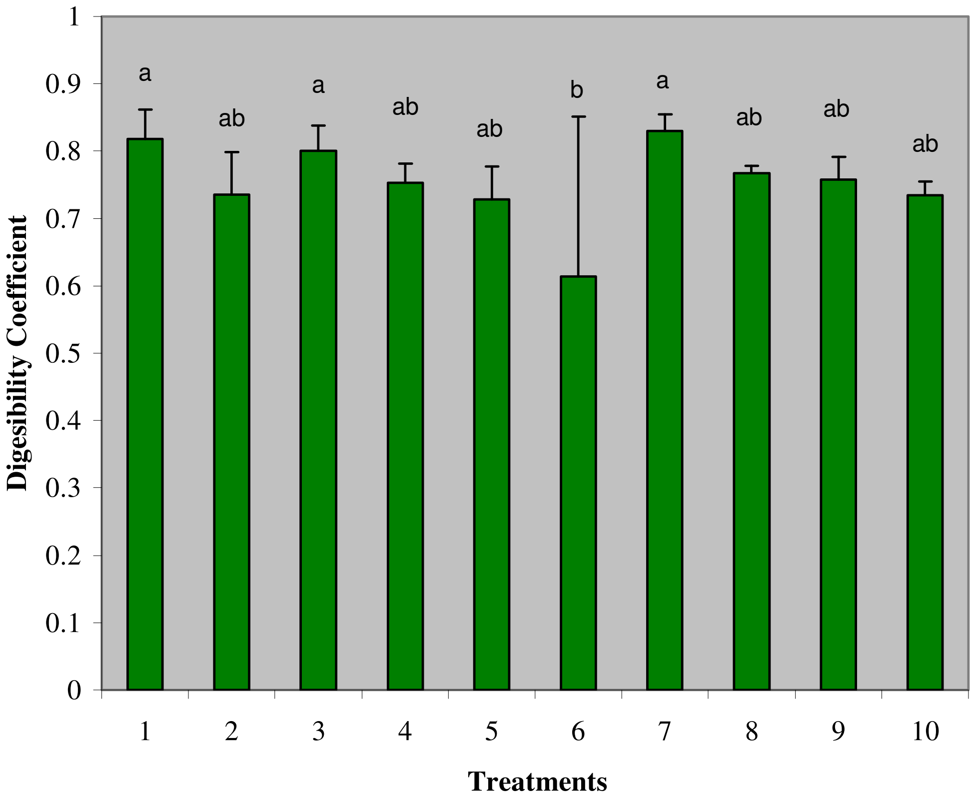


Fig 9. Ileal dry matter digestibility of the different diets shown with the standard error of the mean per treatment

^{abcdef} means treatments with the same superscript do not differ significantly (P>0.05) as determined by Duncans Multiple Range Test.

Treatments:

1: Maize and oil without enzyme
2: Maize and Fat without enzyme
3: Barley and oil with enzyme
4: Barley and oil without enzyme
5: Barley and fat with enzyme
6: Barley and fat without enzyme
7: Irradiated Barley and oil with enzyme
8: Irradiated Barley and oil without enzyme
9: Irradiated Barley and fat with enzyme
10: Irradiated Barley and fat without enzyme

CHAPTER 5. DISCUSSION

Barley is the preferred grain for cultivation in many areas of the world due to its resistance to drought and the ability to mature in climates with a short growing season. Its use for poultry has been limited by the considerable amounts of fibre in the grain. The soluble fibre fraction, which mainly consists of mixed linked β-(1,3), (1,4)-glucans, is associated with an increased gut viscosity, which in turn inhibits digestion and absorption of nutrients (Svihus and Gullord, 2002). This is confirmed by the higher lipid digestibility coefficients and AME values found in this study for the low NSP (maize) diets when compared with the high NSP (barley) diets. These results were consistent with the results obtained by Danicke *et al*. (1999b).

NSPs may effect the production and activity of digestive enzymes, intestinal morphology (and cell proliferation), the microbial population in various segments of the gut and the secretion of certain hormones, including insulin, glucagons, gastric inhibitory polypeptide and possibly secretin and cholecystokinin (Sakata 1987; Anugwa *et al*., 1989; Chesson, 1990; Smits, 1996).

de Lange (2000) stated that some or part of these effects can be indirect effects of NSP and certain effects can be direct. An example is given of how microbial activity can effect gut secretions and the morphology of the small intestine, independent of the presence of NSPs. This has implications for gut health, the rate and extent of nutrient digestion and absorption, and the metabolic efficiency of nutrient utilisation after absorption, which can be illustrated by the effects of NSP on organ sizes found by Anugwa *et al*. (1989) (de Lange, 2000).

The efficiency of utilisation of energy derived from digested NSP is about 30% lower than those of starch and sugars. This has direct implications for the supply of effective energy to the animal. In various studies, increased dietary NSP levels on the digestibility and rate of absorption of nutrients from starch, protein and fats have been demonstrated (de Lange, 2000).

Svihus and Gullord (2002) found a significant increase in performance when β-glucanases had been added to poultry diets containing high levels of NSP. The cocktail of carbohydrase enzymes comprising of cellulases, xylanases and β-glucanases, improved the AME values of the barley diets significantly in this study. The improvement was more pronounced where a high level of yellow grease was included in the diet as significant positive effects were also noted for lipid digestibility and bird performance. These results are in agreement with Danicke *et al.* (1999b, 2000) who found that exogenous carbohydrase enzymes had a more positive effect where high NSP diets contained more saturated lipids.

Danicke *et al.* (1997a) showed that intestinal viscosity was found to interfere with the digestibility of saturated fatty acids more dramatically than with the digestibility of unsaturated fatty acids in rye based diets. This partially explains the greater effectiveness of carbohydrase addition in high NSP diets with high levels of saturated fatty acids.

The type of lipids included in the diets of this study had a definite effect on the digestibility of lipids and consequently the metabolisable energy of the feed. The lipid digestibility and AME value of the yellow grease diets were much lower than that of the soya oil diets, for both the maize and barley diets respectively. These results verify the work done by Garret and Young (1975), who found that the polarity of the different fatty acids found in fats and oils, affected the fatty acids' absorbability. Generally, absorbability increases according to the following order: C18:0 < C16:0 < C14:0 < C18:1, C18:2 and C18:3 (Garret and Young, 1975).

The results obtained in the performance and digestibility trials with the diets containing the yellow grease, may also partly be due to the higher content of free fatty acids in the fat compared to the soya oil. The greater the free fatty acid content of a fat, the lower the efficiency of absorption of the products of its digestion, this reduction being more pronounced the more saturated the fat and the younger the bird (Blanch *et al.*, 1995).

The high levels of saturated fatty acids associated with the yellow grease diets might have amplified the effects of the NSP on intestinal viscosity (Danicke *et al.*, 2000), thereby hindering nutrient digestion and absorption. Most parameters measured for the barley-fat diets showed significant improvements when the carbohydrase enzymes were added to the diet. For the barley-oil diets, however, the only significant effect was observed for the AME value of the diet.

Pre-irradiation of barley showed similar effects as the addition of carbohydrase enzymes to the barley-based diets, indicating that irradiation might possibly be considered as a substitute for enzyme supplementation, probably due to the degrading of the β-glucans in barley by both treatments.

More parameters were significantly enhanced when barley was irradiated in the diets containing yellow grease, with only a significant difference observed in the AME value for the soya oil diets. However, the positive trends noted for the barley-oil-based diets when enzymes were added could also be observed with irradiation of the barley.

Siddhuraju *et al*. (2002) stated that irradiation appeared to benefit cereals containing soluble or mucilaginous fibre types, as typified by the β-glucan of barley and oats. The fibres appeared prone to irradiation-induced depolymerization, as indicated by the increased β-glucan solubility and reduced extract viscosity of irradiated barley and oat samples, as observed by Campbell *et al*. (1986).

The results found in this experiment support the statement above. Although no literature was found showing the effects of pre-irradiation of barley and its interaction with fat type, it can be assumed that due to the depolymerization of the NSP by irradiation, performance and digestibility results would be similar (at an irradiation level of 10kGy) to the use of a carbohydrase enzyme in the same diet.

The addition of carbohydrase enzymes to the irradiated barley diets resulted in enhanced feed quality and bird performance, suggesting that enzyme supplementation and the irradiation of NSP rich raw materials might contribute additively to its feeding value. This combination of treatments seemed to be of particular value where the diet contained a high level of saturated fatty acids, where significant improvements were noted for both AME value and lipid digestibility, compared with the diets where the treatments were applied separately.

The combined use of irradiated barley and supplementation of exogenous carbohydrase enzymes to a broiler diet containing high levels of soya oil, improved the AME and lipid digestibility to the same level as those of the control maize diet. Even more remarkably, is the significant superior body weight and feed intake figures of this barley diet, compared to the control maize diet.

Where yellow grease was included in the diets, the AME for the irradiated barley diet with enzymes was even significantly higher than the AME of the control maize diet. The combination of irradiation of barley and enzyme supplementation also increased lipid digestibility up to the same level as that of the maize based diet, while the barley fed birds performed significantly better in terms of body weight and feed intake.

CHAPTER 6. CONCLUSION

NSPs have a definite effect on broiler performance as well as digestibility parameters as shown in this study when comparing the maize diets to the barley based diets.

In the current study, these similar trends of NSP effecting nutrient digestibility were clearly demonstrated in depressed ileal and fecal crude fat digestibilities and metabolisability of energy in diets containing high amounts of NSP. In poultry, and more specifically in broiler chickens, many of these negative effects on nutrient digestibilities have been related to the viscosity of digesta when increasing levels of NSPs are fed in the diet.

At low inclusion levels NSPs and oligosaccharides actually stimulate digestive function and improves gut health. Barley at 55% of the total diet as in this study, showed effects on animal performance and therefore, would suggest, that the threshold value was exceeded in this case causing negative effects to be observed. Due to the increasing awareness of gut health in poultry production, animal nutritionists shall have to be aware of the effects of NSPs on gut health, their benefits as well as their detrimental effects to efficient digestion and nutrient utilisation.

This study also demonstrated the effects that high levels of NSP in the diet have on lipid digestibility, and that those effects were dependant on dietary lipid type. Danicke *et al*. (2000) demonstrated that increasing saturated fatty acids to diets containing high NSP levels, a higher depression in liveweight gain, increased feed conversion ratios as well as reduced lipid digestibilities and metabolisability of gross energy were seen. Similar results were demonstrated in this study. Therefore, not only is the determination of the NSP content important to ensure an efficiently utilised poultry feed, but also other dietary components such as dietary lipid type.

The study subsequently investigated methods that could be used to aid the digestive system in utilising a feed with high NSP and high saturated lipid types, and to what degree these methods would improve the utilisation of the feed.

This study shows that the positive effects brought about by irradiation of barley and carbohydrase addition to barley based diets, were additive and dependent on dietary lipid type. Irradiation and carbohydrase supplementation might be of more significance where NSP diets are fed to broilers that are rich in saturated fatty acids. The combination of the two treatments enhanced the feeding value of barley as a raw material in broiler diets to the same level, or even superior, than maize.

With the resistance growing towards the use of anti-microbials in poultry feeds and in certain countries where certain anti-microbial use has been banned, further studies are required to determine methods to improve gut health and feed efficiency of high NSP containing feeds. High NSP diets with high levels of saturated fatty acids does effect feed efficiency negatively as shown in this trial. This trial does not address the effect of irradiation and carbohydrase enzyme addition to diets high in NSP and saturated lipid type, on gut health. No financial feasibility of the use of carbohydrase addition and irradiation as methods to improve feed efficiency was conducted, and further cost feasibility studies should be conducted to determine the cost effectiveness of the methods described in this trial. As technology progresses, studies to improve feed utilisation and gut health should investigate new technological possibilities of achieving this.

The results of this study would be useful to a nutritionist looking for tools to negate the negative effects of diets containing high NSP levels and where high saturated lipid types are used in the diet. Therefore, to ensure that diets based on barley, supplemented with a saturated dietary lipid type, are utilised efficiently, carbohydrases and or pre-irradiation of barley should be used.

CHAPTER 7. REFERENCES

Alimirall, M., Esteve-Garcia, E. 1994. Rate of passage of barley diets with chromium oxide. Influence of age and poultry strain and effect of β-glucanase supplementation. Poult. Sci. 73, 1433-1440.

Alimirall, M. Francesch, M., Perez-Vendrell, A.M., Brufau, J., Esteve-Garcia, E. 1995. The differences in intestinal viscosity produced by barley and β-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chickens than in cocks. J. Nutr. 125, 947-955.

Alvarez, A. 1994. Sepiolite: properties and uses. In: Singer, A., Glan, E. (Eds), Palygorskite-sepiolite Occurences, Genesis and Uses, Elsevier, Amsterdam, 253-287.

Angulo, E., Brufau, J., Esteve-Garcia, E. 1995. Effect of sepiolite on pellet durability in feeds differing in fat and fibre content. Anim. Feed Sci. Technol. 53, 233-241.

Annison, G. 1993. The role of wheat NSP in broiler nutrition. Aust. J. Agric. Sci. 44, 405-422.

Anugwa, F. O. I., Varel, V. H., Dickson, J. S., Pond, W. G., Krook, P. 1989. Effects of dietary fibre and protein concentration on growth, feed efficiency, visceral organ weighs and large intestine microbial population of swine. J. Nutr. 119, 879 - 886.

AOAC. 2000. Official method of Analysis. !7th Edition. Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

Bach-Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant material used in animal feeding. Anim. Feed Sci. Technol. 67, 319 - 338.

Bedford, M.R. 1996. Reduced viscosity of intestinal digesta and enhanced nutrient digestibility in chickens given exogenous enzymes. In:

Proceedings of the first Chinese symposium on feed enzymes, Najing Agric. University, People's Rep. of China, 19 - 28.

Bedford, M.R. and Classen, H.L. 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentrations is effected through changes in carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency in broiler chickens. J. Nutr. 122, 560-569.

Blanch, A., Barroeta, A.C., Baucells, M. D., Puchal, F. 1995. The nutritive value of dietary fats in relation to their chemical composition. Apparent fat availability and metabolizable energy in two week-old chicks. Poult. Sci. 74, 1335 - 1340.

Bourdillon, A., Carre, B., Conan, L., Francesch, M., Fuentes, M., Huyghebaert, G., Janssen, W. M. M. A., Leclercq, B., Lessire, M., McNab, J., Rigoni, M., Wiseman, J. 1990. European reference method of *in vivo* determination of metabolisable energy in poultry: reproducibility, effect of age, comparison with predicted values. Br. Poult. Sci. 31, 567-576.

Brown,W. 1979. Interactions of small molecules with hydrated polymer networks. In G. E. Inglett & S. I. Falkehog (Eds.). Chemistry and Nutrition. New York: Academic Press, 1 - 13.

Campbell, G. L., Classen, H. L., Reichert, R. D., Campbell, L. D. 1983. Improvement of the nutritive value of rye for broiler chickens by gamma irradiation induced viscosity reduction. Brit. Poult. Sci. 24, 205 - 212.

Campbell, G. L., Classen, H. L., Rossnagel, B. G., Bhatty, R. Reichert, R. D. 1985. Studies in the use of hull-less barley in chicken diets: deleterious effects and methods of alleviation. Can. J. Anim. Sci. 65, 725 - 733.

Campbell, G. L., Classen, H. L., Balance, G. M. 1986. Gamma irradiation treatment of cereal grains for chick diets. J. Nutr. 116, 560 - 569.

Campbell, G. L., Sosulski, F. W., Classen, H. L., Balance, G. M. 1987. Nutritive value of irradiation and β-glucanase treated wild oat groats (Avena fatta L) for broiler chickens. Poult. Sci. 16, 243 – 252.

Carre, B. 1992. Les polysaccharides non amylaces hydrosolubles. In Les contaminants et facteurs antinutritionels dans les aliments des volailles; vrais ou faux problemes. Cahier du Simavip, WPSA, Nouzilly, France, 49 - 58.

Carre, B., Gomez, J., Melcion, J.P., Gioulot, B. 1994. La viscosite des aliments destines a l'aviculture. Utilisation pour predire la consommation et l' excretion d'eau. INRA Prod. Anim. 7, 369 - 379.

Carre, B., Mohanna, C., Nys, Y. 1999. Incidence of dietary viscosity on growth performance and zinc and manganese bioavailability in broilers. Anim. Feed Sci. technol. 77, 255 - 265.

Chesson, A. 1990. Nutritional significance ad nutritional value of plant polysaccharides. In: Wiseman, J. and Cole, D. J. A. (Eds). Feedstuff Evaluation. Butterworths, London, 41 - 55.

Choct, M. and Annison, G. 1992 Anti-nutritive effect of wheat pentosans in broiler chickens: role of viscosity and gut microflora. Br. Poult. Sci. 33, 821 - 834.

Choct, M., Hughes, R.G., Wang, J., Bedford, M.R., Morgan, A.J., Annison, G., 1995. Feed enzymes eliminate the anti-nutritive effect of NSP and modify fermentation in broilers. In: Proceedings of the Australian Poultry Science Symposium. 7, 121 - 125.

Choct, M., Hughes, R.J., Bedford, M.R., Morgan, A.J., Annison, G. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Brit. Poult. Sci. 37, 609 - 621.

Danicke, S., Jeroch, H., Bottcher, W., Bedford, M.R., Simon, O. 1999a. Effects of dietary fat type, pentosan level and xylanases on digestibility of fatty acids, liver lipids and vitamin E in broilers. Fett/Lipid 101, 90 - 100.

Danicke, S., Jeroch, H., Bottcher, W., Simon, O. 2000. Interactions between dietary fat type and enzyme supplementation in broiler diets with high pentosan contents: effects on precaecal and total tract digestibility of fatty acids, metabolisability of gross energy, digesta viscosity and weights of small intestine. Anim. Feed Sci. Technol. 84, 279 - 294.

Danicke, S., Simon, O., Jeroch, H. 1999b. Effects of supplementation of xylanase or β-glucanase containing enzyme preparations to either rye- or barley- based broiler diets on performance and nutrient digestibility. Archive fur Geflugelkunde 63, 252-259.

Danicke, S., Simon, O., Jeroch, H. and Bedford, M. 1997a. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. Physio-chemical chyme features. Brit. Poult. Sci. 38, 537 - 545.

Danicke, S., Simon, O., Jeroch, H. and Bedford, M. 1997b. Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 2. Performance, nutrient digestibility and the fat soluble vitamin status of livers. Brit. Poult. Sci. 38, 546 - 556.

Danicke, S., Simon, O., Jeroch, H. and Bedford, M. 1999c. Interactions between dietary fat type and exogenous enzyme supplementation of broiler diets based on maize, wheat, triticale and barley. Anim. Feed Sci. Technol. 8, 467 - 483.

Danicke, S., Simon, O., Jeroch, H. and Bedford, M. 1995. Effect of fat source and xylanase supplementation on the performance and intestinal viscosity in rye fed birds. Proceedings of 2nd European Symposium on Feed Enzymes, Netherlands, 102-106.

de Lange, C. F. M. 2000. Characterisation of the non-starch polysaccharides. In: Feed Evaluation – Principles and Practice. 2000. P. J.

Moughan, M. W. A. Verstegen, M. I. Visser (Eds). Wageningen Press. Chp. 5.

Dierick, N. A., Vervaeke, I. J., Demeyer, D. I., Decuypere, J. A. 1989. Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in he overall energy supply. Anim. Feed Sci. Technol. 23, 141 - 167.

Dierick, N.A. and Decuypere, J. A. 1994. Enzymes and growth in pigs. In: Cole, D., Wiseman, J., Varley. M (Eds). Principles of Pig Science, Nottingham University Press, 168.

Evans, A.J., Cheung, P.C.K., Cheetham, W.H., 1993. The carbohydrate composition of cotyledons and hulls of cultivars of *Lupins angustifolius* from Western Australia. J. Sci. Food Agric. 61, 189 - 194.

Favier, M.L., Bost, P.E., Guittard, C., Demigne, C., Remesy, C. 1997. The cholesterol-lowering effect of guar gum is not the result of a simple diversion of bile acids toward faecal excretion. Lipids 32, 953 - 959.

FDA. 1986. Irradiation in the production and handling of food. Federal Register. 51, 1337.

Fengler, A.I. and Marquardt, R.R. 1988. Water-soluble pentosans from rye. II. Effects on rate of dialysis and on the rate of retention of nutrients by the chick. Cereal Chem. 69, 298 - 302.

Fisher, C. and McNab, J. 1987. Techniques for determining the metabolisable energy of poultry feeds. In: Haresign, W. and Cole, D. J. A. (Eds). Recent Advances in Animal Nutrition. Butterworths, London, 3 - 18.

Fedde, M. R., Waibel, P. E., Burger, R. E. 1960. Factors affecting the absorbability of certain dietary fats in the chick. J. Nutr. 70, 447.

Frape, D. L. and Tuck, M. G. 1977. Determining the energy values of ingredients in pig feeds. Proc. Nutr. Soc. 36 (2), 179 – 187.

Garret, R. L and Young, R. J. 1975. Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. J. Nutr. 105, 827 - 838.

Gasa, J., Ouhida, I., Perez, J.F., Piedrafita, J. 2000. The effects of sepiolite in broiler chicken diets of high, medium and low viscosity. Productive performance and nutritive value. Anim. Feed Sci. Technol. 85, 183 - 194.

Guillaume, J. and Summers, J. D. 1970. Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolisable energy. Can. J. Anim. Sci. 50, 363 - 369.

Harris, L. E. 1966. Biological energy interrelationships and glossary of energy terms. N. A. S – N. R. C. Publication 1411, Washington.

Iji, P.A., Saki, A.A., Tivey, D.R. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Anim. Feed Sci. Technol. 89, 175 - 188.

Inarrea, O., Simon, M., Manzano, M., Palacios, J. 1989. Changes in the concentration and composition of biliary and serum bile acids in the young domestic fowl. Brit. Poult. Sci. 30, 353 - 359.

Jamroz, D., Jakobsen, K., Bach Knudsen, K. E., Wiliczkiewicz, A., Orada, J. 2002. Digestibility and energy value of non-starch polysaccharides in young chickens, ducks and geese, fed diets containing high amounts of barley. Comp. Biochem. Physiol. Part A 131, 657 – 668.

Krogdahl, A. 1985. Digestion and absorption of lipids in poultry. J. Nutr. 115, 675 - 685.

Kyriazakis, I. and Emmans, G. C. 1995. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. Brit. J. Nutr. 73, 191 - 207.

Langhout, D. J., Schutte, J. B., Geerse, C., Kies, A. K., De Jong, J., Verstegen, M. W. A. 1997. Effects on chick performance and nutrient digestibility of an endo-xylanase added to a wheat- and rye- based diet in relation to fat source. Brit. Poult. Sci. 38, 557 – 563.

Leeson, S. and Summers, J. D. 1976. Fat ME Values: the effect of fatty acid saturation. Feedstuffs. 48, (46), 26 - 28.

Levrat, M.A., Moundras, C., Younes, H., Morand, C., Demigne, C., Remesy, C. 1996. Effectiveness of resistant starch, compared to guar gum, in depressing cholesterol and enhancing faecal steroid excretion. Lipids. 31, 1069 - 1075.

Lo, G.S. and Cole, T.G. 1990. Soy cotyledon fiber products reduce plasma lipids. Arteriosclerosis 82, 59 - 67.

Lund, E. K., Gee, J. M., Brown, J. C., Wood, P. J., Johnson, I. T. 1989. Effect of oat gum on the physical properties of the gastro intestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine *in vitro*. Br. J. Nutr. 62, 91 – 101.

MacNab, J. and Fisher, C. 1982. The choice between apparent and true metabolisable energy systems – recent evidence. Proceedings of the third European Symposium on Poultry Nutrition. 374 - 376.

McNab, J. 1990. Apparent and true meabolisability of poultry diets. In: Wiseman, J. and Cole, D. J. A. (Eds). Feedstuff Evaluation. Butterworths, London, 41 - 55.

Mohanna, C., Carre, B., Nys, Y. 1999. Incidence of dietary viscosity on growth performance and zinc and manganese bio-availability in broilers. Anim. Feed Sci. Technol. 77, 255 - 266.

Molins, R. A. 2001. Food irradiation: principles and applications. John Wiley and Sons. New York.

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., Price, J. 1981. Concentration and shear rate dependance of viscosity in random coil polysaccharide solutions. Carbohydr. Polymers. 1, 5 – 11.

Naumann, C., Bassler, R. 1993. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag Darmstadt, Germany.

Nene, S. P., Vakil, U. K., Sreenivasan, A. 1975. Effect of gamma radiation on physio-chemical characteristics of red gram (*Cajanus cajan)* starch. Anim. Feed Sci. Technol. 40, 943 - 947.

Noblet, J., Fortune, H., Dupire, C., Dubois, S. 1993. Digestible, metabolisable and net energy values of 13 feedstuffs for growing pigs: effect of energy system. Anim. Feed Sci. Technol. 42, 131 - 149.

Noy, Y. and Sklan, D. 1995. Digestion and absorption in the young chick. Poult. Sci. 74, 366 - 373.

Ockner, R. K., Manning, J. A., Poppenhausen, R. B., Ho, W. K. 1972. A binding protein for fatty acids in the cytosol of intestinal mucosa, liver, myocardium and other tissues. Science. 177, 56 – 58.

Pasquier, B., Armand, M., Castelain, C., Guillon, F., Borel, P., Lafont, H., Lairon, D. 1996. Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fiber in acidic gastric medium in vitro. Biochem. J. 314, 269 - 275.

Polin, D. 1980. Increased absorption of tallow with lecithin. Poult. Sci. 59, 1652.

Rackis, J.J. 1974. Biological and physiological factors in soyabeans. J. Am. Oil Chem. Soc. 51, 161A - 174A.

Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and NSP content in different soyabean products. Anim. Feed Sci. Technol. 79, 331-345.

Reydan, P., and Selvendran, R. R. 1993. Phytic acid: properties and determination. In: Macrae, R., Robinson, R. K., Sadler, M. J (Eds). Encyclopaedia of food science, food technology and nutrition. Academic Press, London, 3582 - 3587.

Sakata, T. 1987. Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Brit. J. Nutr. 58, 95 - 103.

Sattar, A., Neelofar, X., Akhtar, M. A. 1990. Effect of radiation and soaking on phytate content of soyabean. Aca Alimentaria. 19, 331–336.

Schutte, J.B. and Langhout, D.J. 1998. Effect of dietary inclusion of sepiolite in wheat based diets on broiler performance. In: 10th European Poultry Conference, Jerusalem, 95.

Short, F. J., Gorton, P., Wiseman, J., Boorman, K. N. 1996. Determination of titanium oxide added as inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59, 215 - 221.

Sibbald, I. R. 1976. A bioassay for true metabolisable energy in feedstuffs. Poult. Sci. 55: 303 - 308.

Siddhuraju, P., Osoniyi, O., Makkar, H. P. S., Becker, K. 2002. Effect of soaking and ionising radiation on various anti-nutritional factors of seeds from different species of an unconventional legume, green gam (*Vigna radiata*). Food Chem.79, 273 - 281.

Smits, C. H. M. 1996. Viscosity of dietary fibre in relation to lipid digestibility in broiler chickens. PhD thesis. Department of Animal Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.

Smits, C.H.M. and Annison, G. 1996. Non-starch polysaccharides in broiler nutrition - towards a physiologically valid approach to their determination. Wld. Poult. Sci. J. 52, 203 - 221.

Smits, C.H.M., Veldman, A., Verstegen, M.W.A., Beynen, A.C. 1997. Dietary carboxy-methyl cellulose with high instead of low viscosity reduced macronutrient digestion in broiler chickens. J. Nutr. 127, 483 - 487.

Statistical Analysis Systems. 1994. SAS User's Guide: Statistics Version 6. SAS Institute Inc. Cary, NC, USA.

Svihus, B. and Gullord, M. 2002. Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. Anim. Feed Sci. Technol. 102, 71 - 92.

Svihus, B., Newman, R.K., Newman, C.W. 1997. Effect of soaking, germination and enzyme treatment of whole barley on nutritional value and digestive tract parameters of broiler chickens. Brit. Poult. Sci. 38, 390 - 396.

Thompson, D.B., Huang, C., Sieglaff, C. 1987. Rheological behaviour of soluble polysaccharide fractions from soyabeans. Food Hydrocoll. 1, 333 - 337.

Tortuero, F.F., Gonzalez, E.M., Martin, L. 1992. Efectos de la sepiolita en la dieta sobre el crecimiento las medidas viscerales y el transito intestinal en pollos. Arch. De Zootecnia 41, 209 - 217.

Van der Klis, J.D. and Van Woorst, A. 1993. The effect of carboxy-methyl cellulose on the rate of marker excretion from the gastrointestinal tract of a broiler. Poult. Sci. 72, 503 - 512.

Vermorel, M. and Martin-Rosset, W. 1997. Concepts, scienific bases, structure and validation of the French horse ne energy system (UFC). Livstck. Prod. Sci. 47, 261 - 275.

Wang, G. J., Marquardt, R. R., Guenter, W., Zhang, Z., Han, Z. 1997. Effects of enzyme supplementation and irradiation of rice bran on the performance of growing leghorn and broiler chickens. Anim. Feed Sci. Technol. 66, 47 - 61.

Wenk, C. and Messikommer, R. 1991. Utilisation of the energy of soyabean and sorghum hulls for growth in growing chickens. Proc. 12th EAAP Symp, Kartause Ittingen, Switzerland, 197 - 197.

Westergaard, H. and Dietschy, J. M. 1976. The mechanism whereby bile micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. J. Clin. Invest. 58 (1), 97 – 108.

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C., Marlett, J.A. 1981. The viscosity interaction of barley β-glucan with *Tricoderma viride* cellulase in the chicken intestine. Poult. Sci. 62, 853 - 862.

WHO. 1981. Wholesomness of irradiated foods. Report on joint FAO/IAEA/WHO Expert committee, Technical report series no. 659. Geneva, Switzerland: World Health Organisation.

WHO. 1999. High Dose Irradiation: wholesomeness of foods irradiated with doses above 10 kGy. Report of a joint FAO/IAEA/WHO Study Group, technical report series no. 890. Geneva Switzerland: World Health Organisation.

Wiseman, J. 2000. Correlation between physical measurements and dietary energy values of wheat for poultry and pigs. Anim. Feed Sci. Technol. 84, 1 - 11.

Wiseman, J. and Lessire, M. 1987. Interactions between fats of differing chemical content, apparent metabolisable energy values and apparent fat availability. Br. Poult. Sci. 28, 663 - 676.

Wiseman, J., Salvador, F., Craigon, J. 1991. Prediction of the apparent metabolisable energy content of fats fed to broiler chickens. Poult. Sci. 70, 1527 - 1533.

Yin, Y. L., McEnvoy, J. D. G., Schulze, H., Hennig, U., Souffrant, W. B., McCracken, K. J. 2000. Apparent digestibility (ileal and overall) of nutrients and endogenous losses in growing pigs fed wheat (Var. Soissons) or its by-products without or with xylanase supplementation. Livstk. Prod. Sci. 62, 119 - 132.

Yin, Y. L., McEvoy, J., Schulze, H. and McCracken, K. J. 2000. Studies on Cannulation method and alternative indigestible markers and the effects of food enzyme supplementation in barley-based diets on ileal and overall apparent digestibility in growing pigs. Anim. Sci.. 70, 63 - 72.

Filename:	Final thesis
Directory:	C:\DOCUME~1\user\LOCALS~1\Temp\XPgrpwise
Template:	C:\Documents and Settings\user\Application Data\Microsoft\Templates\Normal.dot
Title:	Abstract
Subject:	
Author:	PETER
Keywords:	
Comments:	
Creation Date:	7/9/2008 9:31:00 AM
Change Number:	4
Last Saved On:	7/9/2008 11:02:00 AM
Last Saved By:	UPuser
Total Editing Time:	45 Minutes
Last Printed On:	7/10/2008 8:53:00 AM
As of Last Complete Printing	
Number of Pages:	83
Number of Words:	19,612 (approx.)
Number of Characters:	111,789 (approx.)