Nutritional Evaluation of Wheat Distillers Dried Grains with Solubles for Broiler Chickens

Thesis submitted for the degree of Doctor of Philosophy

By

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DECLARATION

I hereby declare that this thesis is my original research work and to the best of my knowledge and belief it contains no material which was previously submitted or accepted for the award of any qualification of any academic institution. This thesis does not contain collaborative work, whether published or not.

Studies completed during candidature, some of which are reported in this thesis have been presented in the following conference proceedings:

N.B. Rano, A.S. Chaudhry and S.A. Edwards. Effects of feeding Distiller's Dried Grains with soluble in broiler diets on performance and digestibility. Annual Conference of British Society of Animal Science (BSAS) April 24th -25th 2012 University of Nottingham, UK

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ABSTRACT

Nutritional Evaluation of Wheat Distillers Dried Grains with Solubles for Broiler Chickens

Wheat distillers dried grains with soluble (wDDGS) are a by-product from bioenergy production and available in increasing quantities. Three experiments were carried out to evaluate the effects of feeding wDDGS in the presence or absence of enzymes on the productive performance, carcass characteristics, digestibility, and behaviour of broiler chickens. Experiment 1 evaluated the effect of wDDGS inclusion on the acceptability of different inclusion levels (0, 10, 20, & 40%) of wDDGS when substituting for wheat and soybean meal in four isonitrogenous diets differing in slightly amino acid but mainly NDF and energy contents. Results showed significantly lower feed intake in broilers consuming the 40% wDDGS diet that might have been due to the high percentage of fibre and perhaps poor palatability of the diet. The best FCR was recorded from birds fed the 0 or 10% wDDGS diet compared to 20 and 40% wDDGS. Birds fed 40% wDDGS diet had poorer ($P<0.05$) crude protein digestibility, possibly due to the poor lysine digestibility of wDDGS as affected by the drying of insoluble residues after the fermentation of wheat. This effect was reflected in the linear decrease in the digestibility of diets with increasing wDDGS level. Total LWG of birds was lower with the 40% wDDGS diet which resulted in lower carcass weight. However, birds fed 10% wDDGS had a higher LWG and cold carcass weight, with similar edible carcass components comprising breast with bone, thighs, drumsticks neck and back to the 0% wDDGS diet.

Experiment 2 tested the addition of an enzyme into the diets containing intermediate levels of the previously tested wDDGS to improve nutrient utilization. The study involved 168 broiler chicks in a completely randomized design with 3 X 2 factorial arrangement (3 wDDGS levels of 0, 15, 30% and 2 enzyme levels; No enzyme (NE) and with enzyme (E)). Six isonitrogenous diets were formulated and manufactured with and without enzyme inclusion (endo-1, 4-beta-xylanase 9200 U/g, alpha-amylase 1600 U/g and subtilisin as protease 16000 U/g). There were significant ($P<0.05$) decreases in digestibility of nutrients with increasing levels of wDDGS inclusion in broiler diets. A reduction in performance was also observed with increasing inclusion level of wDDGS up to 30%. This experiment provided an indication that 15% wDDGS could be used in practical broiler diets. At 30% wDDGS both feed intake and weight gain were lower in

the presence and absence of enzyme which resulted in similar FCR than other treatments. The same trend for wDDGS and enzyme inclusion was seen at starter, finisher and overall periods of this experiment. Enzyme addition had no effect on dry matter and protein digestibility, or on growth and feed intake. Enzyme addition increased only the digestibility of calcium but reduced the digestibility of protein. Inclusion of 15% wDDGS with enzyme did not affect ($P>0.05$) the carcass composition of broiler chickens, but 30% inclusion gave a poorer carcass and impaired performance. 30% wDDGS caused gizzard weight to increase, possibly due to accumulation of fibres which resulted in a slower passage rate of fibre fractions. The breast meat is considered to be the best meat, being the largest area of muscle concentration in broiler chickens, and was higher at 0 and 15% wDDGS level. Higher protein percentage of breast muscle at 0 and 15% wDDGS apparently revealed higher quality meat than in birds given the 30% wDDGS level. Enzyme supplementation showed a positive effect on some chemical components of broiler muscles where it increased the protein concentration in breast meat. Enzyme supplementation gave no performance benefits when added at manufacturer's recommended level of 0.25kg/tonne in wDDGS-based diets, so increased inclusion rates for this enzyme and another enzyme mixture added to diets with moderate levels of wDDGS were investigated in experiment 3.

Finally, experiment 3 compared the effects of the increased levels of two enzymes (A & B) on broiler performance, carcass characteristics, digestibility, fatty acids, and the gut microbial population. A total of 180 broiler chicks were used in a completely randomized design with 2 X 3 factorial arrangement by using 2 wDDGS levels (0, 15% in isonitrogenous diets) and 3 enzyme levels (no enzyme, NE and enzyme A= EA or B= EB). Here enzyme A contained 9200 U/g endo-1, 4-beta-xylanase, 1600 U/g alpha-amylase and 16000 U/g subtilisin as protease) & enzyme B contained 12200 U/g endo-1, 4-beta-xylanase, 1520 U/g endo-1,3(4)-beta-glucanase. The results demonstrated that neither the starter nor the finisher phase of the broilers showed any noteworthy differences in growth performance (daily feed intake, live weight gain and FCR) when fed 0 or 15% levels of wDDGS with or without the inclusion of enzymes. An increased digestibility of calcium was observed in the previous study as well as calcium and phosphorus in the present study. Improved digestibility of fibre fractions with addition of enzyme was not observed in this experiment. Some digestive organs such as the empty crop, total gut, empty gizzard and liver have had a higher weight due to the 15%

wDDGS's higher fibre content but this has no economic benefits. This study has concluded that the heightened quantities of enzyme A and the use of the improved enzyme B did not produce the desired effect on digestibility. The inconsistent results caused by these enzymes remain a great concern in this area of broiler production.

In summary, these results indicated that feeding wDDGS-based diets with up to 10% inclusion in broiler production resulted in no negative impacts to broiler performance or carcass characteristics. 15% wDDGS inclusion resulted in increased protein content and reduced the ether extract content in broiler meat. Future studies should continue to investigate this technology of enhancing the nutritive availability to broiler chicken through the development and use of specific enzymes for wDDGS especially at high levels of their dietary inclusion. This should also investigate the optimum levels of enzyme to be applied as well as to develop a specific enzyme that would break the various structures of cell walls of NSP in wDDGS. There are currently no commercially prepared exogenous enzymes which target wDDGS NSP and these are needed to enhance the feeding values of wDDGS in terms of improved nutrient digestibility and performance of broiler chickens. Moreover, special emphasis should also be given to the economic aspect, broiler welfare and environmental impact concerning the use of enzymes together with wDDGS to replace wheat and soybean meal in broiler diets.

Keywords: wDDGS, enzymes, broiler chickens

LIST OF ABBREVIATIONS

AAFCO	Association of American Feed Control Officials	LW	Live Weight
AD	Acid Detergent	LWG	Live Weight Gain
ADF	Acid Detergent Fibre	MCP	Moncalcium Phosphate
AIA	Acid Insoluble Ash	ME	Metabolisable Energy
AOAC	Association of Official Analytical Chemists	mEq	Milli Equivalent
BDG	Brewer's Dried Grains	mg	Milligram
Ca	Calcium	Mg	Magnesium
CFUs	Colony Forming Units	MJ	Mega Joules
CIGI	Canadian International Grains Institute	ml	Millilitre
CO2	Carbon dioxide	MUFA	Mono Unsaturated Fatty Acids
CP	Crude Protein	N	Nitrogen
CRD	Completely Randomized Design	ND	Neutral Detergent
Cu	Copper (Cuprous)	NDF	Neutral Detergent Fibre
DF	Dietary Fibre	NE	No Enzyme
DM	Dry Matter	NRC	National Research Council
DWG	Daily Weight Gain	NSP	Non-Starch Polysaccharides
E	Enzyme	^{o}C	Degree celcius
EA	Enzyme A	PBS	Phosphate Buffer Saline
EB	Enzyme B	PHY	Phytase
EE	Ether Extract	ppm	parts per million
FAME	Fatty Acids Methyl Esters	PUFA	Poly Unsaturated Fatty Acids
FAO	Food and Agriculture Organisation	SBM	Soybean Meal
FCR	Feed Conversion Ratio	SCFA	Short Chain Fatty Acids
FI	Feed Intake	SEM	Standard Error of Means
FLW	Final Live Weight	SFA	Saturated Fatty Acids
FTU	Fibre Terminating Unit	TFA	Total Fatty Acids
g	gram	U	Units
GIT	Gastro Intestinal Tract	UNDP	United Nations Development Project
HCl	Hydrochloric Acid	UNICEF	United Nations Children's Fund
IU	International Units	VFAs	Volatile Fatty Acids
Kcal	Kilo calories	wDDGS	wheat Distillers Dried Grains with Solubles
KCl	Potassium Chloride	WG	Weight Gain
Kg	Kilogram	XAP	Xylanase, Amylase and Protease
kpa	kilo pascal	Zn	Zinc

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CHAPTER 1:
GENERAL INTRODUCTION

1.1 Background information

The pressure for nutritionists to focus on the possible use of alternative feedstuffs in non-ruminant animals is increasing. Research into how best to use as favourably as possible, alternative sources of feedstuffs in order to achieve sustainable diets for poultry production is required. The price of grain in the world is increasing sharply and the industry is facing several challenges in relation to producing high-quality animal products for consumers at a reasonable price (Ibitoye *et al.*, 2012; Hossan, 2013).

The search for alternative feeds is necessitated by the high cost of conventional feeds, and the scarcity of feeds on occasions. This has been further exacerbated by the underproduction of grains and keen competition amongst humans, industries and livestock for the grains and oil seeds that are in short supply. As a result, the industry requires alternative feed sources to maintain animal production (Anyanwu *et al.*, 2008; Nalle *et al.*, 2011; Khan and Chaudhry, 2012). The prohibitive cost and scarcity of conventional feed ingredients on occasions has forced agro-industrial by-products to be used in animal feeds (Olorede *et al.*, 2002; Ibitoye *et al.*, 2012).

Presently, the agricultural crop production system generates large quantities of crop residues, which could be used as raw materials for a wide range of new purposes as waste products from the primary function (UNDP, 2002). Distillers dried grains with solubles (DDGS) is a by-product, which is increasingly used for animal feed. This raw material is suitable for feeding many animal species, and offers high levels of crude protein and certain minerals, due to the concentrating effect of the ethanol processing. Thus, the utilization of these materials can assist in solving problems of global importance such as resource recovery, waste utilization, and improved environment management (UNDP, 2002).

Nowadays, nutritionists are hesitant to use high levels of DDGS in poultry diets, largely due to the lower energy (less starch) and higher fibre content, which is of great concern, and moreover, as high dietary levels may limit the intake of high performance broilers (Noll *et al.*, 2001). According to Weeks *et al.* (2000), broiler chickens are probably the most abundant species which is farmed globally, and have been subjected to increasingly intensive genetic selection for growth rate and food conversion efficiency

over the last four decades. In consequence, they attain practically adult body size whilst still very immature in many aspects of development and have high metabolic demands for extremely rapid growth.

In view of the continued growth of ethanol production and the availability and usage of DDGS by broiler producers, coupled with an associated paucity of research and new developments in production of DDGS of variable quality, inclusion levels and digestibility characteristics need to be reconsidered, with an emphasis on productivity, behaviour and the carcass characteristics of broiler chickens.

1.2 Problem statement

The need to reduce the competition between humans and animals for the limited quantities of grains and oilseeds cannot be understated. Agro-industrial by-products offer unique opportunities for animal scientists and livestock farmers to achieve this objective, in addition to their role in increasing the production of essentially required animal protein for human consumption. Poultry meat and eggs can be produced within a considerable short period of time, due to the rapid generation time of chickens (Okoli *et al.*, 2005).

1.2.1 The problem of feeding

Poultry production in developing countries, especially Nigeria, largely depends on erratic or inadequate production of maize, which is a major source of energy. Utilization of cereal/grains such as wheat, maize etc. is very expensive. Intensive research into poultry nutrition concentrates mainly on finding alternative feedstuffs such as agricultural/industrial by-products for poultry. Feed accounts for about 70-80% of the total cost of poultry production in Nigeria. The shortage and high cost of conventional feed ingredients in Nigeria is due to the high cost of feeds for livestock production in competition with other uses (Anyanwu *et al.*, 2008).

A considerable number of Nigerians are malnourished, particularly because of animal protein deficiency in their diet. Scientific evidence shows that optimum animal protein intake is required for optimum physical and mental development. The Food and Agriculture Organisation (FAO) of the United Nations recommends a minimum of about 60g of protein intake per person per day to be consistent with a good quality life, and that a minimum of 50% of this 30g should come from animal proteins such as meat, milk, eggs and fish. The remaining 50% can come from vegetable sources such as oil seeds and legumes. Nigerians consume less than 10g of protein per person per day, out

of which only about 3.2g is animal protein (Abu *et al.*, 2008; Barwa, 2009). Therefore, it is not surprising that UNICEF (2001) statistics demonstrated that Nigeria was ranked 15th highest in the world for the mortality rate of children under 5 years of age. This was attributed to animal protein malnutrition. Hence, research into animal production and nutrition, and more importantly broiler chicken production, is imperative in order to produce superior quality poultry meat at a reduced cost.

Agro-industrial by-products, consisting principally of straw, husks, skins, trimmings, cobs, bran and Brewer's Dried Grains (BDG), are increasingly abundant in Nigeria. These residues, which often are either burned or ploughed into the soil, are suitable for both ruminant and non-ruminant livestock. Nigeria is blessed with abundant land and favourable agro-ecological factors, which are suitable for rapid agricultural development. An increase in the production of crops often leads to a profusion of crop residues and agro-industrial by-products. Thus, farmers are earning revenue from crop residues now, which was never the case before, through utilization of farm wastes such as agro-industrial by-products by farm animals. The processing, packaging and marketing of these products, offers opportunities for an industrial revolution that can lead to job creation and employment opportunities for many Nigerians.

1.3 Overview of wheat DDGS and the concept of its nutrient content

Wheat DDGS is a by-product, or simply a dried residue remaining after the starch portion located in the endosperm of the cereal grain (wheat) is fermented in the ethanol production process to produce ethanol and carbon dioxide. The majority of the starch is removed and it gives an aroma of alcohol. Thus, with the removal of the predominant nutrient in the wheat, all other nutrients in the resulting by-product, the wDDGS, increase their concentration up to three times more than in the original grain (Widyaratne and Zijlstra, 2007a; Min *et al.*, 2009). The remaining grain nutrients are protein, fibre and oil. After the completed fermentation, the alcohol is removed by distillation and the remaining fermentation residues are dried. wDDS is an excellent digestible protein and energy source for ruminants, especially beef cattle. It is rich in cereal and residual yeast protein, minerals and vitamins. However, it could also be a potential feedstuff for poultry (Kluth and Rodehutscord, 2010) due to its high nutrient quality relating to gentle drying and processing (Świątkiewicz and Koreleski, 2007). The continued increase in ethanol demand and its production, especially in the US and European countries, has led to production of enormous amounts of DDGS flooding into

the animal feed industry. Meanwhile, the major constraint associated with the use of DDGS for poultry is the high variability of the nutrient content and availability, as well as the poor digestibility of some amino acids such as lysine, tryptophan and arginine (Choct, 2006), in addition to the presence of high fibre, possible mycotoxins and some anti-nutrients e.g. phytate. However, using enzymes to improve the nutrient availability of wDDGS, in particular the protein quality and the release of metabolisable energy (ME) could result in an improved product for poultry.

1.4 The physical characteristics and chemical composition of wDDGS

Being a by-product produced by means of dry mill ethanol production, wDDGS is predominantly characterized by a dark brown colour. In the past, there was no relationship between the colour and nutritional value of DDGS; nevertheless, recent studies with swine and poultry (Cromwell *et al.*, 1993; Fastinger *et al.*, 2006) have revealed a lower nutritional value for dark-coloured DDGS. Usually more severe processing, especially overheating during drying, results in the dark brown colour, which leads to the binding and possible destruction of nutrients, especially lysine. Several researchers (Pedersen *et al.*, 2007; Widyaratne and Zijlstra, 2007b; Nyachoti *et al.*, 2005) have reported that wDDGS were characterized by a high crude fibre and fat content of approximately 7–7.6%.

It was reported by Dong *et al.* (1987) that the proximate composition of wDDGS contained very low concentrations of starch due to the fact that the majority of the starch in the grains had been converted to ethanol. However, the crude protein content of wDDGS stands at around 37% as shown in Table 1.1 for the proximate composition of wDDGS.

Table 1.1 Proximate composition of wDDGS (%Dry Matter basis)

Nutrient	Composition (% DM)
Dry Matter (% As fed)	91
Crude Protein	37
Crude Fat (Ether Extract)	5
Crude Fibre	8
NDF	34
Ash	6

Source: Heuze et al. (2013)

1.5 The utilization of brewer's dried grains in livestock and poultry feeds in Nigeria

Ethanol production in Nigeria is at its embryonic stage; however, it mainly comes from cassava and sugarcane. The establishment of a mini-ethanol plant is seen as one of the ways of utilizing agricultural produce to generate employment and provide value added goods for the use of people and industries. The beverage alcohol industry also produces grain by- products, which are nutritionally different and have different economic values in various types of animal and poultry feeds**.** Although wheat is produced in considerable quantities in Nigeria, it is mainly used in the production of beer, the by-product of which is BDG. This is rich in its nutrient content, although its utilization by poultry is limited due to its high crude fibre content. BDG is quite similar to wDDGS in the sense that its nutrient contents are higher than those of sorghum or corn, which are conventionally used as an energy source in poultry diets in Nigeria. The utilization of BDG is hindered by the high cost of drying the wet grain, as well as transporting it from breweries to the drying centres (Sonaiya, 1995). Table 1.2 shows the comparison of the nutrient composition of wDDGS and BDG.

Table 1.2 Nutrient compositions of wDDGS and BDG

Component (%)	wDDGS[1]	BDG[2]
Crude Protein	38	29
Ether Extract	4	9*
Ash	5	4[a]
NDF	25	53
Total Fibre	31	-
Lysine	0.9	1.2
Methionine	0.7	0.5
Threonine	1.2	1.03
Tryptophan	0.4	0.3
Calcium	0.1	0.4
Phosphorus	0.96	0.6

***Sources**: [1]CIGI (2011), [2]NRC (1998),*NRC (1984),[a]Onwudike (1986).*

1.6 Comparison between wheat DDGS and corn DDGS

The composition of nutrients in DDGS may vary considerably. As a result, the quality of DDGS may be diminished, adversely affecting its market value (Belyea *et al.*, 1989; Belyea *et al.*, 2004). Ethanol is being produced from wheat and corn, although corn is generally preferred due to its superior ethanol yield. Currently, wDDGS is offered as a feed for monogastric animals due to increased production levels of ethanol; however,

there is a lack of nutritional data relating to wDDGS originating from contemporary ethanol production facilities (Nuez Ortín and Yu, 2009; Cozannet *et al.*, 2010a). In contrast, a considerable amount of data relating to the nutritional value of corn-based DDGS (cDDGS) is available, especially relating to its use in poultry diets (Batal and Dale, 2006; Fastinger *et al.*, 2006) and the effects of increased intensity of cDDGS used for broilers has been assessed (Wang *et al.*, 2007). DDGS possesses a chemical composition distinguished by the presence of higher levels of fibre and protein and lower levels of fat, sodium, phosphorus, ash and lysine compared to previous data published (Cromwell *et al.*, 1993; Spiehs *et al.*, 2002). Such variation is attributed to the use of different techniques in processing DDGS and not the composition of the corn itself (Belyea *et al.*, 2004). Significant variation in corn composition was also discovered by Cromwell *et al.* (1993), and later by Spiehs *et al.* (2002) observing high levels of variation in total amounts of minerals, methionine and lysine, but significantly less variation in levels of fat, protein and fibre, in a variety of DDGS samples.

Table 1.3 shows a higher content of crude protein in wDDGS (39%) than in cDDGS (30%). Results showing protein content of 44.5% in wDDGS and 30.3% in cDDGS were also reported by Widyaratne and Zijlstra (2007). The use of too much heat in the drying process can result in reduced protein fraction availability (Kleinschmit *et al.*, 2006). Nonetheless, the variation in chemical formation of wDDGS is greater than the variation found between the source cereals and there also exists considerable variation among ethanol production facilities based on whether grain preparation procedures incorporate decortication or not; the process of fermentation; quantity of soluble fractions which are mixed with distillers grains; drying time and temperature; and the possibility of additional protein separation (Belyea *et al.*, 2004).

Table 1.3 Comparison of the proximate composition of wDDGS and cDDGS

	Composition (% DM)	
Nutrient	**wDDGS[a]**	**cDDGS[b]**
Dry Matter (% As fed)	94	89
Crude Protein	39	30
Crude Fat (Ether Extract)	5	11
NDF	48	42
ADF	11	16
Ash	6*	6
Starch	6	4
Calcium	0.2	0.1
Phosphorus	0.9	0.9

*Source: * Heuze et al. (2013), [a] Nuez-Ortin and Yu (2009), [b]Spiehs et al. (2002)*

Levels of crude protein content in cDDGS of 29% to 32% have been recorded (Spiehs *et al.*, 2002). With regard to wDDGS, an increase of 25% was recorded in wDDGS (DM basis) to 39% in comparison with the original 14% in wheat grain, demonstrating a greater protein content in wDDGS as opposed to cDDGS (Nuez Ortín and Yu, 2009).

An extra source of protein can be found in the yeasts used in fermenting starch. The amount of soluble fractions mixed with distillers' grains affects both the amino acid (AA) profile and level of protein. The primary limiting AA in cDDGS is Lysine, with levels of 24.6 to 33.1g kg^{-1} of CP in cDDGS (CV=17.3) having been recorded (Spiehs *et al.*, 2002). Levels of lysine in DDGS are affected by the degree of drying used on the DDGS, as lysine may be adversely affected by excess heat during the drying process (Kleinschmit *et al.*, 2007). Table 1.4 shows average values for essential AA found in both cDDGS and wDDGS. It is found that cDDGS possesses a higher concentrated level of essential AAs than wDDGS, with the exception of arginine and tryptophan, both of which show increased levels in wDDGS. The high degree of variance found in the content levels of lysine and arginine gives rise to concern with respect to the use of wheat and corn DDGS for poultry feeding, due to the diminished levels when compared to those found in the source grain.

Table 1.4 Concentration of essential amino acids (AA) in wDDGS and cDDGS

	wDDGS[a]	cDDGS[b]
Essential AA		
Arginine	4.3	3.7
Histidine	2.1	2.3
Lysine	2.3	2.4
Phenylalanine	4.5	4.7
Leusine	6.5	10.8
Isoleusine	3.5	3.5
Valine	4.3	4.6
Methionine	1.5	1.7
Threonine	3	3.4
Tryptopan	1.1	0.9
Total	**33**	**38**

[a]*Cozannet et al. 2009* [b] *Fastinger et al. 2006*

The overall quality, and especially the composition of nutrients in distillers grains, may be affected by production constraints imposed, or conditions which exist, at the ethanol facility, such as initial processing, starch types used in fermentation and effectiveness of the extraction process (Spiehs *et al.*, 2002), quantity of solubles mixed with distillers grains and drying time and temperature (Kleinschmit *et al.*, 2006). The composition of

nutrients in distillers grains can also be affected by the original raw material, the variety of grain used and its nutrient content (Spiehs *et al.*, 2002) and also the mixing of different grains to form the raw material (Köster, 2007). Nonetheless, as noted by Belyea *et al.* (2004), the principal influencing factor on the final nutrient content of DDGS (using similar grain) is in fact the impact of the ethanol production plant and its processing techniques.

Several methods are being employed in DDGS preparation, resulting in frequent variation in the final product in terms of its chemical composition and physical appearance as a result of the types of grains and the specific processing and drying techniques used. As previously mentioned, heat damage can lead to diminished lysine availability (Maillard reaction) and also fractional damage of cystine. The extent of the impact caused by processing and drying techniques on the nutrient value of DDGS is unknown. Exact causes of variation in DDGS composition have not been clearly defined (Belyea *et al.*, 2004). Ethanol is produced by a process of either wet or dry milling with fermentation grains. The product of dry milling is DDGS, while wet milling results in gluten meal, gluten feed, wheat-germ meal and fermentation solubles. Of the ethanol fuel available, 60% is obtained by wet milling and 40% by dry milling. Figure 1.1 shows a step-by-step guide to the process of dry milling as used for the production of DDGS. The process begins with the cereal being harvested and dehulled before passing through a hammer mill where it is ground; revealing the starch and enlarging the area for processes such as enzymatic digestion and fermentation. The primary enzyme in the process of fermentation, alpha amylase, is then blended with the milled wheat, allowing the conversion of wheat starch to light carbohydrate (dextrin) through hydrolysis. The resulting substance is then cooked in order to destroy any bacteria producing lactic acid, before being mixed with glucoamylase and yeast and entering the fermentation process, which lasts up to 50 hours. It is within the tank used for the fermentation process that ethanol, CO_2 and other distillery by-products are created. Through the process of distillation, the ethanol and distillery by-products are separated. The resulting wet substance (whole stillage) then passes through a process of centrifugation in order to extract the liquid, resulting in only coarse solids remaining. These solids are known as distillers wet grains, alternatively they may be dried to produce DDG (distillers dried grains). Meanwhile, the liquid may undergo an evaporation process, resulting in the production of distillers dried solubles (DDS). The

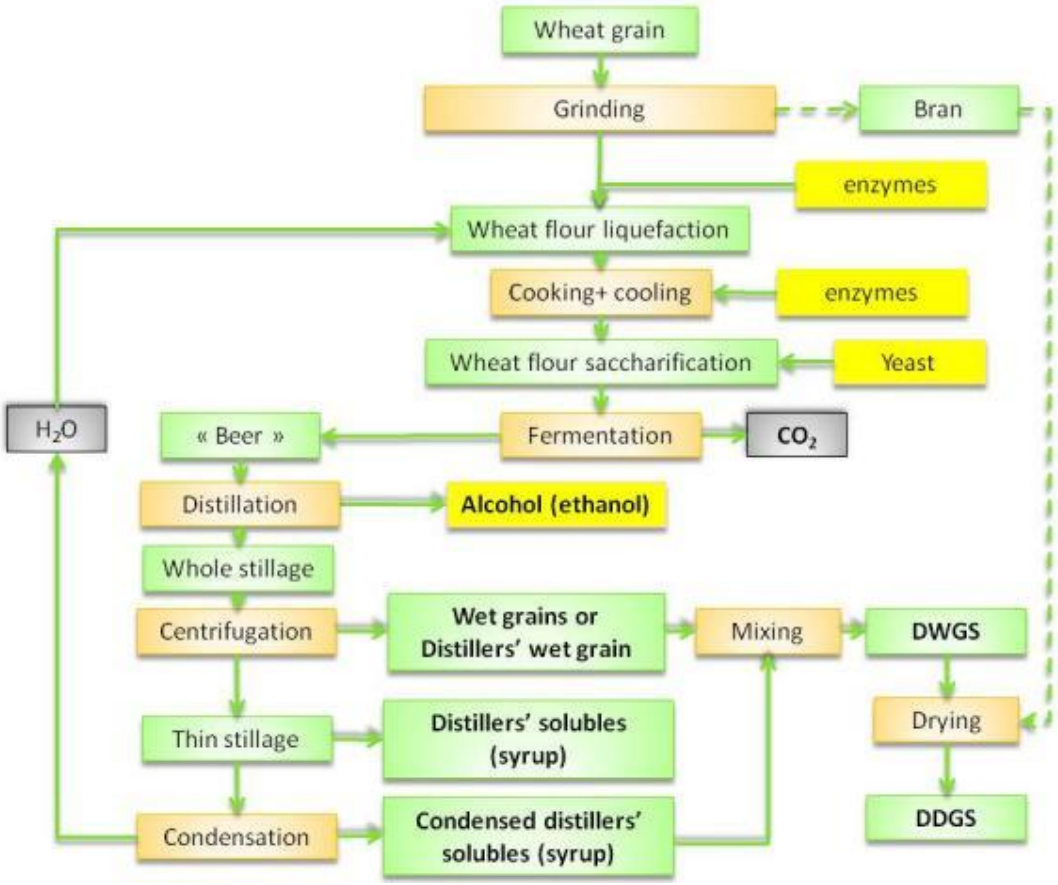
resulting DDS may also be mixed with the original coarse solids, dried and processed as DDGS (distillers dried grains with solubles) (Murphy and Power, 2008). Such reduced solubles are rich in energy, protein and vitamins, and are frequently mixed with distillers' grains in order to produce WDGS and DDGS (Heuzé *et al.*, 2013).

The starch content of distillers' grains is increased through the reintroduction of bran in the final processing stage. The process of fermentation also results in the production of yeast (3%) and glycerol (4%), apart from the ethanol (Hazzledine *et al.*, 2009). wDDGS composition varies far greater than the composition of original grains, which in themselves vary due to inherent factors such as cultivation conditions. There also exists variation among ethanol production facilities based on the various processing techniques which may be used as well as factors such as whether milling or grinding for grains is used, types of yeast and enzymes used in fermentation, the exact mix of protein rich soluble with distillers grains, and time and temperature of the drying process, among others (Cozannet *et al.*, 2010b). The previously mentioned reintroduction of bran in the final stage of processing produces a final product richer in starch but lower in fibre, whilst the addition of solubles produces a product with greater levels of fat content but lower levels of neutral detergent fibre. Levels of neutral detergent fibre (NDF) in wDDGS also vary, for example, 32% dry matter (DM) in wDDGS produced using whole grains compared with 27% DM in wDDGS produced using milled grains.

WDDGS has a low fat content (5% DM), which is considerably less than that found in cDDGS. Due to the fact that wheat solubles have low NDF levels and high fat content (max 34%), combining a greater amount of solubles with wDDGS results in distillers grain containing lower levels of NDF and higher levels of fat (Heuzé *et al.*, 2013). The final composition of DDGS is also influenced by the raw material combination. Certain ethanol production facilities mix corn and wheat before the distilling process. This in turn changes the content levels of protein, energy and phytate found in the final product composition (Heuzé *et al.*, 2013). Compared with wDDGS, cDDGS contains higher levels of lipids. The final by-product's energy value may also be affected by the high levels of protein typically found in DDGS. With regard to the level of oil content, wheat contains approximately half the concentration of oil compared to corn grain and corn distilled by-products.

Heat damage as a result of the process of drying is also considered a cause of variation. The use of high drying temperatures with wDDGS results in diminished availability of amino acids in non-ruminants. The process of drying, and the composition, can also

affect the colour, which may range from a dark brown to pale yellow. Noblet *et al.* (2012) suggested based on this that wDDGS with paler colour would be more suitable for non-ruminants because they would have a higher level of amino acids.



Source: http://www.feedipedia.org/content/wheat-distillers-processing

Figure 1.1 Illustration of the process used to produce wDDGS/cDDGS

The process of fermentation results in distillers grains being relatively free of starch (Stein and Shurson, 2009); however, they retain high levels of other nutrients such as lipids, fibre, protein, phosphorus and sulphur (Spiehs *et al.*, 2002). The overall quality, and especially the composition of nutrients in distillers grains, may be affected by production constraints imposed, or conditions which exist, at the ethanol facility, such as initial processing, starch types used in fermentation and effectiveness of the extraction process (Spiehs *et al.*, 2002) as well as temperature levels and length of time during the drying process and quantity the mix composition of distillers grains and solubles (Stock *et al.*, 2000). The composition of nutrients in distillers grains is also affected by the type of original raw material and its own nutrient composition (Spiehs *et al.*, 2002) and will also depend on if the original raw material was of a single or blended

grain type (Köster, 2007). Nonetheless, as Belyea *et al.* (2004) noted, based on low correlation between the chemical makeup of the final DDGS product and the original raw material, the primary cause of variation in nutrient composition in DDGS is in fact the conditions which exist at the ethanol production facility.

Following the extraction of starch, the remaining principal component of DDGS is fibre. In the study by Widyaratne and Zijlstra (2007), NDF (treated with sodium sulphite) differed little from corn (31%) to wheat DDGS (30%). However, it was noted that wDDGS had a significantly greater value of ADF (21%) than cDDGS (15%) using 4:1 wheat: corn intermediate DDGS. Similar levels of ADF and N-modified NDF (NDFn) were also discovered in cDDGS (15% and 50%) and wDDGS (11% and 48%) by Nuez Ortín and Yu (2009). DDGS with high fibre content is a source of concern for both manufacturers and nutritionists as cereal grains containing ADF are not easily digested by animals.

A study comparing nutrient levels in cDDGS among contemporary ethanol production facilities in Minnesota and South Dakota found a small degree of variation (CV<10%) in CP, DE, DM, crude fibre and crude fat, while a high degree of variation was found in levels of minerals (Spiehs *et al.*, 2002). Phosphorous was discovered to have the lowest CV value (average 0.89%). The highest CV value was that of Zinc (80.4% with average 97.5ppm). It has been suggested that such differences may be caused by variation in levels of minerals found in the original raw material, which in turn are affected by the variation in mineral levels of the area of cultivation. This notion is further supported by Spiehs *et al.* (2002) based on the variation in levels of minerals found in plants, specifically phosphorous, from one year to the next, suggesting this would in turn impact the mineral levels of DDGS. Moreover, DDGS have comparatively low levels of calcium; wDDGS with an average value of 0.15% and cDDGS with an average of 0.06% (Spiehs *et al.*, 2002). Average phosphorous levels in DDGS, as previously mentioned, are 0.89% for cDDGS and 1.07% for wDDGS and it has been demonstrated that DDGS offers greater availability of phosphorous than found in original grains, resulting from the fermentation process. This is crucial in the design of non-ruminant diets (Spiehs *et al.*, 2002). This is supported by findings by Widyaratne and Zijlstra (2007) where a comparison between cDDGS and wDDGS was carried out using pigs which consumed almost half as much more wDDGS than cDDGS, resulting in greater

phosphorous consumption, but exhibited comparable excretion levels to those which consumed only cDDGS.

The larger variation in nutrient levels of DDGS as poultry feed raw material remain the principal challenge for its utilization as a feed resource as well as different blends added. Therefore, research is being carried out to strategically manage the variation while exploring new ways of using DDGS as animal feed.

1.7 Justification for the study

A review of recent research on DDGS indicated that the performance of broiler chickens is reduced when diets containing higher levels of wDDGS are fed to them (Wang *et al*., 2007). Since a combination of enzymes was found in some studies to improve bird performance, especially live weight gain and feed efficiency, there is a need to explore the possibilities of utilizing this technology to improve the raw material and incorporate higher levels of wDDGS in broiler chicken diets.

In recent years, there has been a vast increase in ethanol production from corn, wheat, sorghum, rye, etc., and a significant amount of research has been directed towards determining how best to utilize ethanol by-products such as DDGS. It is therefore apparent that a means to improve the nutrient use of wDDGS will be increasingly valuable. The use of exogenous enzymes as a tool to improve the value of wDDGS is of paramount interest to broiler producers. There is evidence that enzymes designed to improve utilization of nutrients may have added benefits for improving health and body weight uniformity. Before the use of wDDGS can become effective, adequate research must be conducted to optimize their tolerable inclusion levels, and report all possible detriments that might be accrued in the course of feeding them to poultry.

1.8 Aims and objectives of the study

The primary aims of the study are:

1. To determine the optimum levels for inclusion of wDDGS in broiler diets.
2. To establish the effects of feeding wDDGS on behavioural changes.
3. To determine the effects of feeding wDDGS supplemented with enzymes on productivity, carcass characteristics and the organ weights of the broiler chickens.
4. To determine the interaction between wDDGS levels and enzyme supplementation.

5. To compare the effects of different enzymes on broiler performances, carcass characteristics, digestibility, muscle chemical composition, fatty acids and gut microbial population.

To achieve these objectives, a literature review was first conducted to identify knowledge gaps and inform the design and conduct a series of broiler experiments.

CHAPTER 2:
LITERATURE REVIEW

This literature review was conducted in order to identify gaps in the existing knowledge and to inform the design of experiments to achieve the objectives stated in chapter 1.

2.1 Nutrient requirements of broiler chicken

The nutrients that broiler chickens require from their diets to ensure that they develop and grow healthily include energy, water, protein, vitamins and minerals (Smith, 2001). However, no standard nutrient requirements for broiler chickens have yet been produced to cover all possible environmental conditions (Oluyemi and Roberts, 1998), which are attributed to the different conditions of temperate and tropical environments (NRC, 1994).

2.1.1 Energy requirement

Daghir (2008) discussed the requirements for broiler chickens and explained that they differ based on the environmental temperature. The ME requirement was found to decrease as temperatures increase, especially above 21^{o}C. This reduction is associated with a decreased demand for energy for maintenance; the environmental temperature therefore does not affect the requirements for production. Much research supports the claim that energy requirements tend to decrease throughout summer months, compared with winter months; typically, there is a 10-15% reduction in the requirement for nutrients during the summer. Hurwitz *et al*. (1980), in their study with broilers demonstrated that the energy requirements for maintenance were reduced when the environmental temperature reached below of 27^{o}C, followed by an increase to 34^{o}C.

Poultry adjust their intake of feed in order to satisfy their requirements, hence an absolute value for the number of kilocalories per kilogram (kcal ME/kg) cannot be easily expressed (NRC, 1994). Oluyemi and Roberts (1998) discussed how these birds undertake such adjustment when they are provided with feed *ad-libitum*. Chickens that are provided with low energy diets, such as those with around 2600 kcal ME/kg, tended to eat more feed overall (30% more) than birds provided with high energy diets, of around 3200 kcal ME/kg (NRC, 1984). Within the tropics, an energy intake of 2800-3200 kcal ME/kg and a standard dietary intake of 3200 kcal ME/kg were suitable for all stages of broiler chicken development as suggested by Oluyemi and Roberts (1998).

2.1.2 Protein and amino acid requirement

Broiler chickens need to consume a sufficient amount of protein, as the component amino acids are necessary building blocks for the synthesis of different molecules (NRC, 1984). Different strains and breeds of poultry require different amounts of protein for their development (NRC, 1994), this value also differs with their age. The amount of protein that is required is determined by the amino acids that are necessary for growth, as well as the nitrogenous compounds required for maintenance (Smith, 2001). Pond *et al.* (1995) and Bartov (1998) explained how chickens gain weight differently, depending on the provision of amino acids. Amongst the essential amino acids that cannot be synthesised by the bird, Methionine is often included in diets when standard proteins do not provide enough of this amino acid which is required for feather formation (Kim *et al.*, 2006). Yalcin *et al.* (1999) aimed to optimize the efficiency of converting feed and found that broilers that grew from a weight of 1.8kg to 2.6kg require 0.72% of sulphur-based amino acids. The research used concentrations of 0.8% and found this to be suitable as well. Abasiekong and Tyopat (2000) suggested a lysine and methionine concentration of 2.4% in a combined supplementation, in order to maximise broiler chicken performance.

2.1.3 Mineral requirement

Minerals are required for skeletal development of chickens. They are also necessary to activate enzymes and maintain water-balancing processes within the body (NRC, 1994). The main minerals that are required for growth are potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg), calcium (Ca) and phosphorus. Applegate (2007) demonstrated how phosphorus is fundamental to metabolism within cells, as well as providing energy for different regulatory processes, particularly within the skeleton. Ca is most commonly used for the development of the skeleton throughout growth (NRC, 1984). Extreme levels of Ca in the diet can affect the uptake of other nutrients, especially zinc and manganese (NRC, 1994). Limestone, as a source of Ca, can be used to satisfy the Ca: Phosphorus ratio that is required for growth (Nuez-Ortín and Yu, 2010). Phosphorus is necessary for nutritional provision, although much contention surrounds how it should be provided to animals (Applegate, 2007). A substantial amount of phosphorus is excreted by the animal, although it is fundamental for growth; therefore, there is much debate about how much should be provided to a developing

animal (Karimi, 2006). Economic and environmental factors are also considered, due to the cost of phosphorus, as well as how it can pollute the environment. K is a potent electrolyte that serves the purpose of operating with Cl and Na to ensure optimal growth and development, as well as the utilization of nutrients and other minerals.

2.1.4 Vitamin requirement

Vitamins are required by broiler chickens to maximize performance. Vitamin C can be produced by poultry, although all other vitamins need to be included in their diet. Vitamins are required in very small amounts within an organism, highlighting the intricacy of producing suitable diets (Oluyemi and Roberts, 1998). There are two major headings for the classification of vitamins: 1) fat-soluble vitamins (A, D, E and K), and 2) water-soluble vitamins (B-complex vitamins, C). Typically, vitamin requirements are provided on a scale of mg/kg of diet, except for A, D and E, which are measured instead in international units (IU) for standard quantification. Leeson *et al.* (2001) explained how vitamins are not always readily available in normal feed sources, therefore needing to be provided by supplements and artificial premixes. This is especially true for the natural diets of poultry. Interestingly, the rate of growth and the feed conversion efficiency were not affected by a withdrawal of these vitamin supplements after 42 days (Skinner *et al.*, 1992), not at 35 days (Farrell, 2005).

Table 2.1 Requirements of broiler chickens for selected nutrients as units per kilogramme of diet

		Age (Weeks)		
	Unit	**0-3**	**3-6**	**6-8**
Component				
Energy*	(Kcal/Kg)	3,200	3,200	3,200
Protein	%	23.0	20.0	18.0
Lysine	%	1.2	1.0	0.9
Methionine	%	0.5	0.4	0.3
Calcium	%	1.0	0.9	0.8
Phosphorus (available)	%	0.5	0.4	0.4
Potassium	%	0.4	0.4	0.3
Sodium	%	0.2	0.2	0.2
Chlorine	%	0.2	0.2	0.2
Magnesium	mg	600	600	600
Zinc	mg	40	40	40
Iron	mg	80	80	80
Iodine	mg	0.4	0.4	0.4
Vitamin A	IU	1,500	1,500	1,500
Vitamin D	IU	200	200	200
Vitamin E	mg	10	10	10
Vitamin K	mg	0.5	0.5	0.5
Niacin	mg	27	27	11
Thiamine	mg	1.8	1.8	1.8

*These are typical dietary energy concentrations.

Source: NRC (1984)

2.2 Feed intake, water consumption and body weight changes in broiler chickens

A variety of different factors are associated with the intake of feed and the performance of birds. The intake of feed can be greatly affected by the ambient temperature that broiler chickens are developing within (Hurwitz *et al.*, 1980). The suggestions made in this report assume that the chickens are developing in a standard condition of 18-24ºC. Temperatures outside of this range influence the feed intake process, whereby low temperatures result in greater feeding and high temperatures result in lessened feeding (NRC, 1984). Feed intake in poultry decreases by 1.5% for every 1ºC increase in ambient temperature over the thermal neutral zone. Thus, temperatures over 30ºC result in a decrease in feed intake of 2.5-4g per 1ºC increase. Within the range of 32-38ºC each 1ºC rise in temperature results in a 5% reduction in feed intake (Donkoh, 1989; Singleton, 2004; Faria Filho *et al.*, 2005).

Generally, poultry can adjust the amount of feed they consume in order to satisfy a minimal intake of energy that is required from their diets. However, there is debate

about the precision of these energy adjustments (NRC, 1994). Increasing the energy concentration within a diet tends to reduce the amount of feed being consumed overall (NRC, 1984; Smith, 2001). Diets deficient in particular nutrients result in a suppression of appetite and a reduction in the growth and activity of broiler chickens (Smith, 2001). Research has found that some increase in dietary fibre can improve overall performance of chickens being reared (Sklan *et al.*, 2003). A study by Jørgensen *et al.* (1996) found that fibre can help with the development of the gastrointestinal tract as well as the metabolism of energy among populations of broiler chickens. However, fibre also helps feed to pass through the digestive tract, which limits the time of enzyme exposure, as well as the nutrient absorption rate across membranes (Shalash *et al.*, 2009).

Based on weight, birds consume twice as much water as they do feed, highlighting its importance for development (Coetzee, 2005). Research by Vieira and Lima (2005) found that birds feeding on a solely vegetarian diet tended to have an increased water intake and more excreta, associated with an increase in moisture. Feed and water consumption have been found to be dynamically linked together and fibre has been found to bind some water during digestion (Chaplin, 2003). Additionally, it was found that a 6% increase in water consumption occurs for every 1^{o}C increase over 20^{o}C environmental temperature; this has been found to be between 1.83 and 2 times as much, in quantity, as the feed intake.

Other factors have been found to be associated with reduced feed intake , such as the diseases and other infections from *E. coli* and *Campylobacter* (Faria Filho *et al.*, 2005), as well as housing and care, such as ventilation, stocking density and environmental regulation systems.

2.3 Broiler behaviour

A relationship between feed intake and exhibited behaviour has been found to exist within animals (Johannson, 2008). Much research has aimed to investigate how animals behave and why they do so. Fraser and Broom (1997) and Broom (1991) explored abnormal behaviour and defined it as that which differs in frequency, context or pattern compared with the majority of individuals within a species, particularly in an environment where a full range of behaviours can be exhibited. Behavioural problems, such as feather pecking and aggressiveness, have been found to be suppressed by the provision of fibre to the diet of chickens. This method of feeding results in a larger amount of time and energy being focused on feeding thereby distracts them from aggression. The provision of oat hulls, silage and whole cereal grains has been

suggested in this regard by researchers (Hetland and Svihus, 2001; De Jong *et al.*, 2005; Steenfeldt *et al.*, 2007).

Much research has been focussed on how gut health can be improved with fibre provision. Additionally, studies have investigated its effects on behaviour. Birds that were provided with high quantities of insoluble fibre tended to be calmer, often spending a lot of time feeding and behaving neutrally, as opposed to aggressively (Hetland *et al.*, 2004). Research by Hetland and Svihus (2001) found that reduced aggression in broiler chickens could be achieved through the provision of high fibre diets. The birds were considered to behave more calmly as they were sated and tended to not compete for resources, due to the small stable structure of their groups. Further research by Steenfeldt *et al.* (2007) found that the supplementation of fermentation by-products to egg laying hens resulted in an increase in the size of their gizzards, as well as an overall increase in their weight. Furthermore, these birds displayed reduced feather pecking, as a result of their calmer attitudes.

Duncan (1981) expressed how behaviours can be used as indicators for emotions such as pain, frustration and fear. Additionally, the theory continued to explain how playful, explorative or luxury behaviours are associated with positive emotions and feelings.

Broiler chickens were found to feed half as often as egg laying chickens, but tended to eat more meals of a larger size, often for shorter amounts of time (Masic *et al.*, 1974). Additionally, although both types of chicken were found to display the same pattern of feed consumption, they tended to have different feeding activity. Broilers ate about 3 times more feed than pullets but spent the same amount of time at the feeders. This research found no changes in behaviour as a result of the different feed consumption levels. Preston *et al.* (1983) found that broilers kept in small pens displayed the following behavioural time budgets: 73% of the time lying down, 6% consuming feed, and 2% drinking water. Hall (2001) found the following composition: 65.4% lying down, 7.4% eating, and 4.1% drinking.

Research by Sosnowka-Czajka *et al.* (2005) explained how a deep litter system to house chickens resulted in better welfare and a potentially increased yield in breast meat. However, there was a reported decrease in the space for movement, potentially creating problems within the legs of the chickens (Hall, 2001). Similar studies found that broiler chickens display different behaviours as a result of their ages, often through displaying more lying and sitting behaviours. Genetic selection has contributed to an increase in breast meat yield, resulting in them sitting more often than standing.

2.4 Litter quality and broiler performance

Bedding material needs to be well suited to the needs of the birds to ensure proper welfare, especially within intensive broiler chicken rearing systems. Litter helps to provide a cushioning support against the hard flooring, while also insulating the environment and providing absorption for urine, faeces and water. Low moisture levels need to be maintained for good quality litter; it is recommended to maintain a moisture level between 25-35% (Butcher and Miles 2011). Excess moisture levels can saturate the litter and make it unsuitable as demonstrated in some studies by Mayne *et al.* (2007). There is much variation in the reported moisture levels of different kinds of litter. Wyatt and Goodman (1992) reported that a moisture level of 6.5% was found in gypsum litter, while Brake *et al.* (1992) found that pine shavings litter held a moisture level of 44%. Carr *et al.* (1990) explained how litter moisture levels should be maintained below 30%, in order to aid with ammonia control by reducing its emission.

High litter moisture levels can result from diarrhoea, which can be stimulated by nutritional changes that result in the aggravation of polyuria and the compromising of water retention, or it may be a result of enteritis within the gastrointestinal tract. Manipulating the nutrients within the diet can help to control these levels, especially with respect to electrolyte quantities. Additionally, the health of the bird, the age of the bird and different environmental factors can contribute to the aggravation of this phenomenon (Patterson *et al.*, 1998).

Additional factors can contribute to high levels of litter moisture such as increased water spillage from drinkers (Manning *et al.*, 2007). Furthermore, increased humidity reduces the ability of the litter to dry. Moisture levels within litter have been found to increase as chickens age (Eichner *et al.*, 2007). The formulation of the feed can also contribute to increased moisture levels. Moisture contents of the litter may be altered by excreta composition, which can affect water retention and limit evaporative water losses (Bedford, 2000; Francesch and Brufau, 2004). High fibre diets have been found to reduce overall water intake, thereby reducing water retention within the litter. Hocking (2006) found a decrease in litter moisture which was associated with an increase in the provision of oat hulls, and consequently a comparable decrease in the number of litter changes required.

The provision of wheat, barley, oats, rye and triticale in the diet of broiler chickens results in wet and viscous faeces, consequently increasing litter moisture (Collett, 2012). These grains are high in water-soluble non-starch polysaccharides (NSP), which

increase moisture levels within faeces and result in wet litter (Bedford, 2000; Bedford and Perry, 2006). NSP cause flushing of the gut, which is associated with digestive problems that cause an efflux of water into the lumen of the intestines.

Changes in diet composition that increase water intake by birds consequently result in increased faecal output and an increase in moisture levels of litter. Feeds that have been found to increase water intake by broiler chickens tend to have high electrolyte levels (Eichner *et al.*, 2007). Borges *et al*. (2003) found that litter moisture levels over 21% were associated with dietary electrolyte balance (DEB) levels of 360 mEq/kg which were typical for the first week of life of broiler chickens. At six weeks, this level had increased to 55%. Borges *et al.* (2004) continued to support these findings and found that older birds being fed with a 340 mEq/kg diet DEB resulted in increased litter moisture content. Increased K levels in diets resulted in changes in the litter quality and water content.

2.5 Digestive tract and carcass composition

The broiler chicken digestive system is fundamental to ensuring the absorption of nutrients and their conversion into energy, which helps to produce eggs and meat quantity. The system involves the intake of feed, its storage and digestion, the absorption of nutrients, and the excretion of waste. Raw materials are metabolised once they have entered the system; their conversion helps the animal to grow and develop. Feed enters the digestive tract through the mouth, which prompts the start of the process. It then moves through the oesophagus, past the crop, and down the lower oesophagus. It then enters the proventriculus, which is the glandular and enzymatic stomach, before entering the gizzard, which is the muscular stomach. It then travels through the small intestine, the caeca and the large intestine, before reaching the cloaca at the end. The caeca is the location of fibre degradation and absorption.

Figure 2.1 illustrates the gastro intestinal tract (GIT), which is a short, yet well adapted, system for the conversion of dietary materials into nutrients, whatever the avian species. The rate of passage determines the digestion and absorption of different nutrients. Mammals and birds differ in the structure of their GIT based on the following parameters. Whereas mammals have lips, birds have beaks. There are two main distinct stomachs: 1) the proventriculus, which provides glandular and chemical components for digestion, and 2) the gizzard, which is the muscular and mechanical stomach for the physical grinding of feed (Svihus, 2011). The cloaca is located at the end of the digestive tract and provides the chamber for urinary and excretory processes, as well as

reproduction. Identical digestive tracts are found among all poultry species, including guinea fowl, partridges, turkeys and pheasants, as well as chickens (Gallinaceous).

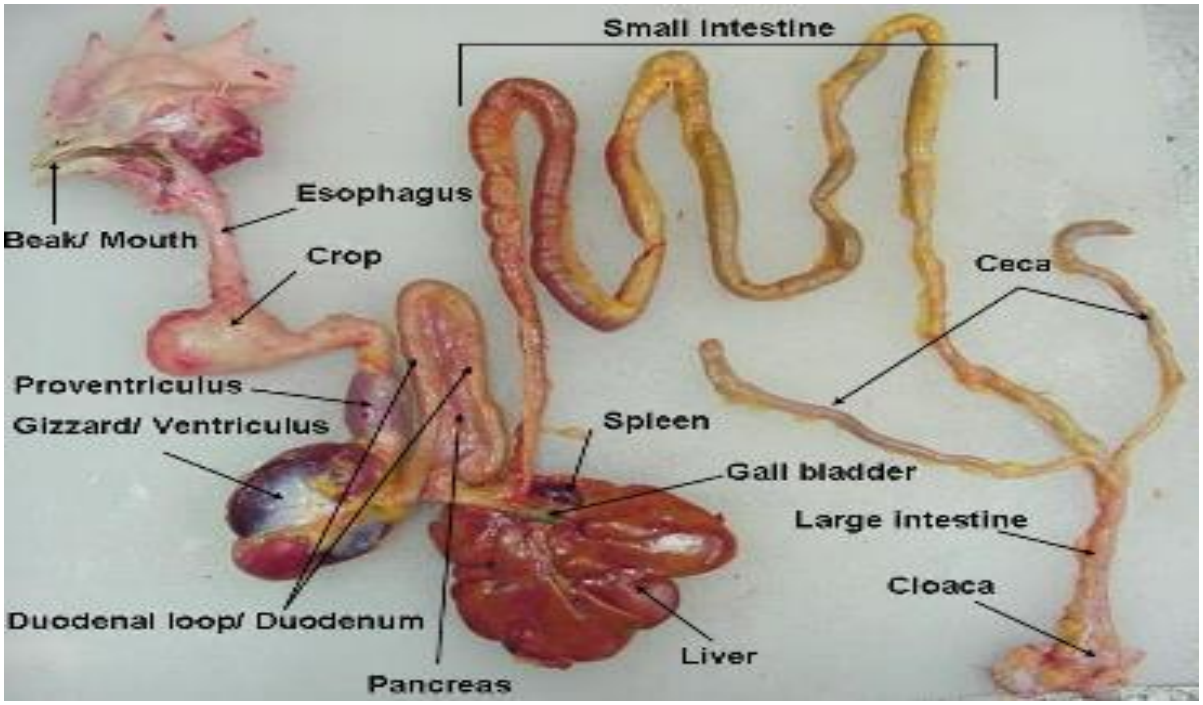


Plate 2.1 Illustration of the digestive system of a broiler chicken

Source: www2.ca.uky.edu

Much research has investigated the composition of the carcass in chickens. Jourdain (1980) found that eight-week old broiler chickens have carcass yields of 64%, with regard to those raised in the tropics. Salami *et al.* (2004) found that the most ideal carcass yield for table birds should be between 65-70%. Previous research by Oluyemi and Roberts (1998) dissected broiler chicken meat and found yield levels of 17.4%, 12.95%, 11.67%, 8.21% and 4.38% for the breast, thighs, drumsticks, wings and neck, respectively.

Manipulating the diets and nutrients of broiler chickens has been found to affect the composition of the carcass, muscles and organs. Additionally, it affects the quality features in meat chickens at the finisher stage (Juzl *et al.*, 2012). The dietary protein level changes the carcass composition; low protein diets were found to reduce the carcass yield, as well as the yield of cut up meat (Salami *et al.*, 2004). Further research by Yalcin *et al.* (1999) found that breast meat yields increased while abdominal fat decreased through the provision of methionine into the diet of broiler chickens. A high fibre diet has been found to affect the mass and size of digestive organs (Sundu *et al.*, 2008), thereby affecting the quality of the carcass.

2.6 Meat quality of broiler chickens

Poultry meat quality is related to the growth of chicken, which thereby affects the muscular development of broilers (Zhang *et al.*, 2012). Suchy *et al.* (2002) found that the overall quality of the meat that is produced from broiler chickens is related to the quality of their muscles. Increasing the level of dietary protein has been associated with an increase in the protein content of broiler meat overall (Snezana *et al.*, 2010). Additionally, the protein and lipid contents of muscles in broiler chickens can be used as an indicator of overall meat quality. Dietary fatty acids are absorbed by the intestine before being included into tissue lipids during lipid synthesis. Polyunsaturated fatty acids, such as linoleic and a-linoleic, are not synthesised by the organism; their relative concentrations may differ based on dietary changes. Monounsaturated and saturated fatty acids, however, are synthesised by the organism and therefore tend not to be included in diets.

Reducing PUFA tends not to be favoured by consumers, although it is considered to improve the overall quality of the meat. PUFA are generally more sensitive to oxidation, compared with SFA (Bonoli *et al.*, 2007; Corzo *et al.*, 2009; Nkukwana *et al.*, 2014). Consumers are recommended to increase their uptake of such molecules due to the health benefits that they can provide upon digestion (Rymer and Givens, 2006). They also stated that broiler diet composition can affect the overall content of fatty acids within tissues of broiler meat. MUFA is an important category of fats which does not influence blood cholesterol in the way that SFA does (Schreiner *et al.*, 2005). Increasing the palmitic and arachidonic acid content of feeds is favoured by consumers, as these compounds can assist blood clotting and bind to endothelial cells, thereby improving the ability of heal wounds (Rahnan *et al.*, 1995). Dietary palmitic acid has been found to generally reduce the serum cholesterol content within humans upon digestion (Sundram *et al.*, 1994).

Further research by Kinsella and Lokesh (1990) found that increasing the content of n-3 PUFA in relation to the increase of n-6 PUFA results in an alteration in the production of eicosanoid, which is necessary for maintaining immune function. Therefore, these compounds are associated with blood clotting and inflammatory responses in both humans and animals. Maintaining the full range of effects of eicosanoid can be achieved through the controlled intake of n-3 and n-6 PUFA; this is a recommended dietary change to ensure proper health (NRC, 1994).

2.7 Wheat distillers dried grains

2.7.1 Definition

As defined by AAFCO (2002), DDGS are a by-product of the ethanol industry created by the fermentation process of cereal grain starch in dry mill ethanol plants. It is the product obtained after the removal of ethyl alcohol by distillation from the yeast fermentation of grain. Alternatively, it can be produced as a grain mixture, through the condensing and drying of stillage, often in large quantities (75% of all stillage produced) by methods employed in the grain distilling industry.

2.7.2 Production of DDGS

Ethanol production is increasing in popularity, which increases the overall amount of DDGS that can be used as feed. Approximately 60 million tonnes of DDGS are produced every year, largely coming from the USA, which produces 9 million tonnes each year (Dien *et al.*, 2002; Choct, 2006; Dien *et al.*, 2008). Distillers dried grains most commonly produced in North America come from maize, whereas European DDGS production stems from the fermentation process of rye and wheat.

DDGS have been used extensively as a source of livestock feed ever since it emerged as a by-product of the beverage industry. It has been fed mostly to ruminants, although it has since been fed to non-ruminant animals, most commonly within the diets of poultry (Cozannet *et al.*, 2011). An increased demand for ethanol due to the growth of bio-ethanol industry is resulting in a mass production of DDGS, which can be used for feeding livestock (Kleinschmit *et al.*, 2007). Therefore, these materials can be used to try and solve agricultural problems, such as resource recovery, improved environment management and the utilization of waste (UNDP, 2002).

As the concentration of many nutrients (especially crude protein and minerals) in DDGS is greater (usually up to 3 folds) than other products that are used within the industry, DDGS that are sourced from the ethanol industry can be used to provide nutrients to monogastric animals that have higher dietary requirements. These higher levels may provide a niche product within the highly competitive feed market. However, there is a large element of variation in nutrient composition that exists within DDGS, thereby limiting its potential applications (Świątkiewicz and Koreleski, 2007). They are used sparingly in practical poultry diets, often contributing 5% or less of the overall meal composition.

One benefit of DDGS is that they have a very long shelf life, making them suitable for transport around the world. Previously, DDGS have been fed to ruminants that utilise the higher bypass protein levels in DDGS and can extract these nutrients. An increase in freight costs of both internal and external trade have resulted in DDGS being restricted for use only in regions where ethanol is manufactured.

Previous literature suggests that research on the inclusion of DDGS into the diets of broiler chickens tends to involve corn-based diets (Leytem *et al.*, 2008). Therefore, there is a substantial lack of information about the use of wDDGS within the diets of broiler chickens. The availability of wheat in the UK for the ethanol production process, results in much wDDGS being produced for use in broiler diets.

2.7.3 Nutritional value and nutrient composition of DDGS

WDDGS have a different composition to other cereal products, largely due to the variability in ethanol plants that are used for their production. Different methods of production such as variable fermentation times, drying temperatures, soluble blending and duration of drying result in the development of different physical characteristics within the final product. Recently, research has highlighted the extent of variability within DDGS products (Belyea *et al.*, 2010). Świątkiewicz and Koreleski (2007) reported how the fermentation of grain starch to produce CO_2 and ethyl alcohol consequently increases the concentration of other nutrients by up to three-fold. DDGS often contains high levels of crude protein, phosphorus, amino acids and a variety of other nutrients that are necessary for poultry. These additional nutrients, such as fats and carbohydrates, are also considered in assessment of raw material value. WDDGS have an energy value lower than that of wheat. Anti-nutritional factors, such as trypsin inhibitors within soybean meals, are considered to reduce the performance of animals. However, such anti-nutritional factors that are commonly found in most plant protein sources are not present in distillers' grains products.

It can be difficult to establish the true composition of DDGS (Shurson *et al.*, 2000; Belyea *et al.*, 2004). This makes it difficult to formulate different diet plans for poultry. Contention surrounding the use of DDGS in these diets relate to the high degree of variability within the physical and nutritional composition of the products, as well as how it is used within the diet as a whole (Noll, 2007). Belyea *et al*. (2010) found that DDGS nutrient information can be incorporated into feed ingredient tables and computer diet formulation software; an example of such databases is (NRC, 1984). In

their research, Arosemena *et al.* (1995) reported high calcium concentrations in DDGS, although the reason for this is not known.

Typically, DDGS diets are formulated in order to satisfy nutrient standards, such as amino acid content (Lumpkins, 2004). Further research by Wang *et al.* (2007) found that DDGS could be effectively supplemented into the diets of broiler chickens at a proportion of 15%, in order to provide the satisfactory level of digestible amino acids. It was noted that digestibility of several amino acids in DDGS is relatively poor, especially Lysine and Threonine (Batal and Dale, 2003; Fiene *et al.*, 2006; Parsons *et al.*, 2006; Waldroup *et al.*, 2007). The overall content of protein within wheat varies greatly, thereby resulting in different lysine content both within and between wheat varieties and their by-products such as wDDGS.

Table 2.2Composition of wDDGS (data on as fed basis)

Nutrient	Percent (%) (unless otherwise stated)
Dry Matter	93
ME(MJ/kg)	10.4
TME(Kcal/kg)	3,097
Ether Extract	9
Protein	27
Crude Fibre	9.1
Calcium	0.2
Total Phosphorus	1.7
Non-Phytate Phosphorus	0.4
Potassium	0.7
Chlorine	0.2

Source: NRC (1994)

The principal method of supplying the demand for amino acid consumption in poultry is to incorporate easily digestible protein as part of their diet. This should contain amino acids which match the profile of amino acids found in grains. The evaluation of AA and the ease of digestion of poultry feed is crucial due to the role it plays in the successful design of diets which contain precise measures of the nutrients necessary for desired optimal production targets (Williams, 1995). While all ingredients in poultry diets are important, particular focus is given to dietary AAs in the form of protein because around 25% of the expense associated with a poultry diet consists of these (Borin *et al.*, 2002). Performance may be affected if the diet lacks or is deficient in any of the essential AAs required. Moreover, if AAs are supplied in excess in the diet, then whatever the poultry do not absorb and utilize is excreted, which may lead to

contamination and various other conditions which may affect their wellbeing (McNab and D'Mello, 1994).

The required level of crude protein (CP) in animal diets is actually evaluated using the protein's amino acid content. The amino acids supplied in the diet as required at different stages of production (Table 2.3) must be in the correct quantities and balance depending on the bird in question. Any excess of amino acids will result in the deamination of the least limiting acid, most probably through being utilized for energy as opposed to providing body protein. A 'limiting' amino acid is considered to be one which when supplied does not fully satisfy the animal's need for that particular amino acid.

Table 2.3 Amino Acids requirement for broilers

	Weeks of age		
	0-3	**3-6**	**6-8**
Nutrient, %			
Arginine	1.25	1.10	1.00
Histidine	0.35	0.32	0.27
Isoleucine	0.80	0.73	0.62
Leucine	1.20	1.09	0.93
Lysine	1.10	1.00	0.85
Methionine	0.50	0.38	0.32
Phenylalanine	0.72	0.65	0.56
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Valine	0.90	0.82	0.70

Source: NRC (1994)

Amino acids are assessed within a protein source according to their essentialness, digestibility and bioavailability. Amino acid availability, especially with regards to lysine, depends on the processing conditions that are being undertaken, particularly pertaining to drying. For example, high levels of heating and moisture throughout the drying process tend to result in Maillard reactions between sugar and amino acids molecules. These ultimately disturb the carbohydrate moieties and lysine residues, consequently producing a darker colour within the distiller products (Parsons *et al.*, 1992; Hodgkinson, 2006). The lysine digestibility is reduced, along with other essential amino acids, producing especially dark DDGS (Fastinger *et al.*, 2006) on precision-fed cecectomised roosters (Nuez Ortín and Yu, 2009; Nuez-Ortín and Yu, 2010) confirming colour as an indicator of amino acid digestibility.

Table 2.4 Essential amino acids composition of wDDGS

Essential amino acid (%)	wDDGS
Arginine	4
Histidine	2
Isoleucine	4
Leucine	7
Lysine	2
Methionine	2
Phenylalanine	5
Threonine	3
Tryptophan	1
Valine	4

Source: Heuze et al. (2013)

Solubles are high in fat (up to 34%) and low in ADF, thus, the more solubles that are added to wDDGS, the higher the levels of fat and the lower the NDF content. Typically, wDDGS have a high phosphorus content and a low calcium content, as well as an increased lipid concentration but reduced amino acid contents (histidine, arginine, threonine and lysine), when compared with soybean meals.

2.7.4 Effect of DDGS on performance and carcass quality

Wang *et al.* (2007) reported that chickens that were provided with diets containing 30% DDGS had an overall reduction in feed intake and weight gain, which was measured at the 35 and 42 day stages, when compared against chickens that were not provided with DDGS. Research by Angeles and Gomez (2010) found that the daily weight gain of chickens was not majorly different after the provision of 15% DDGS, suggesting this concentration is acceptable. In contrast, Min *et al.* (2011) reported that FCR and feed consumption was not significantly affected by 30% DDGS at the starter stage of development. Parsons *et al.* (1983) found that an adjustment in lysine concentration can allow for the substitution of DDGS for proteins in soybean-based meals.

The effect of DDGS on the yield and quality of the carcass is relatively negligible although the dressing percentage can be reduced by increasing the size of the viscera, through the provision of high fibre. The use of different dietary supplements, such as the provision of DDGS, has been found to affect the chemical components of the meat that was produced; this is either directly associated with the nutrient content, or indirectly associated to the consequent performance. Further studies have found that increased DDGS content within diets could negatively affect the yield of breast meat

(Wang *et al.*, 2007). There is a lack of information relating to the effects of DDGS on the overall chicken meat quality (Łukasiewicz *et al.*, 2012). However, this could have been the result of a lack of studies that provided DDGS to broiler chickens throughout their development (Corzo *et al.*, 2009). Researchers found that including DDGS into the diet of broiler chickens resulted in no negative effect and or an increase in the quality of meat and the performance of the chickens (Lu and Chen, 2005; Corzo *et al.*, 2009). Corzo *et al.* (2009) found that increasing DDGS resulted in changes to the quality of the meat. Schilling *et al.* (2010) reported on the effects of DDGS on broiler meat quality, where all the treatments yielded high quality breast meat, and the thigh meat quality was similar for treatments containing 0-12% DDGS. Cowieson *et al.* (2003) found similar results, through the provision of a cocktail of α-amylase, pectinase and cellulase enzyme to a diet consisting largely of wheat; this resulted in increased retention of nitrogen-based compounds.

It has been demonstrated by (Szczurek, 2008) that supplementing diets with a commercial enzyme preparation containing beta-glucanase, cellulase, xylanase, alpha-amylase and protease significantly reduced fat content of breast and thigh muscles, which is reasonably similar to the findings of Suchy *et al.* (2002) in their research on hybrid broilers, as well as a study on pheasants conducted by Vecerek *et al.* (2005).

Increasing levels of DDGS have been associated with oxidation in thigh muscles, which reduces overall quality. Wheat germ and corn oil contain high levels of polyunsaturated fatty acids (Nuez Ortín and Yu, 2009; Nuez-Ortín and Yu, 2010). For example, linoleic acid (18:2, n-6) and a-linolenic acid (18:3, n-3) are associated with metabolism, as they are considered to be essential fatty acids, according to NRC (1994). Linoleic acid is especially important, as it is the only fatty acid that is considered to be essential for dietary regulation. Adult birds can grow and develop at a better rate, when provided with 1% linoleic acid to their diets. High levels of linoleic acid in DDGS (6.88%) are considered to result in a disturbance in the unsaturated fatty acid concentrations recorded in thigh meat. Additionally, NRC (1994) expressed how these levels may range between 4.55% and 6.88% supporting the results reported by Choi *et al.* (2008) who further stated that it is very difficult to explain the observed linear reduction in oleic acid in thigh meat that resulted from an increase in DDGS.

2.7.5 The problems of using wDDGS in broiler diet

There are many problems associated with the use of DDGS within diets, including the variability of the product, which is associated with the processing stages. Parsons *et al.* (1992) found that DDGS could be the main source of protein within certain diets, although these tended to have reduced lysine, arginine and tryptophan content. Shalash *et al.* (2009) found that reduced protein content within DDGS could hinder their use in poultry diets. Wang *et al.* (2007) found that low levels of energy density in DDGS could reduce the overall energy provision of DDGS-based diets, especially for developing chicks. Earlier research Wang *et al.*, (2005) stated that the digestibility of particular amino acids within DDGS was relatively low, particularly with regards to threonine and lysine (Batal and Dale, 2006; Fastinger *et al.*, 2006; Fiene *et al.*, 2006; Parsons *et al.*, 2006; Waldroup *et al.*, 2007). An inability to compensate for reduced amino acid digestibility when producing new diets for poultry could hinder the applications of DDGS, especially for broiler chickens.

The lack of research surrounding the applications of processing conditions within the production of DDGS makes it difficult to scientifically establish the protein availability of diets for monogastric animals, particularly with regards to lysine content (Belyea *et al.*, 2004). A review on the existing literature studied the metabolism of DDGS into energy that could be acquired by monogastric animals, highlighting the scope for suitable enzymes. However, current enzymes are not able to degrade NSP into sugars, especially not within the conditions of the chicken digestive system. Therefore, pre-treatment may be necessary in order to provide sugars to the chicken upon digestion of NSP (Choct, 2006).

There are many obstacles that the ethanol industry needs to overcome, in order to satisfy DDGS requirements of poultry. Manangi (2007) discussed how poultry farms require large volumes of DDGS, as well as outlining current problems that exist within the industry: variation in the availability of energy, variability within and between plant species, variability in amino acid digestibility, variability in phosphorus content, variation in sodium content, presence of toxins, and inconsistency in the quality of products.

2.8 Anti-nutrients and broiler performance

DDGS are commonly included within animal diets; however, due to their high fibre content and the inclusion of anti-nutrients, such as non-starch polysaccharides (NSP) and phytate, they may be less suitable for monogastric animals. Fermentation that occurs within the small intestine is often implicated in the anti-nutrient activity that occurs as a result of NSP digestion by chickens. Such fermentation activity is attributed to the impacts of bacteria within the gut of the chicken (Smits and Annison, 1996).

Many metabolic processes require phosphorus; however, this is usually bound to phytate, as found in 50-70% of all vegetable-based phosphorus which is used in poultry feed (Reddy *et al.*, 1982). The NRC (1994) discussed how phosphorus availability from vegetable sources or plant sources constituted only 30% in broiler diets. Additionally, Rao *et al.* (1999) associated the poor phosphorus availability in phytate-fed chickens to the reduction in available phytase within their gut, which is necessary for the hydrolysis of phytic acid into 6-inorganic phosphate and myo-inositol. Phytic acid ultimately reduces the availability of phosphorus upon digestion by the chicken and also reduces digestibility of other nutrients that exist within the diets of poultry. Hence it is highly influential on the absorption and digestion of both phosphorus and calcium upon digestion within poultry (Leeson *et al.* (2001). Supplementation with phytase improves overall digestion of protein, phosphorus and calcium and hence reduces the overall excretion of phosphorus by broilers. This reduction highlights how phytate-bound phosphorus has been made available for absorption and metabolism, due to hydrolysis. The use of phytase increases the availability of phytate-phosphorus; it increases its digestibility from 25% to between 50 and 70% (Kornegay, 2001; Woyengo *et al.*, 2008). Plant sources have had to be used as a source of protein in animal feed, since the EU banned the use of meat and bone meals for animal diets. Phytase is considered to improve nutrient digestibility overall within such diets (Kornegay, 2001; Selle *et al.*, 2009). Microbial phytase can be added to diets to reduce complexes of both phytate-mineral and phytate-protein. This improves the availability of a range of different amino acids and minerals (P, Zn, Cu, Mg, Ca), as well as increasing the apparent metabolisable energy (AME), for broiler chickens upon digestion.

McDougall *et al.* (1996) defined dietary fibre (DF) as a mixture of different carbohydrate polymers that are bound to other non-carbohydrate compounds. DF is found most prominently within the cells walls of different plants ; these contain NSP,

proteins, waxes and fatty acids. Additionally, DF is defined based on its solubility within water. Insoluble fibre, like cellulose, as well as fibres that are partly soluble, like glucans and pectins, contribute to DF (Hetland *et al.*, 2004). Many plant-based ingredients within animal feed contain high levels of fibre; they tend to be relatively insoluble (Knudsen, 1997). Additionally, the monogastric animal industry utilises the nutritional and physiological aspects of insoluble fibre that is found within animal feed (Hetland *et al.*, 2004). Deloreme and Wojcik (1982) discussed how fibre within monogastric animals can reduce overall nutrient absorption, particularly with regards to protein availability.

2.9 Utilization of crude fibre by broiler chickens

Ruminants can efficiently utilize fibre, as there is a large amount of micro-organisms within their gut (Van Soest, 1994). The micro-organisms secrete digestive enzymes that can metabolise cellulose, pectins and hemi-cellulose molecules, in order to extract energy for their own metabolism, while also releasing volatile fatty acids (VFAs). Examples of such by-product acids include butyric, acetic and propionic acids. Next, the animal can absorb the VFAs in order to exploit their energy content, before the micro-organisms are then digested by enzymes within the gut of the animal. The microbial proteins and amino acids can then be utilized by the host.

Non-ruminant animals, however, do not depend on microbial fermentation, as it occurs mainly in the hind gut, where little nutrient absorption occurs; this occurs just prior to excretion of undigested feed material. Therefore, these animals tend not to extract many nutrients from fibre fermentation, consequently reducing their efficiency of fibre digestion as a whole (Van Soest, 1994). Research by Carré *et al.* (1990) found that plant cell walls constitute much of the non-digested material within chicken diets whereas chicken faeces contain high levels of fibre that helps to reduce the digestion and transit time of the animal feed, consequently reducing the time that is available for further nutrient absorption and utilization. Smits and Annison (1996) also reported that fibre influenced the utilization of other nutrients in poultry feed by increasing the rate of passage of feed through the gastrointestinal tract and this affected the digestion and absorption of other nutrients.

Manangi (2007) found that fibre can bind to nutrients and hold these within plant cell walls, while also binding water. This adds to the overall volume of digesta within the gastrointestinal tract and reduces digestibility and metabolism of nutrients throughout digestion. High fibre diets ultimately reduce the overall intake of feed (Duke *et al.*

(1984), as well as the water intake, largely as a result of its water-binding properties (Chaplin, 2003) and relatively low viscosities of insoluble polysaccharides such as cellulose and xylans (Smits and Annison, 1996). Feed with high levels of crude fibre move slowly throughout the digestive tracts of bird species (Sundu *et al.*, 2008).

Arabinoxylans, β-glucans and other soluble fibres that are found in wheat have been found to reduce digestion and nutrient absorption, as has been found in research upon broiler chicken digestion. This is attributed to the NSP content of endosperm cell walls; arabinoxylans have been found to contribute heavily to the overall viscosity of this material (Annison and Choct, 1991; Choct and Annison, 1992). Further research (Hetland *et al.*, 2004) found that the gizzard is suitable for grinding structural aspects of feed. Larger portions of feed, combined with increased DF, can improve the contraction rates of the gizzard, thereby increasing the overall size of the organ (Sundu, 2009). WDDGS research has found that broiler diets should include only 10% of the compound if xylanase is absent or 15% if the enzyme is added (CIGI, 2011). Salim *et al.* (2010) found that fibre that is not digested, which stems from DDGS, is ultimately fermented by microbial activity within the large intestine of poultry.

2.10 Dietary fibre (DF) and intestinal microbes

DF is a significant energy source for animals, as it supports microbial activity within the digestive system (Knudsen *et al.*, 1991; Bach Knudsen and Hansen, 1991; Jensen and Jorgensen, 1994; Jørgensen *et al.*, 1996; Knudsen, 1997). DF has been found to have significant benefits on intestinal health, which is associated with microbial fermentation, as well as to the physicochemical properties of DF. Digesta is largely bulked with the viscosity of DF and its water-retention properties; this helps to improve the development of digestive tracts within developing chicks (Bach Knudsen, 2001).

Non-ruminant animals tend to experience microbial fermentation in their large intestines, especially with regards to chicks. DF fermentation encourages the formation of microbial communities (Knudsen *et al.*, 1991) and stimulates the development of bacterial colonies that provide significant health benefits. Enzymes can also be supplemented to the diets of broiler chickens to develop their nutrient absorption efficiency, as well as stimulating the growth of digestive bacteria within their guts (Anjum and Chaudhry, 2010) and hydrolyse the components of DF, therefore being classified as hydrolases (Classen and Bedford, 1991; Fry, 1995). Research by Xu *et al.* (2003) found that colonies of Salmonella, Lactobacillus and Escherichia coli tended to fluctuate in size and development within broiler chickens' large intestines.

Jørgensen *et al*. (1996) found that sources of fibre were highly influential on GIT development and activity, particularly relating to the metabolism of energy within broiler chickens. Microflora within the intestines of these animals has been found to increase fibre fermentation, gas and SCFA by-products. However, fibre compounds and their influences on gut microflora regulation have yet to be clearly established (Denayrolles *et al*., 2007). Bedford and Apajalahti (2001) found that supplementing bird feed with xylanase can reduce the formation of different bacterial colonies in the small intestines (coliforms, enterococci, lactic acid bacteria etc.) of broiler chickens that were previously fed with wheat-based diets. A further study by Vahjen *et al*. (1998) found that xylanase can reduce the total enterobacteria content, as well as the gram positive cocci species presence, within broilers fed on wheat-based diets. Similarly, Choct (2006) found that xylan can be supplemented to these diets to reduce the activity of Clostridium perfringes in the guts of chickens. Furthermore, exogenous NSP-degrading enzyme can be added to affect the activity of xylanase, thereby reducing the overall size of the digestive microbial composition, in broiler chickens (Torok *et al*., 2005).

2.11 Improving the quality of wDDGS

Exogenous enzymes can be used to upgrade cereal products, but have not always shown positive benefits. Research by King and Moughan (1998) found that a combined supplementation of glucanase, xylanase, amylase, cellulose and pectinase to a standard wheat diet did not affect the feed intake levels, the feed conversion efficiency, or the daily weight gain of chickens at any development stage. Enzyme provision in wheat-based diets was not found to produce any discernable affects in broiler chickens, according to research by Sayyazadeh *et al.* (2006) and Iji *et al.* (2003). Thacker and Widyaratne, (2007) found that DDGS inclusion within a diet did not result in any significant increases in growth among broiler chickens. Mahagna (1995) found similar results in a study that used protease and microbial amylase to supplement diets within broiler chickens of different developmental stages. There were no significantly different observations on growth and development as a result of this enzyme supplementation.

High levels of fibre within DDGS supplements, as well as their general nature of being variable in composition, highlight how NSP-degrading enzymes might be beneficially incorporated into the diets of broiler chickens, especially if they have high affinities for the degradation of insoluble fibre (Cromwell *et al.,* 1993). Additionally, damage results from the drying process, which reduces the overall amino acid content, especially regarding lysine (Lumpkins *et al.,* 2005). Therefore, exogenous enzyme proteases can

be added to the diets of these animals, in order to hydrolyse the glucosamine-based compounds, thereby increasing the nutrient absorption that occurs from DDGS. Exogenous enzymes have been used to supplement poultry diets extensively, in order to increase the utilization of nutrients, while also improving the welfare and health of birds. This improves the quality of products, while also reducing environmental pollution. There has also been research pertaining to the ingredients that should be included in such diets (Acamovic, 2001; Cowieson *et al.*, 2003). Biotechnological advances have aided animal nutrition research, particularly regarding the use of exogenous enzymes such as xylanase, beta-glucanase and phytase, which have been found to improve the overall use of fibrous polysaccharides and anti-nutrients upon digestion, as reported in monogastric animals (Broz and Frigg, 1986; Bedford, 2000).

Supplementing diets that contain high cereal content with such enzymes has already been found to produce more consistent performance and health among poultry (Jøgensen *et al.*, 1996; Bedford and Apajalahti, 2001; Bedford and Cowieson, 2012). Additionally, these enzymes have been found to reduce the overall size of the GIT within broilers, while also improving nutrient partitioning and absorption, via the activity of microbial fermentation. Further, these enzymes need to be optimised and used properly, in order to improve the performance of chicks as they develop. Selle *et al.* (2009) found that the effects of xylanase and phytase, especially if applied simultaneously in wheat based broiler diets, with respect to their digestive abilities, have been found to ameliorate the negative consequences that are associated with poor diets. Protein digestion has been found to increase within such conditions. Annison (1993) and Friesen *et al.* (1992) found that further supplementation of diets with enzymes can help to assist xylan degradation, thereby improving nutritional extraction from wheat-based diets.

Many different types of enzyme have been utilized within the diets of poultry; they have been found to improve nutrient utilization, to effectively increase the health and performance of broiler chickens that are being reared (Anjum and Chaudhry, 2010). Additionally, the use of single or combination enzymes in the diets of broiler chickens depends on the overall suitability and quality of these enzymes, in relation to the production of birds. Enzymes are currently used extensively throughout bird and pig feed. However, their use as a nutrient supplement in combination with DDGS is somewhat under-researched. Furthermore, combining these enzymes with traditional

diets can assist with the degradation of anti-nutrients, which have been found to be present within corn and corn DDGS.

Research by Simon (1998) found that carbohydrase enzymes are suitable for degrading soluble NSP into smaller compounds that consequently reduce the viscosity of digestive material. This improves the utilization and extraction of nutrients, thereby improving overall animal performance. Sundu *et al.* (2006) found that xylanase enzymes, namely Allzyme SSF, increases body growth, the efficiency of feed conversion, and the digestion of DF, while simultaneously reducing the viscosity of digestive material. However, King and Moughan (1998) found that the use of enzymes as supplements to maize/wheat-based diets did not significantly improve the overall performance of the chicken at either stage (starter or finisher). Phytate is present in wheat, soybean and wDDGS; therefore, a combination of a variety of different enzymes (phytase plus pectinase, xylanase, glucanase and cellulose) could be effective in practice as required to completely metabolise pectins and xylans. The supplementation of glucanase to barley-based diets, or xylanase to wheat-based diets, has been found to reduce activity overall, especially when combined with cellulose (Slominski, 2011).

A study by Cowieson *et al.* (2006a) found that improvements in the FCR can be achieved through the use of enzymes that are commercially available as supplements for barley- or wheat-based diets. This is especially true for carbohydrases, which have been found to produce consistent results when used as an enzyme to supplement maize-based diets. Xylanase has also been found to increase the digestion of phosphorus (Kim *et al.*, 2005). This is considered to be attributed to the release of non-phytate phosphorus from DF, which contains high levels of arabinoxylans (Frolich and Asp, 1985; Cowieson *et al.*, 2006a). A study by Selle *et al.* (2009) found that enzymes can be used to assist amino acid and fibre metabolism, consequently influencing the digestion of nutrients and their consequent absorption within the guts of broiler chickens. Annison (1991) and Friesen *et al.* (1992) found that xylanases can be supplemented to wheat-based diets being fed to broiler chickens, in order to increase the nutrient availability of the diets as a whole.

Enzymes can be incorporated into diets to reduce the size of the GIT and associated digestive organs (Wang *et al.*, 2005). Research by Chesson (1987) and Chesson (1993) claimed that enzymes could be used with substrate competitors, in order to increase overall activity of the target substrate degradation. Multiple enzymes are more likely to produce a beneficial result, compared with the use of just one enzyme. However,

phytase and combinations of xylanase, amylase and protease (XAP) were found to have significantly positive yields in broilers. Bedford and Schulze (1998) found that the digestion activity of NSP increases when cell wall degrading enzymes were present within a diet.

Supplementing diets with enzymes has been found to improve overall digestion of fats (Bedford and Schulze, 1998). This activity benefits fats most, as there is little to no fat within cereal endosperms. Therefore, cell wall dissolution tends not to affect the enzyme activity that is being stimulated (Bedford and Schulze, 1998). Additionally, calcium and phosphorus absorption has been found to be increased in the presence of appropriate enzymes (DoskoviĆ *et al.*, 2013).

Some of the results in relation to DDGS supplementation with exogenous enzymes are summarized in Table 2.5 which indicates that majority of research with DDGS was carried out using corn DDGS and no or little work has been done to investigate the effect of feeding wDDGS on broiler chickens meat composition. It was understood and noted that these previous studies have evaluated the potential of using DDGS as a highly acceptable feed ingredient for broiler chickens being rich in protein and some minerals and also contribute energy to the diets but are hindered by the presence of some anti-nutrients, and as such their utilization requires more careful treatment. These studies have also tested various levels of DDGS in feeding poultry and used various exogenous enzymes in an attempt to overcome the effects of anti-nutrients with a view to improve the chicken performance. Nevertheless, many authors have advocated for the use of further studies to ascertain the optimum inclusion level of DDGS with or without new formulations of suitable enzymes in diets to optimise broiler performance. Therefore a series of broiler experiment were conducted to examine the dietary inclusion of newly produced wDDGS – with or without selected enzymes on broiler performance to achieve the stated objectives.

Table 2.5 Summary of experiments showing different inclusion levels of enzymes & DDGS in broiler chicken production

Authors	Enzyme Used/Composition	Level of Inclusion/Unit of Activity	Type of DDGS	Level of Inclusion of DDGS	Basal Diet	Effects
Martinez-Amezcua *et al*. 2006	Microbial phytase	1,000 or 10,000 FTU/kg of Optiphosphytase	Corn DDGS	30 & 40%	Corn starch Dextrose (2:1)	Phytase + Citric acid increased the bioavailability of P in DDGS; phytase @ 1,000FTU/kg had no effect in AA digestibility.
Richter *et al*. 2007	Glucanase, amylase, protease, xylanase	0.25g/kg	Wheat DDGS	5, 10,15, & 20%	Wheat based diet	Enzymes improved feed efficiency by 1.2% and the live weight gain by 97g/b.
Moran and Lehman 2008	Xylanase, amylase, protease & phytase	Amy 800U, protease 8,000u, xylanase 600U (0.05%), Phytase 500FTU (0.01%)	Corn DDGS	10%	Corn-SBM	Enzyme combination led to favourable responses to live production, skinless, boneless, meat yield and skeletal integrity.
Min *et al*. 2009	Amylase, Cellulase, Phytase, Xylanase, β-glucanase, Pectinase & Protease	200g/ton	Corn DDGS	30%	Corn-SBM diets	No enzyme effect. Diets with DDGS not digested.
Oryschak *et al*. 2010	Xylanase, glucanase, amylase, protease & invertase	150 xylanase, 125glucanase, 4,000Amylase, 1,750 protease, 5,000 invertase.	Triticale DDGS	0,5,10,15 & 30%	Wheat – triticale based	Enzyme increased the nutritive value of triticale DDGS, 10% was safe. No adverse effect on performance & breast muscle yield.
Angeles and Gomez 2010	Xylanase, Phytase, Glucanase	175ppm glucanase, 175ppm xylanase & 200ppm phytase	Corn DDGS	0,5,10 & 15%	Sorghum/Corn diet	10 & 15% DDGS inclusion have no negative effects on growth performance of broiler chickens.

Table 2.5: Summary of experiments showing different inclusion levels of enzymes & DDGS in broiler chicken production (continued)

Authors	Enzyme Used/Composition	Level of Inclusion/Unit of Activity	Type of DDGS	Level of Inclusion of DDGS	Basal Diet	Effects
Adeola *et al.*2010	Carbohydrase (Xylanase, Amylase)	2,000U of xylanase & 1,800U of Amylase per kg of diet.	Corn Distillers Grains	0, 300, 600 g/kg	Corn - SBM diet	Carbohydrase supplementation significantly improved the ileal digestible energy & metabolisable energy.
Liu *et al*. 2010	Xylanase	0,1200,2400 & 3600 U/kg	Corn DDGS	10, 20 %	SBM-Corn gluten meal	No negative effects on growth performance & digestibility.
Olukosi *et al*. 2010	Xylanase, Amylase, Protease & Phytase	XAP 0 or 500mg/kg PHY 0 or 1000FTU/kg	Maize DDGS	0 & 10%	Corn - SBM diet	Combination of the enzymes did not produce greater benefit to the chicks than the use of phytase alone but modestly improved nutrient utilization independently of mDDGS addition
Min *et al*. 2011	Xylanase, glucanase, phytase, cellulases	Xylanase 22000U/g, glucanase 2000U/g, phytase 10,000FTU/g	Corn DDGS	0, 30%	Corn-SBM diets	No significant interaction on performance & nutrient utilization. No effect of enzyme addition throughout.
Fernandez *et al*. 2012	Protease	Protease 0 & 200g/tonne	Corn DDGS	30%	Canola meal & DDGS	Amino acid & Energetic value of diets enhanced by addition of protease.
Barekatain, *et al*. 2013	Xylanase	0.25g/kg of diets 1000U	Sorghum DDGS	0, 100, 200 & 300g/kg	Maize-SBM based	Diets with large amounts of DDGS benefits from xylanase supplementation. FCR & BWG were improved.

Table 2.5: Summary of experiments showing different inclusion levels of enzymes & DDGS in broiler chicken production (continued)

Author	Enzyme Used/Composition	Level of Inclusion/Unit of Activity	Type of DDGS	Level of Inclusion of DDGS	Basal Diet	Effects
Barekatain *et al*. 2013	Protease and Xylanase	Xylanase 0.3g/kg and protease 0.2g/kg of experimental diets.	Sorghum DDGS	0, 20%	Wheat, Barley, Sorghum, SBM based	Enzyme addition helped birds maintain feed intake and body weight.
Swiatkiewicz *et al*. 2014	Xylanase, Fungal Xylanase & Phytase	200mg/kg, 200mg/kg & 10,000 (phytase units/g)/kg	Corn DDGS	0, 12 (starter) & 0, 18 (Finisher)	Corn - SBM diet	Xylanase + Phytase & Chitosan can increase the nutrient digestibility & retention of Ca & N of the diets.

U= Units, IU= International Units, FTU= Fibre Terminating Unit, XAP= Xylanase, Amylase, and Protease, PHY= Phytase. FCR= Feed Conversion Ratio, BWG= Body Weight Gain, Ca= Calcium, N= Nitrogen, ppm= parts per million

2.12 Conclusion

In view of the foregoing descriptions, the use of wDDGS in broiler diets merits investigation to ascertain their optimum inclusion levels and impact on broiler performance, including behaviour which is considered to be a good indicator of the broiler wellbeing in response to various diets. Moreover, owing to the growing interest in the behavioural responses of broiler chickens to feed acceptability, and calming or aggression, during feeding of wDDGS or other fibre containing diets, further investigation is warranted. Furthermore, very little information about the influence of wDDGS on the quality of broiler chicken meat and fat containing fatty acids was found in the available literature.

Inclusion of some fibre is presumably beneficial to broiler chickens, but higher levels are known to impair the performance of chickens. The presence of anti-nutrients such as certain non-starch polysaccharides (arabinoxylans) in wDDGS requires the addition of exogenous enzymes to improve their nutritive availability to the chickens. Thus, enzyme supplementation has been effective in some experiments in improving feed intake, growth performance and digestibility of minerals and crude protein. However, many other studies did not find any positive effects of enzyme addition on the utilization of fibrous feeds by the broilers. The discrepancies that existed in the past research about the effects of enzymes on the utilization of wDDGS based diets call for an investigation to test the optimum dietary inclusion levels of wDDGS in the presence and absence of suitable enzymes for broilers. These questions, therefore, formed the basis for the series of 3 experiments conducted in order to achieve the stated objectives for optimum performance of broilers at a reasonable cost.

CHAPTER 3:
PRODUCTIVE PERFORMANCE OF BROILER CHICKENS FED DIETS CONTAINING VARYING LEVELS OF DISTILLER'S DRIED GRAINS WITH SOLUBLES

3.1 Introduction

Wheat distiller's dried grains with solubles (wDDGS) are increasingly available as a by-product of the beverage and bio-fuel industry worldwide. This raw material is suitable for feeding to many animal species, and offers high levels of crude protein and certain minerals. The increased demand for ethanol has resulted in rapid growth of the bioethanol industry and mass production of large quantities of wDDGS available for use in livestock feeding (Kleinschmit *et al.*, 2007). Thus, the utilization of these materials can help in solving problems of global importance such as resource recovery, waste utilization, and better environment management (UNDP, 2002). Inclusion of corn DDGS at higher levels in livestock diets may provide an additional means for the use of increasing amounts available (Noll *et al.*, 2001). One of the concerns about using relatively high levels of this product is the possible effect on performance that might occur (Wang *et al.*, 2007). Over the past few decades wDDGS have become a quite useful feed ingredient in monogastric rations because of its nutritive value. Although it's nutritive value is quite good for poultry performance, its high fibre content and presence of some anti-nutrients (e.g. phytate) and non-starch polysaccharides (e.g. arabinoxylans) has contributed to limitations in its use. However, wDDGS being a source of protein/amino acids and energy, it was incorporated as a replacement of soybean meal (SBM) in a single phase feeding system which is beneficial in terms of reducing diet cost and improving environmental quality. Further, it has been demonstrated that a high fibre diet could affect the size and weight of digestive organs (Sundu *et al.*, 2008) nutrient digestibility and thus the carcass characteristics of relevant birds. Therefore, this study had the following aims:

(1) To determine the acceptable / optimum level of inclusion of wDDGS in broiler diets.

(2) To determine growth performance (feed intake, live weight gain, and feed conversion ratio) of broiler chickens consuming isonitrogenous diets with different levels of wDDGS inclusion with variable ME, NDF, and some amino acid contents.

(3) To determine behavioural changes as a result of receiving diets containing different levels of wDDGS.
(4) To determine the effect of feeding different levels of wDDGS on carcass characteristics.
The hypothesis for this study was that wDDGS, if optimally incorporated in isonitrogenous diets, can be used with even different NDF and ME contents without adverse effect on performance, health, behaviour and carcass characteristics of broiler chickens.

3.2 Materials and Methods

3.2.1 Study Site:

The study was conducted at the Cockle Park farm of Newcastle University between February and March 2011. The experiment was conducted under the ethical guidelines for non-regulated procedures of Newcastle University.

3.2.2 Experimental wDDGS and diets

The wDDGS used in the experiment were purchased from Armstrong Industries (Teesside, TS17 9JT United Kingdom).
Four isonitrogenous diets, with varying metabolisable energy, fibre and some amino acid contents, were formulated for the study. Diet A, which was the control contained 0% wDDGS while diets B, C, and D contained 10, 20 and 40% wDDGS respectively. Table 3.1 shows the composition of the formulated experimental diets, and Figure 3.1 illustrates a sample of the wDDGS used. All the diets were mixed using a mechanical miller, grinder and a mixer which is more efficient than manual mixing of different ingredients of those diets. Hence, all the ingredients were assembled and the estimated quantities were weighed and mixed accordingly.

Table 3.1 Ingredient and calculated chemical composition of the experimental diets

	wDDGS (%)			
	0	**10**	**20**	**40**
Ingredients (g/kg)				
wDDGS	0	100	200	400
Wheat	634	582	529	424
Soybean meal	288	240	193	98
Soybean oil	10	10	10	10
Limestone	40	40	40	40
MCP[2]	10	10	10	10
Salt[3]	2.5	2.5	2.5	2.5
Premix*	12.5	12.5	12.5	12.5
DL-Methionine	1	1	1	1
Lysine	1	1	1	1
Total	1000	1000	1000	1000
[1]Calculated nutrient composition (g/kg)				
Crude Protein	220	220	220	220
ME (MJ/kg)	13.4	13.2	12.9	12.4
Starch	372.4	343.4	313.9	255.4
Crude Fibre	34	62	90	146
Ether Extract	74	69	64	54
Calcium	22	22	22	22
Phosphorus	5	6	6	7
Amino Acids				
Arginine	14.4	13.7	13.1	11.7
Histidine	6.0	5.9	5.8	5.6
Isoleusine	9.5	9.4	9.3	9.0
Leusine	16.9	16.9	16.9	17.0
Lysine	11.3	10.3	9.4	7.5
Methionine	4.3	4.2	4.2	4.0
Phenylanaline	11.4	11.4	11.4	11.5
Threonine	8.1	8.0	8.0	7.8
Tryptophan	3.0	2.9	2.8	2.6
Valine	10.5	10.6	10.7	10.8
Total AA	95.3	93.3	91.5	87.6

[1]Calculated from the composition values
[2]168.5gCa/kg, 229gP/kg; MCP= Monocalcium phosphate, ME= metabolisable energy
[3]39%Na (British salt) contains Sodium hexacyanoferrate (II), AA = Amino Acids
**Vitamin A=12,500 IU; Vitamin D3=3,000 IU; Vitamin E=150mg; Vitamin K=3mg; Vitamin B2=8mg; Vitamin B6=5mg; VitaminB1=2mg; Vitamin B12=30mmg;Nicotinamide 50mg;Pantothenate 10mg;Folic acid 2mg;Biotin 150mmg; Vitamin C 35mg; Choline 300mg; Copper10mg; Manganese 80mg; Zinc 60mg;Iron 30mg; Iodine 2mg; Selenium 0.25mg;Molybdenum 0.2mg; Calcium ($CaCO_3$) limestone 70%.*

Plate 3.1 Illustration of a wDDGS sample

3.2.3 Experimental birds and management

One hundred and forty four day-old Ross broiler chicks were used for the experiment which lasted for 6 weeks (day 1 to day 42). The chicks were supplied by Grampian Country Food Group, Duns, Berwickshire, and were vaccinated at the hatchery against Infectious Bronchitis. The chicks were individually weighed, wing tagged and randomly assigned to 4 groups (treatments) of 36 chicks each. Each group was further divided into 4 sub-groups (replicates) of 9 birds per replicate. Birds of each replicate were randomly assigned to circular floor pens of 1.2 m^2 with wood shavings as litter material (Figure 3.2) at different sides of the experimental room to achieve uniform distribution of bird groups. Each pen was equipped with a suspended infra-red heat lamp, plastic chick feeder and plastic drinker. The birds were started on experimental diets. The experimental diets and clean drinking water were provided *ad libitum.* Ambient temperature was recorded daily and it was maintained at 22^oC on day 1, and then gradually increased to 26^oC by day 42 due to adjustment in setting of brooder lamps of the pens i.e. drawn up as the birds grew older which eventually changed the room temperature.

Table 3.2 Mean weekly house temperature (ºC)

Days	Minimum	Maximum
7	22	24
14	24	26
21	24	26
28	23	26
35	25	27
42	26	29
Overall	**24**	**26**

Birds were observed daily and any clinical health signs were closely monitored and mortality was recorded.

(A) **(B)**

Plate 3.2 Experimental broiler chicks in circular floor pen (A) and layout of the room (B)

3.2.4 Experimental design

In a completely randomized design, 144 day-old broiler chicks were divided into 4 dietary groups for 4 treatments containing four replicates each. Each replicate contained one group of nine birds on each treatment.

3.2.5 Data collection

All data were recorded on data capture forms, and then transferred to Excel spreadsheets. Records of feed consumption, live-weight (LW), behaviour, diet digestibility and carcass characteristics for experimental birds were taken. Further calculations were carried out to derive total and daily live weight gain (LWG), feed conversion ratios (FCR) and digestibility of selected nutrients for the experimental birds consuming various diets.

Feed intake was monitored daily and measurements were used to calculate the weekly intake per group of birds. A weighed quantity of feed was offered daily to ensure continuous presence of feed and the left over weighed at the end of each week. Weekly intake was determined by difference between the leftover and the quantity offered during the previous week. Birds were individually weighed at the start of the experiment and weekly thereafter. Weekly gain per bird was obtained by difference between the weights of two consecutive weeks and daily weight gain (DWG) was calculated by dividing the weekly gain by 7 (number of days in the week) as:

$$DWG = \frac{\text{Final Weight} - \text{Initial Weight}}{7 \text{ days}}$$

At the end of the experiment (Day 43) FCR, which is the amount of feed consumed per unit weight gain, was calculated by dividing the quantity of feed consumed by the weight increase as follows:

$$FCR = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

Where mortality was recorded, the number of dead bird was excluded accordingly in the calculation.

Water intake was measured during a subsequent digestibility trial over a 4 day period, during which a known volume of water was placed in each trough, and the volume of water remaining on the following morning was measured as refusal. Water to feed ratio was later calculated by dividing the average water intake by the average feed intake.

3.2.5.1 Behaviour study

3.2.5.1.1 Recordings

Recordings of bird behaviours were performed weekly using mounted video cameras (Sanyo digital colour CCD with model No VCC-6572P) to film four sets (sets 1, 2, 3 & 4) of pens on consecutive days, where each set comprised one pen per each of the four treatments (A, B, C & D). Recording commenced with the first set on the first day, to record behaviours during adaptation and post handling of chicks, through the second day and stopped at 10am when recording for the second set started, with sets 3 & 4 following consecutively in subsequent days of the week. Thus, each recording of bird behaviours normally started at 10am and stopped at 10am the following day, when all the 4 cameras were simultaneously shifted to the next set of 4 pens representing another batch of the same 4 treatments.

3.2.5.1.2 Measurement of behaviour

Behaviours measured were; Feeding, Drinking, Standing, Walking, Resting, Aggression, Preening, Dust bathing and Pecking, as defined in Table 3.3. Observations commenced in the first week of life and recordings were made for a 24 hour period per pen to observe their behaviours during one hour in each of 3 periods (morning - 10.00am-11.00am, afternoon-4.00pm-5.00pm and night-9.00pm-10.00pm). The light was supplied for 14 hours daily (8.00am-10.00pm). Observations of the defined behaviours were made on all the nine birds per pen by a time sampling methodology (Altmann, 1974) over a one hour period on each occasion. The number of birds engaged in a particular behaviour was recorded every 10 minutes. Data were recorded in excel spreadsheet and expressed as percentages of the total number of birds in the respective pens performing each behaviour prior to statistical analysis.

Table 3.3 Measured behaviours and definitions

Behaviours	Description
Feeding	Eating feed from the feeder
Drinking	Drinking water from the drinker
Exploration	Searching in the litter
Standing	Standing and not performing any other behaviour
Walking	Moving around in the pen or taking steps
Resting	Lying or sitting on the floor and not performing any other behaviour
Aggression	Fighting or pecking other birds in the pen
Preening	Arranging and smoothing/cleaning feathers by beak
Dust bathing	Lying, spreading wings on the leg and bathing in the litter
Pecking	Gentle pecks on non-food objects e.g. pen wall

3.2.5.2 Measurement of litter moisture content

Litter samples from individual pens were collected weekly from day1 to day 42 of age. Collections were performed by using an empty aluminium container to take a 5cm core sample of litter from 3 different areas in each pen, as described by Macklin (2005). Samples included the litter from near the drinker, middle and side of the pen and comprised about 50g each. Samples were oven-dried at 80^{o}C overnight. The litter moisture content was then determined by difference i.e. initial weight minus final weight in grams.

3.2.5.3 Measurement of digestibility

3.2.5.3.1 Digestibility by the total excreta collection method

At the end of the feeding trial, a digestibility trial was conducted to determine the apparent nutrient digestibility. Two birds per replicate were randomly selected and transferred to metabolism cages measuring 60 x 48 x 60 cm for excreta collection and determination of apparent nutrient digestibility (Figure 3.4). The birds were allowed three days to adapt to the cages. Feed and water were provided *ad libitum*. The digestibility trial lasted for 4 days. Polythene sheets were spread underneath the cages for the total excreta collection. Feathers were hand-picked and discarded from the excreta before weighing. The total collection of excreta was dried in a forced air circulation oven at 60^{o}C overnight. The sample was allowed to cool in a glass desiccator to prevent absorption of moisture from the atmosphere. The 4 day samples from each cage were pooled, ground and then analysed for crude protein (CP), NDF, ADF, ether extract (EE) and total ash according to the method of (AOAC, 2005).

Plate 3.3 Experimental broiler chickens housed in two-tier metabolism cages

3.2.5.3.2 Digestibility by the acid insoluble ash method

In addition to the digestibility determination by the total faecal collection method, the digestibility of experimental diets was evaluated by the Acid Insoluble Ash (AIA) method as described by Van Keulen and Young (1977). For this purpose, spot samples of feed were pooled as replicates and those of excreta were pooled in the same way and analysed for AIA. About 5g sample, in duplicate, was placed into a tarred 50ml crucible dried overnight at 100^{o}C and allowed to cool in a desiccator and reweighed, then ashed for 5 hours at 450^{o}C. This ash was transferred to a 600 ml Berzelius beaker to which 100 ml of 2 N HCl was added. The contents were then boiled for 5 minutes on a heating block. The hot hydrolysate was then filtered through Whatman 541 filter paper and washed with hot distilled water. The filter paper was then transferred back into a crucible and ashed for 5 hours at 450^{o}C. The crucible was then placed in a 100^{o}C oven and re-dried, cooled in a desiccator and weighed. The percentage AIA was calculated using the following formula:

$$\frac{(\text{Wt. of Crucible + Ash} - \text{Wt. of Crucible})}{\text{Sample Dry Weight}} \times 100$$

Digestibility by AIA was calculated as the ratio of AIA in feed and faeces using the following equation according to (Vogtmann *et al.*, 1975; Van Keulen and Young, 1977; Osuji *et al*., 1993):

$$Nutrient\ digestibility\ (\%) = 100 - (\frac{100\ x\ \%AIA\ in\ feed}{\%AIA\ in\ faeces}\ x\ \frac{\%Nutrient\ in\ faeces}{\%Nutrient\ in\ feed})$$

3.2.5.4 Carcass measurements

At the end of the experiment, eight birds per treatment (i.e. 2 birds per replicate) were randomly selected for carcass studies. The birds were killed by cervical dislocation and dressed by manually removing the skin along with feathers to obtain their cold carcasses and weighed. The eviscerated chickens were dressed by removing the neck and the shanks and the dressed chicken (carcasses) were weighed. The dressing percent was calculated by dividing the weight of the dressed chicken (cold carcass weight with guts) by the weight of the living bird before killing, and multiplying by 100 as:

$$\text{Dressing percent (\%)} = \frac{\text{Weight of dressed chicken (g)}}{\text{Weight of live chicken (g)}} \times 100$$

Some cut-up parts (thighs, drumsticks, wings, breast muscle + bone) were also obtained by carefully using a butchering knife to remove these parts from each carcass. The cut up parts were then weighed and expressed as percentage of their respective cold carcass weights. The thighs and drumsticks were removed from the carcass by cutting above the thigh, towards the acetabulum and behind the pubic bone (the pelvic/thigh incision). Then the drumsticks were separated from the thighs by cutting perpendicular to the joint between the drumstick and thigh bones. The wings were removed by shoulder incision through the joint (articulation) surface of the scapula. The breasts were separated by rib incision i.e. a cut perpendicular to the ventral joints of ribs. The back-pelvis separations were performed by cutting perpendicularly to the ventral column at the final vertebral level. The internal organs (liver, heart, gizzard, intestines, crop and proventriculus) were also carefully obtained and weighed where gizzard and crop were emptied prior to weighing. Both edible and non-edible components were expressed as percentages of their respective cold carcass weights. The cold carcass weight expressed excludes the weights of internal organs.

3.2.6 Chemical analysis

Feed samples of each of the 4 experimental diets as well as samples of the principal feed ingredients, wheat, wDDGS and soybean, were analysed for proximate composition (AOAC, 2005) as described in the following sections:

3.2.6.1 Dry matter and ash determination

To determine dry matter (DM), about 2 g of the sample was oven dried at 105^{o}C. The weight loss was that of moisture and the DM was calculated as:

$$\%DM = \frac{Dried\ Sample\ weight\ (g)}{Initial\ sample\ weight\ (g)}\ x\ 100$$

The ash or total mineral content was determined by incinerating 10 g of the dried sample in a furnace at 600^{o}C for 3 h. The residue after incineration (ash) was cooled in a desiccator and then weighed. The percentage ash for each sample was calculated as:

$$\%Ash = \frac{weight\ \text{of residue}\ (g)}{weight\ \text{of dried sample}\ (g)}\ x\ 100$$

3.2.6.2 Determination of crude protein

The crude protein content of the experimental diets and faeces was determined using the Elementar Vario Macro Cube method in which 0.1g of each sample was weighed into a tin foil cup, which was carefully folded and squashed into a pellet to expel the air, and analysed by elementar (an instrument which works on the Dumas principle to get N content). The analysis itself was carried out in CN (Carbon-Nitrogen) mode which involved using a combustion, post combustion and reduction tube in the furnace of the analyser. Oxygen was used to burn the sample and the gas was carried off in helium through both the post combustion (900^{o}C) and reduction tubes (830^{o}C) which were also heated to the detectors housed within the analyser. Each element (carbon, nitrogen) was analysed separately and a % figure was then obtained. CP content was obtained by multiplying N content by 6.25 and expressed as CP in g/kg DM.

The ash or total mineral content was determined by incinerating 5g of the dried sample in a furnace at 550^{o}C overnight. The residue after incineration (ash) was cooled in desiccators and then weighed. The percentage ash for each sample was calculated as:

$$\%Ash = \frac{Weight\ of\ Ashed\ residue}{Weight\ of\ dried\ sample}\ x\ 100$$

3.2.6.3 Determination of NDF

The neutral detergent fibre (NDF) content of the samples was determined by the method of Van Soest *et al.* (1991), in which 5g dried ground sample was placed into a conical flask for refluxing. Then, 100ml neutral detergent (ND) solution and 50µl of heat stable α-amylase were added to the sample and heated to boiling in 60 minutes in a heating block (Figure 3.4). The solution was then filtrated through a pre-weighed sintered glass crucible using Whatman K17 (Type 726.3 FT.18) light vacuum suction. After this, the insoluble contents in each crucible were washed by filling two thirds of the crucible with hot (90-100^{o}C) water while stirred with a glass rod, soaked for 15-30 seconds and filtered/drained with the aid of vacuum suction. This process was repeated thrice while rinsing the sides of the crucible with hot water and finally by using small volume of acetone to remove the remaining solubles and pigments from the insoluble fibre. The crucible and its content of residue was dried at 105^{o}C in a forced oven overnight, then cooled in a desiccator and weighed. Finally, the residual content was incinerated at 550^{o}C in a furnace for 5hr, cooled in a desiccator for 30 minutes and weighed.

NDF was calculated as follows:

$$NDF\ (g/kg\ DM) = \frac{R-A}{S}\ x\ 1000$$

Where: R= weight of residue;

A= weight of ash;

S= weight of dried sample.

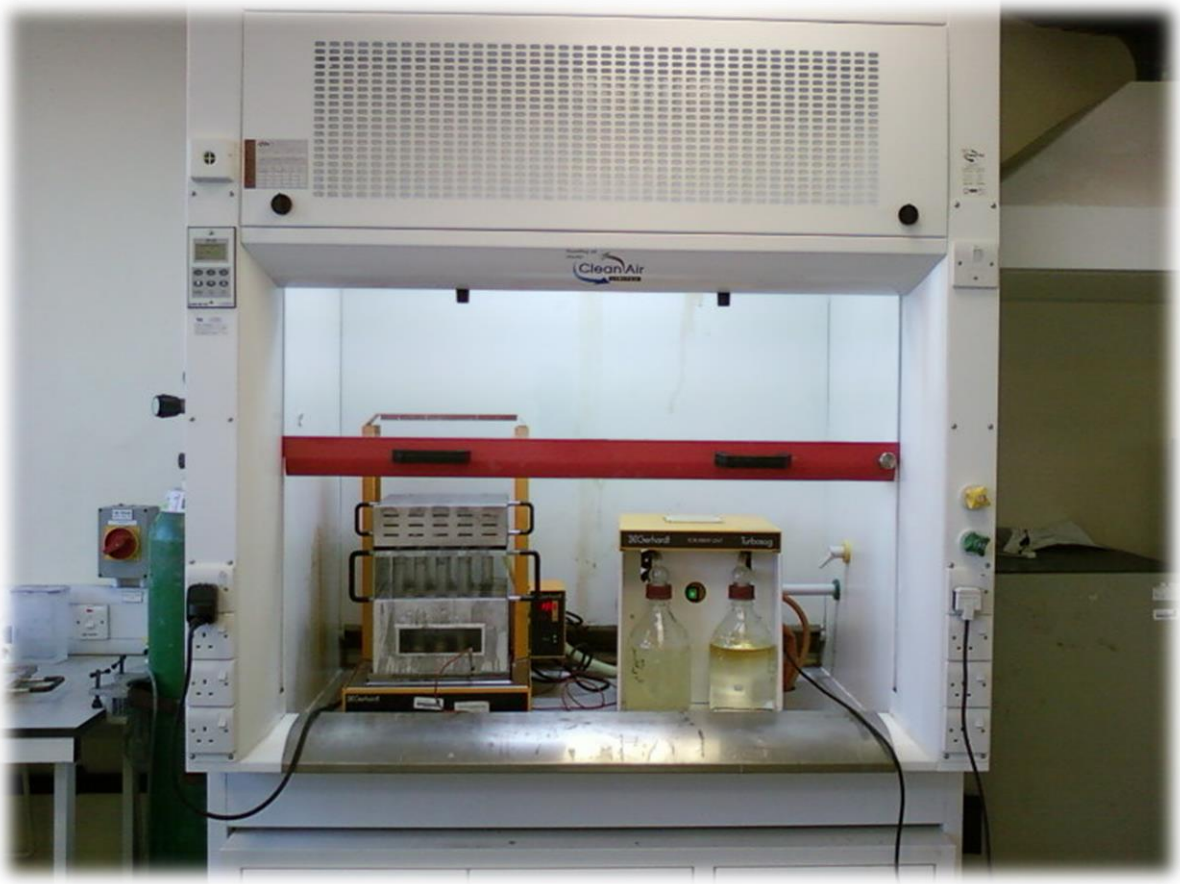


Plate 3.4 Digestion chamber for fibre determination

3.2.6.4 Determination of ADF

The acid detergent fibre (ADF) content of the feed was determined by the Van Soest *et al.* (1991) method of analysis in which 2g dried ground sample was placed into a conical flask for refluxing. Then, 100ml acid detergent (AD) solution were added to the sample and heated to boiling in about 60 minutes (Figure 3.4). The solution was then filtrated through a pre-weighed sintered glass crucible using light vacuum suction. After this, the crucible contents were washed by filling two thirds of the crucible with hot water, stirred, soaked for 15-30 seconds and drained with the aid of vacuum suction. This was repeated while rinsing the sides of the crucible and performing the same

washing with small volume of acetone until no more colour was visible. The crucible and its content of residue was dried at 105^{o}C in a forced oven overnight, then cooled in a desiccator and weighed. Finally, the residual content was incinerated at 550^{o}C in a furnace for 5hr, cooled in a desiccator for 30 minutes and weighed. ADF was calculated as shown below:

$$ADF\ (g/kg\ DM) = \frac{R-A}{S}\ x\ 1000$$

Where: R= weight of residue;

A= weight of ash;

S= weight of dried sample.

3.2.6.5 Determination of Calcium & Phosphorus

The calcium content of feed samples was determined by dry combustion, where the soluble mineral constituents in the ash were dissolved in hydrochloric acid (HCl). Two grams of dried ground sample in a crucible was incinerated in a furnace at 550^{o}C overnight. The ash was then moistened with water and digested in 10ml of 6M HCl which was evaporated on a water bath. About 2ml of concentrated HCl was then added, and the crucible containing the sample covered and the contents boiled for two minutes.10ml of water was also added and boiled for 2 minutes. The contents were transferred through a filter paper into a 50ml volumetric flask while rinsing the filter paper with water to make up to volume, mixed well and transferred into plastic vials until analysis. Using standard concentrations, a standard curve was constructed to establish the relationship between absorbance and the concentration of calcium and phosphorus by (plotting data with the absorbance readings on the vertical axis and concentration on the horizontal axis). The samples were read on a spectrophotometer for phosphorus and on a flame photometer for calcium respectively. The readings were then imported into excel spreadsheet using graph/equation where the values obtained were substituted in the equation to determine the concentration of calcium and phosphorus contents which were expressed as g/kg.

Other minerals were analysed on a Varian Vista-MPX CCD by simultaneous inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian Inc. Australia).

3.2.6.6 Determination of ether extracts (EE)

Fat extractions were carried out by refluxing feed and excreta samples in the Soxhlet apparatus. Two grams of the samples were placed in a thimble and the mouth closed with a piece of cotton wool. The thimbles were placed into an extraction chamber of the Soxhlet system. The chambers were fixed onto an extraction flask (round bottom flask of 500 ml) in which about 200 ml of petroleum ether was placed. The two units were fixed onto a heating mantle and a condenser placed on top of the unit. The heating mantles were turned on at a temperature of 60^{o}C. As the ether in the flask evaporated, it followed the side arm and went to the condenser of the Soxhlet where it condensed and came down into the extraction chamber. As it dripped onto the samples in the thimbles it extracted the fat and, when the chamber was full, the siphon arm filled up also and then poured down in the flask. As the process was repeated by refluxing, the total fat in the sample was removed and received into the flask. After about 5 hours, the ether coming down was received into a beaker, leaving the flask with only extracted fat. The flasks were then oven-dried at 100^{o}C for 1 hour, cooled in desiccators and weighed. Ether extract was then calculated as follows:

$$\% \text{ EE} = \frac{\text{(Weight of oil +flask after extraction) - (weight of empty flask)}}{\text{Weight of dried sample}} \times 100$$

3.2.7 Statistical analysis

At the end of the experiment, data collected were entered in an excel spreadsheet and calculations were performed to determine the averages of feed intake, LWG, FCR as well as the % values of carcass and digestive organs. The pattern of live weight and feed intake per bird was also monitored over 42 days. The data were subjected to Analysis of Variance (ANOVA) using the Minitab 16 statistical package. The command One-way ANOVA was used to assess the effects of dietary treatments on growth performance, digestibility, carcass characteristics and digestive organs parameters. Means of the said parameters were compared using the Tukey's test for their statistical differences at $P<0.05$. Residuals were used to test the normality of the data using Anderson Darling test. For behaviour data, the values were non-parametric, the Kruskal Wallis test was used to analyse the data and the medians were tested for significant differences at $P<0.05$. Where the data were significantly different, a Mann-Witney test was used to determine the differences among the variables. Mean values of variables for different

treatments and days were illustrated graphically using Microsoft Excel to show the pattern of behaviour displayed by birds. A fitted line plot regression analysis was performed to assess the relationship between the dry matter and wDDGS levels and between protein and wDDGS levels.

3.3 Results

3.3.1 Nutrient composition of the experimental diets

The results of proximate composition of the experimental diets and that of the major dietary ingredients are presented in Tables 3.4 and 3.5. The crude protein content varied between 190 and 206g. The DM, EE, NDF, ADF, and phosphorus contents increased as the level of wDDGS increased in the diets while the calcium content varied between 21.8g and 25.2g. However, the contents of other nutrients measured did not follow any consistent trend.

The amino acid (AA) compositions of the experimental diets (Table 3.1) were calculated using the nutrient database reported by Stein (2006) and shows that the total amino acid content decreased linearly with increasing wDDGS levels. Similarly, lysine content decreased with increasing wDDGS levels. The AA concentration in the control diet was highest while the diet containing 40% wDDGS presented the least. The diets contained 8 to 11g lysine and 4g methionine respectively. Concentration of leucine methionine, threonine, tryptophan and valine were equal in all the diets. Leucine had the highest values followed by arginine while tryptophan and methionine presented the lowest values. The phenylalanine content was slightly higher in 40% wDDGS compared to the control, 10 and 20% wDDGS diets. The lysine and methionine contents of the control diet were similar to the recommended level for broilers aged 3-6 weeks by NRC 1994. Lysine content of 20 & 40% wDDGS diets were slightly below the NRC recommended level.

Table 3.4 Proximate compositions of the broiler diets

	wDDGS (%)			
	0 (Control)	10	20	40
Nutrients (g/kg DM)				
Dry Matter	898	902	908	912
Crude Protein	194	190	200	206
Ether Extract	22	28	34	41
Total Ash	93	93	100	98
NDF	90	118	149	221
ADF	39	54	74	101
Calcium	22	23	26	25
Phosphorus	7.1	7.4	7.7	9.4
Copper	0.01	0.01	0.01	0.01
Iron	0.3	0.2	0.3	0.2
Potassium	6.3	6.7	6.8	6.9
Magnesium	1.6	1.7	1.9	2.0
Manganese	0.01	0.01	0.02	0.02
Sodium	1.8	2.1	2.8	3.7
Zinc	0.22	0.16	0.19	0.17

NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre

Table 3.5 Proximate compositions of the major dietary ingredients

	Ingredients		
	Wheat	Soybean	wDDGS
Nutrients (g/kg)			
Dry Matter	887	898	913
Ash	15	74	49
Crude Protein	82	442	311
Ether Extract	16	19	49
Neutral Detergent Fibre	109	83	469
Acid Detergent Fibre	27	60	145
Calcium	4	3	2
Phosphorus	7	9	9

3.3.2 Growth performance of broiler chickens

Analysis of the performance of broiler chickens (Table 3.6) showed that there were significant differences in feed intake, live weight gain and water intake between diets with different wDDGS inclusion. Birds fed a 10% wDDGS diet had significantly ($P < 0.05$) higher feed intake than did birds fed 0, 20 and 40% wDDGS diet. Similarly, they gained more weight than those fed 20 & 40% wDDGS though similar to birds fed control (0% wDDGS) diet. Feed conversion ratio also showed a significant treatment effect, with poorer feed conversion on the highest inclusion level.

Table 3.6 Performance of broiler chickens fed varying levels of wDDGS over a 42 day period

	wDDGS (%)					
	0	**10**	**20**	**40**	**SEM**	**P value**
Parameters						
Feed Intake (g/b/d)	85^{b}	93^{a}	82^{b}	35^{c}	1.4	0.005
Live Weight Gain (g/b/d)	43^{ab}	49^{a}	41^{b}	16^{c}	1.4	0.001
FCR (g feed/g gain)	1.99^{ab}	1.94^{b}	2.04^{ab}	2.14^{a}	0.08	0.013
Water Intake (ml/b/d) *	372^{a}	347^{a}	322^{ab}	259^{b}	17.9	0.005
Water to Feed Ratio	2.6	2.7	2.6	2.9	0.20	0.710

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of Means, FCR = Feed Conversion Ratio,
**=Water Intake was measured for 4 days during the digestibility trial.*

Significant differences were also observed in water intake, which decreased as inclusion level of wDDGS increased but water to feed ratio was similar among treatments.

The productive performance of broiler chicks fed varying levels (0, 10, 20, and 40%) of wDDGS is summarized for different periods of the experiment in Table 3.7. The feed intake was significantly lower for the 40% wDDGS diet throughout the phases of production. Feed intake was significantly higher for 10% wDDGS at 15-35 days than for other treatments

Table 3.7 Productive performance of broiler chickens fed varying levels of wDDGS over different periods of the 42 day experiment

	Feed Intake(g/b/day)			Weight Gain (g/b/day)			FCR (g feed/g live weight)		
	0-14 d	15-35 d	35-42 d	0-14 d	15-35 d	35-42 d	0-14 d	15-35 d	35-42 d
Treatments									
0%wDDGS	18[a]	108[b]	166[a]	15[b]	52[ab]	70[a]	1.17	2.07[b]	2.37
10%wDDGS	24[a]	117[a]	177[a]	20[a]	58[a]	76[a]	1.17	2.02[b]	2.36
20%wDDGS	18[a]	102[b]	171[a]	17[b]	47[b]	68[a]	1.05	2.18[ab]	2.52
40%wDDGS	6[b]	44[c]	76[b]	8[c]	18[c]	26[b]	0.79	2.43[a]	2.98
SEM	1.7	2.0	9.5	0.6	1.8	3.8	0.090	0.07	0.386
P value	0.001	0.001	0.001	0.001	0.001	0.001	0.056	0.007	0.652

a,b,c Means bearing different letter superscripts within columns are significantly different (P<0.05), SEM=Standard Error of means, d = days, FCR = Feed Conversion Ratio

Similarly, there were significant differences in weight gain throughout the feeding trial in which birds on 10% wDDGS gained more weight than the other groups. At an early age of 0-14 days, the weight gain was higher in 10% wDDGS, though in the finishing phase 10% wDDGS was not significantly different to treatments 0% and 20%. However, the live weight gain of 40% wDDGS was always lower than the other groups. The feed conversion ratio was significantly poorer for 40% wDDGS diet in the middle phase of the experiment. The litter moisture content (Table 3.8 and Figure 3.3) decreased with increasing level of wDDGS, where 40% wDDGS treatment presented the lowest moisture content throughout the experimental period. Same pattern of feed intake and subsequent live weight gain was observed among birds in all the treatments (Figures 3.1 and 3.2).

Table 3.8 Litter moisture content (%) of broilers at different phases of production

	wDDGS (%)					
	0	**10**	**20**	**40**	**SEM**	**P value**
Production Phase						
Starter (0-14 days)	42^{a}	41^{ab}	40^{ab}	32^{b}	1.1	0.022
Grower(21-35 days)	43^{a}	43^{a}	41^{ab}	36^{b}	0.8	0.032
Finisher(35-42 days)	43^{a}	43^{a}	43^{a}	34^{b}	0.9	0.012
Overall (1-42 days)	43^{a}	42^{a}	41^{a}	34^{b}	0.5	0.001

a,b, Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means

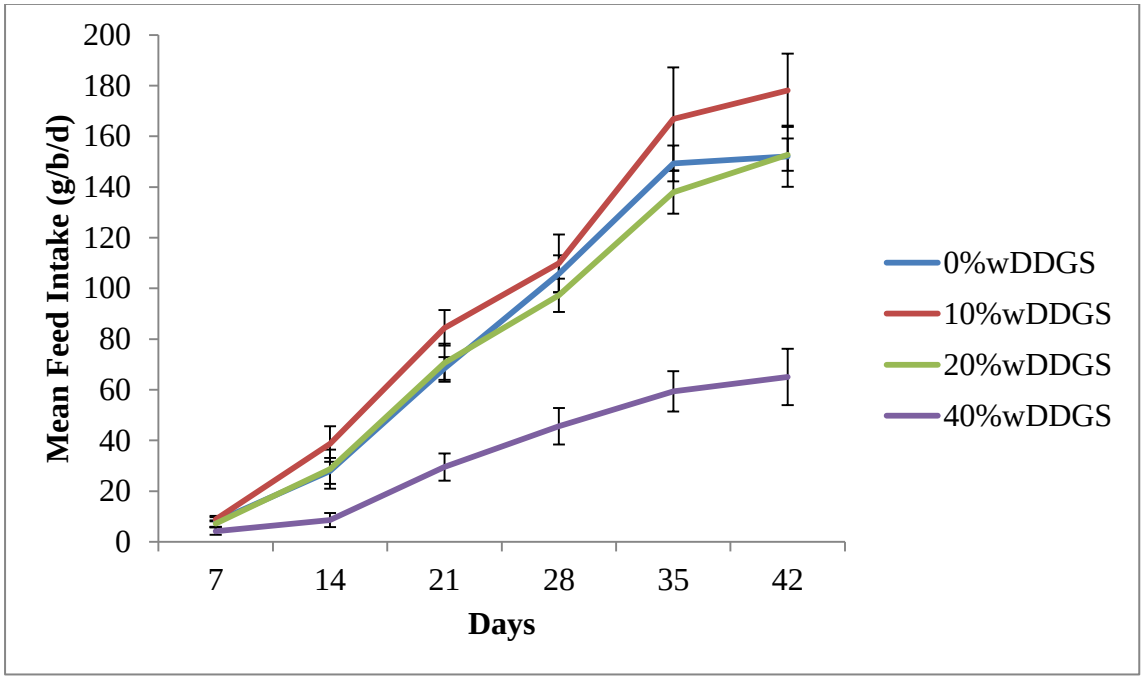


Figure 3.1 Mean bird's feed intake increase with age

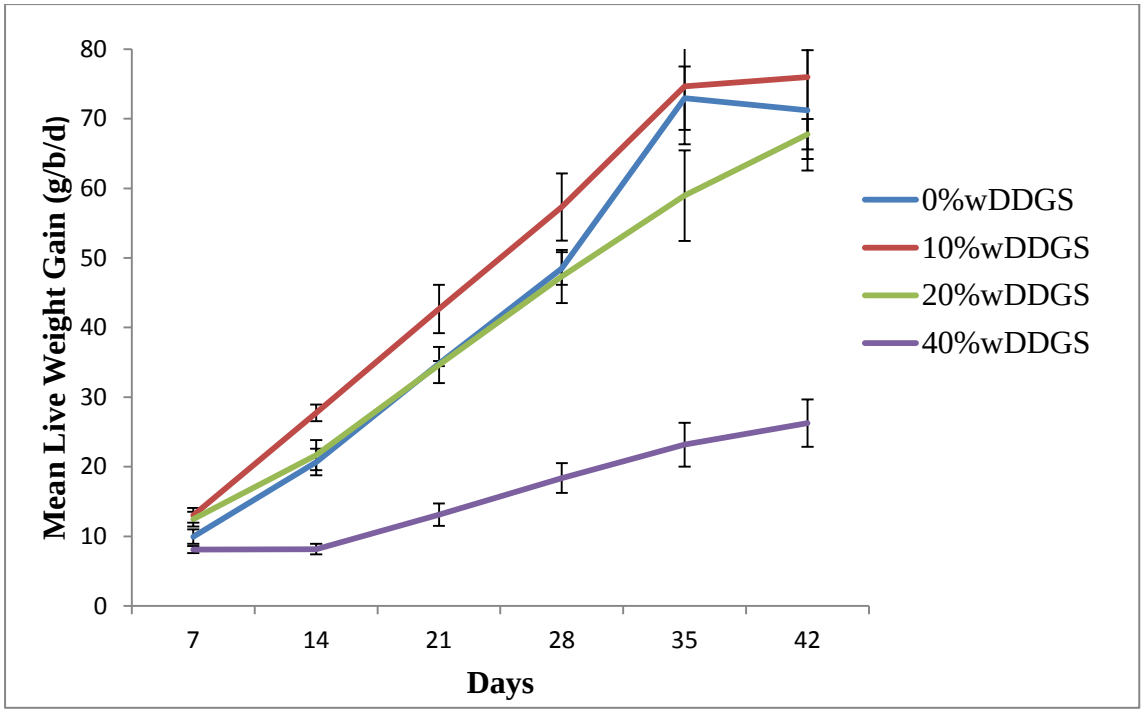


Figure 3.2 Mean bird's live weight increase with age

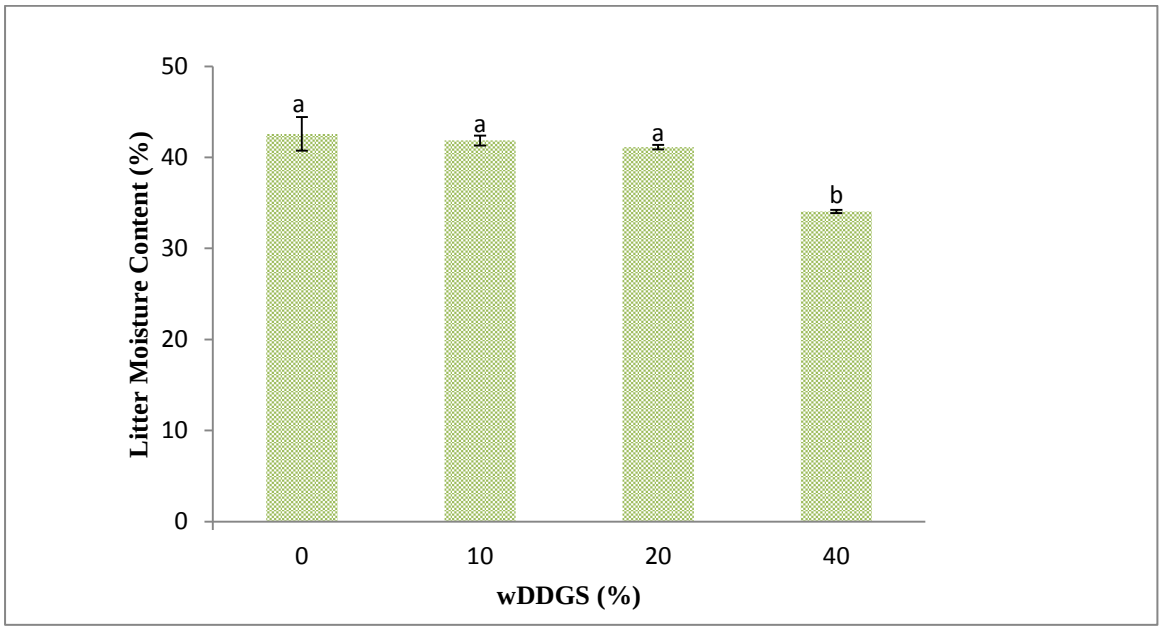


Figure 3.3 Litter Moisture Content

3.3.3 Behavioural responses of broiler chickens fed varying levels of wDDGS

Different behaviours of broiler chickens were significantly affected by age (Table 3.9). No resting behaviour was observed in the first seven days of life, while the medians of birds feeding significantly reduced with increasing age of the birds and more birds were exploring in the periods of 14 and 21 days. During the 28 day period, birds reduced drinking significantly and walking behaviour. At the age of 35 and 42 days, birds reduced feeding and exploring as well and more were found to be resting (Table 3.9).

Different levels of wDDGS had no significant effect on the behaviour of birds, with the same number of birds performing feeding, drinking, exploration, walking and resting at different levels of wDDGS (Table 3.10).

Figure 3.4 shows that most of the birds were engaged in feeding in day 7. From day 14 to day 42, the trend remained similar and treatment did not show any difference for feeding behaviour in any of these days. Similarly, the drinking behaviour (Figure 3.5) also showed a higher value at day 7 for all birds and then remained similar from day 14 to day 42, and there was no difference among the treatments. The exploration behaviour (Figure 3.6) was reduced after day 28; it was lower in days 35 and 42 than other days, while wDDGS treatment had no any effect. Walking behaviour (Figure 3.7) was also slightly higher at day 7 for all levels of wDDGS then remained constant from day 14 to day 42. In the first 7 days, birds did not show the resting behaviour. From days 14 to day 28 about 30% of birds were resting for all the wDDGS levels, and in day 35 and day 42 more birds were resting (Figure 3.8). WDDGS levels did not show any difference for the resting behaviour.

Table 3.9 The medians of observations of different behaviours shown by birds in different weeks of the experiment

	Feeding	Drinking	Exploration	Walking	Resting
Days					
7	56^{a}	22^{a}	33^{c}	22^{a}	0
14	33^{b}	22^{a}	56^{a}	22^{a}	67^{b}
21	33^{b}	22^{a}	50^{b}	22^{a}	56^{c}
28	33^{b}	11^{b}	44^{b}	11^{b}	56^{c}
35	22^{c}	11^{b}	22^{d}	11^{b}	78^{a}
42	22^{c}	11^{b}	22^{d}	11^{b}	67^{b}
P value	0.001	0.001	0.001	0.005	0.001

Broiler behaviours were not affected (P>0.05) by dietary wDDGS inclusion (Table 3.10).

Table 3.10 The medians of observations of different behaviours shown by birds in different treatments for wDDGS based diets (0 to 40%) of the experiment

	Feeding	Drinking	Exploration	Walking	Resting
wDDGS (%)					
0	33	22	33	11	67
10	33	22	44	22	67
20	33	22	33	22	67
40	33	22	44	22	67
P value	0.097	0.243	0.272	0.372	0.058

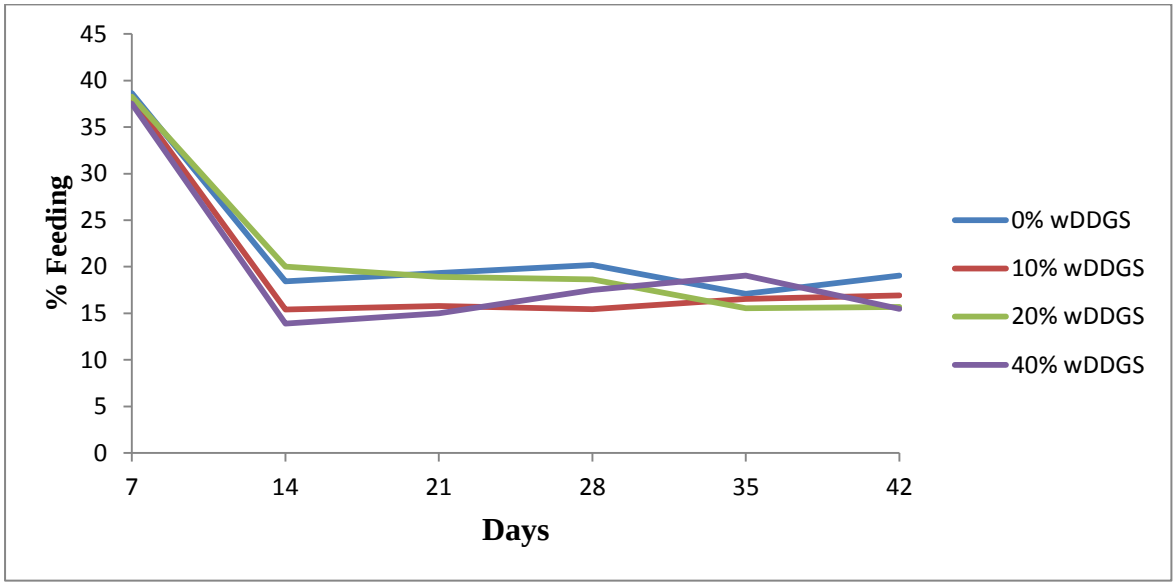


Figure 3.4 Proportion of birds showing feeding behaviour

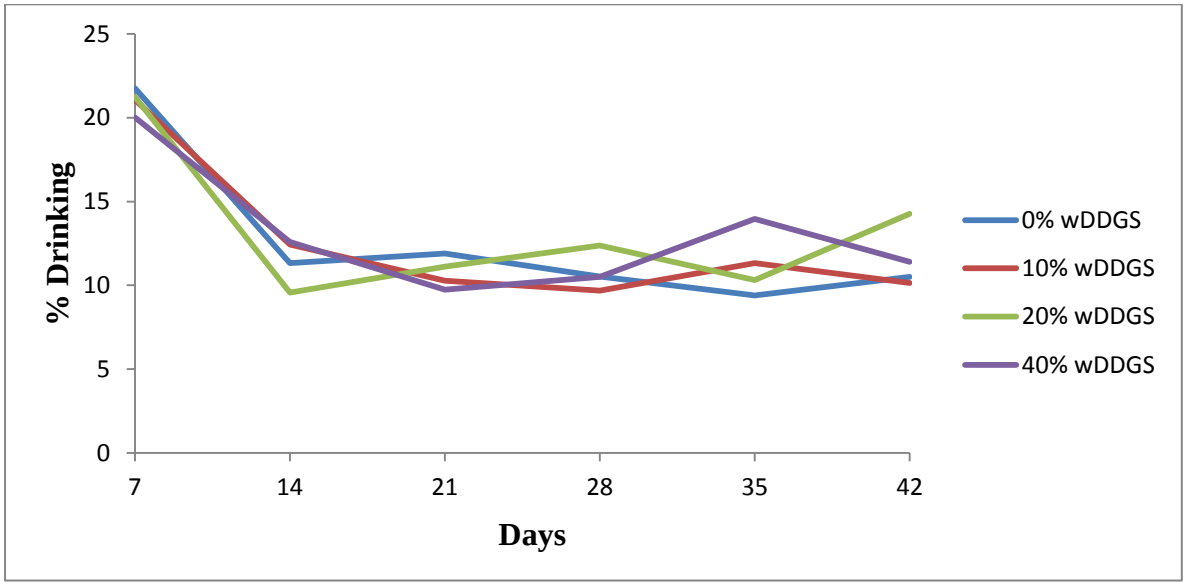


Figure 3.5 Proportion of birds showing drinking behaviour

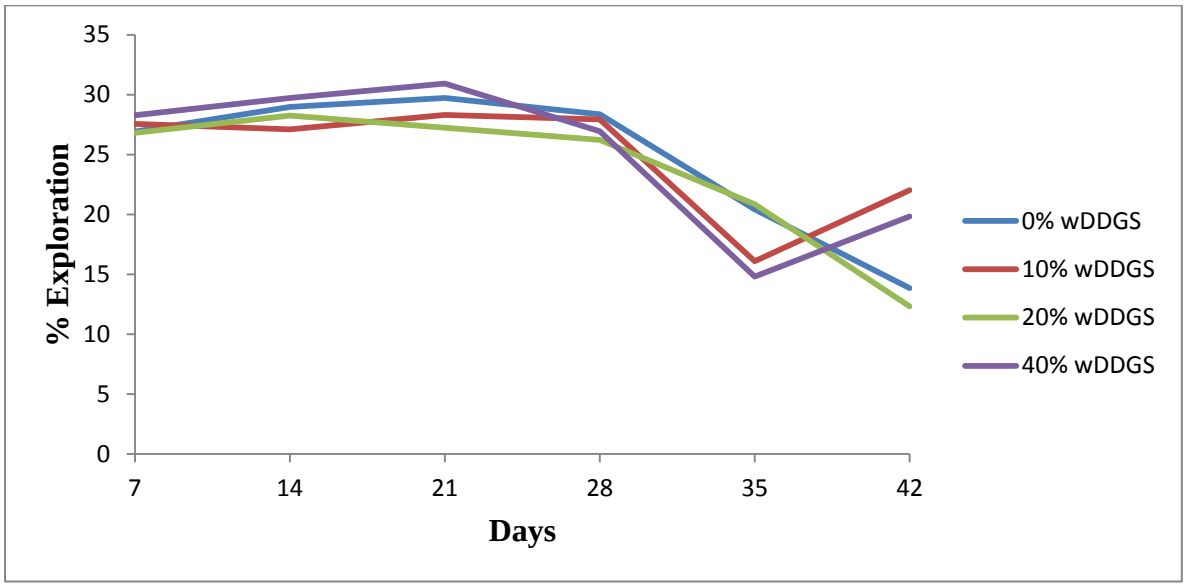


Figure 3.6 Proportion of birds showing exploration behaviour

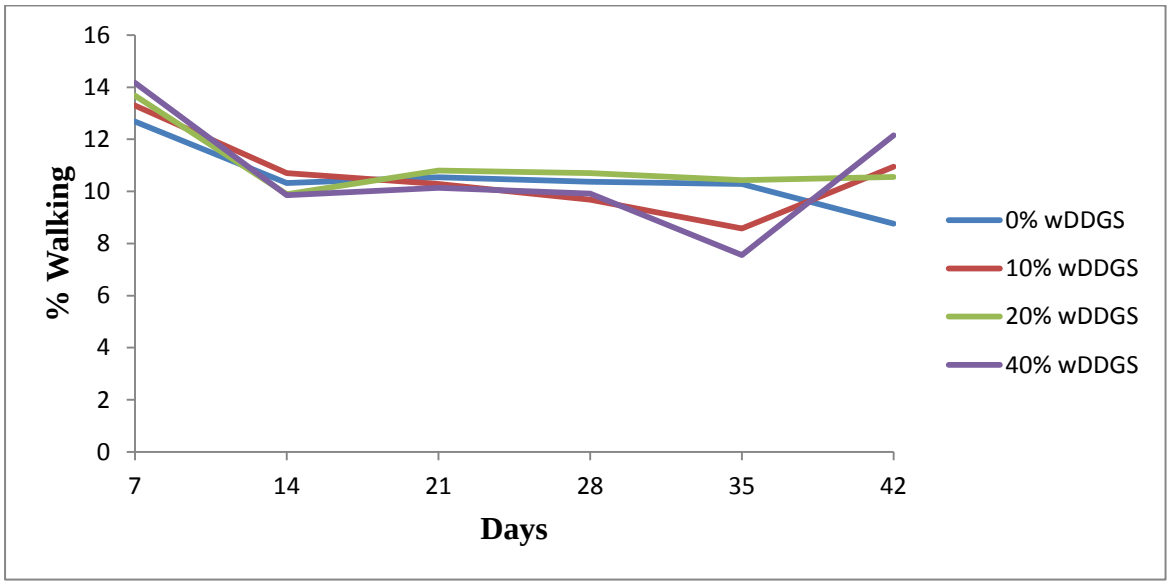


Figure 3.7 Proportion of birds showing walking behaviour

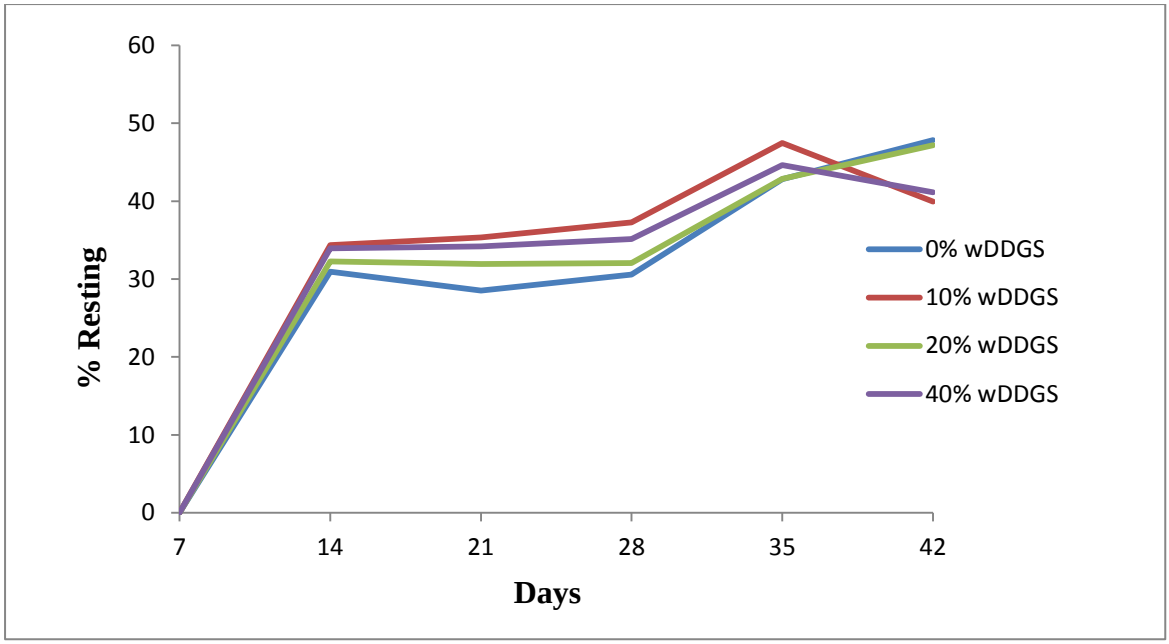


Figure 3.8 Proportion of birds showing resting behaviour

3.3.4 Nutrient digestibility of the experimental diets

Table 3.11 shows the nutrient digestibility of the experimental diets determined by total collection method. The DM, protein, EE, and phosphorus digestibility were significantly affected by wDDGS levels. The digestibility of NDF at 40% wDDGS inclusion was significantly higher than other treatments while ADF and calcium were not affected (P>0.05) by wDDGS inclusion. The control (0% wDDGS) diet had the highest digestibility value for dry matter, protein and phosphorus compared to other treatments while diet D (40% wDDGS) presented the lowest.

Table 3.11 Nutrient digestibility of the diets (g /kg) determined by total collection method

	wDDGS (%)					
	0	**10**	**20**	**40**	**SEM**	**P value**
Nutrient (g/kg)						
Dry Matter	691^{a}	651^{ab}	620^{bc}	560^{c}	13.9	0.001
Protein	525^{a}	442^{a}	399^{ab}	259^{b}	30.8	0.004
Ether Extract	770^{b}	812^{ab}	838^{a}	826^{ab}	9.4	0.034
NDF	127^{b}	141^{b}	145^{b}	271^{a}	9.9	0.001
ADF	93	91	104	109	6.2	0.761
Calcium	239	250	259	236	19.0	0.818
Phosphorus	385^{a}	275^{b}	263^{b}	219^{c}	10.5	0.001

a,b,c Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of Means, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre

The dry matter and protein digestibility decreased with increasing level of wDDGS in all the treatments. Figures 3.9 and 3.10 showed the relationship between wDDGS inclusion and DM and protein digestibility respectively. It shows that for every 10% increase in wDDGS inclusion, DM digestibility decreased by 3.2%. However, the ether extract digestibility significantly increased with increasing levels of wDDGS. Meanwhile, the NDF and ADF digestibility were slightly higher in 40% wDDGS diet but were statistically similar among the treatments (P>0.05). Calcium digestibility was not affected by wDDGS inclusion. However, phosphorus digestibility decreased linearly with increasing level of wDDGS. The digestibility was highest in 0% wDDGS followed by 10 and 20% wDDGS while 40% wDDGS presented the lowest value.

The results of nutrient digestibility of the diets as determined by the AIA method (Table 3.12) also show that the protein digestibility decreased with increasing wDDGS level, as in the total collection method. Calcium digestibility did not follow any definite trend, however, phosphorus digestibility significantly (P<0.05) decreased with increasing level of wDDGS as in the total collection method.

Regression analysis shows a strong positive relationship between total collection and AIA methods for digestibility of protein, ether extract, NDF and phosphorus (Figures 3.8, 3.9, 3.10).

Table 3.12 Nutrients digestibility (g/kg) of the diets determined by the AIA method

	wDDGS (%)					
	0	**10**	**20**	**40**	**SEM**	**P value**
Nutrient						
Dry Matter	929^{a}	921^{a}	883^{ab}	849^{b}	9.6	0.001
Protein	481^{a}	442^{a}	379^{a}	198^{b}	30.8	0.001
Ether Extract	749^{b}	811^{a}	833^{a}	811^{a}	9.9	0.003
Neutral Detergent Fibre	104^{b}	170^{ab}	141^{ab}	214^{a}	20.6	0.017
Acid Detergent Fibre	78	88	84	101	5.1	0.452
Calcium	262^{b}	317^{ab}	407^{a}	323^{ab}	28.9	0.028
Phosphorus	377^{a}	368^{a}	298^{b}	235^{c}	5.5	0.001

a,b, Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means

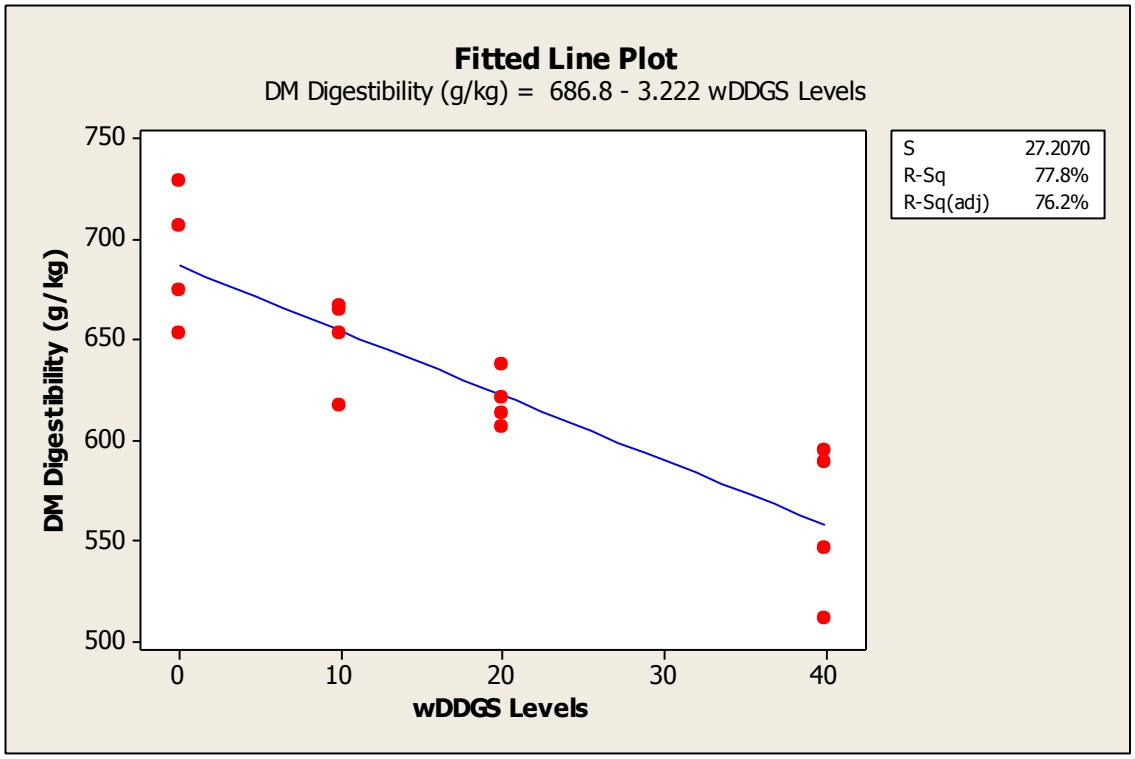


Figure 3.9 Relationship between wDDGS inclusion and DM digestibility

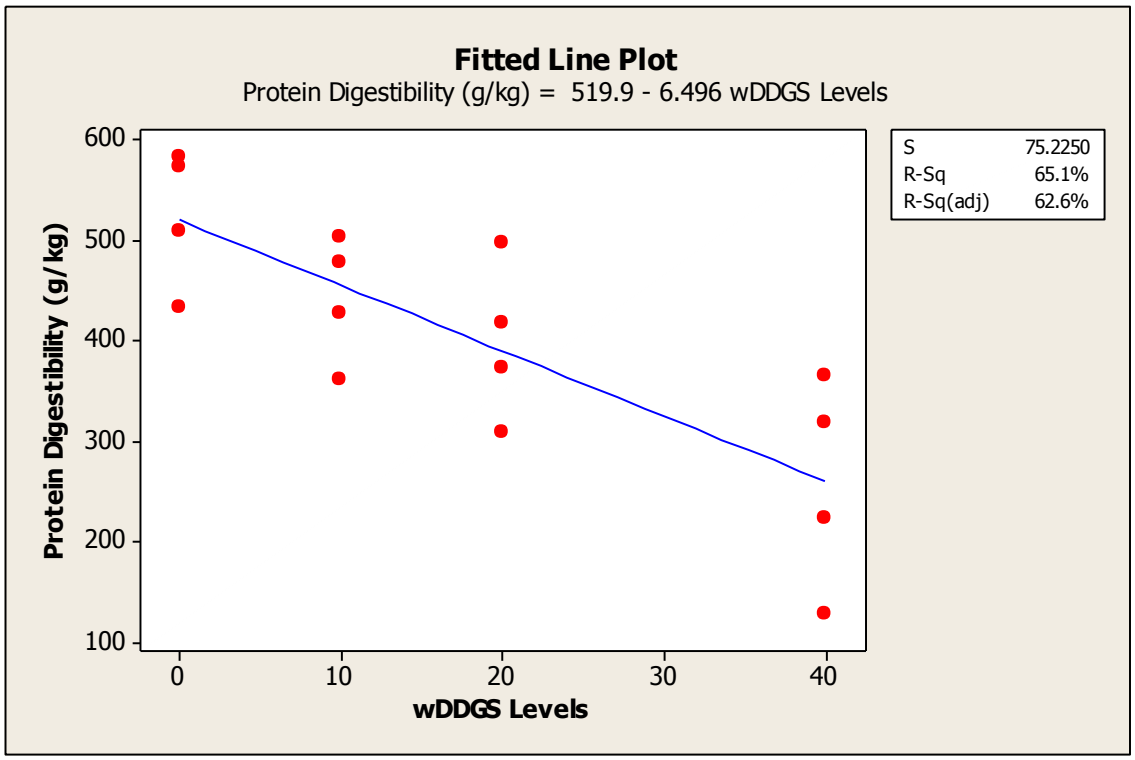


Figure 3.10 Relationship between wDDGS inclusion and protein digestibility

3.3.5 Carcass characteristics and digestive organs of broiler chickens

The effect of feeding varying levels of wDDGS on carcass characteristics and digestive organs of broilers is shown in Table 3.13. There was a significant (P<0.05) influence of the wDDGS levels on final live weight of broiler chickens. Similarly, both carcass and digestive organs were significantly affected by inclusion of wDDGS levels except drumsticks, wings, heart and empty crop. Inclusion of 40% wDDGS significantly reduced the carcass weight and dressing percent of birds as well as breast with bone weight and thighs compared to other treatments. Breast with bone weight decreased linearly with increasing levels of wDDGS. On the contrary, neck and back, liver, empty gizzard, total gut and proventriculus were significantly increased by inclusion of 40% wDDGS. Proventriculus weight and empty gizzard weight increased with increasing levels of wDDGS. Using 20% DDGS did not affect most parameters when compared to the control diet.

Table 3.13 Effect of feeding varying levels of wDDGS on carcass characteristics and digestive organs of broiler chickens (% Carcass weight)

	wDDGS (%)					
	0	**10**	**20**	**40**	**SEM**	**P value**
Parameters (%)*						
Final live weight (g)	1874.6^{a}	2131.1^{a}	1987.5^{a}	957.9^{b}	135.0	0.001
Cold Carcass weight (g)	1382.0^{a}	1562.2^{a}	1435.4^{a}	647.4^{b}	105.7	0.001
Dressing percent	73.7^{a}	73.3^{a}	72.1^{a}	67.5^{b}	0.92	0.002
Major carcass components						
Breast + Bone	32.1^{a}	30.7^{a}	27.5^{a}	19.5^{b}	1.51	0.002
Thighs	13.7^{ab}	14.2^{a}	14.1^{a}	13.0^{b}	0.15	0.014
Drumsticks	12.4	13.1	13.0	12.5	0.32	0.347
Neck + Back	17.3^{b}	17.8^{b}	19.5^{ab}	21.5^{a}	0.51	0.002
Wings	10	10	10	9	0.2	0.209
Edible Organs						
Liver	3.1^{b}	3.3^{b}	3.3^{b}	4.2^{a}	0.15	0.011
Heart	0.8	0.9	0.9	1.1	0.04	0.059
Empty Gizzard	1.6^{c}	1.7^{c}	2.4^{b}	3.0^{a}	0.15	0.001
Non-Edible Organs						
Total gut	7.5^{b}	6.9^{b}	8.2^{b}	14.1^{a}	1.20	0.004
Empty Crop	1.0	0.7	0.8	1.1	0.12	0.152
Proventriculus	0.6^{b}	0.6^{b}	0.7^{b}	1.0^{a}	0.04	0.001

*a,b,c, Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means, *= unless otherwise stated*

3.4 Discussion

Diets were formulated to contain 22% crude protein for the entire period of growth (single phase) to cover all stages of growth and to meet the requirements of the bird and NRC (1994) nutrient recommendations being iso-nitrogenous with varying ME. A control wheat-based diet was formulated to contain 13.4 ME (MJ/kg) whereas the 40% wDDGS diet was formulated to contain 12.4 ME (MJ/kg). The varying energy contents were meant to test the response of the birds based on that varying levels as any attempt to unify the energy content by adding more oil to the diets may act as a source of variation and can deter the palatability of the diets which may affect the birds feed intake as opposed to the report of Leeson and Summers, (1991) that varying the energy levels of a broiler diets today has less of an effect on feed intake being governed by the energy requirements. Inclusion of wheat, soybean meal and oil served as major sources of energy to meet the energy requirements of the birds for maintenance and growth. The oil was to complement the energy supply as well as to improve the physical consistency of diets and dispersion of micro-ingredients in feed mixtures. It may as well increase the energy utilization when birds attain maturity as stated by NRC (1994). The use of soybean meal in the diets was due to the fact that it is a high quality protein source/supplemental protein in poultry diets but its protein is deficient in methionine and hence the supplementation with synthetic methionine was needed. Previous studies have indicated that the residual starch content of wDDGS is low which is likely associated with the Maillard reaction producing brown compounds and a lower availability of amino acids and energy (Cozannet *et al*., 2010).

The ideal diet for broilers consists of certain nutrients in order to provide the means of maintaining maximum muscle synthesis. This requires the diet to contain crude protein, fat, carbohydrates, vitamins and minerals. Maximum poultry performance is achieved when these ingredients are supplied in the diet in correct quantities and proportions. The ingredients utilized in the experimental diets were mixed so as to provide all necessary nutrients. Although each individual ingredient in the feed has its own exclusive composition which permits it to be added to diets for birds, there is a wide degree of variation regarding how easy it is to digest each nutrient of different ingredients. It is common to add more nutrients than required to animal diets based on the fact that there exists variation among different animals as to the exact quantity of nutrients necessary to meet optimal production conditions. According to the NRC (1994), broilers need a comparatively high level of amino acids in their diet in order to maintain their growth rate, as gains in body weight are typically fifty

times that of their weight at birth. This illustrates how essential it is that sufficient amino acids are supplied in the diet in order to achieve optimal broiler production. Results of this study show that concentration of some amino acids in the control diet was higher than the wDDGS diets. The reason for this higher concentration could probably be due to the higher inclusion of wheat and soybean meal in the diet. The reduced lysine content in 40% wDDGS diet could be due to lower lysine availability in the wDDGS resulting from drying process and colour of the wDDGS. The drying which wDDGS undergoes is thought to have an adverse effect on AA availability as reported in previous studies that excessive heating during drying process can cause Maillard reactions of the protein-bound lysine and available sugars and hence reduced lysine availability (Cromwell *et al.*, 1993; Hodgkinson, 2006; Cozannet *et al*., 2010). Similarly, Lan *et al.* (2008) also reported the lysine in wDDGS was poorly available.

The highest leucine concentration in both wDDGS, wheat and soybean meal explains its higher profile in the diets. The highest phenylalanine content of 40% wDDGS diet could also be traced to the higher content of phenylalanine in soybean meal and wDDGS having the highest mean concentration in wDDGS. The lower lysine values recorded in 40% wDDGS coupled with poor lysine digestibility were in agreement with the findings of Fastinger *et al.* (2006) who reported lowered lysine digestibility in precision-fed cecectomized roosters. The better performance in terms of live weight gain and feed conversion ratio of birds fed 10% wDDGS though statistically similar to control but numerically slightly higher could be as a result of the diets supplied the limiting amino acid that were similar to those of the control diet. Despite the equal methionine content of the diets, the breast with bone weight was similar in birds fed 0 and 10% wDDGS and better than birds fed 40% wDDGS diets.

The crude protein value of wDDGS obtained in this study was higher than that (265.3g/kg) reported by Yousef *et al.* (2009) and lower than that (357g/kg) reported by Thacker and Widyaratne (2007) which is lower than the level found in soybean meal (442g/kg). The ether extract content (48.8g/kg) of wDDGS obtained in this study was comparable with that (43.2g/kg) reported by (Thacker and Widyaratne, 2007).

The significantly lower feed intake on the 40% wDDGS diet might have been due to the high percentage of fibre and perhaps the palatability of the diet. Lower feed intake caused lower nutrient availabilities in birds, which accounts for the lower weight gain with the 40% wDDGS diet. Moreover, a high fibre level may increase the rate of passage of the feed

through the digestive tract, thereby reducing the time of ingesta exposure to enzymatic degradation and the time of nutrient contact with the absorptive membranes (Shalash *et al.*, 2009). Wang *et al.* (2007) also reported that chicks fed diets with 30% corn DDGS had significantly reduced feed intake and live weight gain at 35 and 42 days compared to those fed diets without DDGS. In the current experiment, the highest feed intake and growth were recorded from birds fed the 10% wDDGS diet. The best FCR was also recorded from birds fed the 10% wDDGS diet compared to 20 and 40% wDDGS, but was similar to 0% wDDGS. From an economic point of view, 10% wDDGS will be suggested as it gave higher intake than 0% wDDGS and numerically greater live weight but statistically similar to 0% wDDGS. The cost of diet will be less at 10% wDDGS than 0% wDDGS, so farmers will get more profit margins by using 10% wDDGS at the same FCR as 0% wDDGS.

As fibre can bind some water (Chaplin, 2003) and negatively affect production performance and water intake of broiler chickens (Van Der Klis and De Lange, 1999), a higher fibre diet can also reduce feed intake of broiler chickens. Birds fed 40% wDDGS diet in this study had reduced feed intake. Feed intake had a positive relationship with moisture content of the litter. The high fibre diet reduced water intake and subsequently reduced litter moisture content. This was also demonstrated by Hocking (2006), who reported a significant decline in litter moisture with increasing concentration of oat hulls and a comparable decrease in the number of litter changes required. Results also showed that there was significantly ($P<0.05$) higher water intake in 0% wDDGS compared with 40% wDDGS. Although cereals such as wheat are high in water soluble non-starch polysaccharides (NSP) which have been shown to increase faecal moisture and cause wet litter (Collett, 2012), the decrease in litter moisture content in this experiment could be explained by the significant decrease in water intake with increasing levels of wDDGS.

In the first seven days of the experiment, more birds were engaged in feeding and drinking. At a young age, all animals, including human beings take interest in new things; this is why the exploration behaviour indicating curiosity of birds was higher at a younger age with very few birds performing resting behaviour. When birds grew older, they became more experienced, and used less time for eating and drinking. As they increased body weight, more birds showed resting behaviour and reduced general activity. The greater number of birds performing resting behaviour in the latter part of this experiment (days 35-42) could probably be explained by the birds being housed in a deep litter system, which provides them with much higher welfare levels as reported by Sosnowka-Czajka *et al.* (2005), and possibly due

to the high breast meat yield and decreased space for movement in the pen and perhaps some leg problems (Hall, 2001). Weeks *et al.* (2000) also reported age-related increases in lying and sitting behaviour by broilers which might be attributed to the high breast meat yield that predisposes them to perform increased sitting behaviour. Other behaviours (aggression, dust bathing and preening) were infrequently observed in this study. The lack of aggression observed could be due to the effect of the diets being high in fibre, which might have caused the birds to remain calm due to satiety (Hetland and Svihus, 2001), but probably reflects the small stable groups and lack of competition for resources. The responses observed herein were only affected by the age of the birds, whereas, different levels of wDDGS had no effect on any of the behaviours of the birds.

It is apparent that feeding 40% wDDGS significantly reduced DM and crude protein digestibility compared with the control diet without wDDGS, and 10% wDDGS inclusion level. This finding is in agreement with the results of Barekatain *et al.* (2013) who reported that high inclusion of sorghum DDGS resulted in poor digestibility of protein and amino acids, a factor that contributed to poor feed efficiency of the broilers. Birds fed 40% wDDGS had poorer ($P<0.05$) crude protein digestibility, possibly due to the poor lysine digestibility of wDDGS as affected by the drying process after fermentation, and it followed the same linear decrease with increasing wDDGS level (Kluth and Rodehutscord, 2010). Deloreme and Wojcik (1982) also reported that fibre in the diets of monogastric animals impaired utilization of other nutrients, especially crude protein. The digestibility of ether extract was higher in the 40% wDDGS diet compared to other diets and the reason for this is not fully understood as many researchers like Annison (1993) have reported that water soluble, non-starch polysaccharides (NSP) occurring in wheat, rye and barley are believed to be responsible for the reduction in growth performance and the digestibility of lipids in broiler chickens fed these feedstuffs. It is possible that the more digestible nature of fibre (NDF & ADF) in the wDDGS based diet could be due to the type of fibre that is probably more of soluble fractions and perhaps the presence of bacteria in the gizzard and caecum were the reasons of higher fibre digestibility at higher levels of wDDGS. It has been reported that most feed ingredients of plant origin contain considerable amounts of fibre, with a majority being insoluble (Knudsen, 1997). Fibre was classified based on water solubility as insoluble, such as cellulose, and partly soluble fibres, such as arbinoxylans, glucans and pectins (Hetland *et al.*, 2004). Although several studies have indicated that structural components are beneficial for poultry diets when included at moderate levels, as reported by

Hetland *et al.* (2004) the mechanism by which inclusion of insoluble fibre in poultry diets improves the nutrient digestion is not clearly understood. Both TC and AIA methods of measuring digestibility revealed that the digestibility of NDF was greater ($P<0.05$) in the 40% wDDGS diet compared to 0% wDDGS but similar to 10 and 20% wDDGS diets when measured by the AIA method. The increased digestibility of NDF with 40% wDDGS inclusion could possibly be due to its low feed intake as diets with higher levels of DDGS had a lower bulk density, which may induce the feeling of fullness before meeting their energy needs as reported by Wang *et al.* (2007). There were significant differences among treatments in phosphorus digestibility where 40% DDGS presented the lowest digestibility. The phosphorus digestibility values obtained in this study are lower than those reported by Widyaratne and Zijlstra (2007), who reported that phosphorus digestibility in wDDGS was substantially higher (62%) than in wheat (15%). Meanwhile, the trend of linear decrease could be due to increasing fibre content and possibly the presence of phytase in the higher levels which might have caused the lower digestibility. The values obtained in both methods of assessing nutrients digestibility (total collection and AIA method) followed a similar trend and were close to each other in most measures. As such, both are acceptable in measuring digestibility. However, the AIA method may be a more convenient option to be used for digestibility trials in broiler chickens, since the total collection method is more laborious and time consuming.

The adverse effects of 40% wDDGS on breast with bone yield as well as no significant effect on wings are in agreement with the findings of Wang *et al.* (2007), who obtained the same result when feeding diets with 30% DDGS. The total gut and proventriculus weights were affected by the high wDDGS level (40%), as the weight of these organs of birds fed 40% wDDGS were significantly higher than other treatments. This could be attributed to the high fibre content of the diet. Sundu (2009) reported that higher dietary fibre in the diet increased gizzard contraction and expansion consequent upon which the size and weight of gizzard was increased. High fibre diets increased the activity of total gut, proventriculus, and gizzard that might have caused the higher weight of these organs at this level of wDDGS.

Total weight gain of birds was low with the 40% wDDGS diet, resulting in lower carcass weight. This also might have reduced the dressing percentage. As digestive tract components like the total gut, proventriculus, and gizzards were at higher proportion of body weight with the 40% wDDGS diet, this correspondingly reduced breast with bone percentage and thigh muscles of this group of birds. On the other hand, birds fed 10% wDDGS had a higher weight

gain and cold carcass weight, with similar edible muscles like breast with bone, thighs, drumsticks neck and back to the 0% wDDGS diet.

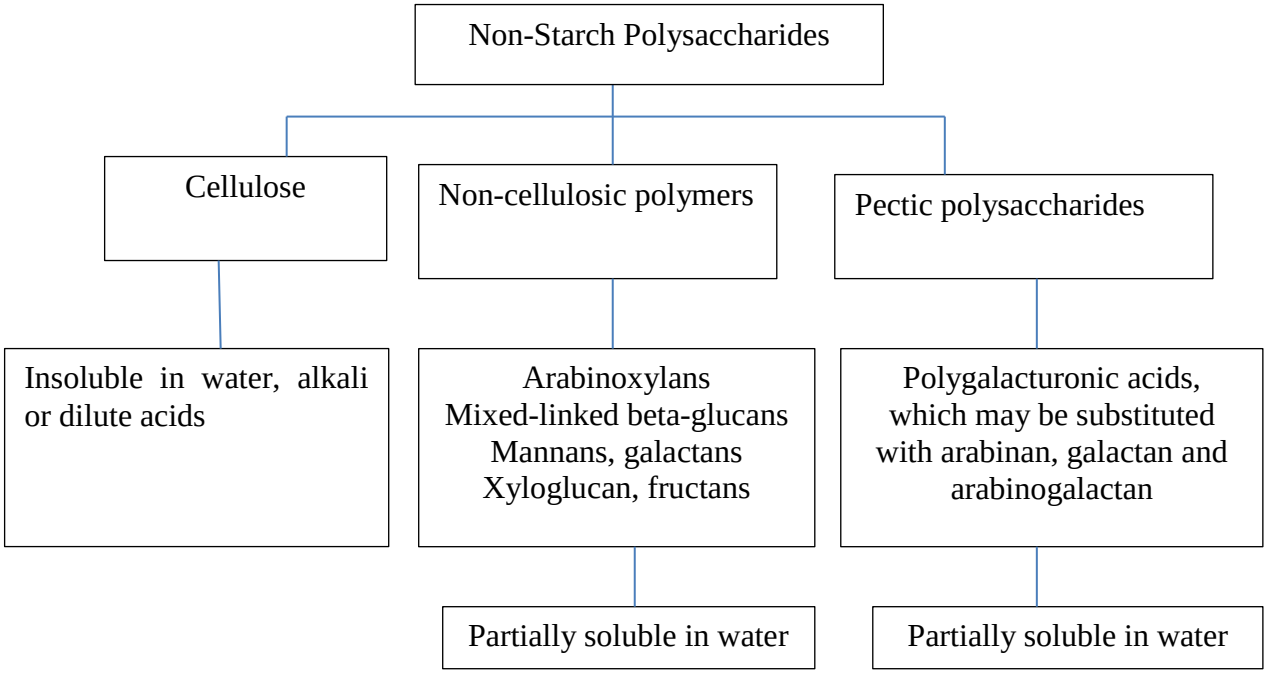
From the foregoing discussion, it was clear that inclusion of 40% wDDGS had detrimental effects on the production performance of broiler chickens. On the other hand, 10% wDDGS inclusion had in some cases a better effect, and in other cases a similar effect, to the control diet with 0% wDDGS. As farmers will gain more profit with inclusion of wDDGS in feeding broiler chickens, so, they can use up to 10% wDDGS level without any apparent risk. The optimum inclusion level of wDDGS could fall between 10-20%, so, more research is suggested over this range to test the effect of enzymes in improving the use of wDDGS in broiler diets.

CHAPTER 4:
UTILIZATION OF DISTILLER'S DRIED GRAINS WITH SOLUBLES SUPPLEMENTED WITH ENZYMES ON PERFORMANCE, DIGESTIBILITY, CARCASS CHARACTERISTICS AND BEHAVIOUR OF BROILERS

4.1 Introduction

From the results of the previous chapter, it was found that the acceptable inclusion level of wDDGS lies between 10-20%. Meanwhile, the lowest feed intake and lowest body weight gain, as well as lowest carcass yield and low organ weights, recorded with a 40 % wDDGS inclusion could be attributed to poor digestibility and poor utilization of feed by birds. Hence, the reduced nutrient digestibility calls for improvement in the utilization of wDDGS. The nature of the raw materials used in diet formulation in this study comprised mainly of cereal grains (wheat, soybean meal) and grain by-product (wDDGS) that contained considerable amounts of anti-nutrients such as non-starch polysaccharides (NSP), including arabinoxylans, and phytate. These NSPs give rise to highly viscous conditions in the small intestine of chickens; depress nutrient utilization and performance. The presence of such anti-nutrients in DDGS has contributed to limitations in its optimal use. The cereal grains provide the energy in the diet. However, the presence of cell wall polysaccharides hampers digestion of all nutrients including starch, depending on the nature of the grain and digestive capacity of the animal (Classen, 1996). The cell walls contain β-glucans and arabinoxylans which act as physical barriers to endogenous enzymes and, therefore, decrease the utilization of starch and protein enclosed within the endospermal cells (Hesselman & Aman, 1986; Khattak *et al* 2006). It has been reported that arabinoxylans can increase the viscosity of wheat- based diets (Annison & Choct, 1991) and corn-based diets (Saki *et al*., 2011), and thus interfere with digestion and absorption of the nutrients. NSP are classified into three main groups namely; cellulose, non-cellulosic polymers and pectic polysaccharides (Fig. 4.1), they are also categorized as soluble and insoluble based on solubility in water. The water soluble NSP generally are stubborn and disrupt the process of digestion by interfering with gut function (Danicke *et al*., 1999; Leeson and Summers, 2001) while insoluble NSP block the access of endogenous enzymes to their substrates by physical entrapment (Danicke *et al*., 1999). NSP are polymeric carbohydrates (Fig. 4.1) which have chemical cross linking among these. Their composition, structure and physiochemical characteristics differ largely, and for this reason,

are not well digested by poultry (Morgan *et al*., 1995; Choct, 1997; Bedford, 2000; Choct and Kocher, 2000). Poultry lack the capability to synthesize enzymes that hydrolyse NSP found in the cell wall of the grains, so they stay un-hydrolyzed in the gut and consequently led to low feed efficiency (Jorgensen *et al*., 1996; Khattak *et al*., 2006). Broiler chickens in particular show problems associated with the lack of s enzymes to digest the NSP (Saki *et al*., 2011); the soluble and viscous arabinoxylans (pentosans) are responsible for these adverse effects including increased water intake of birds resulting in wet and sticky droppings causing litter problems (Khattak *et al*., 2006).



Source: Khattak et al. (2006)

Figure 4.1 Illustration of Polymeric Structure/Linkages of NSP

The wDDGS used in the experimental diets contain a considerable amount of fibre, which traps nutrients within plant cell walls that also hold water. Manangi (2007) and Sundu *et al.* (2008), in their studies with DDGS and copra meal respectively, reported that this adds more bulk in the gastro-intestinal tract, slowing down the movement of feed throughout the gut and reducing feed intake, nutrient digestibility and performance of broiler chickens.

DDGS is known to have lower lysine availability resulting from the drying process which it undergoes during processing. This is also thought to have an adverse effect on energy and colour of the wDDGS as it is reported in previous studies that excessive heat during the drying process can cause Maillard reactions of the protein-bound lysine and available sugars and hence reduced lysine availability and the residual starch content of wDDGS (Cromwell *et al*., 1993; Hodgkinson, 2006; Cozannet *et al*., 2010). Similarly, Lan *et al*. (2008) and Nyachoti *et al*. (2005) reported that the lysine in wDDGS was poorly available. This poses a major concern in the use of wDDGS for feeding non-ruminant animals, as reported by Bandegan *et al*. (2009).

It is also worthy of note that the physico-chemical structure of starch in the cereals and the interactions between starch and other components is responsible for poor digestibility of starch in young broilers, as reported by Wiseman (2006). However, Zanella *et al*. (1999) and Jiang *et al*. (2008) suggested that exogenous amylase may improve starch digestion in young broiler chickens. Weurding *et al*. (2001) also demonstrated that exogenous enzymes improved starch digestibility greatly and resulted in improved performance of birds fed on wheat and barley-based diets.

In view of the aforementioned observations, and in order to target the anti-nutrients present in the major ingredients of the experimental diets, several previous studies have proven the use of exogenous enzymes to give a potential improvement in the utilization of wDDGS-based diets. Therefore, inclusion of NSP hydrolyzing enzymes in the experimental diets was considered. Several authors (Kocher *et al*., 2000; Montanhini *et al*., 2012) have suggested that the addition of exogenous enzymes to diets can be used as a measure to enhance the usage of NSP rich raw materials. These enzymes hydrolyse NSP from cereal ingredients and enhance the production efficiency of animals by increasing the digestion of by-products and reducing nutrient loss through the excreta (Kim *et al* 2005; Shirmohammad and Mehr, 2011).

Choct & Annison (1992) reported that exogenous xylanase can partially hydrolyse the arabinoxylans and release the enclosed nutrients for the birds to use. Consequently, birds can digest the nutrients more easily and achieve better growth performance. Xylanolytic enzymes have been shown to significantly alter the fermentation profiles in both ileum and caecum. Hence, fermentation is encouraged in the caeca and a reduction of ileal fermentation could be beneficial since it is evident that the material being fermented in this region is predominantly undigested starch and protein, which would ordinarily be available to the bird (Bedford, 1996). Bird performance was also seen to be enhanced through the use of xylanase in diets

based on lupins (Naveed *et al.*, 1999). Choct *et al.* (1999) also reported that supplementation of wheat-based diets with xylanase resulted in improvements in starch digestibility in the jejunum and ileum. Further, Meng and Slominski (2005) reported that NSP-degrading enzymes are capable of ameliorating the lower starch digestibility in higher inclusion corn diets with high NSP contents. Thus, enzymes improved starch digestibility considerably in corn-based diets. Adeola and Bedford (2004) demonstrated that the nutritional value of wheat-based diets for ducks could be improved by supplementation of the diet with exogenous xylanase. In broilers, supplementation of a diet formulated with lupins (Rubio *et al.*, 2003) with protease alone increased feed intake and final live weight of birds, whilst supplement of a soybean diet (Ghazi *et al.*, 2002) led to improved protein digestibility. Moreover, some other benefits of enzyme supplementation include reduction in the variation of nutrient quality of feed ingredients and reduction of the incidence of wet litter when feeding diets rich in viscous grains (Bedford 2000).

In the past two decades, researchers like Annison (1991), Friesen *et al.* (1992) and Steenfeldt *et al*. (1998) have shown that supplementation of diets with appropriate enzymes capable of degrading the xylan improves the nutritive value of DDGS and wheat-based diets for broiler chickens, resulting in improved performance. Nejib *et al.* (2002) also reported that exogenous enzymes supplementation to wheat-based diets can overcome the anti-nutritive effect of water soluble NSP. The efficacy of commercial enzymes on wheat, sorghum, and lupin-based poultry diets with high NSP content have been reported by (Hughes *et al.*, 2000; Selle *et al.*, 2010) where enzymes enhanced the digestibility of NSP. Selle *et al.* (2009) reported the ability of enzymes to increase digestibility of fibre and amino acids in DDGS, thereby ameliorating the negative influences on protein digestibility. Gracia *et al*. (2003) reported that α-amylase supplementation of corn-SBM diets increased the average daily gain, feed intake and apparent metabolisable energy for nitrogen (AMEn) through 42 days. The enzyme was formulated to improve energy utilization in corn-SBM and sorghum-SBM diets.

A single enzyme can be useful for enhancing the digestibility of individual nutrients e.g. NSP by the use of xylanase or starch by the use of amylase and protein by the use of protease. However, as the digestibility of one nutrient affects the digestion of the other nutrients, multi-enzyme techniques are considered to give more reliable results. Further, when an enzyme combination with multiple activities is used in a broiler diet, it is more likely to achieve greater effect than when enzymes are added individually. It has been reported that supplementation of poultry diets with an enzyme mixture including protease and amylase

produced significant improvement in growth performance in broilers (Gracia *et al*. 2003). Supplementation with exogenous enzymes such as xylanase, amylase and protease (XAP) mixtures have been shown to lower the viscosity of intestinal contents and to improve digestibility of starch, protein, fat and apparent metabolisable energy (AME) in broilers fed wheat-based diets (Annison and Choct, 1991; Bedford, 1995; Bedford and Schulze, 1998). Previous studies with corn/soybean diet have reported enhanced digestibility of nitrogen, amino acids and energy as a consequence of the use of XAP mixture (Douglas *et al.*, 2000). It has also been reported that the enzymes' affinity for soluble and insoluble fibre, the susceptibility to inhibition and its ability to bind to their substrate (Fontes *et al.*, 2004) using attachment modules, increased the efficacy of the enzyme especially associated with the hydrolysis of insoluble carbohydrate. The enzyme product containing XAP derived from species of bacteria promotes the breakdown of starch, cell walls, storage proteins and proteinaceous anti-nutritional factors (ANF), respectively. Bi Yu and Chung (2004) demonstrated that exogenous enzymes addition appeared to be beneficial for reduced-energy diets.

Furthermore, previous studies by Zanella *et al.* (1999); Yu and Chung (2004); Scheideler *et al.* (2005) have shown that an enzyme complex containing XAP improved the digestibility of starch, DM and energy as well as improving nutrient utilization, and totally compensated for the reduced energy in the diets. Hong *et al.* (2002) demonstrated that Pekin ducks fed corn-SBM diets supplemented with this enzyme experienced a 6-8% increase in BWG as well as improvements in feed efficiency over a 42 day period, compared with ducks on an unsupplemented control diet.

In this experiment, measurement of digestibility as performed in chapter 3 by the AIA method was repeated alongside the total faecal collection method in order to reassure its reproducibility.

Table 4.1 summarises the beneficial effects of various exogenous enzymes in different substrates (cereal grains) on the performance of various species of poultry, which proves the success in the use of these enzymes.

Table 4.1 Effect of supplementation of poultry diets with either xylanase/amylase/protease or in combination on the performance of broiler chickens

Diet	Enzyme	Level of Inclusion	Species	Effect/Remark	Reference
Yellow corn/SBM	Amylase & Xylanase	1.1g/kg	Male Turkeys	Villus length within jejunum & ileum increased. Amylase increased growth during 0-3 week.	Ritz *et al.* (1995)
Wheat/SBM	Xylanase	0.50g/kg	Broilers	Increased performance & reduced intestinal viscosity. Improved digestibility of total NSP, Arabinose & Xylose residues. Break down the cell wall NSP to a certain extent	Steenfeldt *et al.* (1998)
Corn/SBM/Lupin	XPC	0.2, 0.05 & 0.5g/kg	Broilers	XC improved broiler performance	Naveed *et al.* 1999
Corn/SBM	XAP	0.1% of diet	Broilers	Improved overall CP digestibility by 2.9%	Zanella *et al.* (1999)
SBM	XAP	0.10% of diet	Broilers	Improved growth & ileal digestible energy	Douglas *et al.* (2000)
Corn/SBM/Wheat midlings	XAP	0, 0.375 & 0.50g/kg	Ducks	Improved ileal amino acid digestibility & apparent amino acid retention	Hong *et al.* (2002)
Rye	Xylanase	10, 30g/kg	Broilers	Improved performance, 25% increase in the digestibility of nitrogen & a doubling of the digestibility of fat.	Silva and Smithard (2002)
Sweet lupin meal	Protease	1g/kg	Broilers	BW, FI tended to increase	Rubio *et al.* (2003)
Corn-Soy based	XAP	0, 0.075g/kg	Layers	Positive influences on energy & lysine (protein) utilization in hens impacting EP, FI & BW.	Sohail *et al.* (2003)
Corn-Soy based	α-amylase	40ppm, 1720 Units/kg diet	Broiler chickens	Improved digestibility of nutrients & performance	Gracia *et al.* (2003)
Sweet lupin seed meal	Protease	1g/kg	Broilers	BW, FI of birds tended to increase	Rubio et al. 2003

Table 4.1 Effect of supplementation of poultry diets with either xylanase/amylase/protease or in combination on the performance of broiler chickens (continued)

Diet	Enzyme	Level of Inclusion	Species	Effect/Remark	Reference
Corn/SBM	XAP	0.05% of diet	Broilers	Improved utilization of SBM by improving nutrient digestibility and reducing the losses of endogenous amino acids	Yu and Chung (2004)
Wheat	Xylanase	0.50g/kg	Broilers	BWG about 10%, FCR about 6%	Scott (2005)
Wheat	Xylanase	0, 1.5 or 3.0g/kg	Ducks	BWG about 10%, FCR about 6%	Adeola and Bedford (2004)
Corn-Soy based	XAP	0, 0.075g/kg	Layers	Significant positive effect on protein & calcium retention	Scheideler *et al.* (2005)
Corn/SBM	XAP	100, 200mg/kg	Broilers	6-7% BWG improvement	Cowieson and Adeola (2005)
Corn/Wheat	Xylanase & Protease	0.1g/kg	Broilers	Reduction in the amount of maize needed in the diet	Iyayi and Davies (2005)
Corn	XAP&Phytase	300, 400, 4000 & 500U/kg	Broilers	Maintained performance Improved digestibility of nutrients	Cowieson *et al.* (2006a) Cowieson *et al.* (2006b)
Wheat/SBM	Xylanase	0.05% of diet	Broilers	Stabilized diet containing wDDGS for broilers	Thacker & Widyaratne 2007
Wheat	Xylanase	150mg/kg	Broilers	FI & DG increased	Parsaie *et al.* (2007)
Corn/SBM/Wheat	XAP	250, 500, 750g/tonne	Turkey	Improved energy & protein ileal digestibility	Troche *et al.* (2007)
Corn/SBM	XAP	125, 500mg/kg	Broilers	BWG improved by enzyme addition	Yu *et al.* (2007)
Corn/Soybean	XAP	0.075% of diet	Layers	Positive interaction effect on egg production within strain of birds	Jalal *et al.* (2007)
Corn/Soybean	XAP	0.05, 0.0375%	Layers	FCR improved by enzyme supplementation	Novak *et al.* (2007)
Corn	XAP	300,400,4000U/kg	Broilers	BWG,AME, nitrogen retention improved & offered prospect of economic benefit	Cowieson and Ravindran (2008)

Table 4.1 Effect of supplementation of poultry diets with either xylanase/amylase/protease or in combination on the performance of broiler chickens (continued)

Diet	Enzyme	Level of Inclusion	Species	Effect/Remark	Reference
Corn/SBM	XAP	650,1650&4000U/kg	Broilers	Enhanced phosphorus digestibility & chicks benefitted more from enzyme addition at a younger age	Olukosi *et al.* (2007)
Yellow corn/SBM	XAP	180, 360, 720mg/kg	Broilers	180, 360mg inclusions supported better performance of birds	Yuan *et al.* (2008)
Wheat	Xylanase	2000XU/kg	Broilers	Xylanase in combination with phytase was beneficial to nutrient utilization & growth performance	Selle *et al.* (2009)
Wheat/Triticale/DDGS	XAP, Glucanase & Invertase	0.05% of diet	Broilers	Increased the nutritive value of triticale DDGS for broilers	Oryschak *et al*. 2010
Corn	Xylanase	0, 1200, 2400, 3600U/kg of diet	Broilers	Xylanase increased digestibility of DM & hemicellulose by 5%	Liu *et al.* (2011)
Corn/Canola	Protease	0, 200g/tonne	Broilers	Enhanced amino acids & energetic value of the diets	Fernández-Tinoco *et al.* (2012)
Corn/SBM	Xylanase	0, 16000U/kg	Broilers	Increased serum peptide concentration & serum insulin levels	Singh *et al.* (2012)
Wheat/SBM	XP	200g/kg	Broilers	Xylanase and protease improved feed conversion	Kalmendal and Tauson (2012)
Corn/SBM/SDDGS	Xylanase	0, 0.25g/kg	Broilers	FCR improved with higher inclusion of SDDGS (300g/kg)	Barekatain *et al.* (2013)

AME= Apparent Metabolisable Energy, BW= Body Weight, BWG= Body Weight Gain, DG= Daily Gain, EP= Egg Production, FCR= Feed Conversion Ratio, FI= Feed Intake, NSP= Non-Starch Polysaccharide, XAP= Xylanase, Amylase and Protease

Researchers like Zhou *et al.* (2012) have reported that dietary manipulations of poultry diets have been extensively studied with a view to improve the quality of poultry meat. Similarly, several studies have been conducted to evaluate fatty acids in various animal products but none of these researches has been concerned with broiler breast meat. Further, breast meat being the most popular segment of poultry meat, its lipids are known to contain considerable amounts of essential fatty acids which are of paramount interest for consumer health implications. Hence, it is worthy to investigate if high fibre wDDGS based diets could influence the concentration of the fatty acids, especially n-3 PUFA levels, in edible broiler breast meat. Therefore, in the present study, 3 inclusion levels of wDDGS i.e. 0, 15, and 30%, as intermediaries of levels tested in the previous experiment were compared with and without an enzyme mixture of XAP incorporated at 0, 0.25g/kg, with the following two objectives:

(1) To determine the effects of feeding diets containing wDDGS supplemented with enzymes on performance, behaviour, digestibility, carcass characteristics and chemical and fatty acid compositions of muscles, of broiler chickens.

(2) To determine the interaction between wDDGS levels and enzyme supplementation.

It is hypothesised that the utilization of agricultural by products such as wDDGS can be improved if optimally incorporated and supplemented with fibrolytic enzymes in broiler chickens' diet, with benefits for performance, carcass yield and behaviour of broiler chickens.

4.2 Materials and Methods

4.2.1 Study site and ethical guidelines

The study was conducted at Cockle Park farm, Newcastle University, between June and August 2012 under the ethical guidelines for non-regulated procedure of Newcastle University.

4.2.2 Experimental enzymes, wDDGS and Diets

4.2.2.1 Description and composition of enzyme used

The enzyme used for the study was donated by Danisco Animal Nutrition, UK. The enzyme was a mixture of xylanase, amylase and protease, marketed to increase the efficiency of wheat-based broiler, turkey and duck feeds. It is specifically developed for use in mixed-grain poultry diets, containing vegetable and other protein sources alongside high fibre by-products. The enzymes were derived from *Bacillus subtilis* and *Trichoderma reesei* fermentation processes. The enzyme mixtures contained wheat flour and calcium propionate as carriers and binders. Each g of enzyme was claimed to contain 9200 U of endo-1, 4-beta-xylanase, 16000 U subtilisin as protease and 1600 U of alpha – amylase.

4.2.2.2 Source and composition of wDDGS

The wDDGS used in this experiment were from the same batch as the product used in experiment 1. Table 4.1 presents the nutrient composition of wDDGS used in this study while the wheat and soybean meal used in this experiment were obtained from North East Grains Ltd (Grain Merchants), Longhirst, Morpeth, Northumberland, UK.

Table 4.2 Nutrient composition of wDDGS

Nutrient	g/kg DM (unless otherwise stated)
Dry Matter	913
Ash	49
Crude Protein	311
Ether Extract	49
Neutral Detergent Fibre	469
Acid Detergent Fibre	145
Calcium	2
Phosphorus	9
ME (MJ/kg)	13.7

4.2.2.3 Experimental diets and design

Six isonitrogenous diets, with metabolisable energy and fibre content allowed to vary, were formulated for the study by substituting wDDGS for coarsely ground wheat and soybean meal. The inclusion of wDDGS and enzymes in the experimental diets is summarized in Table 4.2. Enzyme addition was done by initially mixing its required amount in a bucket with about 2 kg of coarsely ground wheat which was then remixed alongside other ingredients and the premix where applicable.

A completely randomized design with a 3 X 2 factorial arrangement using 3 wDDGS levels of 0, 15, 30% and 2 enzyme levels of -E, +E was used.

Table 4.3 Description of diets containing different amounts of wDDGS and enzyme

Diet	wDDGS inclusion (%)	Enzyme inclusion
A	0	No
B	0	Yes
C	15	No
D	15	Yes
E	30	No
F	30	Yes

Table 4.4 Ingredient composition (kg/tonne) and calculated analysis of the experimental broiler diets

	Diets (wDDGS %)					
	0		15		30	
Ingredients	**No Enzyme**	**Enzyme**	**No Enzyme**	**Enzyme**	**No Enzyme**	**Enzyme**
Diet ID	**A**	**B**	**C**	**D**	**E**	**F**
wDDGS	0	0	150	150	300	300
Wheat	649	648.8	575	574	498	497.8
Soybean meal	294	294	218	218.8	143	143
Soybean oil	10	10	10	10	12	12
Limestone	20	20	20	20	20	20
MCP	10	10	10	10	10	10
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Premix*	12.5	12.5	12.5	12.5	12.5	12.5
Methionine	1	1	1	1	1	1
Lysine	1	1	1	1	1	1
Enzyme	0	0.25	0	0.25	0	0.25
Total	1000	1000	1000	1000	1000	1000
Calculated nutrient composition (g/kg) (unless otherwise stated)						
Crude Protein	225	225	223	223	221	221
ME (MJ/kg)	13.7	13.7	13.3	13.3	13.0	13.0
Starch	380.6	380.6	339.4	338.8	296.4	296.4
Crude Fibre	34	34	76	76	118	118
Ether Extract	75	75	67	67	61	61
Calcium	11	11	10	10	10	10
Phosphorus	5	5	6	6	7	7
Amino Acids						
Arginine	14.7	14.7	13.6	13.6	12.5	12.5
Histidine	6.1	6.1	5.9	6.0	5.8	5.8
Isoleusine	9.7	9.7	9.4	9.4	9.2	9.2
Leusine	17.3	17.3	17.1	17.2	17.1	17.1
Lysine	11.5	11.5	10.0	10.0	8.4	8.4
Methionine	4.4	4.4	4.3	4.3	4.1	4.1
Phenylanaline	11.6	11.6	11.6	11.6	11.5	11.5
Threonine	8.2	8.2	8.1	8.1	7.9	7.9
Tryptophan	3.1	3.1	2.9	2.9	2.7	2.7
Valine	10.7	10.7	10.8	10.8	10.8	10.8
Total AA	97.3	97.3	93.7	93.9	90	90

ME = Metabolisable Energy, MCP= Monocalcium Phosphate, AA = Amino Acids,
**Premix of 12.5kg contained Vitamin A12,500iu,Vitamin D3 3,000iu,Vitamin E-150g,Vitamin K 3g,Vitamin B1 2g,Vitamin B2 8g, Vitamin B6 5g, Vitamin B12 30mg,Nicotinamide50g,D-cal Pan 10g,Folic Acid 2g,Biotin 150mg,Vitamin C 4g, Choline 300g, Copper (Copper Sulphate pentahydrate) 10g, Manganese (Manganese Sulphate monohydrate) 80g, Zinc (Zinc Oxide) 60g, Iron (Ferrous Sulphate monohydrate) 30g, Iodine (Calcium Iodate) 2g, Selenium (Sodium Selenite 0.25mg, Selenium rich yeast 0.08mg),Molybdenum (Sodium molybdate) 0.20mg, Calcium (30%),Sodium Chloride (8%)*

4.2.3 Experimental birds and management

One hundred and sixty eight day-old Ross broiler chicks, vaccinated at day old at the hatchery against Infectious Bronchitis, were used for the experiment which lasted for 6 weeks (day 5 to day 46 of the birds' age). After arrival at the experimental site, the chicks were fed a commercial starter diet as crumbs for 4 days before initial weighing and allocation to the experimental diets. The crumbs were purchased from ABN Peterborough, UK and supplied crude protein 18%, crude fibre 5.3%, crude fat 4.0%, crude ash 5.3%, calcium 0.9%, phosphorus 0.6%, sodium 0.14% lysine 0.90%, and methionine 0.29%. After the 4-day adaptation period, on day 5, the chicks were individually weighed, wing tagged and assigned to 6 groups (treatments) of 28 chicks each. Each group was further divided into 4 sub-groups (replicates) of 7 birds per replicate. Birds were managed the same way in the same house and facilities used in experiment one. The diets and clean drinking water were provided *ad libitum.* Room light was provided for 16 hours daily and hanging lamps were used to provide heat in each pen throughout the experiment.

4.2.4 Data collection and measurements

Different datasets were collected as previously described in chapter 3 section 3.2.5. However, brief descriptions of various measurements for broilers are summarised in the following sections:

4.2.4.1 Feed intake and growth

Feed intake, weight gain feed conversion ratio (FCR) and faecal moisture of broilers were measured as described in chapter 3.

4.2.4.2 Behaviour records

Bird behaviours were video recorded as described in chapter 3 section 3.2.7.1. Video recordings of bird behaviours in this experiment were performed at only the beginning (day 5-12), middle (day19-26) and last part (day 39-46) of the experiment using the same mounted video cameras to film six sets (sets 1, 2, 3, 4, 5 & 6) of pens on consecutive days. Table 4.4 shows the treatment and replicate combinations of bird pens that were observed per camera set. The video recording commenced with the first set on the fourth day of the trial through the fifth day and stopped at 10:00am when the

recordings for the second set were started followed by the recordings for sets 3, 4, 5 & 6 in the subsequent days of the seven days.

Table 4.5 List of treatment & replicate bird combinations that were observed simultaneously per camera sets

	Camera numbers per set to observe birds in different pens			
Set	1	2	3	4
1	B1	C1	D1	E1
2	A1	B2	D2	F1
3	A2	C2	E2	F2
4	A3	B3	C3	F3
5	B4	D3	E3	E4
6	A4	C4	D4	F4

Letters and figures denotes treatment and replicate combination per camera

4.2.4.3 Digestibility measurements

At the end of the feeding trial, two birds per pen were randomly selected and transferred to metabolism cages for their total excreta collection in order to determine the apparent nutrient digestibility as described in chapter 3.2.9. The nutrient digestibility values of wDDGS based diets were also determined by using the AIA method according to procedures described by Van Keulen and Young (1977) as described in chapter 3 section 3.2.11.

4.2.4.4 Carcass measurements

At the end of the feeding trial, eight birds per treatment (i.e. 2 birds per pen) were randomly selected based on similar LW for carcass studies. The birds were killed by cervical dislocation and processed as described in chapter 3 section 3.2.10, in this case, to monitor the effect of enzyme on the organ weights. The cold carcass weight expressed includes the weights of internal organs. Samples of muscle tissues from the breast, thighs and drumsticks were collected for chemical analyses. The samples collected were stored in a freezer at -20^{o}C until analysis.

4.2.5 Chemical analysis

Feed samples of each of the 6 experimental diets as well as faecal samples and samples of breast, thigh and drumstick muscles were analysed for proximate composition according to AOAC (2005) as well as the fatty acid composition of ether extract from

breast muscles. The dry matter, ash, crude protein, NDF, ADF, ether extract, as well as calcium and phosphorus contents of both feed and excreta were determined as described in chapter 3 section 3.2.12.

Chemical analysis of breast, thigh and drumstick muscles without skin was carried out in the same way as the feed and faecal samples were analysed. Following evisceration, the same procedure was followed as described in chapter 3 section 3.2.10 to collect internal organs and cut parts of carcass. Thereafter, about 10g was cut from the dorsal position of breast, thigh and drumsticks as samples and stored at -20^{o}C until freeze drying and subsequent chemical and fatty acid analyses. Five grams of each skinless muscle sample were weighed and then freeze dried in a freeze dryer (LYOLAB G, Lyophilization Systems Inc. USA). The freeze drying process involved placing the collected samples in clean labelled plastic bags. These were placed in the shelves of the sample chamber of a freeze dryer at -25^{o}C, with the condenser chamber at -54^{o}C and the pressure at 0.04 – 0.05mbar, before the vacuum was applied and the samples were considered dried on average after 4-5 days. The samples were thereafter removed and reweighed to determine their moisture and dry matter contents by difference. The meat samples were homogenised in an electric food processor (Kenwood 8D16). The organic matter and ash content of the muscle samples were determined by burning the organic components in a furnace (Carbolite AAF 1100) at 550^{o}C overnight.

The Nitrogen content of muscle samples was determined by using the Dumas method in Cube (Elementar vario macro cube N mode AN-A-020609-E-01) and ether extract content also was determined as described in chapter three.

4.2.5.1 Determination of fatty acids composition of broiler breast muscles

Fatty acid composition of ether extract from broiler breast muscles was determined according to a modified version of the method described by Sukhija and Palmquist (1988). The chemicals and the procedure that were used for the fatty acid analysis are briefly described in the following sections:

4.2.5.2 Chemicals

All chemicals were purchased from either Fisher Scientific Ltd or Sigma-Aldrich Ltd, UK.

Methanol: toluene (4:1 v/v) solution was prepared by mixing 400 ml methanol ≥ 99.8 %, and 100 ml toluene puriss (≥ 99.5% purity, in a volumetric flask before transferring

the solution into a screw capped glass Duran bottle. Potassium chloride (5% w/v) was prepared by dissolving 25g potassium chloride (KCL; > 99%) in distilled water in a 500ml volumetric flask. Acetyl chloride (>99%) was also used when it was needed

4.2.5.3 Ether extraction and gas chromatography procedure

The ether extracts collected from the representative samples of breast meat of birds from each replicate were esterified before their fatty acid analysis by using a Gas Chromatograph (Shimadzu, GC – 2014, Japan) equipped with a Varian CP – SIL 88 fused silica capillary column (100m x 0.25mm ID x 0.2um film thickness). Individual peaks were identified by comparing sample peaks with the standard peaks from known 52 FAME standards. Each 1ml of ether extract was pipetted into a 10 ml reaction vial to which 1 mg of heptadecanoic acid methyl ester (C17) was added as an internal standard. Residual air in the vials was expelled in a stream of nitrogen; the vials were closed tightly with Teflon-lined screw caps and heated at 100^{o}C for one hour in a heating block (TECHNE DRI-BLOCK DB.3D). The tubes were then removed from the block and cooled in room temperature for 30 minutes. After that, 5ml of 5% KCl were mixed and centrifuged at 1000xG for 5 minutes at 6^{o}C. 0.25ml of acetyl chloride was added to the tubes slowly (drop by drop) in the fume cupboard and vortexed slightly by using a 250ul pipette and different tip for each tube. The vials were then cooled to collect the supernatant of which 400ul were transferred into the screw-cap brown GC vials which were stored at -20^{o}C until the required volume was injected into the gas chromatograph. Helium gas was used as a carrier with a head pressure of about 109.9 kPa and a column flow of 0.31 ml/minute. The injector and detector temperature were maintained at 250^{o}C and 275^{o}C respectively while 1µl sample was injected when the initial column temperature was at 50^{o}C and held for 1 minute. It was then raised at 2^{o}C/minute to 188^{o}C which was held for 10 minutes. The temperature was similarly increased to 240^{o}C and held for about 45 minutes to yield a final gradient with the total runtime of 2.5 hours. Gas chromatographic analyses were carried out where fatty acids were quantified by comparing their peaks with the relevant peak areas of the corresponding standard fatty acids. Each fatty acid was then expressed as a percentage of the total fatty acids quantified. Categories of fatty acids (Saturated or SFA, monounsaturated or MUFA and polyunsaturated or PUFA) were also determined by summation of respective fatty acids that have no double bonds as SFA and those that have 1 double bond as MUFA while those that have more than 1 double bond as PUFA.

4.2.6 Statistical analysis

Collected performance and carcass data were statistically analysed by using general linear model (GLM) in the Minitab 16 statistical package, to study the effects of wDDGS level, enzyme inclusion and their interaction on the above mentioned measurements. Normal distribution of the data was determined by analysis of the residuals using the Anderson Darling test. As some of the data for fatty acids were not normally distributed, a log transformation was carried out before the statistical analysis was performed on this data set. Tukey's post-hoc test was used if there were more than two means to compare for significance at $P<0.05$. The behaviour data were analysed non-parametrically in a similar way as described in chapter 3 section 3.2.13. A fitted line plot regression analysis was performed to assess the relationship between the dry matter digestibility and wDDGS levels.

4.3 Results

4.3.1 Amino acids composition of the experimental diets

The calculated AA concentration of the experimental diets is shown in Table 4.4. It appeared that concentration of amino acids decreased with increasing wDDGS inclusion. While the concentration of histidine, methionine, phenylalanine, threonine, tryptophan and valine was identical, the relative concentration of arginine and lysine varied and decreased with increasing wDDGS levels. The lysine content of the diets was reduced from 12 to 8g. The methionine concentration was same in all the diets. The 30% wDDGS diet contained the lowest lysine than the control and 15% wDDGS.

4.3.2 Growth performance of broiler chickens

The main effects of wDDGS and enzyme inclusion on performance of broiler chickens in the starter phase (1-3 weeks of age) are shown in Table 4.6. There were significant differences in feed intake among different wDDGS levels where the highest daily feed intake was observed in the control diet containing 0% wDDGS, while the lowest was found in the 30% wDDGS diet. However, the feed intakes observed with and without enzyme inclusion in this study were statistically similar. There were significant

differences in weight gain between wDDGS levels, but no effect of enzyme inclusion. The FCR was not influenced by the wDDGS levels or enzyme inclusion.

Table 4.6 Means for the main effects of wDDGS and enzyme on performance of broilers fed the experimental diets during the starter phase (5-26 days of age)

	DIETS								
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	No Enzyme	Enzyme	SEM	P value
Parameters									
FI (g/b/d)	62[a]	56[a]	35[b]	2.2	0.001	51	51	1.8	0.920
LWG (g/b/d)	63[a]	63[a]	36[b]	1.6	0.001	55	52	1.3	0.228
FCR	1.00	0.90	0.99	0.041	0.232	0.95	0.97	0.033	0.743

a,b,c Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means FI= Feed Intake, WG= Weight Gain, FCR= Feed Conversion Ratio

Table 4.7 shows the interaction of wDDGS levels and enzyme inclusion on performance of broilers in the starter phase. There was no significant interaction of wDDGS levels and enzyme inclusion for feed intake. However, there was significant interaction in weight gain of broilers where birds on 15% wDDGS with no enzyme gained more weight relative to this treatment when enzymes were included. There was no significant interaction of wDDGS levels and enzyme inclusion in FCR. At 30% wDDGS, feed intake and weight gain were lower in both the presence and absence of enzyme that resulted in a similar FCR to that of the other treatments.

Table 4.7 Interaction effect of wDDGS levels x enzyme inclusion on performance of broilers fed the experimental diets in the starter phase (5-26 days of age)

	Enzyme Inclusion							
	No Enzyme			+Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Parameters								
FI (g/b/d)	61	58	34	63	53	36	2.2	0.517
LWG (g/b/d)	60[ab]	70[a]	34[c]	65[a]	55[b]	37[c]	2.3	0.001
FCR	1.02	0.83	1.01	0.97	0.97	0.96	0.058	0.163

a,b,c Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means FI= Feed Intake, WG= Weight Gain, FCR= Feed Conversion Ratio

In the finisher phase, the main effects (Table 4.8) show that there were significant effects of wDDGS levels on growth performance (feed consumption, live weight gain and FCR) with a lower feed consumption by birds on 30% wDDGS, as in the starter phase. Birds on 0% wDDGS (control group) consumed more feed, gained higher weight and had better FCR than birds fed 30% wDDGS.

Table 4.8 Means for the main effects of wDDGS levels and enzyme inclusion on performance of broilers fed the experimental diets in the finisher phase (27-46 days of age)

DIETS									
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Parameters									
FI (g/b/d)	162^{a}	182^{a}	116^{b}	7.6	0.001	151	155	6.2	0.650
LWG (g/b/d)	82^{a}	80^{a}	43^{b}	3.1	0.001	70	67	2.5	0.432
FCR	1.97^{b}	2.33^{ab}	2.73^{a}	0.139	0.005	2.25	2.43	0.114	0.257

a, b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E = Enzyme, FI= Feed Intake, LWG= Live Weight Gain, FCR= Feed Conversion Ratio

Table 4.9 presents the effect of interaction of wDDGS levels x enzyme inclusion on growth performance of broilers in the finisher phase. Inclusion of 30% wDDGS significantly reduced the live weight gain of birds compared to 0 and 15% wDDGS when averaged over both levels of enzyme inclusion, as well as with or without enzyme. There was no significant interaction on average between enzyme addition and wDDGS level.

Table 4.9 Interaction effect of wDDGS levels x enzyme inclusion on performance of broilers fed the experimental diets in the finisher phase (27-46 days of age)

Enzyme Inclusion								
No Enzyme			+Enzyme					
wDDGS (%)								
	0	15	30	0	15	30	SEM	P value
Parameters								
FI (g/b/d)	152	193	109	172	170	123	10.8	0.127
LWG (g/b/d)	78^{a}	88^{a}	43^{b}	86^{a}	71^{a}	43^{b}	4.4	0.028
FCR	1.94	2.21	2.59	2.00	2.44	2.86	0.197	0.855

a, b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, FI= Feed Intake, LWG=Live Weight Gain, FCR= Feed Conversion Ratio

When considering the main effects of wDDGS level and enzyme inclusion on the overall growth performance of broilers (Table 4.10), a lower feed intake and growth rate, and poorer FCR was observed with the 30% wDDGS diet. However, there was no significant effect of enzyme inclusion on any of the parameters measured.

Table 4.10 Means for the main effects of wDDGS levels and enzyme inclusion on the overall growth performance of broilers fed the experimental diets (5-46 days of age)

	DIETS								
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Parameters									
FI (g/b/d)	112[a]	119[a]	76[b]	4.5	0.001	101	103	3.7	0.718
LWG (g/b/d)	73[a]	71[a]	39[b]	2.1	0.001	62	60	1.7	0.294
FCR	1.54[b]	1.69[b]	1.93[a]	0.078	0.001	1.67	1.77	0.064	0.060

a,b Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means, NE = No Enzyme, E = Enzyme FI= Feed Intake, LWG=Live Weight Gain, FCR= Feed Conversion Ratio

The interaction of wDDGS x enzyme inclusion (Table 4.11) was not significant for feed consumption or feed conversion ratio. As expected from results from the starter and finisher phase, there was a significant interaction (P<0.05) for live weight gain. Without enzyme, birds on the 15% wDDGS diet gained relatively more weight than those receiving this diet with added enzyme. Figure 4.1 shows the mean live weight of birds by days, showing the reduced gain on the 30% diet.

Table 4.11 Interaction effects of wDDGS levels x enzyme inclusion on the overall growth performance of broilers fed the experimental diets (5-46 days of age)

	Enzyme Inclusion							
	No Enzyme			+Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Parameters								
Feed Intake(g/b/d)	106	125	72	118	112	79	6.4	0.135
LiveWeight Gain(g/b/d)	69^{ab}	79^{a}	38^{c}	76^{ab}	63^{b}	40^{c}	2.9	0.003
Feed Conversion Ratio	1.53	1.59	1.88	1.55	1.79	1.98	0.111	0.390

a,b,c Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means

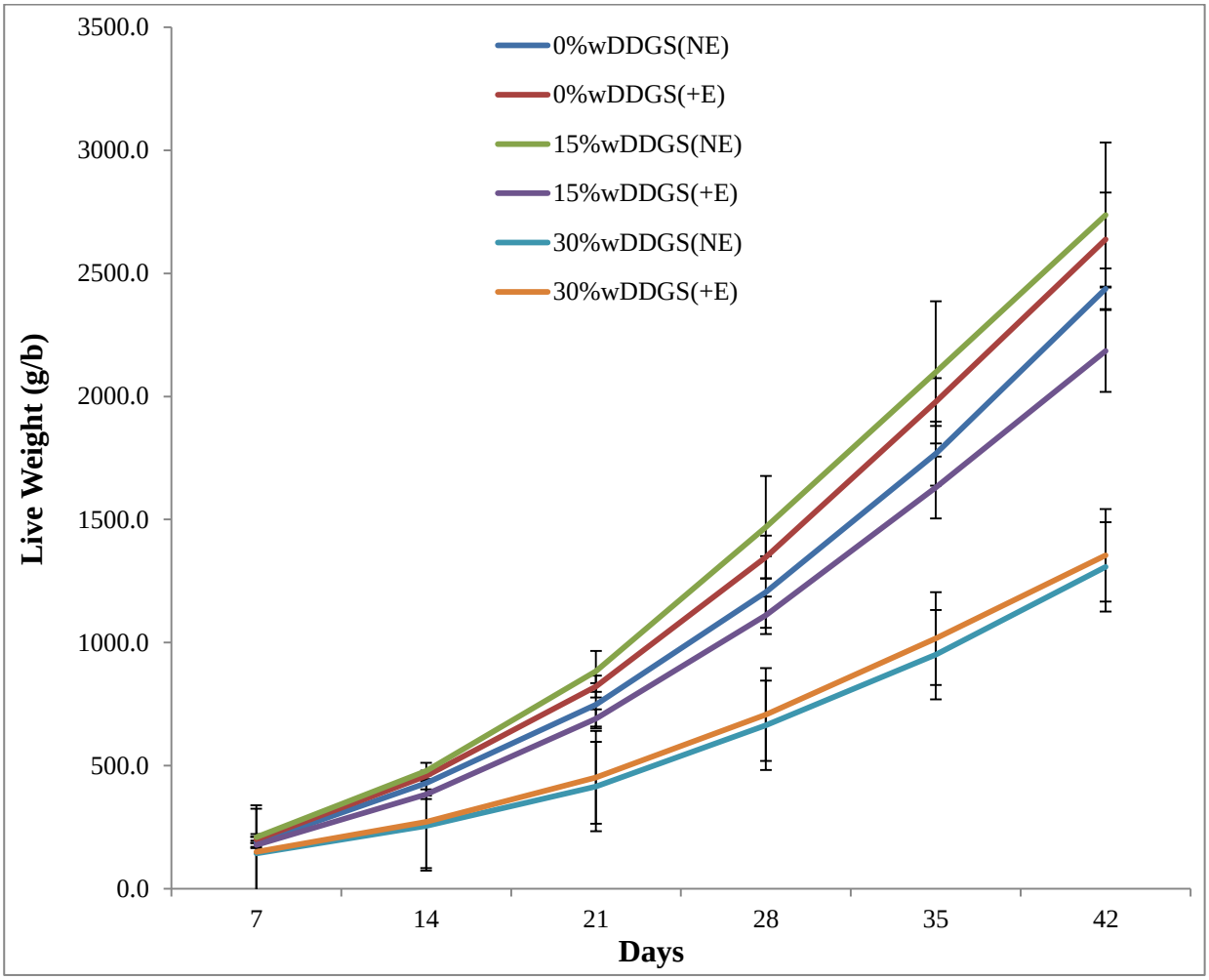


Figure 4.2 Mean birds' live weight increase with age

Inclusion of 30% wDDGS (Table 4.12) significantly (P<0.001) reduced the individual bird's live weight and weight gain in all the 42 days. Bird's LW and LWG were not affected by enzyme addition at all.

Table 4.12 Means for the main effects of wDDGS and enzyme inclusion on bird live weight (g) and weight gain (g /bird/7days)

	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Days									
Live weight									
7	193[a]	193[a]	147[b]	3.9	0.001	180	175	3.2	0.312
14	444[a]	430[a]	263[b]	10.5	0.001	387	371	8.5	0.177
21	785[a]	786[a]	434[b]	20.4	0.001	680	655	16.7	0.232
28	1277[a]	1290[a]	686[b]	34.3	0.001	1109	1055	28.0	0.139
35	1874[a]	1865[a]	984[b]	47.8	0.001	1601	1535	38.9	0.202
42	2537[a]	2462[a]	1331[b]	60.6	0.001	2148	2049	49.3	0.121
Weight gain									
7	89[a]	87[a]	48[b]	3.0	0.001	77	72	2.4	0.149
14	250[a]	238[a]	117[b]	7.3	0.001	207	196	5.9	0.163
21	341[a]	356[a]	170[b]	11.2	0.001	293	283	9.2	0.391
28	491[a]	504[a]	252[b]	16.1	0.001	429	401	13.1	0.101
35	587[a]	576[a]	298[b]	16.7	0.001	486	483	13.7	0.818
42	647[a]	597[a]	347[b]	19.0	0.001	540	514	15.5	0.192

a,b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE= No Enzyme, E= Enzyme

There were highly significant (P<0.001) interaction effects of wDDGS and enzyme (Table 4.13) on bird live weight and weight gain in all the 42 days except for weight gain at day 42 (P>0.05). Birds on 30% wDDGS with NE had similar LW with those on 30E throughout the 42 days. Enzyme addition significantly reduced the LW of birds on 15% wDDGS compared with NE in all the days. The weight gain of birds followed the same pattern as LW except at 42 days which was similar among treatments.

Table 4.13 Interaction effects of wDDGS x enzyme inclusion on bird live weight (g /bird) and weight gain (g /bird /7days)

	Enzyme Inclusion							
	No Enzyme			+ Enzyme				
	wDDGS (%)							
	0NE	15NE	30NE	0E	15E	30E	SEM	P value
Days								
Live weight								
7	188^{ab}	208^{a}	144^{c}	199^{ab}	177^{b}	150^{c}	5.5	0.001
14	429^{ab}	479^{a}	255^{c}	458^{a}	383^{b}	272^{c}	14.8	0.001
21	748^{bc}	885^{a}	415^{d}	822^{ab}	690^{c}	452^{d}	28.6	0.001
28	1204^{bc}	1475^{a}	664^{d}	1347^{ab}	1111^{c}	708^{d}	48.1	0.001
35	1772^{bc}	2110^{a}	951^{d}	1973^{ab}	1630^{c}	1017^{d}	66.9	0.001
42	2439^{ab}	2751^{a}	1307^{c}	2628^{a}	2185^{b}	1354^{c}	84.9	0.001
Weight gain								
7	86^{ab}	99^{a}	47^{c}	93^{a}	76^{b}	48^{c}	4.2	0.001
14	241^{ab}	271^{a}	111^{c}	259^{a}	206^{b}	122^{c}	10.3	0.001
21	317^{b}	407^{a}	160^{c}	364^{ab}	307^{b}	180^{c}	15.8	0.001
28	456^{bc}	589^{a}	249^{d}	526^{ab}	421^{c}	255^{d}	22.6	0.001
35	545^{ab}	635^{a}	287^{c}	626^{a}	519^{b}	309^{c}	23.5	0.001
42	638	641	356	656	555	338	26.7	0.155

a,b,c,d Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE= No Enzyme, E= Enzyme

4.3.3 Behavioural responses of broiler chickens fed different levels of wDDGS supplemented with enzyme

Table 4.14 presents the medians of observations of different behaviours exhibited by broiler chickens in different days of the experiment. Feeding, walking, exploration, and resting behaviours were significantly ($P<0.05$) affected by age. More birds were engaged in feeding and walking behaviours during the middle (days 14-21) of the experiment. Resting behaviour increased with increasing age as more birds were engaged in resting at the latter part (days 35-42) of the experiment while exploration behaviour was decreased as birds grew older. Comfort behaviours (preening and dust bathing) as well as agonistic behaviours (aggression and pecking) were infrequently performed and were not affected by age.

Table 4.14 The medians of observations of different behaviours shown by birds in different periods of the experiment

Days	Feeding	Drinking	Walking	Exploration	Standing	Resting	Aggression	Preening	Dust bathing	Pecking
1-7	33^{b}	19	10^{b}	43^{a}	14	36^{b}	0	0	0	0
14-21	43^{a}	17	14^{a}	43^{a}	14	57^{a}	14	14	14	24
35-42	33^{b}	20	11^{ab}	22^{b}	13	60^{a}	50	14	14	20
P value	0.001	0.067	0.016	0.001	0.243	0.001	0.317	0.828	1.000	0.607

Medians within columns that do not share a letter are significantly different

Table 4.15 presents the medians of observations of different behaviours shown by broiler chickens in different treatments of the experiment. Significant differences were found in drinking, exploration and resting behaviours among treatments in this experiment. Birds on 15% wDDGS with enzyme performed more drinking behaviour than birds on other treatments, while fewer birds on the 30% wDDGS diets engaged in drinking behaviour. Birds on 0% wDDGS without enzyme performed least exploration compared to those on other treatments. On the contrary, they performed more resting behaviour along side birds on 0% wDDGS with no enzyme followed by those on 30% wDDGS with and without enzymes but significantly different from birds on 15% wDDGS with enzyme. There were no significant effects of wDDGS and enzyme

inclusion on feeding, walking, standing, aggression, preening, dust bathing as well as pecking behaviours.

Table 4.15 The medians of observations of different behaviours shown by birds in different treatments of the experiment

wDDGS (%)/Enzyme Inclusion	Feeding	Drinking	Walking	Exploration	Standing	Resting	Aggression	Preening	Dust bathing	Pecking
0NE	33	22[b]	14	29[c]	14	55[a]	0	15	12	9
0E	39	22[b]	14	43[a]	8	57[a]	0	14	8	20
15NE	43	15[c]	14	43[a]	14	50[b]	14	13	14	0
15E	38	25[a]	14	40[b]	14	42[c]	0	21	17	35
30NE	33	14[c]	14	43[a]	14	50[b]	50	50	24	25
30E	43	14[c]	12	43[a]	14	50[b]	0	12	15	31
P value	0.382	0.013	0.640	0.001	0.754	0.001	0.317	0.352	0.711	0.621

Medians within columns that do not share a letter are significantly different, NE= No Enzyme, E= Enzyme

Figure 4.2 shows that feeding behaviour of birds in all the treatments followed a similar pattern as almost the same percentage of birds were feeding in the first seven days and feeding time decreased between days 14-21 and 35-42. The exploration behaviour (Figure 4.3) followed a similar trend as in feeding but was higher at days 1-7 and continued to decrease through days 14-21 and 35-42. The resting behaviour (Figure 4.4) was rather inconsistent in this experiment as birds in some treatments increased their resting behaviour while this behaviour decreased in other treatments. Birds fed 0% wDDGS without enzyme performed less resting behaviour at days 1-7 compared to other treatments but gradually increased through days 14-21 and 35-42. Similarly, birds on 15% wDDGS with enzyme followed the same trend as they increased resting through to days 35-42. Conversely, birds on 0% wDDGS with enzyme decreased their resting behaviour at day 21 and later increased through 35-42 days. Furthermore, birds fed 15% wDDGS without enzyme and those fed 30% wDDGS with enzyme as well as those on 30% wDDGS with no enzyme slightly decreased the resting behaviour. The walking behaviour (Figure 4.5) also did not follow any consistent pattern.

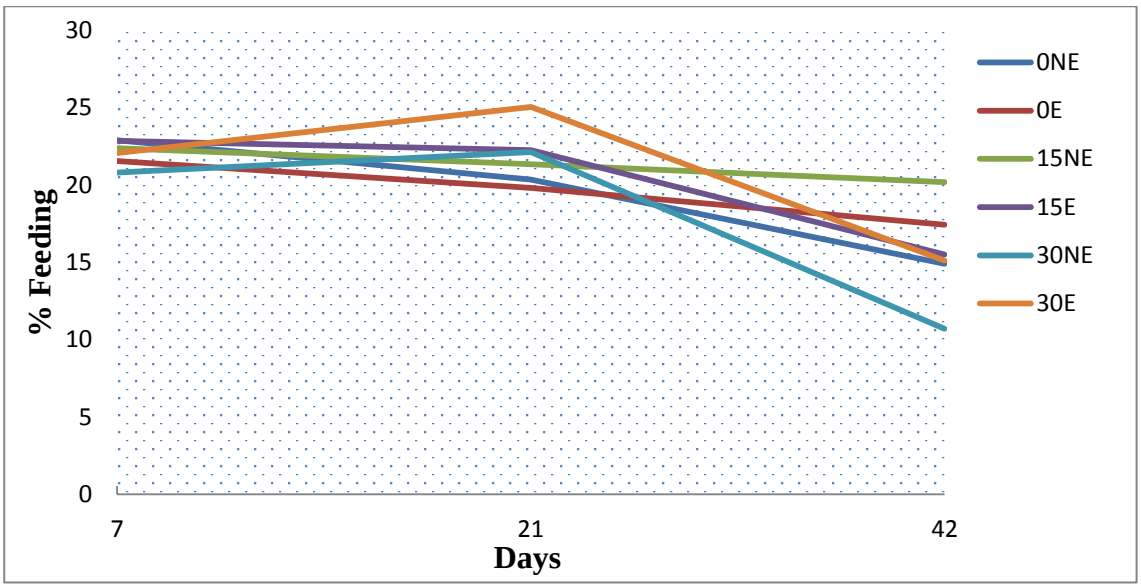


Figure 4.3 Feeding behaviour of broiler chickens fed wDDGS and enzyme

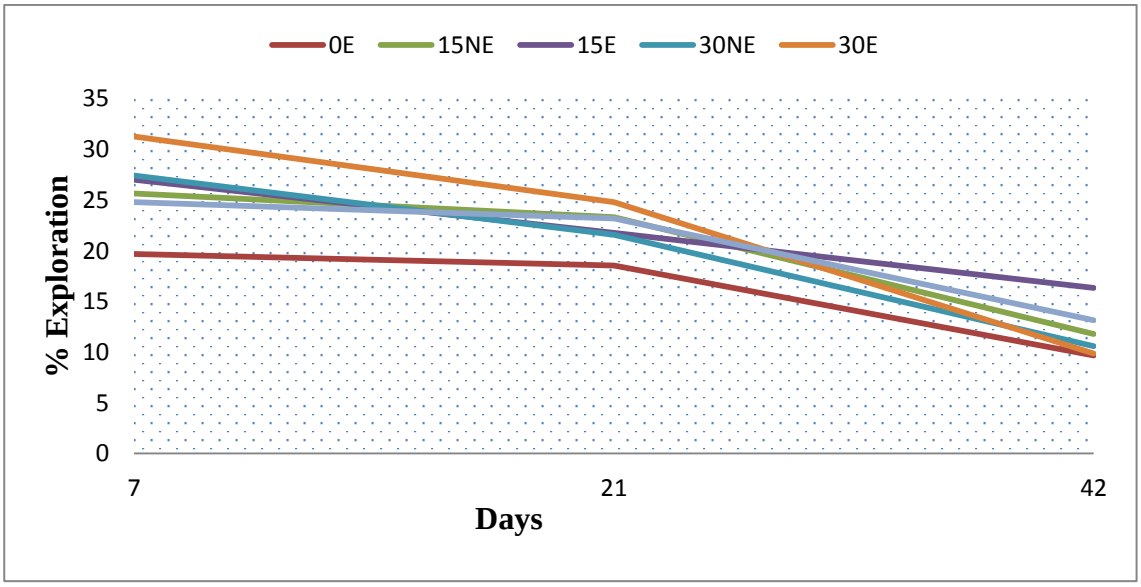


Figure 4.4 Exploration behaviour of broiler chickens fed wDDGS and enzyme

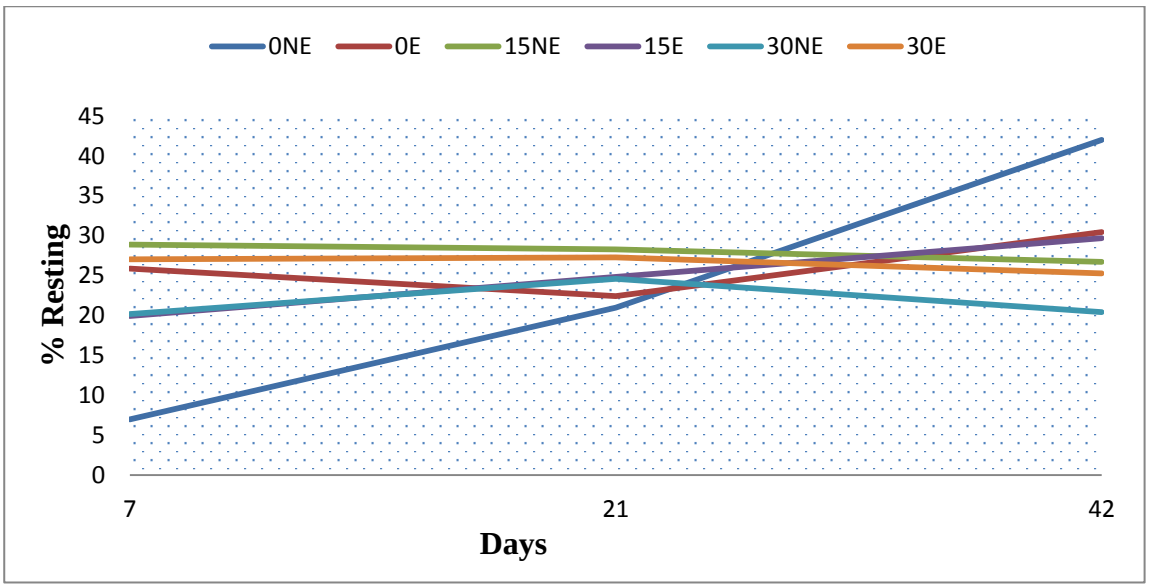


Figure 4.5 Resting behaviour of broiler chickens fed wDDGS and enzyme

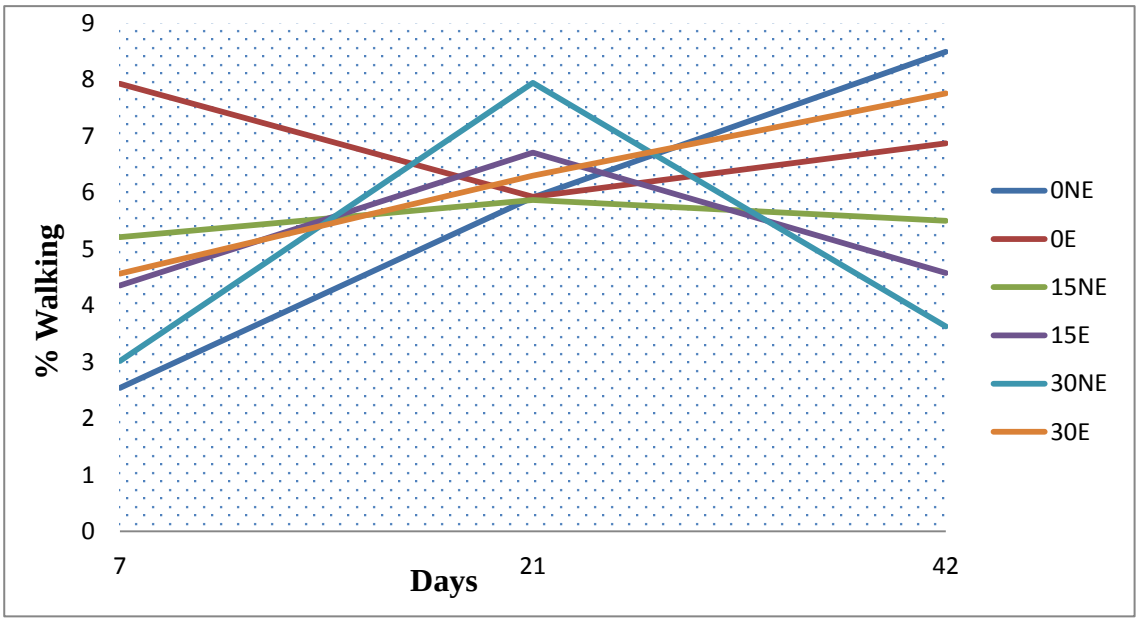


Figure 4.6 Walking behaviour of broiler chickens fed wDDGS and enzyme

4.3.4 The effect of different levels of wDDGS and enzyme inclusion on nutrient digestibility by total collection method

Table 4.16 shows the main effects of treatment on nutrient digestibility of the experimental diets. The digestibility of nutrients differed significantly with wDDGS inclusion except for calcium digestibility, with the 30% wDDGS diet having significantly lower dry matter (DM) and protein digestibility than the control diet. Mean NDF digestibility was higher with inclusion of wDDGS. Figure 4.6 shows the relationship between wDDGS and DM digestibility. It showed that for every 15% increase in wDDGS inclusion, DM digestibility decreased by 2.7 %.

Table 4.16 Means for the main effects of wDDGS levels and enzyme inclusion on nutrient digestibility of the diets (g/kg)

DIETS									
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Digestibility (g/kg)									
Dry Matter	687[a]	659[ab]	631[b]	10.5	0.005	662	655	8.6	0.534
Protein	529[a]	480[a]	270[b]	21.9	0.001	430	422	17.9	0.756
EE	657[b]	655[b]	765[a]	15.4	0.001	684	700	12.6	0.374
NDF	337[b]	471[a]	448[a]	22.9	0.001	373	464	18.7	0.003
ADF	112[ab]	107[b]	144[a]	9.1	0.019	126	115	7.5	0.326
Calcium	263	253	278	12.1	0.387	253	276	9.9	0.123
Phosphorus	388[a]	357[b]	354[b]	5.8	0.001	358	375	4.7	0.020

a,b Means bearing different letters within rows are significantly different (P<0.05)
NE = No Enzyme, E = With Enzyme, EE = Ether Extract, NDF= Neutral Detergent Fibre, ADF = Acid Detergent Fibre

Supplementation with enzyme improved the digestibility of NDF and phosphorus. Meanwhile, digestibilities of other nutrients were not affected by enzyme addition.

The wDDGS levels x enzyme interaction (Table 4.17) showed significant effects on fibre and mineral digestibility which resulted from greater increments in digestibility from enzyme addition at higher wDDGS inclusion levels.

Table 4.17 Interaction effects of wDDGS levels x enzyme inclusion on nutrient digestibility of the diets (g/kg)

	Enzyme Inclusion							
	No Enzyme			+ Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Nutrient (g/kg)								
Dry Matter	693	657	637	680	661	624	14.9	0.799
Protein	567	470	253	490	489	287	30.1	0.179
Ether Extract	638	647	767	676	662	762	21.8	0.624
NDF	364^{b}	414^{ab}	342^{b}	310^{b}	528^{a}	553^{a}	32.4	0.002
ADF	151^{a}	88^{bc}	139^{ab}	72^{c}	125^{abc}	149^{a}	12.9	0.001
Calcium	270^{ab}	252^{ab}	239^{b}	257^{ab}	255^{ab}	317^{a}	17.1	0.036
Phosphorus	386^{a}	385^{a}	301^{b}	389^{a}	329^{b}	406^{a}	8.1	0.001

a,b,c Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means ,NDF= Neutral Detergent Fibre, ADF = Acid Detergent Fibre

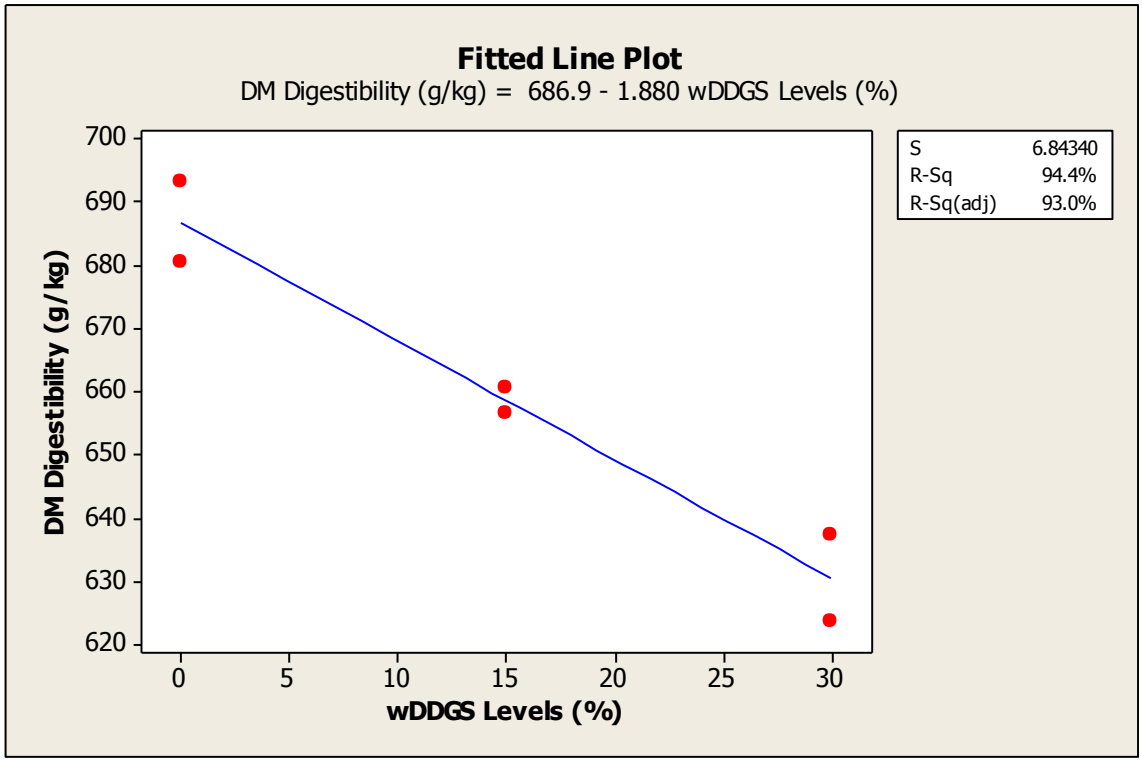


Figure 4.7 Relationship between wDDGS levels & DM digestibility

4.3.5 The effect of different levels of wDDGS and enzyme inclusion on nutrient digestibility by the AIA method

Table 4.18 shows the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets as determined by the AIA method. Ether extract, ADF and calcium digestibilities were not (P>0.05) affected by either wDDGS inclusion or enzyme addition. However, 15% wDDGS had significantly higher NDF digestibility than 0% wDDGS.

Table 4.18 Means for the main effects of wDDGS and enzyme on nutrient digestibility of the diets by AIA Method

	DIETS								
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Nutrients (g/kg)									
Protein	499[a]	510[a]	297[b]	30.6	0.001	504[a]	367[b]	24.1	0.001
Ether Extract	641	681	701	20.4	0.130	670	678	16.7	0.718
NDF	344[b]	513[a]	373[b]	35.5	0.007	377	443	28.9	0.121
ADF	259	274	297	12.2	0.121	266	288	10.0	0.133
Calcium	253	284	295	18.0	0.254	257	297	14.7	0.070
Phosphorus	332	329	313	8.3	0.216	342	308	6.8	0.002

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE= No Enzyme, E= Enzyme.

The effect of wDDGS x enzyme interaction on nutrient digestibility of the diets, as determined by the AIA method, is presented in Table 4.19. It showed that there was a significant (P<0.05) interaction effect on fibre fractions digestibility. Addition of enzyme significantly increased NDF digestibility only in the 30% wDDGS, whilst ADF digestibility also was increased at 30% wDDGS with no enzyme compared to 0% wDDGS without enzyme.

Table 4.19 Means for the wDDGS x enzyme on nutrient digestibility of the diets by AIA method

	Enzyme Inclusion							
	No Enzyme			+ Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Nutrients (g/kg)								
Protein	586[a]	519[a]	406[a]	412[a]	501[a]	187[b]	43.3	0.078
EE	655	692	662	626	669	740	28.8	0.143
NDF	394[ab]	491[a]	245[c]	293[bc]	535[a]	501[a]	61.5	0.001
ADF	239[b]	241[ab]	317[a]	280[ab]	307[ab]	276[ab]	17.2	0.016
Calcium	229	257	286	277	311	303	25.4	0.740
Phosphorus	346	339	340	320	319	285	11.8	0.314

a, b, c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means. NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, EE = Ether Extract.

4.3.6 The effect of different levels of wDDGS and enzyme inclusion on carcass characteristics and digestive organs of broilers

In the main effects (Table 4.20) there was a significant (P<0.05) influence of the wDDGS levels on final live weight, cold carcass weight, dressing percent, as well as on breast with bone proportion and wings weight (P<0.01) which were lower on the 30% wDDGS than 0% wDDGS diets. Similarly, there was significant (P<0.05) effect of wDDGS levels on the proportional weight of empty gizzard, empty crop as well as on total gut and proventriculus (P<0.01). These organs were heavier on the 30% wDDGS than 0% wDDGS diets. However, there were no significant effect (P>0.05) of enzyme inclusion on any of the parameters measured.

Table 4.20 Main effects of wDDGS levels and enzyme inclusion on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (5-46 days of age)

	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Parameters (%)*									
FLW (g)	2531[a]	2525[a]	1547[b]	98.6	0.001	2230	2172	80.5	0.613
Cold Carcass (g)	2357[a]	2304[a]	1422[b]	95.0	0.001	2053	2002	77.6	0.651
Dressing percent	65[a]	63[a]	56[b]	0.8	0.001	61	61	0.6	0.512
Major carcass components									
Breast + bone	27.1[a]	25.9[a]	18.6[b]	0.82	0.001	24	24	0.7	0.952
Thighs	19	20	19	0.2	0.121	19	20	0.2	0.164
Drumsticks	10	10	10	0.2	0.125	10	10	0.1	0.187
Neck & Back	17	17	17	0.5	0.856	16	17	0.4	0.694
Wings	6.5[a]	6.3[ab]	5.9[b]	0.15	0.021	6.1	6.3	0.13	0.454
Edible organ components									
Liver	3.1	3.2	3.3	0.11	0.347	3.2	3.2	0.09	0.541
Heart	0.8	0.8	0.8	0.04	0.997	0.8	0.7	0.03	0.114
Empty Gizzard	1.3[b]	1.4[b]	2.1[a]	0.08	0.001	1.6	1.6	0.06	0.663
Non-edible components									
Total Gut	16[b]	16[b]	24[a]	0.5	0.001	18	18	0.4	0.166
Empty Crop	0.38[b]	0.44[ab]	0.54[a]	0.041	0.041	0.49	0.42	0.034	0.138
Proventriculus	0.42[b]	0.46[b]	0.63[a]	0.024	0.001	0.49	0.52	0.020	0.354

*a,b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, *****unless otherwise stated, FLW= Final Live Weight, NE= No Enzyme, E= Enzyme*

The wDDGS levels x enzyme interaction (Table 4.21) showed a significant interaction effect on breast with bone weight, empty gizzard, and total gut of broiler chickens. Enzyme significantly increased the weight of empty gizzard and total gut at 30 % wDDGS (Figure 4.12).

Inclusion of 30% wDDGS with no enzyme significantly reduced breast with bone weight compared to 0 and 15% wDDGS but reduction was less for 30% wDDGS with enzyme.

Table 4.21 Interaction effects of wDDGS levels x enzyme inclusion on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (5-46 days of age)

	Enzyme Inclusion							
	No Enzyme			+Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Parameters (%)*								
Final live weight(g)	2442^{a}	2776^{a}	1472^{b}	2620^{a}	2273^{a}	1622^{b}	139.4	0.041
CC weight(g)	2269	2529	1360	2445	2078	1483	134.4	0.058
Dressing percent	64^{a}	63^{a}	55^{c}	65^{a}	62^{ab}	57^{bc}	1.1	0.166
Major Carcass components								
Breast with bone	26.5^{a}	28.1^{a}	17.2^{c}	27.8^{a}	23.7^{ab}	20.1^{bc}	1.15	0.012
Thighs	19	20	19	19	20	19	0.3	0.688
Drumsticks	9.5	9.5	9.8	9.4	10.1	10.1	0.24	0.311
Neck & Back	16	16	17	17	17	16	0.8	0.542
Wings	6.7	5.9	5.8	6.3	6.6	5.9	0.22	0.078
Edible organ components								
Liver	3.1	3.2	3.4	3.1	3.1	3.2	0.16	0.785
Heart	0.8	0.8	0.8	0.7	0.7	0.7	0.05	0.823
Empty Gizzard	1.4^{b}	1.2^{b}	2.1^{a}	1.2^{b}	1.6^{ab}	2.0^{a}	0.11	0.024
Non-edible components								
Total Gut	15.9^{b}	15.3^{b}	23.5^{b}	15.2^{b}	16.3^{b}	23.5^{a}	0.66	0.045
Empty Crop	0.43	0.42	0.62	0.33	0.47	0.46	0.06	0.206
Proventriculus	0.43	0.45	0.68	0.41	0.48	0.59	0.035	0.221

a,b,c Means bearing different letters within rows are 0ly different (P<0.05), SEM=Standard Error of means, CC=Cold Carcass,
unless otherwise stated

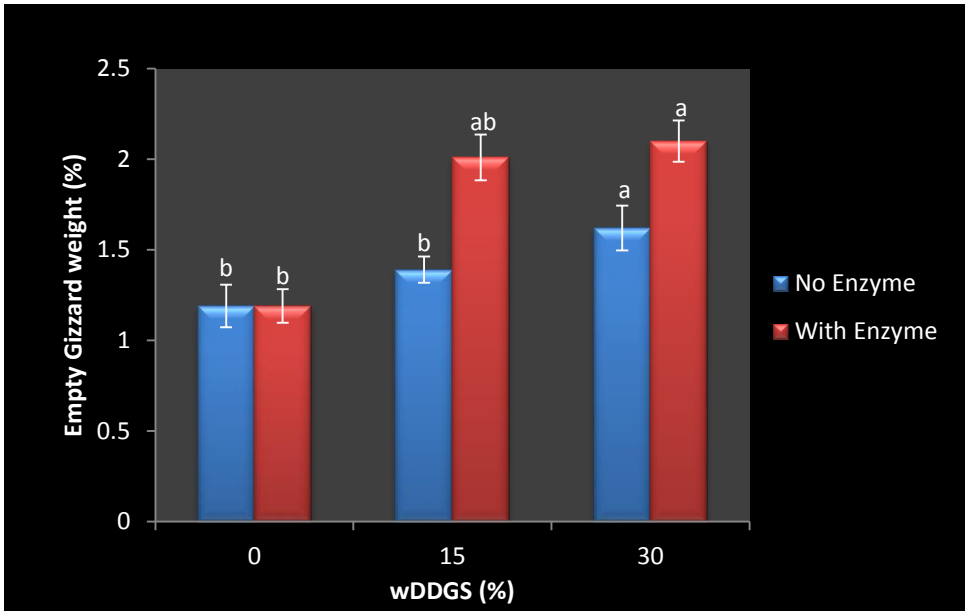


Figure 4.8 Interaction effects of wDDGS and enzyme on empty gizzard weight

4.3.7 The effect of different levels of wDDGS and enzyme inclusion on chemical composition of broiler muscles

The main effects of wDDGS and enzyme on the chemical composition of broiler muscles are presented in Table 4.22. There were significant ($P<0.05$) effects of wDDGS and enzyme on broiler breast muscle where 30% wDDGS reduced the protein content. Meanwhile, enzyme addition significantly ($P<0.05$) increased the protein content of breast muscles. Enzyme addition also significantly reduced the fat content of breast and thigh muscles. The ash content of thigh and breast muscles was significantly increased by addition of 15 and 30% wDDGS respectively. Enzyme addition also increased the ash content of breast muscles only but did not influence that of thigh muscles. The drumstick muscles were not however affected by either wDDGS or enzyme addition.

Table 4.22 Mean values for the main effects of wDDGS & enzyme on the chemical composition of different broiler muscles

	DIETS								
	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Composition (g/kg)									
Breast Muscles									
Moisture	755^{b}	759 ^{b}	783^{a}	2.9	0.001	765	766	2.4	0.713
Protein	803^{a}	816^{a}	758^{b}	4.2	0.001	779	805	3.4	0.001
Ether Extract	35	34	53	5.6	0.055	48	33	4.6	0.033
Ash	45^{c}	48^{b}	51^{a}	0.7	0.001	47	49	0.5	0.031
Thigh Muscles									
Moisture	728	742	754	7.7	0.084	740	742	6.3	0.747
Protein	682	690	690	17.8	0.929	678	696	14.5	0.394
Ether Extract	193^{ab}	179^{b}	243^{a}	17.4	0.044	228	182	14.2	0.035
Ash	40^{b}	44^{a}	41^{b}	0.7	0.001	41	41	0.6	1.000
Drumstick Muscles									
Moisture	770	771	773	3.2	0.784	770	773	2.6	0.527
Protein	745	737	707	10.9	0.054	736	723	8.9	0.290
Ether Extract	104	96	140	13.8	0.084	107	119	11.3	0.453
Ash	46	49	45	1.4	0.094	47	46	1.1	0.396

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), NE= No Enzyme, E= Enzyme, SEM=Standard Error of means

The wDDGS x enzyme interaction (Table 4.23) had no significant (P>0.05) effect on the composition of drumstick muscles. However, there was significant interaction for breast muscle protein, ether extract and ash contents of thigh muscles. At 30% wDDGS, enzyme significantly increased the protein content of broiler breast muscle compared with 30% wDDGS with no enzyme. The enzyme also reduced the ether extract content of thigh muscle at 30% wDDGS inclusion.

Table 4.23 Means for wDDGS x enzyme on chemical composition of broiler muscles

	Enzyme Inclusion							
	No Enzyme			+ Enzyme				
	wDDGS (%)							
	0	15	30	0	15	30	SEM	P value
Composition(g/kg)								
Breast Muscles								
Moisture	752	758	784	757	760	781	4.1	0.575
Protein	794^{bc}	811^{ab}	733^{d}	812^{ab}	821^{a}	783^{c}	5.9	0.008
EE	36	42	66	34	26	39	7.9	0.315
Ash	43	46	51	46	49	51	0.9	0.282
Thigh Muscles								
Moisture	735	731	753	720	753	754	10.9	0.258
Protein	683	695	656	680	684	724	25.1	0.250
EE	181^{b}	182^{b}	320^{a}	204^{b}	175^{b}	166^{b}	24.6	0.004
Ash	39^{b}	46^{a}	39^{b}	40^{b}	42^{ab}	42^{ab}	1.0	0.025
Drumstick Muscles								
Moisture	770	767	773	770	775	773	4.5	0.570
Protein	763	740	706	727	734	707	15.4	0.458
EE	87	96	137	121	95	142	19.6	0.628
Ash	46	52	44	46	46	45	1.9	0.117

a,b,c Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard Error of means, EE = Ether Extract

4.3.8 The effect of different levels of wDDGS and enzyme inclusion on fatty acids composition of lipids from broiler breast meat

The effects of different levels of wDDGS and enzyme inclusion on fatty acids composition of lipids from broiler breast meat are presented in Table 4.24. Wheat DDGS had a significant effect on fatty acid composition of breast meat of broiler chickens. Pentadecanoic, Palmitic, oleic, linoleic and C18:1n11c were highest in 0% wDDGS, while tridecanoic, stearic, palmitoleic, elaidic and cis-8, 11, 14 -eicosatrienoic and erucic acid were highest in 15% wDDGS. Similarly, myristic, α-linolenic and ɣ-linolenic acids, cis-5-eicosenoic, cis-8-eicosenoic and docosapentaenoic fatty acids were higher in 30% wDDGS. Higher values were observed in palmitic, and palmitoleic acids with No enzyme. Enzyme supplementation significantly (P<0.05) increased the concentration of stearic, linoelaidic, linoleic, cis 8-Eicosenoic and docosapentaenoic acids. Enzyme also reduced the concentration of tridecanoic, palmitic and palmitoleic acids.

There was also a significant interaction effect of wDDGS and enzyme (Table 4.25) of broiler breast meat where enzyme supplementation significantly reduced the concentration of palmitic acid at 15% wDDGS. It also reduced the concentration of myristic and oleic acid for 15% wDDGS and increased linoleic acid at 15% wDDGS.

Table 4.24 Means for the main effects of wDDGS and enzyme on fatty acids profile of breast meat from broiler chickens

		wDDGS (%)					Enzyme Inclusion			
	Structure	0	15	30	SEM	P value	NE	E	SEM	P value
Fatty Acid (g/100g TFA)										
Tridecanoic	C13:0	0.083^{b}	0.351^{a}	0.042^{b}	0.0987	0.001	0.169	0.148	0.0806	0.001
Myristic	C14:0	0.080^{c}	1.714^{b}	4.764^{a}	0.1326	0.001	2.094	2.278	0.1083	0.247
Myristoleic	C14:1	0.227	0.445	0.584	0.1122	0.103	0.418	0.419	0.0916	1.000
Pentadecanoic	C15:0	0.173^{a}	0.000^{b}	0.145^{b}	0.0929	0.001	0.058	0.154	0.0758	0.896
Palmitic	C16:0	24.087^{a}	19.015^{b}	21.105^{b}	0.7230	0.001	22.317	20.488	0.5903	0.042
Palmitelaidic	C16:1t	0.095	0.435	0.050	0.1615	0.211	0.332	0.055	0.1318	0.155
Palmitoleic	C16:1	3.777^{b}	11.498^{a}	4.261^{b}	0.3301	0.001	7.539	5.484	0.2695	0.002
cis-10-Heptadecenoic	C17:1	0.127^{b}	0.348^{ab}	0.463^{a}	0.0878	0.042	0.244	0.381	0.0717	0.192
Stearic	C18:0	7.103^{b}	9.588^{a}	7.858^{b}	0.4182	0.001	7.923	8.442	0.3415	0.001
Elaidic	C18:1n9t	0.157^{b}	0.535^{a}	0.238^{ab}	0.0870	0.016	0.338	0.282	0.0710	0.585
Vaccenic	C18:1n11t	0.069	0.000	0.000	0.0191	0.029	0.037	0.009	0.0156	0.228
C18:1n6c	C18:1n6c	0.969^{a}	0.010^{b}	0.009^{b}	0.1844	0.002	0.318	0.341	0.1505	0.914
Oleic	C18:1n9c	26.530^{a}	19.622^{b}	19.949^{c}	0.5287	0.001	22.743	21.324	0.4317	0.092
C18:1n11c	C18:1n11c	2.064^{a}	0.223^{b}	0.000^{c}	0.0607	0.001	0.799	0.725	0.0495	0.304
Linoelaidic	C18:2n6t	0.002^{b}	0.000^{b}	0.118^{a}	0.0259	0.006	0.001	0.078	0.0211	0.018
Nonadecanoic	C19:0	0.005^{b}	0.347^{a}	0.000^{b}	0.0524	0.001	0.066	0.168	0.0428	0.108
Linoleic	C18:2n6c	25.483^{a}	19.877^{b}	18.988^{b}	0.6285	0.001	19.592	23.306	0.5131	0.007
Nonadecenoic	C19:1	0.023	0.000	0.000	0.0070	0.055	0.003	0.012	0.0057	0.318
ɣ-linolenic	C18:3n6	0.247^{b}	0.375^{b}	0.879^{a}	0.1244	0.005	0.397	0.603	0.1016	0.167
α-linolenic	C18:3n3	0.199^{b}	0.506^{b}	1.108^{a}	0.1643	0.003	0.746	0.462	0.1342	0.152
Tricosanoic	C23:0	0.762	1.045	1.872	0.3787	0.127	1.091	1.361	0.3092	0.545
Arachidic	C20:0	0.000	0.093	0.212	0.0654	0.100	0.076	0.127	0.0534	0.509
cis-5-Eicosenoic	C20:1n5c	0.040^{b}	0.219^{ab}	0.395^{a}	0.0580	0.002	0.238	0.198	0.0474	0.526
cis-8-Eicosenoic	C20:1n8c	0.835^{b}	0.919^{b}	1.785^{a}	0.1806	0.003	0.794	1.565	0.1474	0.002
cis-11-Eicosenoic	C20:1n11c	1.737^{a}	0.552^{b}	0.063^{b}	0.1537	0.001	0.809	0.758	0.1255	0.781
cis-11-14-Eicosadienoic	C20:2	0.375	1.776	3.585	0.2124	0.001	1.863	1.961	0.1735	0.696

Table 4.24: Mean main effects of wDDGS and enzyme on fatty acids profile of breast meat from broiler chickens (continued)

		wDDGS (%)					Enzyme Inclusion			
	Structure	0	15	30	SEM	P value	NE	E	SEM	P value
Fatty Acid (g/100g TFA)										
cis-8,11,14-Eicosatrienoic	C20:3n6	0.250^{b}	1.154^{a}	0.624^{b}	0.1434	0.001	0.955	0.397	0.1171	0.003
Arachidonic	C20:4n6	0.309	0.386	0.272	0.0994	0.716	0.218	0.426	0.0811	0.086
Behenic	C22:0	0.210^{b}	0.617^{ab}	0.987^{a}	0.1889	0.031	0.512	0.697	0.1542	0.408
Erucic	C22:1n9	0.213^{b}	0.844^{a}	0.640^{ab}	0.1624	0.038	0.673	0.458	0.1326	0.268
cis-13,16-Docosadienoic	C22:2	0.063^{b}	0.973^{a}	0.267^{b}	0.1403	0.001	0.710	0.159	0.1145	0.003
cis5,8,11,14,17-Eicosapentanoic	C20:5n3	0.078^{c}	0.383^{b}	0.637^{a}	0.0668	0.001	0.380	0.351	0.0546	0.710
Lignoceric	C24:0	0.081^{b}	0.349^{a}	0.434^{a}	0.0713	0.007	0.308	0.268	0.0582	0.633
Docosatetraenoic	C22:4	0.220^{b}	0.519^{b}	0.892^{a}	0.0872	0.001	0.454	0.633	0.0712	0.094
Nervonic	C24:1	0.137^{b}	0.714^{a}	0.222^{b}	0.0849	0.001	0.337	0.378	0.0693	0.682
Docosapentaenoic	C22:5	0.828^{b}	1.370^{b}	3.195^{a}	0.0624	0.001	1.513	2.083	0.0509	0.003
Docosahexaenoate	C22:6	0.631^{b}	1.931^{a}	2.570^{a}	0.2518	0.001	1.617	1.804	0.2056	0.529
Others		1.739	1.275	0.795	0.2658	0.067	1.320	1.219	0.2171	0.746
∑n-6		26.664^{a}	11.816^{b}	10.464^{b}	0.5166	0.001	15.192	17.437	0.4218	0.001
∑n-3		1.734^{c}	4.255^{b}	7.509^{a}	0.5556	0.001	4.255	4.698	0.4537	0.498
n-6:n-3		0.064^{c}	0.359^{b}	0.719^{a}	0.0415	0.001	0.405	0.356	0.0339	0.317

a,b,c Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E = Enzyme, TFA= Total Fatty Acid

Table 4.25 Interaction effect of wDDGS x enzyme on fatty acid profiles of breast meat from broiler chickens

		Enzyme Inclusion							
		No enzyme			+ Enzyme				
		wDDGS (%)							
	Structure	0 NE	15 NE	30 NE	0 E	15 E	30 E	SEM	P value
Fatty Acid (g/100g TFA)									
Tridecanoic	C13:0	0.083^{ab}	0.423^{a}	0.000^{b}	0.083^{ab}	0.278^{ab}	0.083^{ab}	0.1396	0.001
Myristic	C14:0	0.160^{c}	0.423^{c}	5.700^{a}	0.000^{c}	3.005^{b}	3.828^{b}	0.1876	0.001
Myritoleic	C14:1	0.105	0.370	0.780	0.348	0.520	0.388	0.1586	0.125
Pentadecanoic	C15:0	0.173	0.000	0.000	0.173	0.000	0.290	0.1313	0.228
Palmitic	C16:0	25.700^{a}	21.500^{ab}	19.750^{bc}	22.473^{ab}	16.530^{c}	22.460^{ab}	1.0225	0.035
Palmitelaidic	C16:1t	0.025	0.870	0.100	0.165	0.000	0.000	0.2283	0.096
Palmitoleic	C16:1	5.115^{b}	11.330^{a}	6.173^{b}	2.438^{c}	11.665^{a}	2.348^{c}	0.4668	0.001
cis-10-Heptadecenoic	C17:1	0.058	0.250	0.423	0.195	0.445	0.503	0.1241	0.899
Stearic	C18:0	7.125	9.770	6.875	7.080	9.405	8.840	0.5914	0.131
Elaidic	C18:1n9t	0.118	0.545	0.350	0.195	0.525	0.125	0.1230	0.470
Vaccenic	C18:1n11t	0.110	0.000	0.000	0.028	0.000	0.000	0.0270	0.238
C18:1n6c	C18:1n6c	0.943	0.010	0.000	0.995	0.010	0.018	0.2608	0.995
Oleic	C18:1n9c	26.740^{a}	22.238^{b}	19.250^{bc}	26.320^{a}	17.005^{c}	20.648^{b}	0.7477	0.001
C18:1n11c	C18:1n11c	2.398^{a}	0.000^{d}	0.000^{d}	1.730^{b}	0.445^{c}	0.000^{d}	0.0858	0.001
Linoelaidic	C18:2n6t	0.003^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	0.235^{a}	0.0366	0.006
Nonadecanoic	C19:0	0.005^{b}	0.193^{ab}	0.000^{b}	0.005^{b}	0.500^{a}	0.000^{b}	0.0741	0.083
Linoleic	C18:2n6c	25.050^{ab}	17.500^{c}	16.225^{c}	25.915^{a}	22.253^{ab}	21.750^{b}	0.8888	0.038
Nonadecenoic	C19:1	0.010	0.000	0.000	0.035	0.000	0.000	0.0099	0.368
ɣ-linolenic	C18:3n6	0.283^{ab}	0.000^{b}	0.908^{a}	0.210^{ab}	0.750^{ab}	0.850^{a}	0.1759	0.049
α-linolenic	C18:3n3	0.365	0.743	1.130	0.033	0.268	1.085	0.2324	0.648
Tricosanoic	C23:0	0.793	0.668	1.813	0.730	1.422	1.930	0.5355	0.730
Arachidic	C20:0	0.000	0.000	0.228	0.000	0.185	0.195	0.0925	0.462

Table 4.25: Interaction effect of wDDGS x enzyme on fatty acids profile of breast meat from broiler chickens (continued)

		Enzyme Inclusion							
		No enzyme			+ Enzyme				
		wDDGS (%)							
	Structure	0 NE	15 NE	30 NE	0 E	15 E	30 E	SEM	P value
Fatty Acid (g/100g TFA)									
cis-5-Eicosenoic	C20:1n5c	0.018^{b}	0.100^{b}	0.605^{a}	0.070^{b}	0.338^{ab}	0.185^{b}	0.0820	0.002
cis-8-Eicosenoic	C20:1n8c	0.350^{b}	0.303^{b}	1.730^{a}	1.320^{ab}	1.535^{a}	1.840^{a}	0.2553	0.001
cis-11-Eicosenoic	C20:1n11c	1.823	0.603	0.000	1.650	0.500	0.125	0.2174	0.777
cis-11-14-Eicosadienoic	C20:2	0.230^{d}	1.365^{cd}	3.995^{a}	0.520^{d}	2.187^{bc}	3.175^{ab}	0.3004	0.040
cis-8,11,14-Eicosatrienoic	C20:3n6	0.010^{b}	2.045^{a}	0.810^{b}	0.490^{b}	0.263^{b}	0.438^{b}	0.2028	0.001
Arachidonic	C20:4n6	0.080	0.348	0.225	0.538	0.423	0.318	0.1405	0.330
Behenic	C22:0	0.070	0.315	1.150	0.350	0.918	0.823	0.2672	0.237
Erucic	C22:1n9	0.070^{b}	0.668^{ab}	1.280^{a}	0.355^{ab}	1.020^{ab}	0.000^{b}	0.2296	0.003
cis-13,16-Docosadienoic	C22:2	0.013^{b}	1.793^{a}	0.323^{b}	0.113^{b}	0.153^{b}	0.210^{b}	0.1984	0.001
cis5,8,11,14,17-Eicosapentanoic	C20:5n3	0.108^{c}	0.125^{c}	0.908^{a}	0.048^{c}	0.640^{ab}	0.365^{bc}	0.0945	0.001
Lignoceric	C24:0	0.038	0.310	0.575	0.123	0.388	0.293	0.1008	0.143
Docosatetraenoic	C22:4	0.075	0.283	1.005	0.365	0.755	0.778	0.1233	0.029
Nervonic	C24:1	0.070	0.620	0.320	0.203	0.808	0.123	0.1201	0.249
Docosapentaenoic	C22:5	0.223^{d}	0.620^{cd}	3.695^{a}	1.433^{bc}	2.120^{ab}	2.695^{ab}	0.0882	0.003
Docosahexaenoate	C22:6	0.173	1.713	2.965	1.088	2.148	2.175	0.3561	0.072
Others		1.295	1.956	0.709	2.183	0.593	0.881	0.4235	0.052
∑n-6		25.655	9.758	10.163	27.672	13.874	10.765	0.7306	0.079
∑n-3		0.868^{d}	3.200^{bcd}	8.698^{a}	2.600^{cd}	5.175^{abc}	6.320^{ab}	0.7858	0.021
n-6:n-3		0.034^{d}	0.331^{bc}	0.851^{a}	0.094^{cd}	0.387^{b}	0.586^{b}	0.0587	0.018

a,b,c,d, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means
NE = No Enzyme, E= Enzyme, TFA= Total Fatty Acid

4.3.9 The effect of different levels of wDDGS and enzyme inclusion on different categories of fatty acids of lipids from broiler breast meat

The effects of different levels of wDDGS and enzyme inclusion on fatty acids composition of lipids from broiler breast meat are presented in 3 categories in Table 4.26. The highest values of SFA and PUFA were observed at 30% wDDGS level while 0% wDDGS had the highest MUFA. Similarly, enzyme supplementation significantly (P<0.05) increased PUFA and also significantly reduced SFA and MUFA.

Table 4.26 Mean for the main effects of wDDGS and enzyme on the main categories of fatty acids of breast meat from broiler chickens

	wDDGS (%)					Enzyme Inclusion			
	0	15	30	SEM	P value	NE	E	SEM	P value
Fatty acid (%)									
SFA	33^{b}	34^{b}	38^{a}	0.9	0.001	35^{a}	34^{b}	0.8	0.023
MUFA	37^{b}	36^{a}	27^{b}	0.7	0.001	35^{a}	31^{b}	0.6	0.001
PUFA	31^{b}	32^{b}	36^{a}	0.8	0.001	30^{b}	35^{a}	0.6	0.025

a, b, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E= Enzyme,
SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids,
PUFA= Polyunsaturated Fatty Acids

The interaction of wDDGS levels and enzyme supplementation (Table 4.27) indicated that enzyme supplementation changed the SFA and PUFA proportions more at lower wDDGS levels than at 30% wDDGS.

Table 4.27 Interaction effect of wDDGS x enzyme on categories of fatty acids of breast meat from broiler chickens

	Enzyme Inclusion							
	No enzyme			+ Enzyme				
	wDDGS (%)							
	0NE	15NE	30NE	0E	15E	30E	SEM	P value
Fatty acid (%)								
SFA	34^{c}	34^{c}	37^{a}	31^{c}	33^{bc}	38^{a}	1.3	0.001
MUFA	38	38	29	35	33	25	1.0	0.067
PUFA	28^{cd}	29^{c}	34^{c}	34^{c}	34^{c}	38^{a}	1.1	0.014

a,b,c Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE = No Enzyme, E= Enzyme,
SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids,
PUFA= Polyunsaturated Fatty Acids

4.4 Discussion

In the previous experiment, 40% inclusion of wDDGS adversely affected growth performance of broilers while 20% wDDGS did not show positive effects. This was attributed to low feed intake and the inability of birds to process the feed very well which resulted in low nutrient digestibility and absorption. However, 10% wDDGS proved beneficial to broiler performance. This study investigated the supplementation of intermediate levels of the previously tested diets with an enzyme preparation to improve the nutrient availability of these diets to broilers. In this study, at 30% wDDGS the feed intake was lower at starter, finisher and the entire periods of this study. In view of the aforementioned result, wDDGS can be incorporated up to 15% level without showing significant negative effect on dry matter and protein digestibility, feed intake, live weight gain and FCR. Angeles and Gomez (2010) also reported no significant difference in daily weight gain of chickens when 15% wDDGS diets were compared with the 0% wDDGS diets. At 30% wDDGS, both feed intake and weight gain were lower in the presence and absence of enzyme which resulted in similar FCR to other treatments. This was corroborated by individual bird's LW and LWG. In contrast, Min *et al.* (2011) reported no significant difference in the feed consumption and FCR of starter broilers fed DDGS at up to 30% level.

Just as enzyme addition had no effect on dry matter and protein digestibility, it also had no effect on growth and feed intake. The same trend for wDDGS and enzyme inclusion took place at starter, finisher and overall phases of this study. Enzyme showed a negative effect at 15% wDDGS for weight gain as well as FCR. King and Moughan (1998) reported no discernible effects of enzyme (xylanase, amylase, pectinase and glucanase) supplementation of wheat based diets on daily feed intake, daily weight gain and FCR for any of the growth periods. Enzyme slightly increased feed intake at the finisher phase for 30% wDDGS level, but it was still lower than 0 and 15% levels. The reduced performance might be related to the lower lysine content in the 30% wDDGS diets and lower protein digestibility and perhaps the quality of wDDGS which is known to have poor lysine digestibility (Lan *et al.*, 2008; Nyachoti *et al.*, 2005), a factor that will compromise its' nutritive value for the non-ruminant animal and will be a major concern in the use of wDDGS as reported by Bandegan *et al.* (2009). Other researchers, like Parsons (1983), reported that if the lysine content is adjusted, the wDDGS can to a large extent replace soybean meal protein.

As feeding behaviour was more performed by birds given 0% wDDGS with no enzyme and 15% wDDGS with the enzyme at days 21, this agrees with the finding herein that they consumed more feed to meet their energy requirements for production (Hetland and Svihus, 2001). The decrease in exploration behaviour of birds over time was consistent with the previous experiment and could be due to the birds being engaged in other behaviours such as resting. Steenfeldt *et al.* (2007) reported that supplementing laying hens with by-products of fermentation stimulates gizzard function and consequently caused gizzard size and weights to increase. Due to slow passage rate that occurred as a result of consuming these fibrous material, the gizzard remain full and is likely to make the birds feel more satiated resulting in birds appearing more calm, which in turn, may contribute to a lower aggression behaviour. As the negative behaviours of aggression and pecking were very infrequently performed throughout the experiment, this might be linked to the effect of the fibrous diets on internal organs and its subsequent effect on the bird as mentioned above. Similarly, comfort behaviours such as preening and dust-bathing were performed infrequently as more birds were engaged in resting in the middle and latter part of this experiment, in agreement with the findings reported in the previous chapter.

From the results of this study, it is clear that protein digestibility was higher in 0% wDDGS than 30% wDDGS diets. At 15% wDDGS inclusion, protein digestibility remained in between these and was statistically similar to the control. The relative decrease of dry matter digestibility with increasing wDDGS level in both this and the previous experiment means that digestibility of wDDGS especially at higher inclusion is poor. Birds at 15 and 30% wDDGS diets consumed more fibre but NDF and ADF digestibility was improved, possibly due to a higher soluble fibre fraction content which is more easily fermentable. The enzymes used in this study were derived from 2 bacteria (*Bacillus* and *Trichoderma*) which might prove beneficial in these situations in increasing fibre digestibility. NDF digestibility was higher in the presence of enzyme which could be due to enhanced bacterial activity as reported by Smits and Annison (1996). Enzyme addition increased the digestibility of minerals in both methods; calcium for AIA method, and phosphorus for total collection method. Conversely, enzyme supplementation reduced the digestibility of protein in both methods.

The lower lysine content of 30% wDDGS diet is a reflection of lower lysine content of wDDGS. Thacker and Widyaratne (2007) reported that wDDGS contain less lysine and threonine than soybean meal. However, it should be noted that the amount of soybean

meal incorporated in 30% wDDGS diet was also lower than that of the control and 15% wDDGS that is probably the reason for the lower lysine in the diet. The AA analysis of the experimental diets confirmed that the diets met the requirements of growing to finishing broiler chickens though the diet with 30% wDDGS had slightly lower values birds of 3-6 weeks of age (NRC 1994). Meanwhile, the crude protein levels of the experimental ingredients were expected to meet the AA requirements of the birds. However, it should be noted that lysine and methionine were added to the diets at the same level of inclusion in order to supplement the AA content of the test ingredients. The reduced BWG of birds fed 30% wDDGS as well as the reduced digestibility of protein of their diet and their subsequent lower breast meat yield could be attributed to lower lysine content and perhaps its poor digestibility as previous studies have noted that digestibility of several AAs in wDDGS was relatively poor especially lysine and threonine (Batal and Dale, 2006; Waldroup *et al.*, 2007). Stein (2006) also reported that lysine is the most variable amino acid in DDGS in terms of digestibility. The reduced breast with bone weight in this study was in agreement with the findings of Wang *et al.* (2007) who reported lower breast meat yield of broilers fed 30% cDDGS compared to those fed no wDDGS.

It is apparent that nutrient digestibility by both total collection and AIA methods consistently followed similar trend and gave results which were mostly close to each other in some nutrients but different in few others as in the previous experiment. Thus, both methods can be used for measuring digestibility in broiler chickens. However, the AIA method might be more useful to predict total tract digestibility due to its time saving and cost benefit as opposed to the total collection method which is more laborious and time consuming.

As body weight gain was higher at 0 and 15% wDDGS level, the cold carcass weight was also higher in birds fed these two levels than 30% inclusion diets. Even dressing percentages were also higher at 0 and 15% than 30% wDDGS levels which are more important to the consumer. The 30% wDDGS diets contained more fibre to digest so total gut weight was larger at 30%, but this has no economic benefit to the consumer. As wDDGS are an insoluble residue from wheat fermentation, 30% wDDGS might have caused gizzard weight to increase, possibly due to accumulation of fibre which resulted in a slower passage rate of fibre fractions as reported by Hetland *et al.* (2004). Due to the high fibre, other digestive organs like proventriculus and empty crop were also larger in 30% wDDGS treatments which also have no economic benefit.

Among the meat, the breast meat is considered by humans to be the best meat which was also higher at 0 and 15% wDDGS levels. As enzyme reduced body weight gain of broilers at 15% wDDGS, it also reduced cold carcass weight at the same level of wDDGS. Like feed intake, enzyme slightly increased cold carcass weight of birds for 30% wDDGS level, though the weights of these groups of birds were still lower than 0 and 15% wDDGS levels. As the ultimate outcome for a broiler is its carcass weight, low carcass weight at 30% wDDGS level will reduce the profit margin of farmers and even the enzyme supplementation will not be able to recover the profit.

A higher protein percentage of breast muscle at 0 and 15% wDDGS apparently revealed higher quality meat than in birds given the 30% wDDGS level. A higher wDDGS level also caused higher fat in the breast and thigh muscle that can reduce the quality of the meat. The inclusion level of wDDGS significantly ($P<0.05$) affected the content of some components of broiler breast and thigh muscles. 30% inclusion of wDDGS significantly increased the ash content, which may be attributed to the lower nutrient intake of birds in this group and subsequent reduced accumulation of muscles in the breast. Enzyme supplementation showed a positive effect for chemical composition of broiler muscles where it increased the protein concentration in breast meat, as previously demonstrated by Szczurek (2008) who reported that enzyme supplementation to barley based diets increased the crude protein content and reduced the proportion of fat in broiler chickens. A similar finding was reported by Cowieson *et al.* (2003) who observed a significant improvement in the retention of nitrogen compounds in broiler carcasses as influenced by enzyme addition to a wheat-based diet. Enzyme addition also significantly ($P<0.05$) reduced the fat content of breast and thigh muscles which is quite similar to the findings of Szczurek (2008). However, the drumstick muscles were not affected ($P>0.05$) by either wDDGS or enzyme inclusion in any of the parameters measured.

Numerically, the chemical composition of breast and thigh muscles indicated that breast muscles contained more protein than thigh and drumstick muscles (Table 4.19) while both thigh and drumstick muscles contained more fat than breast muscles. This followed the same pattern as results obtained by Suchy *et al.*, (2002) in commercial hybrid broilers and Vecerek *et al.* (2005) in pheasant meat. Similarly, Juzl *et al.* (2012) reported highly significant differences between the breast and thigh muscles of Chukar partridge for crude protein, fat, ash and gross energy. Breast meat is the largest area of muscle concentration in broiler chickens. Having increased protein content, as found

herein, will increase its acceptability or preference by the consumer as it yields products with healthier implications (high protein and low fat compared to beef or pork products).

Fatty acid composition is very important for consumers for quality meat. The reduced concentrations of oleic acid in wDDGS treatments could not be clearly understood and this corroborates the report of NRC (1994) and Choi *et al.* (2008) that it was impossible to explain why the concentration of oleic acid in broiler thigh meats decreased (linear, $P<0.05$) as the DDGS level increased. Corzo *et al.* (2009) also reported similar findings of high concentration of oleic acid in the control treatment. Corzo *et al.* (2009) also found elevated linoleic acid in 8% DDGS diets fed to broilers in comparison with the control treatment. Conversely, both 15 and 30% wDDGS in this study had a lower concentration of linoleic acid than the control (0% wDDGS) which is quite similar to the findings of Stark (1994) who found the potentiality of this fatty acid as a biomarker of susceptibility to skin cancer.

Higher inclusion of wDDGS had higher SFA and lower PUFA which indicated that this higher wDDGS (30%) level had detrimental effect on the quality of the meat. On the other hand 15% inclusion level had similar composition for type of fatty acids. Moreover, 15% wDDGS level reduced palmitic acid of breast meat which in turn has been associated with increased risk of coronary heart disease and some tumours (Fattore and Fanelli, 2013). Though SFA was higher at 30% inclusion of wDDGS than other two inclusion levels, stearic acid was higher at 15% inclusion which had less health hazard than palmitic acid and considered as a low-hazard ingredient (Nick, 2011). Inclusion of 15% wDDGS also increased many types of MUFA; however essential fatty acid linoleic acid was lower in 15% inclusion of wDDGS than 0% inclusion of wDDGS. Higher inclusion of wDDGS influenced higher SFA and PUFA. From the aspect of the fatty acid composition, it could be proclaimed that wDDGS could be used in broiler diet up to 15% level of inclusion without showing any detrimental effect or with minimum effect of quality of broiler breast meat.

Since the dietary concentrations of nutrients are translated in the edible tissues, and since the experimental diets contained an increased level of wDDGS (Table 4.4), this elevated composition of PUFA could be expected due to these differences. Therefore, feeding wDDGS based diets to broiler chickens can positively affect breast meat by increasing the long chain PUFA composition which consumers are encouraged to increase their daily intake of it for health benefits (Rymer and Givens, 2005). The

higher PUFA values obtained in this study confirmed the findings of different researchers (Bonoli *et al.*, 2007; Corzo *et al.*, 2009; Nkukwana *et al.*, 2014) who described these as nutritionally undesirable to the consumers as these render the meat more susceptible to oxidation than SFA and consequently negatively affects meat flavour. Thus, the keeping quality especially in tropical countries where humidity is usually high remains a serious concern over long term keeping quality of meat which is essential as not everybody can afford facilities such as freezers to preserve meat. Enzyme supplementation increased the meat quality of breast muscle of broiler by increasing PUFA and decreasing SFA. Enzyme supplementation also reduced palmitic acid which has a detrimental effect in human health (Fattore and Fanelli, 2013). It is therefore worthwhile to note that enzyme supplementation had to some extent some positive effect in improving the quality of broiler breast meat.

To conclude, the results of this experiment indicated that, whilst 15% wDDGS could be used in broiler diets, the enzyme used was either not appropriate to improve utilization of the protein, ether extract, ADF and calcium in the diets, or the amount incorporated was not sufficient enough to show considerable desired effects on wDDGS digestibility and performance of broilers. Therefore, an increased amount of this enzyme was tested alongside another enzyme mixture in a subsequent experiment to determine whether this could improve the utilization of wDDGS based diets by broilers.

CHAPTER 5:
THE USE OF EXOGENOUS ENZYMES TO IMPROVE THE NUTRITIVE AVAILABILITY OF WHEAT BASED DISTILLERS DRIED GRAINS WITH SOLUBLES TO BROILER CHICKENS

5.1 Introduction

In the previous experiments, inclusion of 30% wDDGS in broiler diets resulted in reduced growth rate and feed efficiency of chickens. However, wDDGS, as reported by Thacker and Widyaratne (2007), could be incorporated into broiler diets at an inclusion of up to 15% without negative effects on performance as long as the diet composition can compensate for the low lysine and energy content. Experiment 1 returned crude protein digestibility results of the diets as– 525g/kg for 0% wDDGS and 442g/kg for 10% wDDGS while experiment 2 returned 528g/kg for 0% wDDGS and 479g/kg for 15% which were lower than the values reported by Youssef *et al.* (2008) who reported values of 838g/kg for 0% wDDGS and 773g/kg for 15% wDDGS and Thacker (2006) also reported 824g/kg for 0% wDDGS and 743g/kg for 15% wDDGS. Applegate (2007) further stated that, in order to meet limiting amino acid needs, diets containing increasing inclusion of wDDGS should contain an increased amount of synthetic lysine to account for imbalances and for the reduced digestibility. However, an alternative approach to the supplementation with synthetic amino acids is to increase digestibility by use of exogenous enzymes that specifically target the indigestible fibres of wDDGS. Despite the combination of the enzymes (XAP mixture) tested in experiment 2 (chapter 4) at 0.25g/kg inclusion, no noteworthy positive effect of enzyme inclusion on protein, ether extract, and ADF as well as calcium digestibility was seen at any wDDGS inclusion level, likewise in the overall performance of the birds, meaning the enzymes did not exhibit adequate activity to improve the overall performance of the birds and nutrient digestibility of the diets. This might also suggest that the enzyme was unable to disrupt or modify the chemical or structural components of wDDGS, possibly due to the presence of perhaps Maillard products in wDDGS. This result indicates that the enzyme mix which was used may be not the most appropriate enzyme combination for the wDDGS diets. The protein in the diets was not well digested and made readily available for utilization by the birds in order to grow optimally. Another possible reason for lack of an enzyme effect could be that the level included was too low to show an effect on

the digestibility of wDDGS based diets. Slominski (2000) reported that supplementation of wheat-based diets with a low level of xylanase resulted in a less pronounced response. However, literature showed that an increased amount of enzyme can enhance the digestibility and performance of broilers. Marquardt *et al.* (1994) reported an improvement obtained in growing chicks when five different cereals were supplemented with an enzyme preparation high in xylanase and β-glucanase activity and later (in 1996) the authors also reported the presence of a linear relationship between the quantity of enzyme introduced to the diet and the resulting improvements in digestibility by broiler chicks.

Wheat is an important source of energy in poultry diets and, in addition to NSP, it contains considerable amounts of β-glucan and cellulose (Steenfeldt *et al.*, 1995). The presence of these β-glucans adversely affects utilization of all nutrients, especially protein and starch utilization, and they are known to give rise to highly viscous conditions in the small intestine of the chicks (Hesselman and Aman, 1986). They further reported that the use of enzymes may actually increase the digestibility and utilization of fibre through preparing it for microbial fermentation into volatile fatty acids in the caeca, where it may be available for chick absorption/utilization. It has been established that the β-glucans are degraded by glucanase. Hence, the use of a combination of xylanase and β-glucanase in various cereal-based diets incorporated at different levels has been proved to be beneficial to growth performance of broilers. In the past decades, studies by Pettersson and Åman (1989) tested the addition of an enzyme cocktail, consisting of xylanase and β-glucanase to rye and wheat-based poultry diets where the enzyme addition increased the digestibility of organic matter, crude protein and starch, which resulted in significant increase in body weight and feed intake of broilers. It is worthy of note that soybean meal was used in the formulation of experimental diets in this study which is also known to contain a considerable quantity of xylans and β-glucans as structural components of cell walls. Bach-Knudsen (1997) reported that it contains approximately 180 to 210g/kg NSP of which 25 to 30g/kg are soluble.

The NSP degrading carbohydrases have been used in wheat-based diets for young poultry and their benefits have been credited to the partial breakdown of water-soluble and viscous arabinoxylans, which inhibit nutrient digestion and absorption by raising intestinal viscosity (Bedford and Classen, 1992). Theander *et al.*, (1989); Wiseman *et al.*, (2000) suggested that the entrapment of wheat starch and protein by cell wall

polysaccharides is an important consideration by which NSP exercise their anti-nutritive properties. Therefore, the use of xylanase and glucanase preparations to target various fractions of wheat NSP may provide a potential for further improvements in the nutritive value of the diet being wheat-based. These enzymes are also used because they help to offset any negative effects of NSP and help reduce variation in energy which can be metabolized, as well as variation in performance related to viscous-grain based diets for poultry. In addition, benefits in a wheat-based diet with a combination of ingredients including plants by-products and various types of NSP may be obtained through a mix of carbohydrases, where each one possesses a different substrate preference and method of targeting different forms of polysaccharides in the cell walls. Such NSP-degrading enzymes were considered suitable for addition to grains with considerable amounts of NSP based on their ability to degrade certain chemical bonds found in raw materials which are not usually broken down by the bird's own enzymes. This eliminates or decreases the presence of anti-nutritional activity and increase the availability of nutrient components such as starch and proteins that are enclosed within fibre-rich cell walls and, therefore, not as accessible to endogenous digestive enzymes. Brenes *et al.* (1993) demonstrated benefits for broilers through using cellulase and xylanase as supplements in research on protein-rich legumes. Zanella *et al*. (1999) also showed that commercial carbohydrase products improved weight gain and FCR in broilers fed a corn/soya diet as a result of increased ileal digestibility of protein and NSP. Enzymes here were able to solubulise parts of the insoluble NSP, which improved the overall NSP digestibility.

Furthermore, the use of glycanases (xylanase and β-glucanase) is common throughout the poultry industry in order to combat adverse effects in birds fed wheat-based diets. Such beneficial results using glycanases for enhancing the absorption of nutrients and general poultry performance are well documented (Annison and Choct, 1991; Bedford and Schulze, 1998). These enzymes are considered important due to their affinity to cereal wheat grains substrate which contain considerable amounts of soluble arabinoxylans that behave in the same manner to β-glucans from barley and presently most of the glycanases used in poultry industry target the soluble carbohydrates in an endo-active manner i.e. cleaving the molecules from the middle to reduce the molecular size with little or no monomeric sugars released (Bhat and Hazlewood, 2001).

Dietary supplementation with exogenous enzymes brings potential advantages in terms of improved digestion and reduced levels of excretion. Supplementing poultry diets

with xylanase and β-glucanase can result in gains in bodyweight, feed-weight conversion and nutrient absorption in poultry. It should be noted, however, that reactions to such enzymes may vary according to the type of enzyme used and also levels of non-starch polysaccharides in the diet (Woyengo and Nyachoti, 2011). Studies by Cowieson *et al.* (2003) examined the impact of using amylase-resistant starch in pea-based diets, utilising an exogenous enzyme. They observed that the addition of the exogenous enzymes resulted in enhanced weight gain, higher feed conversion, and improved digestion of nutrients.

It has been demonstrated that composition of the diet affects litter moisture (Mushtaq *et al.*, 2014) and the gut micro-flora in broiler chickens and the presence of viscous polysaccharides has been shown to increase the intestinal microbial activity (Parsaie *et al.*, 2007). The fibre component of the diet can serve as a substrate for microbial fermentation and can change the intestinal microbial population and its environment. Further, the microbial fermentation of dietary fibre can promote the development of specific bacterial populations that are considered to be beneficial for degrading both xylan and β-glucans (Pieper *et al.*, 2008). Moreover, exogenous enzymes are characterized by their ability to reduce the viscosity of intestinal contents, stabilizing the composition of the intestinal microbial community (Choct and Annison, 1992; Choct *et al.*, 1996) and so enhancing nutrient delivery to the host. Consequently, these reduce excreta moisture, which in turn improves litter quality as reported by Lee *et al*., (2010). Since the previous and present experiments involved the feeding of wDDGS-based diets supplemented with these enzymes, it is worthwhile to assess the litter moisture content and bacterial populations as influenced by the formulation of the experimental diets.

Literature showed that the process of digestion of feed materials involves the action of both enzymes produced by the bird itself or by the microbes in its gastro intestinal tract (Munir and Maqsood, 2012). Since the digestion process in monogastric animals, especially swine and poultry, is not perfectly efficient with inability to digest about 25% of the feed they consume, the supplementation of the feed with appropriate enzymes to increase the efficiency of digestion can be viewed as the prolongation of the animal's digestive process (Carre *et al*., 1995; Pariza and Cook, 2010). Sheppy (2003) and Munir and Maqsood (2012) enumerated: (i) That the ability of the chicken to digest various component parts of the feed raw material differently, particularly fibre, is an important consideration in diet formulation.In order to achieve the potential nutritional value of feed ingredients, there is thus a need for supplementation of diets with appropriate

enzyme combinations to break down the anti-nutritional factors present in the raw materials. Many substances therein are not prone to digestion by the animal's endogenous digestive enzymes, can affect the normal digestion process and result in poor performance. However, Choct, (1997) and Scott *et al*. (2001) demonstrated that enzyme supplementation, especially an XG mixture to hydrolyse the NSP in the diet, reduces the ability of NSP to form highly viscous digesta and therefore, aids birds to increase their feed intake, achieve higher digestibility and absorption for greater growth and feed efficiency. (ii) That enzymes can increase the nutritive availability of starches, proteins and minerals that are enclosed within the fibre-rich cell wall, and which are beyond the reach of the animal's own digestive enzymes. (iii) That enzymes can break down specific chemical bonds in raw materials that are not usually broken by animal's own enzymes, thus releasing more nutrients.

(iv) That enzymes are particularly beneficial to supplement the enzymes produced by young animals due to immaturity of their own digestive system, as endogenous enzymes production may be insufficient. Overall, Sheppy (2003) demonstrated that enzyme addition can reduce the variability in nutritive value between feed ingredients and improve the accuracy of feed formulations and diet utilization.

Another consideration from the previous experiment was the lack of noteworthy positive effect of the XAP enzyme mixture at 0.25g/kg inclusion rate. Therefore, an increased amount of this enzyme (twice the amount added in the previous experiment) was considered alongside another enzyme mixture (xylanase and β-glucanase) which may be of help to determine whether this could improve the utilization of wDDGS based diets by broilers in this experiment.

Table 5.1 summarises the effects of β-glucanase alone or in combination with xylanase enzyme in different substrates (cereal grains) on the performance of various species of poultry, which proved to be beneficial to poultry in terms of improved nutrient digestibility and/or performance of the birds.

Table 5.1 Effect of supplementation of poultry diets with beta-glucanase and xylanase on the performance of broiler chickens

Diet	Enzyme	Level of Inclusion	Species	Effects/Remarks	Reference
Barley	β-glucanase	0, 0.05, 0.1 & 0.5g/kg	Broilers	Improved FI, LW & FCR	Hesselman *et al.* (1982)
Barley	β-glucanase	0.5g/kg	Broilers	Improved BWG, FC, DM of S.I. digesta, and starch & nitrogen digestibility. β-glucans better degraded	(Hesselman and Åman, 1986)
Rye/Wheat	XG	0.11, 0.22, 0.44, 0.88g/kg	Broilers	BW increased by 27 & 15%, FI increased by 15 & 8%. Improved digestibility of OM, CP, & Starch.	(Pettersson and Åman, 1989)
Barley	β-glucanase	250g/kg	Broilers	BWG enhanced of growers & improved LW of 6-week old broilers	Yu *et al.* (1998)
Barley/Wheat	XG	1500XU, 500GU/kg	Broilers	In barley, β-glucanase reduced intestinal viscosity. In wheat, xylanase improved FE, & reduced intestinal viscosity as well	Esteve-Garcia *et al.* (1997)
Barley/Wheat	XAP, G & Cellulase	350g/tonne	Broilers	β-glucanase overall improved performance of birds fed barley	Leeson *et al.* (2000)
Rye	XG	20mg/kg	Broilers	BWG, FI, FE improved but decreased water intake. Increased villus size, villus height to crypt depth ratio & concentration of conjugated bile acids	Nejib *et al.* (2002)
Wheat/Barley	XG	20mg/kg of diet	Broilers	Improved digestibility of wheat & barley-based diets by reducing the viscosity of the intestine content & by impeding the growth of bacteria	Mathlouthi *et al.* 2002
SBM	β-glucanase, Protease, Hemicellulase, Pectinase	365g/kg	Broilers	Improved AMEn, reduced excreta moisture and improved ileal protein digestibility	Kocher *et al.* (2002)
Wheat/Barley/Rye	XG	0, 250, 1250, 2500mg/kg	Layers	Reduced intestinal viscosity, incidence of dirty eggs & gave higher nutrient digestibility	Lázaro *et al.* (2003)
Wheat/Barley	XG	560X IU & 2800IU/kg	Male Turkeys	BWG & FE improved by both enzymes	Mathlouthi *et al.* (2003)

Table 5.1 Effect of supplementation of poultry diets with beta-glucanase and xylanase on the performance of broiler chickens (continued)

Diet	Enzyme	Level of Inclusion	Species	Effects/Remarks	Reference
Wheat/Triticale	XG	0, 0.06% of diet	Broilers	AME about 10%	Shakouri and Kermanshahi (2003)
Wheat	XG	0,200, 400,660, 800,1000mg/kg	Broilers	Daily gain & FCR improved linearly with increasing levels of enzyme	Wang *et al.* 2005
Barley	β-glucanase	0, 0.5, 1.0 & 1.5g/kg	Broilers	Significant increase of ME over control	Eila *et al.* (2006)
Triticale/Rye/Wheat	β-glucanase	1g/kg feed	Broilers	Increased lactic acid concentration & lowered the pH of the crop contents	Jozefiak *et al.* (2007)
Barley/Wheat/Triticale	XAP, Glucanase, Pectinase, Cellulase	100, 250, 1000g/tonne	Layers	Improved egg quality of layers fed wheat-based diets	Roberts and Choct (2006)
Corn/Wheat/SBM	XG	200mg/kg	Layers	Reduced the negative influence of 20% inclusion of DDGS on laying rate & DW of eggs	Świątkiewicz and Koreleski (2006)
Corn/SBM	XG	0.1% of NSM diet	Layer Quails	Significant improvement of egg production & fertility rate	Attia *et al.* (2008)
Barley	XG & Protease	1kg/tonne	Broilers	Lowered the counts of enterobacteria, total gram +ve cocci	Torok *et al.* (2008)
Corn/Soy	XG	16000BXU/kg, 30000BU/kg	Broilers	Improved FCR & ileal nutrient digestibility (Combined effect was greater than single effect)	(Cowieson *et al.* (2010))

AMEn= Apparent Metabolisable Energy for nitrogen, BWG= Body Weight Gain, CP= Crude Protein, DG= Daily Gain, DM= Dry Matter, FC= Feed Conversion, FCR= Feed Conversion Ratio, FE= Feed Efficiency, FI= Feed Intake, LW= Live Weight, ME= Metabolisable Energy, NSM= Nigella Seed Meal, OM= Organic Matter, SDDGS= Sorghum Distillers Dried Grains with Solubles, SI= Small Intestine, XAP= Xylanase, Amylase and Protease, XG= Xylanase and β-glucane

The aforementioned observations on various effects of xylanase and β-glucanase combination on various parameters, apparently indicated that enzyme supplementation of cereal based diets can systematically improve the nutrient digestibility and performance of broilers. Thus, an enzyme mix of this type (Xylanase and β-glucanase mixture (XG)) was used as enzyme B in this experiment. Literature showed that the effect of the enzyme is dependent upon their type but also on their inclusion level (Kocher *et al*., 2002). They demonstrated the positive effect of a commercially prepared enzyme mixture containing β-glucanase included at higher dosage, when the previous lower but recommended dosage did not show an effect. Wang *et al*. (2005) also reported that enzyme supplementation (XG mixture) improved performance of broilers where daily gain and feed conversion increased linearly with increasing levels of enzyme supplementation. This experiment therefore, compared the effect of adding an increased level of the previously used enzyme (XAP as enzyme A) with another enzyme mixture (XG as enzyme B), each one incorporated at 0.50g/kg diet, on the utilization by broilers of isonitrogenous diets containing either 0 or 15% wDDGS.

The digestibility of wDDGS-based diets in broilers was measured by both total collection and AIA methods in experiments 1 and 2 of this thesis. In addition to these measurements, digestibility at both starter and finisher phases was also measured by the AIA method in order to test its repeatability, feasibility for digestibility studies in deep litter managed broilers and any possible variation between the two phases of broiler production. The study had two objectives as described below:

(1) To compare the effects of two enzymes on the performance, litter moisture, feed digestibility, gut microbial population and carcass characteristics including chemical and fatty acid composition of broiler meat.

(2) To determine the interaction between wDDGS levels and enzyme supplementation.

It is hypothesized that the nutritional value of wDDGS based diets can be enhanced by using enzyme additions to improve nutrient digestibility and thus improve performance of broiler chickens.

5.2 Materials and Methods

5.2.1 Study Site:

The study was conducted at the Cockle Park farm, Newcastle University between April and May 2013 under the ethical guidelines of the University for non-regulated procedures.

5.2.2 Experimental enzymes, wDDGS and Diets

5.2.2.1 Description and composition of enzymes used

The following description and composition of enzymes were declared by the supplier.

The enzyme A was a mixture of xylanase, amylase and protease and it was designed to increase the efficiency of wheat-based broiler, turkey and duck feeds. It is specifically developed for use in mixed-grain poultry diets, containing vegetable and other protein sources and some high fibre feeds. The enzymes were derived from *Bacillus subtilis* and *Trichoderma reesei* fermentation processes and mixed with wheat flour and calcium propionate as carriers and binders. This enzyme is recommended for its addition to prepared vitamin and mineral premixes that were then mixed at appropriate levels with different broiler feeds. Each gram of this enzyme was expected to have enzymic activities of 9200 U endo-1, 4-beta-xylanase, 16000 U of subtilisin as protease and 1600 U of alpha amylase.

The enzyme B was declared to be derived from *Trichoderma reesei* fermentation and it also contained variable amounts of sodium sulphate, poly-vinyl alcohol, starch, hydrated magnesium silicate and sucrose. Each g of this enzyme was declared to contain activities of 12200 U endo-1, 4-beta-xylanase, and 1520 U of endo-1, 3 (4) -beta-glucanase.

5.2.2.2 Source and Composition of wDDGS

The wDDGS used in the experiment were purchased from Trident Feeds of AB Agri. Ltd, Peterborough Business Park, England, while the wheat and soybean meal used in this experiment were obtained from North East Grains Ltd (Grain Merchants), Longhirst, Morpeth, Northumberland, UK.

Table 5.1 presents the proximate composition of wDDGS as declared by the supplier:

Table 5.2 Nutrient Composition of wDDGS

Nutrient	g/kg DM
Dry Matter (g/kg)	920
Crude Protein	350
Ether Extract	70
Neutral Detergent Fibre	320
Starch	20
Sugar	60
Metabolisable Energy (MJ/Kg)	13.7

5.2.2.3 Experimental diets

Three isonitrogenous diets were formulated by substituting wDDGS for coarsely ground wheat and soybean meal, allowing variation in metabolisable energy and fibre content. The diets were identified by combinations of numbers and letters depending upon the wDDGS levels and enzyme types, as described in Table 5.2. The ingredient and estimated chemical composition of these diets is presented in Table 5.3.

Table 5.3 Description of dietary treatments that were used in the broiler study

Diet	wDDGS inclusion (%)	Enzyme inclusion
A	0	No (NE0)
B	15	No (NE15)
C	0	Yes (EA0)
D	15	Yes (EA15)
E	0	Yes (EB0)
F	15	Yes (EB15)

NE = No Enzyme, EA= Enzyme A, EB= Enzyme

Table 5.4 Ingredient and estimated nutrient composition of broiler diets (kg/ tonne)

	DIETS (%wDDGS Inclusion)					
	0	15	0	15	0	15
	NE0	NE15	EA0	EA15	EB0	EB15
	A	B	C	D	E	F
Ingredients						
wDDGS	0	150	0	150	0	150
Wheat	647	575	646.5	574.5	646.5	574.5
Soybean meal	296	218	296	218	296	218
Soybean oil	10	10	10	10	10	10
Limestone	20	20	20	20	20	20
MCP	10	10	10	10	10	10
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Premix*	12.5	12.5	12.5	12.5	12.5	12.5
Methionine	1	1	1	1	1	1
Lysine	1	1	1	1	1	1
Enzyme	0	0	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000

Table 5.5 Ingredient and estimated nutrient composition of broiler diets (kg/ tonne) (continued)

	DIETS (%wDDGS Inclusion)					
	0	15	0	15	0	15
	NE0	NE15	EA0	EA15	EB0	EB15
	A	B	C	D	E	F
Estimated Analysis (g/kg) (unless otherwise stated)						
Crude Protein	226	223	226	223	226	223
ME (MJ/kg)	14	13	14	13	14	13
Starch	380.1	339.9	380.1	339.9	380.1	339.9
Crude Fibre	34	76	34	76	34	76
Ether Extract	76	67	76	67	76	67
Calcium	11	10	11	10	11	10
Phosphorus	5	6	5	6	5	6
Amino Acids						
Arginine	14.7	13.6	14.7	13.6	14.7	13.6
Histidine	6.1	5.9	6.1	5.9	6.1	5.9
Isoleusine	9.7	9.4	9.7	9.4	9.7	9.4
Leusine	17.3	17.2	17.3	17.2	17.3	17.2
Lysine	11.6	10.0	11.6	10.0	11.6	10.0
Methionine	4.5	4.3	4.5	4.3	4.5	4.3
Phenylanaline	11.7	11.6	11.7	11.6	11.7	11.6
Threonine	8.3	8.1	8.3	8.1	8.3	8.1
Tryptophan	3.1	2.9	3.1	2.9	3.1	2.9
Valine	10.8	10.8	10.8	10.8	10.8	10.8
Total AA	97.7	93.7	97.7	93.7	97.7	93.7

*NE = No Enzyme, EA= Enzyme A, EB= Enzyme B, ME = Metabolisable Energy, MCP= Monocalcium Phosphate, AA = Amino Acids *Premix of 12.5kg contained Vitamin A 12,500 IU, Vitamin D3 3,000iu, Vitamin E-150g, Vitamin K 3g, Vitamin B1 2g, Vitamin B2 8g, Vitamin B6 5g, Vitamin B12 30mg,Nicotinamide50g,D-cal Pan 10g,Folic Acid 2g,Biotin 150mg,Vitamin C 4g, Choline 300g, Copper (Copper Sulphate pentahydrate) 10g, Manganese (Manganese Sulphate monohydrate) 80g, Zinc (Zinc Oxide) 60g, Iron (Ferrous Sulphate monohydrate) 30g, Iodine (Calcium Iodate) 2g, Selenium (Sodium Selenite 0.25mg, Selenium rich yeast 0.08mg), Molybdenum (Sodium molybdate) 0.20mg, Calcium (30%), Sodium chloride (8%)*

Before the feeding of the experimental diets was started, there was a habituation period during which starter crumbs were offered *ad libitum* alongside fresh clean water for 3 days to the chicks. The purpose of feeding crumbs to the chicks was to avoid starvation during the initial adaptation of birds to the pens. Also this was done to ensure that the feed quality was not a limiting factor in the first 72 hours of their life.

5.2.2.4 Experimental birds and management

One hundred and eighty day-old Ross broiler chicks, vaccinated at the hatchery against Infectious Bronchitis, were used for the experiment which lasted for 6 weeks (day 3 to day 45 of the birds' age). After arrival at the experimental site, the chicks were fed a commercial starter diet as crumbs of the same source and composition as described in chapter 4 section 4.2.3 for 2 days before initial weighing and allocation to the experimental diets. On day 3, the chicks were individually weighed; wing tagged and assigned to 6 groups (dietary treatments) of 30 chicks per treatment. Each group was further divided into 5 sub-groups of 6 birds per replicate. The bird groups as replicates were housed in floor pens of 1 m^2 per pen covered with wood shavings. Each pen was equipped with a plastic feeder and plastic drinker. The diets and clean drinking water were provided *ad libitum* to these birds.

The overall mean house temperatures were maintained within the recommended range of 18.9^oC minimum and 23.0^oC maximum. Ambient temperature was recorded daily and it was 22^oC on day 3, and gradually increased to 24^oC by day 45 due to adjustment in setting of brooder lamps of the pens i.e. drawn up as the birds grew older which eventually changed the room temperature.

Table 5.6 Mean weekly house Temperatures during 6 weeks of broiler study

	Average weekly Temperature (^oC)	
	Maximum	Minimum
Day		
7	22	19
14	20	16
21	25	21
28	24	19
35	23	19
42	24	20
Overall	23	19

5.2.3 Experimental Design

This study was conducted as a completely randomized design with a 2 x 3 factorial arrangement comprising 2 wDDGS levels of 0 and 15% and 3 enzyme levels of no enzyme (NE), + enzyme A (EA) and + enzyme B (EB). Each of the 6 dietary treatments (Table 5.3 & 5.4) was then allocated to 5 groups of birds in pens as replicates. The diet allocation was carefully performed to ensure pens were located in all sides of the room for each diet.

5.2.3.1 Feeding routines and measurements of feed Intake and live weight

Feed intake, live weight, and feed conversion ratio as well as nutrient digestibility and carcass composition formed the major response criteria for treatments i.e. major parameters measured. These measurements were performed as described in section 3.2.6 of chapter 3. Feed intake, water intake, live weight, diet digestibility, faecal moisture, were measured weekly. Feed conversion ratio (FCR) was calculated after measurement of feed intake and live weight gain. Water intake was measured for 5 days in every week which commenced from day 21 of the experiment when the variation in water consumption of the birds became apparent and measurable. Microbes and carcass characteristics of the birds were also measured after the experiment.

5.2.3.2 Measurement of litter moisture content

This was performed as described in chapter 3 section 3.2.8.

5.2.3.3 Carcass measurements

At 45 days of age, 10 birds per treatment (2 per replicate) were selected based on their uniform live weights across the pens, sacrificed, processed, weighed and dissected to study the carcass characteristics and organ weights as described in chapter 3 section 3.2.10. The cold carcass weight expressed includes the weights of internal organs.
The birds caged for an additional one week after the feeding trial, being used for the digestibility measurements, were also sacrificed and processed to monitor possible changes in carcass composition.

5.2.3.4 Measurement of digestibility

This was performed for both caged and penned birds by using methods of total excreta collection and AIA respectively, as described in chapter 3 section 3.2.9.

5.2.3.5 Collection of feed samples

Feed samples of about 30g were collected in labelled plastic bags daily from the feed bags and stored in the laboratory at room temperature for 23 days and then pooled to obtain a weekly representative sample of each experimental diet.

5.2.4 Chemical analysis

Feed samples of each of the 6 experimental diets, a sample of the wDDGS as well as meat samples of breast, thigh and drumstick were analysed for proximate composition (AOAC, 2005) as well as fatty acid composition of breast muscles. The crude protein, dry matter, ether extract, ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), calcium and phosphorus contents of the said samples were determined as described in chapters 3 and 4 sections 3.2.12 and 4.3.6.4.

Feed samples for the first 3 weeks were further pooled to obtain the representative sample of starter period and that of week 4-6 to obtain samples of finisher period. The representative samples were ground and analysed to determine the possible similarity or variation due to storage for the period of the trial.

5.2.4.1 Determination of acid insoluble ash (AIA)

Samples of excreta were collected daily from metabolism cages (total collection) and weekly from the pens during the feeding trial and put into labelled polythene bags and stored at -4^{o}C until analysis. Daily collections from each cage and weekly collections from each pen were pooled into starter and finisher phases and analysed to compare the digestibility of each diet at these feeding phases. The same procedure as described in chapter 3 (Section 3.2.11) was applied to determine the digestibility by AIA method.

5.2.4.2 Determination of chemical composition of muscles

This was performed as described in chapter 4 section 4.3.6.

Plate 5.1 Sample of ground broiler meat

5.2.4.3 Determination of fatty acid composition of breast muscles

The chemicals used for the determination of fatty acid composition as well as the sample preparation procedures were the same as described in chapter 4 section 4.3.6.4.

5.2.5 Determination of intestinal microbial population

5.2.5.1 Collection of intestinal digesta sample

While processing representative samples of birds at the end of the feeding trial, a sample of intestinal digesta from the distal end of the duodenum to the ileo-caecal junction was collected under sterile conditions and homogenised. The digesta samples were immediately mixed with phosphate buffer saline (PBS) in plastic containers which were sealed and stored at room temperature to make a stable environment for the microbes before their examination in suitable agar plates.

5.2.5.2 Examining intestinal digesta for microbial enumeration

Serial dilutions of digesta samples were made in a saline solution (9g of sodium chloride dissolved in 1 litre of distilled water) for microbial enumeration. The initial dilution in the saline solution was used as a microbial source for serial dilutions to enumerate bacterial populations. Total bacterial count was determined by a modified standard plate method as reported by Reynolds and Farinha (2005).

Using aseptic techniques, the initial dilution was made by transferring 0.2ml of each digesta sample to a 9.8ml sterile saline solution in a labelled test tube to prepare a 10^{-2}dilution which was then shaken (vortexed) to break up digesta lumps through homogenisation. This mixture was then used to prepare further dilutions of 10^{-4}, 10^{-6} and 10^{-8} by using fresh saline solutions. About 20ml of sterile nutrient agar containing 0.5% peptone, 0.3% yeast extract, 1.5% agar and 0.5% sodium chloride was poured aseptically into each petri plate and cooled so that the agar was hardened. All the tubes, petri dishes, agar, etc. were autoclaved at 121^{o}C and 105kPa pressure for 30 minutes before their inoculation with the diluted digesta samples. The petri dishes containing the agar were inoculated with 0.2 ml of diluted digesta samples, inverted and incubated at 37^{o}C for up to 48 hours. The petri dishes were regularly checked for the microbial growth as colonies until no further growth was visible. At the end of the incubation period, colonies on each plate were counted visually and recorded.

5.2.6 Calculations and statistical analysis

Data collected were entered in an excel spreadsheet and calculations were performed to determine the average feed intake, LWG, and FCR. The pattern of live weight and feed intake per bird was also monitored over 42 days. These data, and that of digestibility by AIA method, were also analysed separately for the starter, finisher and overall phases respectively. Calculation for the bacterial count was performed to obtain number of colony forming units per millilitre of sample by dividing the CFUs by the amount of sample plated and multiplying by the dilution factor by using the following equation:

$$\#\ \text{of bacteria per ml} = \frac{\text{CFU}}{\text{amount plated}}\ x\ \text{dilution.}$$

The 10^{-8} dilutions were not included in the statistical analysis because of too many zero, or too few colonies, counting plates.

The collected data were subjected to the analysis of variance (ANOVA) general linear model (GLM) by using the Minitab 16 statistical package to study the effect of wDDGS level, enzyme inclusion and their interaction on water intake, litter moisture,

digestibility, carcass and digestive organs characteristics as well as muscle chemical composition for significance at $P<0.05$ for the total period. Similarly, the data for CFU were analysed in the same way to test the effects of wDDGS levels and enzyme inclusion on CFUs as well as the interaction of diet x enzyme x dilution. The data were tested for normality by using the Anderson Darling test, with significance set at $P<0.05$. As the CFU data were not normally distributed, a log transformation before the statistical analysis was performed on this data set. Where the effect of a factor was significant for more than 2 treatments, means of individual treatments were compared for significance ($P<0.05$) using the Tukey's test.

5.3 Results

5.3.1 Chemical compositions of major ingredients and experimental diets

The analysed chemical compositions of major ingredients and experimental diets are presented in Tables 5.7 and 5.8 respectively. The CP content of wDDGS was 281g, which was lower than the level found in SBM (421g). The ether extract, NDF and ADF contents of wDDGS were much higher than those of SBM. All diets had approximately the same CP content, while the EE and NDF contents were increased at 15% inclusion level of wDDGS in the diet which is a true reflection of the presence of higher fibre and oil content in wDDGS. Table 5.9 presents the analysed values of both starter and finisher diets that were quite comparable to each other.

Table 5.7 Nutrient composition of the major ingredients used in diet formulation

Nutrients (g/kg DM)	Wheat	Soybean meal	wDDGS
Dry Matter (g/kg)	894	900	931
Crude Protein	90	421	281
Ether Extract	15	17	34
Neutral Detergent Fibre	162	119	441
Acid Detergent Fibre	25	60	95
Ash	15	79	50
Calcium	4	3	2
Phosphorus	7	9	6

Table 5.8 Nutrient composition of the broiler diets

	DIETS (DM Basis)					
	wDDGS (%)					
Nutrients (g/kg DM)	**0NE**	**15NE**	**0EA**	**15EA**	**0EB**	**15EB**
Dry Matter (g/kg)	875	882	875	878	878	882
Crude Protein	219	207	213	217	215	201
Ether Extract	26	29	26	29	25	29
Ash	61	74	71	59	62	63
Neutral Detergent Fibre	128	178	120	180	115	182
Acid Detergent Fibre	42	61	48	69	44	63
Calcium	12	13	13	12	14	13
Phosphorus	6	6	7	6	7	7

Table 5.9 Nutrients composition of starter and finisher period diets

DIETS												
	Starter						Finisher					
Diet ID	**0NE**	**15NE**	**0EA**	**15EA**	**0EA**	**15EB**	**0NE**	**15NE**	**0EA**	**15EA**	**0EA**	**15EB**
Nutrients (g/kg DM)												
Dry Matter (g/kg)	876	881	875	879	878	879	878	881	874	879	877	882
Crude Protein	194	189	197	199	182	202	193	194	191	187	189	206
Ether Extract	25	27	25	28	22	27	25	24	26	29	25	28
Neutral Detergent Fibre	125	185	127	162	120	165	123	181	136	162	122	171
Acid Detergent Fibre	48	52	38	69	42	60	49	51	38	61	41	62
Ash	61	73	72	59	62	63	61	72	72	60	62	63
Calcium	13	14	15	13	13	13	14	14	16	15	13	14
Phosphorus	22	21	21	21	21	20	22	19	21	21	22	21

0 and 15 represent the % wDDGS inclusion levels in the diets whereas NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

The estimated AA concentration of the experimental diets is shown in Table 5.5. It appeared that the concentration of all the AAs were slightly higher than the NRC 1994 recommended level of growing birds. Diets supplemented with enzymes A and B contained slightly lower concentrations of arginine, isoleucine and methionine than no wDDGS diets. The concentration of histidine, leucine, phenylalanine, threonine, tryptophan and valine was identical in all the diets. The lysine content of the diets ranged from 10 to 12g. The methionine concentration was slightly lower in 15% wDDGS diets than the no wDDGS diets.

5.3.2 Growth performance of broilers

The main effects of wDDGS and enzyme inclusion (Table 5.10) on performance of broiler chickens during the starter phase (1-21 days of age) showed no significant (P>0.05) treatment differences in feed intake, live weight gain and FCR.

Table 5.10 Means for the main effects of wDDGS and enzyme on performance of broilers fed the experimental diets during the starter phase

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Parameters									
FI (g/b/d)	72	73	2.0	0.796	72	72	74	2.4	0.894
LWG (g/b)	42	40	0.9	0.174	41	43	40	1.1	0.165
FCR	1.73	1.82	0.046	0.161	1.77	1.69	1.86	0.056	0.148

FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio, SEM = Standard Error of Means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

The interaction of wDDGS levels and enzyme inclusion on performance of broilers in the starter phase is presented in Table 5.11. There was no significant interaction of wDDGS and enzyme inclusion on feed intake, live weight gain and FCR.

Table 5.11 Means for the effect of wDDGS x enzyme on performance of broilers fed the experimental diets during the starter phase

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	wDDGS (%)							
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Parameters								
FI (g/b/d)	71	74	70	74	76	72	3.5	0.504
LWG (g/b)	42	40	42	43	42	38	1.5	0.285
FCR	1.69	1.85	1.67	1.72	1.81	1.90	0.079	0.787

FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio, SEM = Standard Error of Means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

The main effects of wDDGS and enzyme inclusion (Table 5.12) on performance of broiler chickens during the finisher phase (21-42 days of age) showed feed intake was not affected (P>0.05) by wDDGS levels, while live weight gain of birds and FCR were significantly reduced at 15% wDDGS level. There were no significant effects of enzyme inclusion.

Table 5.12 Means for the main effects of wDDGS and enzyme on performance of broilers fed the experimental diets during the finisher phase

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Parameters									
FI (g/b/d)	179	187	3.7	0.111	186	187	176	4.5	0.185
LWG (g/b)	102	93	2.1	0.007	98	101	94	2.6	0.131
FCR	1.75[b]	2.02[a]	0.029	0.001	1.90	1.85	1.89	0.035	0.631

a, b Means bearing different letters within rows are significantly different (P<0.05)
FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio,
NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

Table 5.13 presents the interaction of wDDGS levels x enzyme inclusion on growth performance of broilers in the finisher phase. There were no significant (P>0.05) interactions in all the parameters measured.

Table 5.13 Means for the effects of wDDGS x enzyme on performance of broilers fed the experimental diets during the finisher phase

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15EA	0 EB	15 EB	SEM	P value
Parameters								
FI (g/b/d)	183	188	178	197	175	177	6.3	0.380
LWG (g/b)	102	94	103	100	101	87	3.7	0.350
FCR	1.79	2.02	1.73	1.98	1.73	2.06	0.050	0.631

FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio, SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

The main effects of wDDGS and enzyme inclusion on the overall growth performance of broilers are presented in Table 5.14 and Fig.5.1. Birds live weight consistently and steadily increased with increasing age through 42 days. Birds in all treatments gained almost similar weights. No significant difference for feed intake was seen, however, lower growth rate, and poorer FCR were observed with 15% wDDGS diet. There was also a significant (P<0.05) enzyme effect on live weight gain of birds. Addition of EA significantly increased LWG compared to EB but not significantly different from the control (NE) while addition of EB was slightly lower than NE (Table 5.14).

Table 5.14 Means for the main effects of wDDGS and enzyme on the performance of broilers fed the experimental diets for 42 days

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Parameters									
FI (g/b/d)	124	129	2.43	0.251	127	128	124	3.0	0.597
LWG (g/b)	72	67	1.05	0.002	70^{ab}	72^{a}	67^{b}	1.3	0.026
FCR	1.73^{b}	1.93^{a}	0.026	0.001	1.83	1.78	1.87	0.032	0.192

a, b Means bearing different letters within rows are significantly different (P<0.05)
FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio,
SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

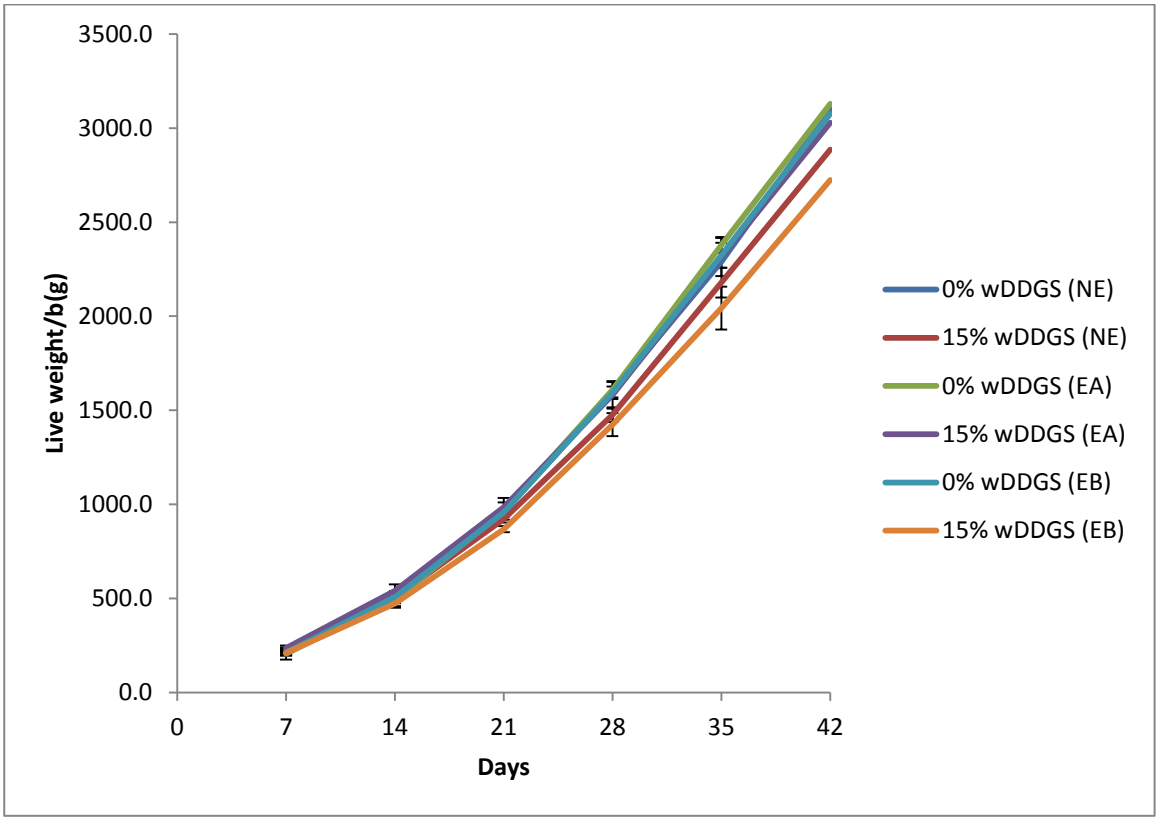


Figure 5.1 Illustration of change in mean birds live weight during 42 days of broiler study

The interaction effect of wDDGS x enzyme inclusion was not significant for feed consumption, live weight gain and FCR (Table 5.15), meaning that the two variables acted independently on the growth performance of broiler chickens throughout the experiment.

Table 5.15 Means for the effect of wDDGS x enzyme on performance of broilers fed the experimental diets for 42 days

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Parameters								
FI (g/b/d)	125	130	124	132	125	123	4.2	0.537
LWG (g/b)	72	67	73	71	71	62	1.8	0.117
FCR	1.73	1.94	1.71	1.86	1.75	1.99	0.046	0.561

FI = Feed Intake, LWG = Live Weight Gain, FCR = Feed Conversion Ratio, SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

Table 5.16 presents the main effects of wDDGS and enzyme inclusion on weekly total live weight of broiler chickens during the 42-day experiment. It showed no significant (P>0.05) treatment differences in live weight in days 7-21. However, there were significant (P<0.05) effects of wDDGS at days 28, 35 and 42 where birds on 0% wDDGS gained more weight than birds on 15% wDDGS. There were no significant (P>0.05) effects of enzyme addition on the live weight of birds in days 7, 14, 21, 28 and 42. Birds supplemented with enzyme A presented significantly higher (P<0.05) live weight than birds supplemented with enzyme B at day 35 though similar to birds on no enzyme treatment.

Table 5.16 Means for the main effects of wDDGS and enzyme on bird weekly live weight (g)

	DIETS								
	wDDGS				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Days									
7	211	223	5.4	0.106	220	220	211	6.6	0.591
14	501	506	11.8	0.769	503	517	489	14.5	0.409
21	960	924	18.7	0.188	940	974	913	22.9	0.189
28	1599^{a}	1494^{b}	20.6	0.001	1536	1595	1508	25.3	0.062
35	2331^{a}	2179^{b}	29.0	0.001	2234^{ab}	2347^{a}	2183^{b}	35.5	0.010
42	3102^{a}	2879^{b}	46.0	0.002	2993	3079	2900	55.8	0.098

a,b Means bearing different letters within rows are significantly different (P<0.05) SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

No significant (P>0.05) interaction (Table 5.17) was observed on weekly live weight of birds throughout the experimental period.

Table 5.17 Means for the interaction effect of wDDGS x enzyme on bird weekly live weight (g)

	Enzyme Inclusion							
	No Enzyme			+Enzyme				
	NE	EA	EB	NE	EA	EB	SEM	P value
Days								
7	215	204	213	225	235	210	9.3	0.219
14	502	494	506	505	540	472	20.4	0.176
21	959	964	958	921	984	868	32.3	0.253
28	1595	1609	1593	1476	1582	1423	35.7	0.149
35	2289	2379	2324	2179	2315	2042	50.1	0.094
42	3100	3129	3076	2885	3028	2724	78.9	0.299

SEM=Standard Error of means, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B.

The result of the main effects of wDDGS and enzyme on water intake of broilers is presented in Table 5.18. It showed that there were no significant (P>0.05) effects of wDDGS and enzyme addition on the water intake of broilers throughout the experiment.

Table 5.18 Means for the main effects of wDDGS and enzyme on water intake (ml/b/d)

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Days									
21	394	366	11.9	0.111	382	384	372	14.6	0.826
28	495	498	13.6	0.868	497	515	477	16.7	0.288
35	537	544	13.1	0.713	538	557	526	16.0	0.413
42	561	543	15.7	0.435	551	552	553	19.2	0.998
Overall	462	447	10.9	0.334	455	463	445	13.4	0.649

SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

Table 5.19 shows the means for the interaction effect of wDDGS and enzyme on the water intake of broilers. No significant (P>0.05) interaction was observed throughout the experimental period.

Table 5.19 Means for the effect of wDDGS x enzyme on water intake (ml/b/d)

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Days								
21	394	371	379	390	408	336	20.7	0.149
28	490	504	498	532	496	457	23.6	0.291
35	523	553	550	563	538	515	22.7	0.497
42	566	536	581	525	535	568	27.1	0.267
Overall	460	451	461	465	466	425	18.9	0.478

SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

Means for the main effects of wDDGS and enzyme on litter moisture are presented in Table 5.20. The results show that there was significantly (P<0.05) lower moisture content at 15% wDDGS than 0% wDDGS at the first seven days, day 28 and day 42. Enzyme inclusion also reduced the moisture content on day 1-7 and 28. Enzyme B presented the least moisture content, while enzyme A presented the least moisture in day 35.

Table 5.20 Means for the main effects of wDDGS and enzyme on litter moisture

	DIETS								
	wDDGS (%)				Enzymes				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Days									
7	24	18	1.9	0.046	27^{a}	19^{b}	17^{b}	2.3	0.009
14	26	26	2.1	0.883	26	24	28	2.6	0.434
21	41	39	0.6	0.052	39	41	40	0.8	0.201
28	35	30	1.4	0.028	34^{a}	36^{a}	27^{b}	1.7	0.004
35	37	35	1.0	0.189	38^{a}	32^{b}	37^{ab}	1.3	0.015
42	35	23	1.3	0.001	34	29	24	1.6	0.445

a,b Means bearing different letters within rows are significantly different (P<0.05)
SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B

There were mainly no significant interactions of wDDGS and enzyme on the litter moisture content (Table 5.21), except in days 35 where enzyme A presented lowest litter moisture at 0% wDDGS while enzyme B presented the lowest moisture at 15% wDDGS level.

Table 5.21 Means for the interaction effects of wDDGS x enzyme on litter moisture

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Days								
7	29^{a}	26^{ab}	23^{ab}	14^{b}	20^{ab}	15^{b}	3.2	0.716
14	24	29	26	22	28	29	3.7	0.561
21	39	38	42	40	41	39	1.1	0.873
28	36^{a}	33^{a}	36^{a}	36^{a}	33^{a}	22^{b}	2.4	0.103
35	38^{ab}	38^{ab}	30^{b}	35^{ab}	42^{a}	32^{b}	1.8	0.001
42	40^{a}	29^{bc}	37^{ab}	21^{cd}	30^{bc}	18^{d}	2.2	0.445

a,b,c,d Means bearing different letters within rows are significantly different (P<0.05)
SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

5.3.3 The effects of different levels of wDDGS and enzyme inclusion on nutrient digestibility of broiler chickens determined by total collection method

Table 5.22 shows the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets. The protein and phosphorus digestibility values were significantly higher in control than the 15% wDDGS inclusion, while fibre and calcium digestibilities were higher in the 15% DDGS diets. Ether extract digestibility was significantly higher with enzyme B than enzyme A (Fig.5.3). Similarly, enzyme B supplementation also improved the digestibility of calcium and phosphorus (Fig.5.4 and 5.5).

Table 5.22 Means for the main effects of wDDGS and enzyme on nutrient digestibility of the diets

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Digestibility (g/kg)									
Protein	556	510	11.7	0.011	531	528	542	14.4	0.766
Ether Extract	799	801	10.4	0.924	796^{ab}	773^{b}	832^{a}	12.7	0.011
NDF	387	477	15.0	0.001	426	431	439	18.3	0.884
ADF	82	157	8.4	0.001	131	120	107	10.3	0.264
Calcium	297	331	9.3	0.016	304^{b}	292^{b}	346^{a}	11.4	0.007
Phosphorus	383	356	9.8	0.001	328^{b}	350^{b}	431^{a}	9.8	0.001

a, b Means bearing different letters within rows for enzyme inclusion are significantly different (P<0.05). SEM =Standard Error of means, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B. NDF = Neutral detergent fibre, ADF = Acid Detergent Fibre

The means for the wDDGS x Enzyme interaction for nutrient digestibility of the diets is presented in Table 5.23. There were no significant (P>0.05) interaction effects on most of the parameters measured, though mineral digestibility showed different diet response patterns to enzyme supplementation.

Table 5.23 Means for the wDDGS x enzyme on nutrient digestibility of the diets

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Digestibility (g/kg)								
Protein	547	514	572	484	550	534	20.3	0.201
Ether Extract	801	790	790	755	806	857	17.9	0.066
NDF	381	471	378	483	401	477	25.9	0.856
ADF	82	180	81	159	82	132	14.5	0.277
Calcium	252^{c}	356^{ab}	337^{ab}	247^{c}	302^{bc}	390^{a}	16.1	0.001
Phosphorus	373^{bc}	282^{d}	350^{c}	350^{c}	427^{ab}	436^{a}	13.8	0.002

a,b,c, Means bearing different letters within rows are significantly different (P<0.05) SEM=Standard Error of means, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B, NDF = Neutral detergent fibre, ADF = Acid Detergent Fibre

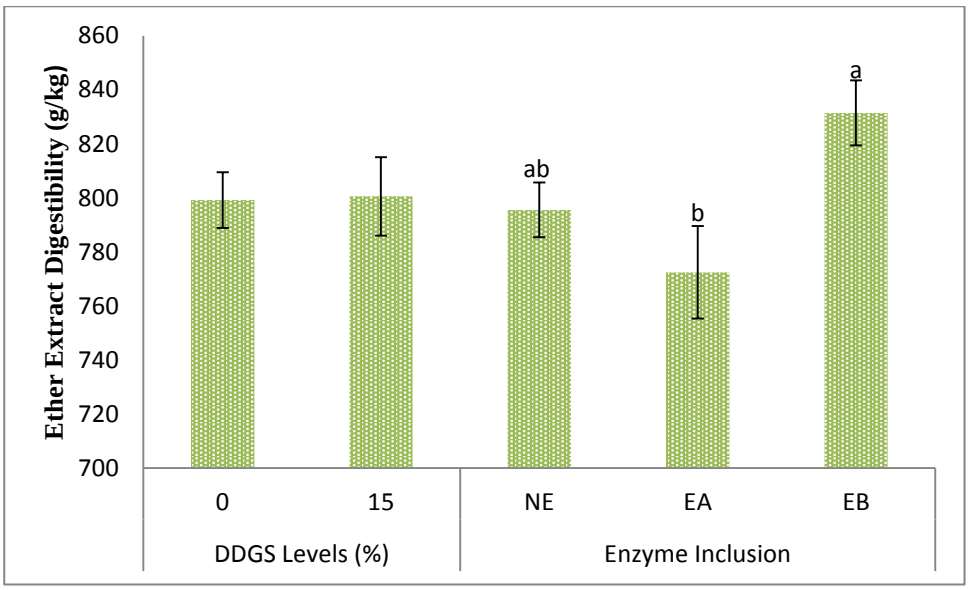


Figure 5.2 Effect of wDDGS and enzyme addition on ether extract digestibility

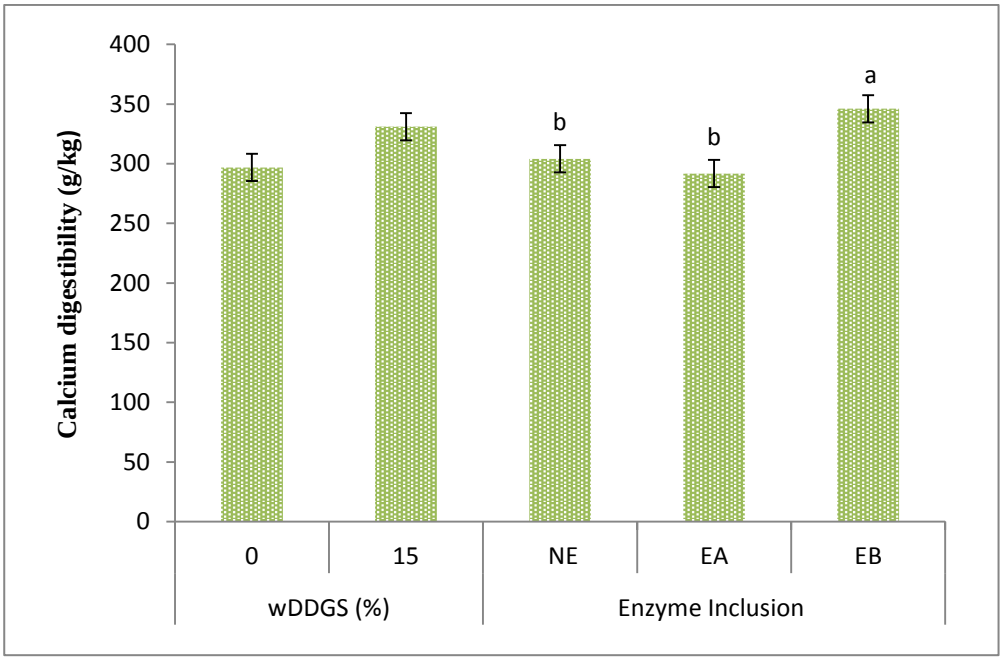


Figure 5.3 Effect of wDDGS and enzyme addition on calcium digestibility

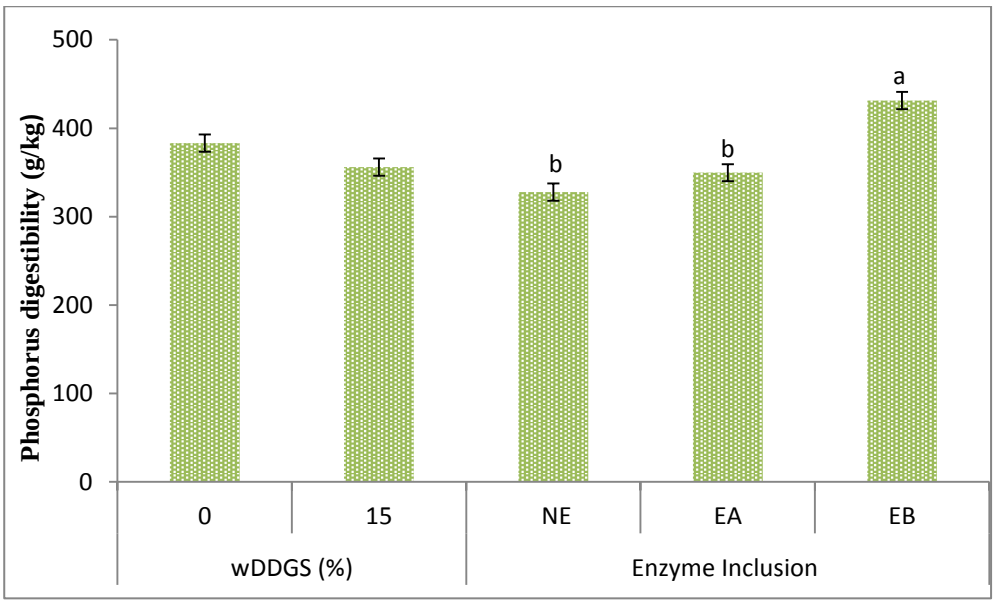


Figure 5.4 Effect of wDDGS and enzyme addition on phosphorus digestibility

5.3.4 The effects of different levels of wDDGS and enzyme inclusion on nutrient digestibility of broiler chickens determined by the AIA method

Table 5.24 shows the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets as determined by the AIA method. Protein, ether extracts and phosphorus digestibilities were not affected by wDDGS inclusion. However, 15% wDDGS had significantly higher NDF, ADF, and calcium digestibilities than 0% wDDGS, as found using the total collection method. Digestibility of ether extract was significantly better with enzyme B than enzyme A, while phosphorus digestibility was significantly better with enzyme B than for the no enzyme treatment.

Table 5.24 Means for the main effects of wDDGS and enzyme on nutrient digestibility of the diets by AIA Method

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	+EA	+EB	SEM	P value
Digestibility (g/kg)									
Protein	463	431	20.7	0.288	500	340	441	25.4	0.033
Ether Extract	787	771	10.9	0.305	783^{ab}	751^{b}	802^{a}	13.4	0.040
NDF	323	434	25.1	0.005	421	317	398	30.7	0.059
ADF	235	318	25.8	0.033	303	269	257	31.6	0.572
Calcium	287	365	23.0	0.026	317	293	368	28.2	0.184
Phosphorus	369	343	18.4	0.336	323^{b}	339^{ab}	406^{a}	22.6	0.035

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means. NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

The effect of wDDGS x enzyme interaction on nutrient digestibility of the diets as determined by AIA method is presented in Table 5.25. It showed that there were no significant (P>0.05) interaction effects on ADF and phosphorus digestibility. However, there were significant (P<0.05) interactions in protein, ether extract, NDF and calcium digestibilities where supplementation of 15% wDDGS with enzyme B gave better improvement than with enzyme A.

Table 5.25 Means for the wDDGS x enzyme on nutrient digestibility of the diets by AIA method

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Digestibility (g/kg)								
Protein	536^{a}	464^{abc}	485^{ab}	448^{c}	368^{bc}	515^{ab}	35.9	0.001
Ether Extract	783	782	779	723	798	806	19.0	0.208
NDF	402^{abc}	441^{ab}	330^{bc}	304^{bc}	239^{c}	557^{a}	43.4	0.001
ADF	291	316	215	323	199	314	44.7	0.541
Calcium	324^{ab}	311^{ab}	283^{b}	304^{b}	255^{b}	480^{a}	39.9	0.013
Phosphorus	367	278	338	340	401	412	31.9	0.249

a, b, c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means. NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

Table 5.26 shows the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets during the starter phase as determined by AIA method. The ether extract and fibre (NDF and ADF) digestibility were significantly higher for 15% wDDGS inclusion than 0% wDDGS. Protein digestibility was higher in NE than enzyme A and enzyme B supplementation. Ether extract digestibility was significantly better with enzyme B than enzyme A, though similar to 0% wDDGS. Calcium and phosphorus digestibilities were not affected by either wDDGS or enzyme addition.

Table 5.26 Means for the main effects of wDDGS and enzyme on nutrient digestibility of the diets by AIA method for the starter phase

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Digestibility (g/kg)									
Protein	539	537	19.7	0.925	610[a]	501[b]	503[b]	24.2	0.005
EE	315	444	33.9	0.013	413[ab]	283[b]	444[a]	41.6	0.026
NDF	247	370	21.2	0.001	368[a]	264[b]	293[ab]	26.0	0.027
ADF	197	295	19.0	0.001	264	242	232	23.3	0.615
Calcium	256	304	25.2	0.190	260	317	263	30.8	0.361
Phosphorus	203	257	27.1	0.062	284	214	193	27.1	0.062

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), NE = No Enzyme, EA= Enzyme A, EB= Enzyme B, SEM= Standard Error of means, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, EE = Ether Extract

The interaction effects of wDDGS x enzyme on nutrient digestibility of the diets during the starter phase, as determined by AIA method, are presented in Table 5.27. It showed that there were no significant (P>0.05) interaction effects on fibre (NDF and ADF), as well as calcium and phosphorus digestibilities. However, protein and ether extract digestibilities were significantly higher in 15% wDDGS with enzyme B than enzyme A, and similar to 0% wDDGS with no enzyme.

Table 5.27 Means for the wDDGS x enzyme on nutrient digestibility of the diets by AIA method for the starter phase

	Enzyme Inclusion							
	No Enzyme			+ Enzyme				
Digestibility (g/kg)	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Protein	627[a]	593[a]	601[a]	401[b]	390[b]	616[a]	34.2	0.001
EE	374[ab]	451[ab]	300[b]	265[b]	271[b]	617[a]	58.8	0.011
NDF	318	417	245	284	179	408	36.8	0.047
ADF	184	343	221	263	185	278	32.9	0.228
Calcium	232	289	322	312	215	312	43.6	0.466
Phosphorus	260	308	204	224	146	240	38.3	0.618

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means, EE= Ether Extract, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

Table 5.28 shows the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets for the finisher phase as determined by the AIA method. NDF and calcium digestibility were significantly higher at 15% wDDGS inclusion than 0% wDDGS. Protein, ether extract, as well as NDF digestibility were reduced by enzyme A. Enzyme supplementation did not improve the digestibility of calcium and phosphorus in this case.

Table 5.28 Means for the main effects of wDDGS and enzyme on nutrient digestibility of the diets by AIA method for the finisher phase

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Digestibility (g/kg)									
Protein	589	579	23.3	0.761	637[a]	520[b]	594[ab]	28.5	0.025
Ether Extract	416	483	29.3	0.116	483[a]	356[b]	510[a]	35.9	0.013
NDF	276	429	23.5	0.001	396[a]	283[b]	377[ab]	28.8	0.023
ADF	215	269	21.6	0.091	276	248	202	26.4	0.156
Calcium	232	320	20.8	0.006	279	240	310	25.5	0.178
Phosphorus	230	277	19.6	0.100	263	237	261	24.0	0.696

a,b Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means, NDF=Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

The effect of wDDGS x enzyme interaction on nutrient digestibility of the diets for the finisher phase as determined by the AIA method is presented in Table 5.29. It showed that there was no significant (P>0.05) interaction effect on ADF, calcium and phosphorus digestibility measured.

However, protein, EE and NDF digestibility were significantly (P<0.05) better at 15% wDDGS when supplemented with enzyme B than enzyme A, and similar to 0% wDDGS.

Table 5.29 Means for the wDDGS x enzyme on nutrient digestibility of the diets by AIA method for the finisher phase

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Digestibility (g/kg)								
Protein	647^{ab}	627^{ab}	622^{ab}	418^{c}	498^{bc}	691^{a}	40.3	0.001
Ether Extract	473^{ab}	493^{ab}	421^{b}	291^{b}	354^{b}	666^{a}	50.8	0.001
NDF	340^{bc}	452^{ab}	260^{c}	306^{bc}	227^{c}	528^{a}	40.8	0.013
ADF	264	289	213	283	169	235	37.4	0.800
Calcium	262	296	181	300	254	365	36.0	0.445
Phosphorus	251	275	216	258	224	299	33.9	0.752

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM= Standard Error of means, NDF= Neutral Detergent Fibre, ADF= Acid Detergent Fibre, NE = No Enzyme, EA= Enzyme A, EB= Enzyme B.

5.3.5 The effects of different levels of wDDGS and enzyme inclusion on carcass characteristics and digestive organs

The effects of different levels of wDDGS and enzyme inclusion on carcass characteristics and digestive organs are shown in Table 5.30. There was no significant (P>0.05) effect on cold carcass weight either for different levels of wDDGS or for enzyme inclusion. Inclusion of 15% wDDGS significantly (P<0.05) reduced the weight of breast with bone and increased the proportional weight of liver and gizzard. Meanwhile, enzyme B addition, significantly (P<0.05) increased the dressing percent and liver weight compared with no enzyme.

Table 5.30 Means for the main effects of wDDGS and enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (45 days of age)

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Parameters (%)*									
FLW(g)	3150	2956	70.8	0.064	3075	3141	2944	86.8	0.283
Cold Carcass (g)	2848	2751	69.2	0.335	2825	2816	2759	84.7	0.836
Dressing percent	70	72	0.9	0.144	69^{b}	71^{ab}	74^{a}	1.10	0.010
Major Carcass Components									
Breast + Bone	33	31	0.57	0.026	32	32	32	0.7	0.945
Thighs	21	22	0.5	0.154	21	22	22	0.6	0.890
Drumsticks	10	10	0.2	0.925	10	10	11	0.2	0.889
Neck & Back	15	16	0.3	0.076	16	16	16	0.3	0.330
Wings	7	7	0.2	0.325	7	7	7	0.2	0.867
Edible Organ Components									
Liver	2.8	3.2	0.05	0.023	2.8^{b}	3.2^{ab}	3.1^{a}	0.06	0.025
Heart	0.74	0.63	0.073	0.287	0.74	0.66	0.64	0.089	0.699
Empty Gizzard	1.24	1.51	0.041	0.001	1.37	1.40	1.36	0.050	0.871
Non-Edible Components									
Total Gut	6.7	6.8	0.26	0.856	7.4	6.4	6.4	0.32	0.086
Empty Crop	0.54	0.59	0.072	0.640	0.51	0.47	0.72	0.088	0.134
Proventriculus	0.44	0.50	0.027	0.101	0.50	0.45	0.50	0.033	0.605

a,b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means,
**unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B, FLW= Final Live Weight*

The wDDGS levels x enzyme interaction (Table 5.31) had no significant (P>0.05) effect on the dressing percent, cold carcass weight, or proportional weight of breast with bone as well as thighs weight.

However, there was a significant (P<0.05) wDDGS level x enzyme interaction for the liver and empty crop weight, although following any consistent trend.

Table 5.31 Means for the interaction effect of wDDGS x enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (45 days of age)

	Enzyme Inclusion							
	No Enzyme				+ Enzyme			
	wDDGS (%)							
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Parameters (%)*								
Final Live Weight (g)	3181	2969	3159	3122	3111	2777	122.7	0.486
Cold Carcass (g)	2885	2764	2803	2828	2855	2662	119.8	0.654
Dressing percent	68	72	71	70	70	77	1.6	0.123
Major Carcass Components								
Breast + Bone	34	32	34	30	32	31	1.0	0.164
Thighs	20	22	21	22	22	22	0.9	0.533
Drumsticks	11	10	10	10	10	11	0.3	0.862
Neck & Back	15	16	15	17	16	16	0.4	0.137
Wings	7	7	7	7	7	7	0.3	0.630
Edible Organ Components								
Liver	2.5^{b}	3.1^{a}	3.1^{a}	3.2^{a}	2.9^{ab}	3.3^{a}	0.09	0.004
Heart	0.89	0.59	0.68	0.65	0.65	0.63	0.126	0.452
Empty Gizzard	1.22	1.52	1.30	1.49	1.21	1.51	0.070	0.674
Non-Edible Components								
Total Gut	7.4	7.3	6.4	6.5	6.4	6.5	0.451	0.968
Empty Crop	0.6^{a}	0.5^{ab}	0.5^{b}	0.4^{ab}	0.4^{ab}	1.0^{a}	0.124	0.018
Proventriculus	0.43	0.51	0.44	0.45	0.44	0.55	0.02	0.517

a,b,c,d Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means,
unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B

The main effects of wDDGS and Enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets for a further week during the digestibility trial are presented in Table 5.32. The digestibility trial took place after the feeding trial and lasted for a week (i.e. 3 days adaptation period and 4 days total collection of excreta). The birds here attained 52 days of age. Inclusion of 15% wDDGS significantly ($P<0.05$) decreased the weights of breast with bone and heart. However, it significantly increased the weights of thighs and wings. There was also a significant ($P<0.05$) increase with the 15% wDDGS in the proportional weight of empty gizzard, total gut (Plate 5.3) as well as empty crop.

Addition of EA significantly ($P<0.05$) increased the cold carcass weight compared with NE (Plate 5.2 vs Plate 5.3) but weight was similar to EB. EA also increased drumstick and heart weights, which were better than EB, and supported highest wing weight but decreased the weight of empty crop. Similarly, addition of EB significantly ($P<0.01$) increased the dressing percentage and breast with bone weight compared with NE and EA. EB also significantly decreased the weight of thighs compared with NE and EA. Both EA and EB significantly reduced the weight of total gut (Plate 5.5 vs Plate 5.6) relative to NE (Plate 5.4).

Table 5.32: Means for the main effects of wDDGS and enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets during the digestibility trial (52 days of age)

DIETS									
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Parameters (%)*									
Final Live weight (g)	3954	4079	0.1	0.001	3867^{c}	4225^{a}	3958^{b}	0.2	0.001
Cold Carcass (g)	2738	2638	38.0	0.075	2582^{b}	2820^{a}	2661^{ab}	46.5	0.005
Dressing percent	61	59	1.0	0.401	58^{b}	58^{b}	66^{a}	1.3	0.001
Major Carcass Components									
Breast + Bone	35.3^{a}	32.3^{b}	0.47	0.001	32.6^{b}	33.2^{b}	35.7^{a}	0.48	0.003
Thighs	13.6^{b}	14.7^{a}	0.29	0.002	14.4^{a}	14.8^{a}	13.2^{b}	0.28	0.001
Drumsticks	11.9	11.8	0.11	0.712	11.8^{ab}	12.2^{a}	11.6^{b}	0.12	0.016
Neck & Back	17	18	0.33	0.251	17	18	17	0.5	0.331
Wings	7.0^{b}	7.3^{a}	0.07	0.021	7.5^{a}	6.8^{c}	7.1^{b}	0.09	0.001
Edible Organ Components									
Liver	2.7	2.5	0.07	0.060	2.7	2.5	2.6	0.08	0.544
Heart	0.7^{a}	0.6^{b}	0.003	0.001	0.7^{a}	0.7^{a}	0.68^{b}	0.004	0.001
Empty Gizzard	1.2^{b}	1.4^{a}	0.02	0.001	1.4^{a}	1.3^{b}	1.2^{b}	0.02	0.001
Non-Edible Components									
Total Gut	10	11	0.2	0.025	11^{a}	10^{b}	10^{b}	0.3	0.002
Empty Crop	0.4^{b}	0.5^{a}	0.03	0.005	0.5^{a}	0.3^{b}	0.5^{ab}	0.04	0.015
Proventriculus	0.3	0.4	0.01	0.056	0.4	0.3	0.4	0.002	0.196

a, b, c, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means
unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B

The wDDGS levels x enzyme interaction (Table 5.33) had no significant effect on cold carcass, thighs as well as breast with bone weights and total gut. The dressing percent was significantly better at 15% wDDGS wnen supplemented with enzyme B than enzyme A. There were significant interactions ($P<0.05$) for the proportional weights of liver, heart, and empty gizzard. Inclusion of 15% wDDGS with EA reduced wings weight of broiler chickens compared to 15% wDDGS with EB, though similar to 0% wDDGS with no enzyme. Similarly, 15% wDDGS supplemented with enzyme A had the best heart weight. It also presented better empty gizzard compared to 15% wDDGS with EB, though similar to 0% wDDGS with no enzyme. Meanwhile, 0% wDDGS with NE presented higher empty crop and proventriculus weights compared to 0% wDDGS with enzyme A.

Table 5.33: Means for the effects of wDDGS x enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets during the digestibility trial (52 days of age)

	Enzyme Inclusion							
	No Enzyme		+ Enzyme					
	wDDGS (%)							
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Parameters (%)*								
Final Live weight (g)	3676^{f}	4057^{c}	4255^{a}	4193^{b}	3931^{e}	3985^{d}	0.2	0.001
Cold Carcass weight (g)	2558	2607	2894	2746	2762	2560	65.7	0.155
Dressing percent	61^{abc}	54^{c}	59^{bc}	56^{bc}	63^{ab}	68^{a}	1.8	0.007
Major Carcass Components								
Breast + Bone	34	32	34	32	38	34	0.8	0.228
Thighs	14	15	14	16	13	13	0.4	0.328
Drumsticks	11.3^{b}	12.2^{a}	12.1^{a}	12.2^{a}	12.2^{a}	11.0^{b}	0.17	0.001
Neck & Back	17	17	18	18	16	18	0.7	0.076
Wings	7.3^{abc}	7.8^{a}	7.0^{bcd}	6.6^{d}	6.8^{cd}	7.4^{ab}	0.12	0.001
Edible Organ Components								
Liver	3.0^{a}	2.3^{b}	2.6^{ab}	2.4^{b}	2.5^{ab}	2.7^{ab}	0.12	0.007
Heart	0.7^{c}	0.7^{d}	0.6^{e}	0.8^{a}	0.8^{b}	0.6^{f}	0.01	0.001
Empty Gizzard	1.4^{a}	1.4^{a}	1.1^{b}	1.4^{a}	1.2^{b}	1.2^{b}	0.03	0.001
Non-Edible Components								
Total Gut	11	11	10	11	9	10	0.4	0.070
Empty Crop	0.5^{a}	0.5^{a}	0.2^{b}	0.5^{a}	0.4^{ab}	0.5^{a}	0.05	0.027
Proventriculus	0.4^{a}	0.3^{ab}	0.3^{b}	0.4^{a}	0.4^{ab}	0.4^{a}	0.02	0.002

a,b,c,d Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means,
unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B.

Plate 5.2 Heavier cold carcass of dressed chicken fed 15% wDDGS with EA.

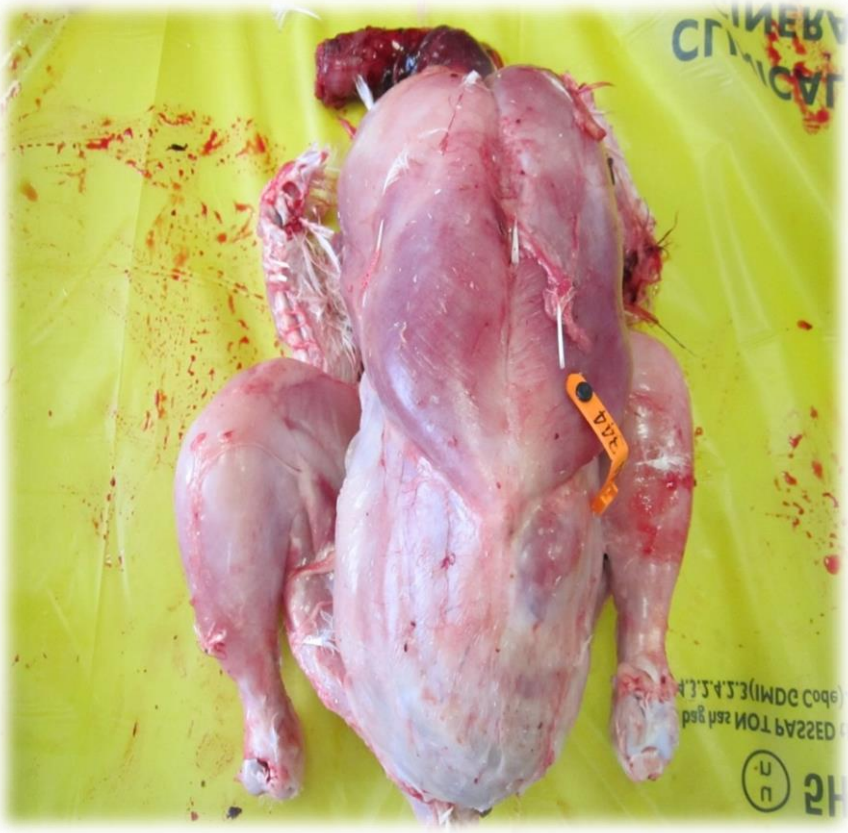


Plate 5.3: Lighter cold carcass of dressed chicken fed 15% wDDGS NE

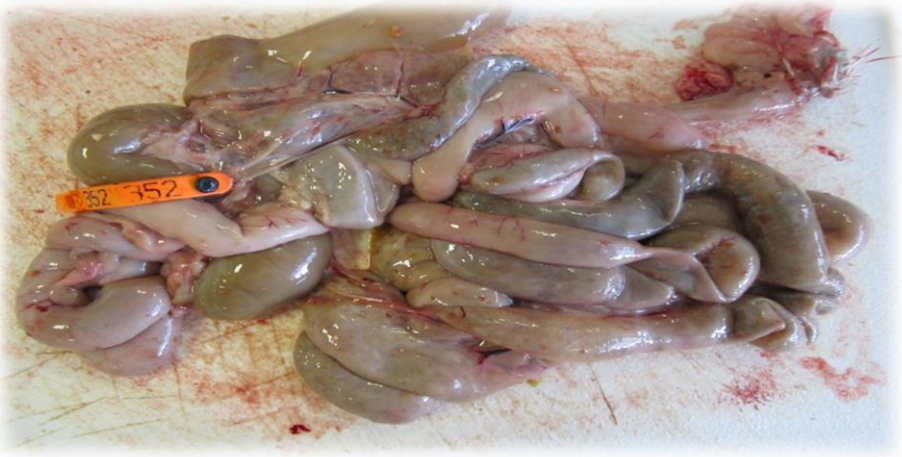


Plate 5.4 Larger Total gut of chicken fed 15% wDDGS NE

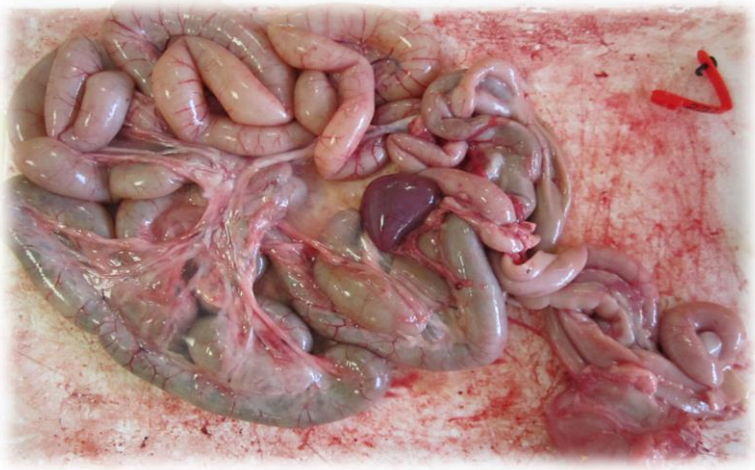


Plate 5.5 Reduced Total gut of chicken fed 15% wDDGS with EA.

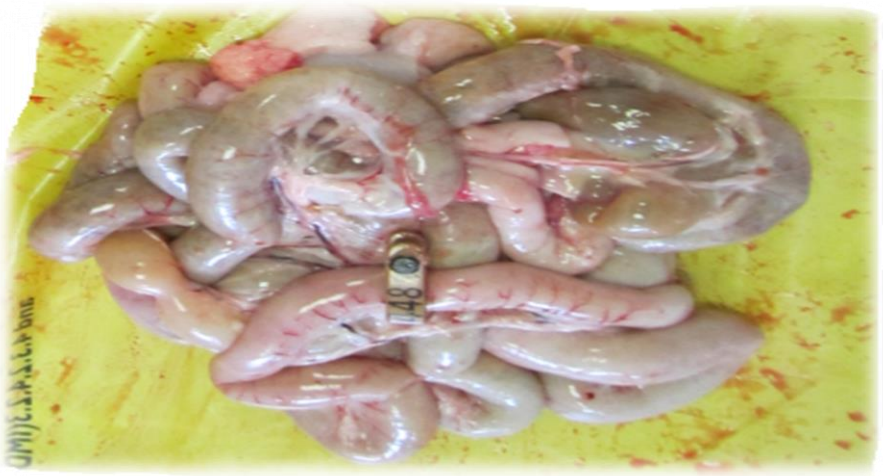


Plate 5.6 Reduced Total gut of chicken fed 15% wDDGS with EB

5.3.6 The effects of different levels of wDDGS and enzyme inclusion on muscle chemical composition of broilers

The main effects of wDDGS and enzyme on the chemical composition of broiler muscles are presented in Table 5.34. Significantly higher ether extract content in thigh and drumstick muscles and lower protein and moisture contents in drumstick muscles of broilers were observed for 15% wDDGS level than 0% wDDGS level.

Enzyme addition, significantly ($P<0.05$) reduced the ether extract content of breast and thigh muscles. The chemical composition values of breast and thigh muscles indicated that breast muscles contained more protein than thigh and drumstick muscles while both thigh and drumstick muscles contained more ether extract than breast muscles. The ash content was not affected any way in any muscle.

Table 5.34 Means for the main effects of wDDGS and enzyme on chemical composition of broiler muscles

	DIETS								
	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	No Enzyme	Enzyme A	Enzyme B	SEM	P value
Composition (g/kg)*									
Breast Muscles									
Moisture	772	761	5.8	0.213	772	767	762	7.1	0.618
Protein	812	812	3.8	0.960	812	817	808	4.7	0.406
Ether Extract	48	44	4.3	0.503	63[a]	39[b]	36[b]	5.3	0.003
Ash	48	48	0.4	0.608	49	48	48	0.5	0.690
Thigh Muscles									
Moisture	755	751	6.7	0.634	757	747	755	8.2	0.664
Protein	695	705	13.6	0.626	699	702	700	16.6	0.992
Ether Extract	80	83	0.1	0.001	141[a]	63[b]	40[c]	0.1	0.001
Ash	42	43	1.0	0.822	43	44	42	1.2	0.583
Drumstick Muscles									
Moisture	768	773	1.0	0.006	777[a]	765[c]	770[b]	1.2	0.001
Protein	810	773	7.2	0.001	783	785	808	8.8	0.092
Ether Extract	75	97	5.9	0.014	84	81	93	7.3	0.524
Ash	48	48	0.3	0.987	48	49	48	0.3	0.103

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means,
** All means are per kg DM except moisture*

There were significant ($P<0.05$) interactions (Table 5.35) of wDDGS and enzyme for the protein content of broiler breast muscles as well as in the ether extract composition of thigh muscle and moisture content of drumstick muscles. Addition of enzyme B at 15% wDDGS increased the protein content of the breast muscles compared to 15NE diets, but a similar effect was not observed with enzyme A.

Table 5.35 Means for the effects of wDDGS x enzyme on chemical composition of broiler muscles

	Enzyme Inclusion							
	No Enzyme					+ Enzyme		
	wDDGS (%)							
	0 NE	15 NE	0 EA	15 EA	0 EB	15 EB	SEM	P value
Composition(g/kg)*								
Breast Muscles								
Moisture	778	765	778	755	759	764	10.1	0.370
Protein	830^{a}	794^{bc}	818^{ab}	816^{abc}	789^{c}	827^{a}	6.6	0.001
Ether Extract	71	54	36	42	37	35	7.5	0.352
Ash	49	48	48	48	47	48	0.8	0.411
Thigh Muscles								
Moisture	752	762	741	753	773	737	11.6	0.078
Protein	701	696	702	702	683	717	23.5	0.675
Ether Extract	132^{b}	150^{a}	73^{c}	53^{d}	35^{f}	45^{e}	0.2	0.001
Ash	42	43	43	44	42	41	1.7	0.927
Drumstick Muscles								
Moisture	780^{ab}	774^{b}	747^{d}	782^{a}	778^{ab}	762^{c}	1.7	0.001
Protein	807	758	786	783	837	779	12.4	0.079
Ether Extract	67	101	75	87	82	103	10.3	0.572
Ash	49	48	49	49	48	48	0.5	0.649

a,b,c, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B.
** All means are per kg DM except moisture*

5.3.7 The effects of different levels of wDDGS and enzyme inclusion on fatty acid concentration of lipids from broiler breast meat

The effects of different levels of wDDGS and enzyme inclusion on fatty acid composition of ether extract from broiler breast meat are presented in Table 5.36. Wheat DDGS significantly decreased the concentration of dodecenoic, tridecanoic, and α-linoleic fatty acids of breast meat of broiler chickens. However, 15% wDDGS supported higher palmitic acid concentration.

On the other hand, addition of enzyme A significantly ($P<0.05$) increased the concentration of palmitic and linoelaidic acids while enzyme B increased the concentrations of arachidonic and pentadecanoate acids. Enzyme B addition also presented a better value of γ-Linoleic acid than 0% wDDGS with no enzyme, as well as better lauric acid concentration compared to EA and better linolenic (b) compared to NE. No significant treatment (wDDGS inclusion or enzyme supplementation) effects were observed on other fatty acids.

There were also significant interaction effects of wDDGS and enzyme on fatty acid composition of broiler breast meat (Table 5.37) where enzyme A supplementation significantly increased the concentration of tridecanoic acid at 0% wDDGS compared to 15% wDDGS with EA and without enzyme and similar to 0% wDDGS NE, while enzyme B increased the concentration of lauric acid for 15% wDDGS, though similar to 0% wDDGS without and with enzyme A. Inclusion of 15 % wDDGS presented greater values of linolenic (b) only in the absence of enzyme. The interaction effect of wDDGS and enzyme on n-6:n3 ratio showed that the 15% wDDGS with EA was significantly ($P<0.05$) better than 15% wDDGS with EB, and statistically similar to control (0% wDDGS) with and with out enzyme addition.

Table 5.36 Means for the main effects of wDDGS and Enzyme on fatty acids profile of breast meat from broiler chickens

		wDDGS (%)				Enzyme Inclusion				
		0	15	SEM	P value	NE	EA	EB	SEM	P value
Fatty Acid (g/100g TFA)	Structure									
Hexanoate	C6:0	0.122	0.071	0.0283	0.218	0.091	0.116	0.082	0.0347	0.782
Heptanoate	C7:0	0.270	0.214	0.0729	0.591	0.263	0.195	0.269	0.0893	0.815
Caprylic	C8:0	0.718	0.564	0.1556	0.494	0.625	0.639	0.660	0.1906	0.992
Nonanoic	C9:0	0.096	0.038	0.0265	0.140	0.098	0.061	0.043	0.0324	0.480
Capric	C10:0	0.077	0.018	0.0427	0.349	0.024	0.108	0.011	0.0523	0.387
Undecanoic	C11:0	0.108	0.115	0.0130	0.714	0.102	0.128	0.105	0.0160	0.474
Dodecenoic	C12+C11:1	0.022	0.014	0.0021	0.019	0.023	0.014	0.017	0.0026	0.065
Tridecanoic	C12:1+C13:0	0.889	0.808	0.2190	0.018	0.828	0.900	0.817	0.0268	0.086
Myristic	C14:0+9C13:1	0.239	0.232	0.0404	0.902	0.173	0.307	0.227	0.0495	0.185
Pentadecanoic	C15:0	29.787	29.142	0.4623	0.336	29.723	29.477	29.194	0.5661	0.806
Pentadecanoate	C15:1	1.683	1.385	0.2763	0.457	0.055^{b}	0.258^{b}	4.289^{a}	0.3385	0.001
Palmitic	C16:0	0.889	1.808	0.2415	0.015	0.912^{b}	2.204^{a}	0.931^{b}	0.2957	0.009
Heptadecanoic	C17:0	0.000	0.000	0.0000	0.000	0.000	0.000	0.000	0.0000	0.000
cis-10-Heptadecenoic	C17:1	0.174	0.092	0.0476	0.243	0.098	0.243	0.058	0.0583	0.089
Stearic	C18:0	30.677	30.653	0.7692	0.981	31.580	31.025	29.390	0.9421	0.260
Linoelaidic	t6t7t8	0.043	0.039	0.0123	0.838	0.000^{b}	0.089^{a}	0.035^{b}	0.0150	0.002
Lauroleic	c9	24.783	25.796	0.7824	0.372	26.676	24.900	24.294	0.9582	0.216
Lauric	c12	0.102	0.076	0.0101	0.086	0.085^{ab}	0.056^{b}	0.127^{a}	0.0124	0.003
Tridecenoic	c13	0.096	0.152	0.0289	0.191	0.176	0.082	0.115	0.0354	0.186
Dihomolinoleic	c14+t16	0.078	0.088	0.0319	0.833	0.098	0.070	0.081	0.0390	0.881
Pentadecanoic	c15	0.085	0.301	0.1711	0.384	0.072	0.450	0.058	0.2096	0.348
Parinaric	t11t15	2.384	2.376	0.1995	0.979	2.672	1.963	2.504	0.2443	0.129
Linoleic (a)	c9t12	0.024	0.049	0.0116	0.149	0.022	0.054	0.034	0.0143	0.296
Linoleic(b)	LA	0.351	0.214	0.1318	0.473	0.216	0.503	0.131	0.1614	0.259

Table 5.36: Mean for the main effects of wDDGS and enzyme on fatty acids profile of breast meat from broiler chickens (continued)

		wDDGS (%)				Enzyme Inclusion				
		0	15	SEM	P value	NE	EA	EB	SEM	P value
Fatty Acid (g/100gTFA)	**Structure**									
Stearidonic	c12c16	0.310	0.375	0.0825	0.588	0.172	0.393	0.463	0.1011	0.134
Arachidic	C20:0	0.168	0.289	0.0902	0.354	0.075	0.431	0.179	0.1105	0.090
Linoleic©	GLA	1.504	1.022	0.3034	0.275	1.211	0.929	1.650	0.3716	0.403
Cis-11,14-Eicosadienoic	aLN+c11 C20:2	0.033	0.035	0.0119	0.860	0.017	0.049	0.037	0.0145	0.301
Linolenic(a)	CLA9	0.050	0.154	0.7815	0.357	0.028	0.217	0.062	0.0957	0.350
Linolenic(b)	t11c13 (exCLA7)	0.078	0.075	0.0094	0.812	0.046^{b}	0.082^{ab}	0.101^{a}	0.0115	0.010
Linolenic(c)	CLA2 (t,t)	0.073	0.136	0.0362	0.234	0.055	0.058	0.200	0.0443	0.052
Linolenic(d)	c9c13c15 (ex CLA4)	0.101	0.063	0.0145	0.085	0.088	0.073	0.085	0.0178	0.822
cis-11-14-Eicosadienoic	C20:2	0.160	0.176	0.0266	0.677	0.163	0.153	0.190	0.0326	0.710
γ-Linoleic	c9c11c15(exCLA5)	0.020	0.017	0.0046	0.622	0.010^{b}	0.015^{ab}	0.031^{a}	0.0056	0.043
α-Linoleic	C20:3 n6	0.522	0.239	0.0721	0.012	0.489	0.387	0.266	0.0883	0.229
cis-8,11,14-Eicosatrienoic	C20:3 n3	0.091	0.074	0.0332	0.702	0.095	0.130	0.023	0.0407	0.196
Arachidonic	C20:4 n6	0.014	0.016	0.0030	0.572	0.007^{b}	0.010^{b}	0.028^{a}	0.0037	0.002
Tricosanoic	C23:0	0.416	0.293	0.0439	0.064	0.362	0.342	0.360	0.0538	0.958
cis-13,16-Docosadienoic	C22:2	1.148	0.936	0.2343	0.530	0.901	0.970	1.257	0.2869	0.654
EPA	C20:5	0.096	0.303	0.1205	0.240	0.124	0.391	0.084	0.1476	0.301
DHA	C22:6	0.016	0.052	0.0157	0.115	0.056	0.032	0.015	0.0192	0.323
Others		1.510^{a}	1.501^{b}	0.0008	0.001	1.498^{c}	1.504^{b}	1.515^{a}	0.0010	0.001
∑n-6		0.286	0.283	0.0464	0.963	0.274	0.308	0.272	0.0568	0.884
∑n-3		0.944	0.969	0.1725	0.920	0.840	1.203	0.826	0.2113	0.380
n-6:n-3		3.967	4.781	0.9090	0.535	3.626	6.488	3.008	1.1133	0.089

a,b, Means bearing different letters within rows are significantly different (P<0.05), SEM = Standard Error of Means, TFA = Total Fatty Acids, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B

Table 5.37 Means for the interaction of wDDGS x enzyme on fatty acids profile of breast meat from broiler chickens

		Enzyme Inclusion							
		No enzyme				+ Enzyme			
		wDDGS (%)							
		0NE	15NE	0 EA	15EA	0 EB	15EB	SEM	P value
Fatty Acid (g/100gTFA)	Structure								
Hexanoate	C6:0	0.115	0.067	0.176	0.055	0.074	0.090	0.0490	0.396
Heptanoate	C7:0	0.369	0.156	0.179	0.211	0.263	0.274	0.1263	0.570
Caprylic	C8:0	0.842	0.408	0.678	0.600	0.635	0.684	0.2696	0.656
Nonanoic	C9:0	0.173	0.023	0.064	0.058	0.051	0.034	0.0458	0.245
Capric	C10:0	0.025	0.023	0.195	0.020	0.010	0.012	0.0740	0.413
Undecanoic	C11:0	0.121	0.083	0.116	0.140	0.088	0.122	0.0226	0.241
Dodecenoic	C12+C11:1	0.032^{a}	0.014^{b}	0.015^{b}	0.013^{b}	0.018^{ab}	0.015^{b}	0.0037	0.063
Tridecanoic	C12:1+C13:0	0.862^{ab}	0.793^{b}	0.993^{a}	0.807^{b}	0.811^{b}	0.823^{ab}	0.0379	0.054
Myristic	C14:0+9C13:1	0.124	0.221	0.395	0.218	0.197	0.257	0.0700	0.135
Pentadecanoic	C15:0	30.259	29.186	29.850	29.104	29.253	29.135	0.8006	0.834
Pentadecanoate	C15:1	0.060^{b}	0.050^{b}	0.041^{b}	0.475^{b}	4.947^{a}	3.631^{a}	0.4786	0.193
Palmitic	C16:0	0.720^{b}	1.103^{b}	1.379^{ab}	3.028^{a}	0.568^{b}	1.293^{ab}	0.4182	0.317
Heptadecanoic	C17:0	0.000	0.000	0.000	0.000	0.000	0.000	0.0000	0.00
cis-10-Heptadecenoic	C17:1	0.104	0.092	0.370	0.116	0.047	0.069	0.0825	0.217
Stearic	C18:0	30.850	32.310	32.150	29.900	29.030	29.750	1.3323	0.358
Linoelaidic	t6t7t8	0.000	0.000	0.097	0.081	0.032	0.037	0.0212	0.870
Lauroleic	c9	26.665	26.686	23.770	26.029	23.915	24.673	1.3551	0.707
Lauric	c12	0.129^{a}	0.040^{b}	0.061^{ab}	0.050^{b}	0.115^{ab}	0.138^{a}	0.0176	0.015
Tridecenoic	c13	0.104	0.248	0.074	0.089	0.111	0.118	0.0501	0.333
Dihomolinoleic	c14+t16	0.064	0.131	0.092	0.048	0.077	0.084	0.0552	0.612
Pentadecanoic	c15	0.096	0.047	0.090	0.809	0.069	0.047	0.2964	0.360
Parinaric	t11t15	2.536	2.808	2.066	1.860	2.549	2.459	0.3456	0.774
Linoleic (a)	c9t12	0.015	0.029	0.031	0.077	0.026	0.041	0.0202	0.665

Table 5.37: Means for the interaction effect of wDDGS x enzyme on fatty acids profile of breast meat from broiler chickens (continued)

		Enzyme Inclusion							
		No enzyme		+ Enzyme					
		wDDGS (%)							
		0NE	15NE	0 EA	15EA	0 EB	15EB	SEM	P value
Fatty Acid (g/100gTFA)	**Structure**								
Linoleic(b)	LA	0.263	0.168	0.715	0.290	0.076	0.185	0.2283	0.511
Stearidonic	c12c15	0.103	0.240	0.382	0.404	0.445	0.480	0.1430	0.908
Arachidic	C20:0	0.101	0.049	0.177	0.685	0.225	0.133	0.1563	0.129
Linoleic©	CLA9	1.165	1.256	1.368	0.490	1.980	1.319	0.5255	0.633
Cis-11,14-Eicosadienoic	t11c13 (exCLA7)	0.000	0.033	0.074	0.024	0.024	0.049	0.0206	0.112
Linolenic(a)	CLA2 (t,t)	0.030	0.025	0.054	0.380	0.066	0.058	0.1354	0.384
Linolenic(b)	c9c13c15 (ex CLA4)	0.000^{b}	0.091^{a}	0.116^{a}	0.048^{ab}	0.117^{a}	0.085^{a}	0.0163	0.001
Linolenic(c)	C20:2	0.000	0.109	0.022	0.094	0.196	0.204	0.0627	0.721
Linolenic(d)	c9c11c15(exCLA5)	0.111	0.064	0.063	0.083	0.128	0.042	0.0252	0.132
cis-11-14-Eicosadienoic	C20:3 n6	0.160	0.165	0.168	0.137	0.153	0.227	0.0460	0.530
γ-Linoleic	C20:3 n3	0.000	0.020	0.030	0.000	0.031	0.031	0.0080	0.019
α-Linoleic	C20:4 n6	0.693^{a}	0.285^{ab}	0.374^{ab}	0.400^{ab}	0.499^{ab}	0.032^{b}	0.1249	0.127
cis-8,11,14-Eicosatrienoic	C23:0	0.056	0.133	0.202	0.058	0.016	0.030	0.0576	0.170
Arachidonic	C22:2	0.011^{ab}	0.003^{b}	0.003^{b}	0.017^{ab}	0.027^{a}	0.028^{a}	0.0053	0.144
Tricosanoic	C20:5	0.460	0.263	0.396	0.288	0.391	0.329	0.0760	0.673
cis-13,16-Docosadienoic	C22:6	1.018	0.783	1.270	0.669	1.157	1.356	0.4057	0.622
EPA		0.062	0.185	0.161	0.621	0.064	0.103	0.2087	0.574
DHA		0.000	0.112	0.034	0.029	0.013	0.016	0.0271	0.081
Others		1.506^{c}	1.490^{e}	1.509^{bc}	1.498^{d}	1.514^{ab}	1.515^{a}	0.0015	0.001
∑n-6		0.227	0.321	0.403	0.212	0.228	0.316	0.0803	0.157
∑n-3		0.859	0.822	0.951	1.454	1.021	0.630	0.2988	0.344
n-6:n-3		4.288^{ab}	2.965^{ab}	3.575^{ab}	9.401^{a}	4.039^{ab}	1.976^{b}	1.5744	0.041

a,b, Means bearing different letters within rows are significantly different (P<0.05), SEM = Standard Error of Means, TFA = Total Fatty Acids, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B

5.3.8 The effect of different levels of wDDGS and enzyme inclusion on different categories of fatty acids of broiler breast meat

The effects of different levels of wDDGS and enzyme inclusion on different categories of fatty acids of broiler breast meat are presented in Table 5.38 where wDDGS inclusion did not affect the concentration of SFA, MUFA and PUFA. However, enzyme B supplementation significantly (P<0.05) increased the MUFA composition**.**

Table 5.38 Means for the main effects of wDDGS and enzymes on categories of fatty acids of breast meat from broiler chickens

	wDDGS (%)				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Fatty Acid (%)									
SFA	61	61	1	0.854	63	61	60	1.6	0.419
MUFA	1.9	1.5	0.3	0.351	0.2^{b}	0.5^{b}	4.4^{a}	0.34	0.001
PUFA	37	37	1	0.982	37	39	35	1.7	0.307

a, b =Means bearing different letters within rows are significantly different (P<0.05). SEM=Standard error of means, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B, SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids

Table 5.39 showed no significant interaction effects of wDDGS levels and enzyme supplementation on any category of fatty acids.

Table 5.39 Means for the interaction effects of wDDGS x enzyme on various categories of fatty acids of breast meat from broiler chickens

	Enzyme Inclusion							
	No Enzyme		+ Enzyme					
	wDDGS (%)							
	0NE	15NE	0EA	15EA	0EB	15EB	SEM	P value
Fatty Acid (%)								
SFA	63	63	60	61	60	61	2.2	0.903
MUFA	0.2	0.1	0.4	0.6	5.0	3.7	0.48	0.281
PUFA	37	37	40	38	35	36	2.3	0.869

SEM=Standard Error of means, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B, SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA=Polyunsaturated Fatty Acids

5.3.9 The effects of different levels of wDDGS and enzyme inclusion on bacterial counts of intestinal digesta of broiler chickens

There was no significant influence of wDDGS on bacterial count (Table 5.40). However, the 15% wDDGS count exceeded the control (0%wDDGS) by 21.5%. Enzyme supplementation did not influence the total CFUs counts. As expected, dilution 10 showed significantly ($P<0.05$) greater bacterial count compared to dilution 1000, though it was statistically similar to dilution 100.

Table 5.40 Means for the total bacterial colony forming units (CFUs/ml) from intestinal digesta of broilers fed wDDGS diets

	Mean Counts (CFU/ml)	SEM	P value
Item			
wDDGS (%)			
0	38509	10168.0	
15	49076	10781.0	0.479
Enzyme Inclusion			
No Enzyme	44793	14642.0	
Enzyme A	61556	11234.0	
Enzyme B	25029	12392.0	0.102
Dilution			
10	64933[a]	10890.0	
100	49306[ab]	16312.0	
1000	17138[b]	10463.0	0.009

Means bearing different letters within columns are significantly different ($P<0.05$), SEM = Standard Error of Means.

There were no significant ($P>0.05$) interaction (Table 5.41) of wDDGS, enzyme and dilution on total bacterial count.

Table 5.41 Means for the effect of wDDGS x enzyme x dilution on total bacterial colony forming units (CFUs/ml) from intestinal digesta of broilers fed wDDGS based diets

	Enzyme inclusion					
	NE		EA		EB	
	wDDGS (%)					
	0	15	0	15	0	15
Dilution						
10	70875	76600	90500	88900	34125	28600
100	20000	65000	37500	19230	40000	25000
1000	18100	18180	24870	10833	10610	11840
SEM- wDDGS	28654.0	25629.0	25629.0	25629.0	28654.0	25629.0
SEM- Enzyme	40523.0	57308.0	28654.0	33087.0	40523.0	33087.0
SEM- Dilution	25629.0	25629.0	25629.0	25629.0	25629.0	25629.0
P value- wDDGS x Enzyme x Dilution 0.858						

SEM = Standard Error of Means, NE = No Enzyme, EA = Enzyme A, EB = Enzyme B

Plates 5.5 - 5.10 illustrate the typical colonies formed from the digesta of broilers fed wDDGS diets. The colonies that were formed appeared in various shapes as some star shape colonies resembling rhizoid (Plate 5.5) were observed in a few plates while others appeared as creamy (Plate 5.6, 5.7 and 5.10) and majority of those appeared as small dot shape (Plate 5.8 and 5.9). The colonies were not re-plated in differential agar media to determine the specificity of different bacterial strains as beneficial or pathogenic.

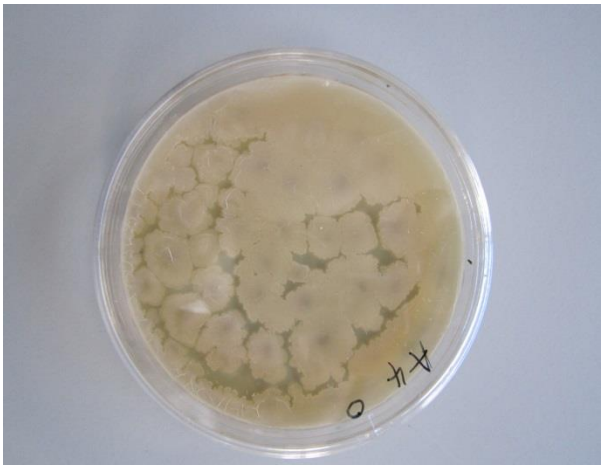


Plate 5.7 Star shaped creamy bacterial growth from digesta of broilers having 15% wDDGS with no enzyme

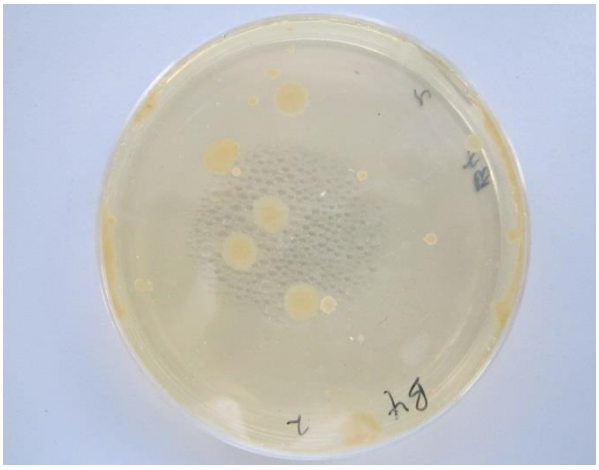


Plate 5.8 Scanty creamy round shaped colonies in digesta of broilers having 0 %wDDGS with no enzyme

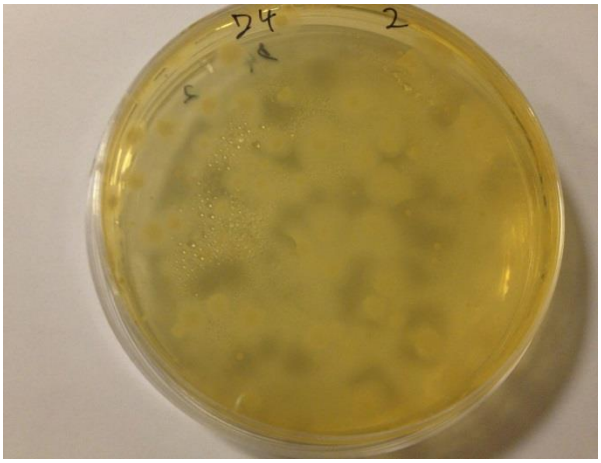


Plate 5.9 Round shaped creamy bacterial growth in digesta of broilers having 15% wDDGS and enzyme A

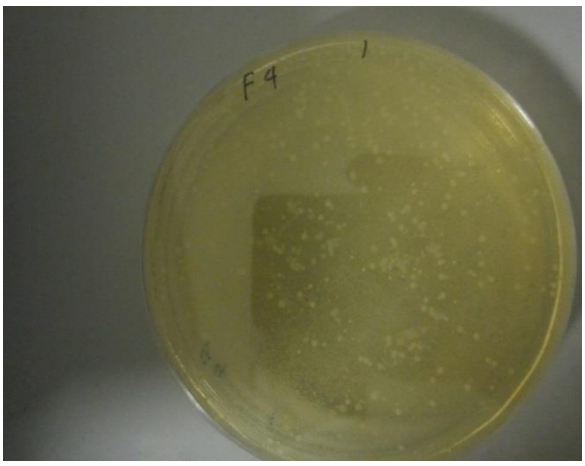


Plate 5.10 Small dot shaped bacterial growth in digesta of broilers having 15% wDDGS and enzyme B

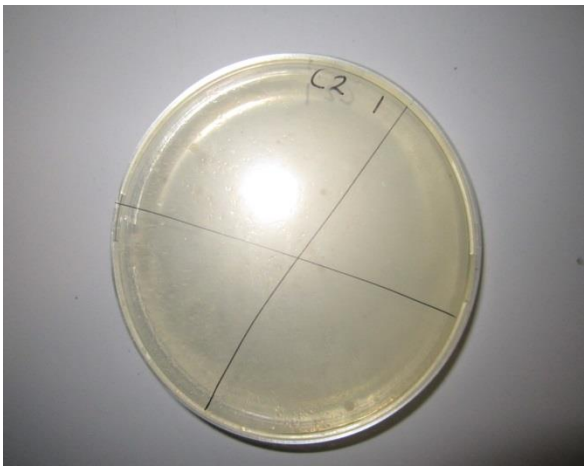


Plate 5.11 Small dot shaped bacterial growth in digesta of broilers having 0% wDDGS and enzyme A

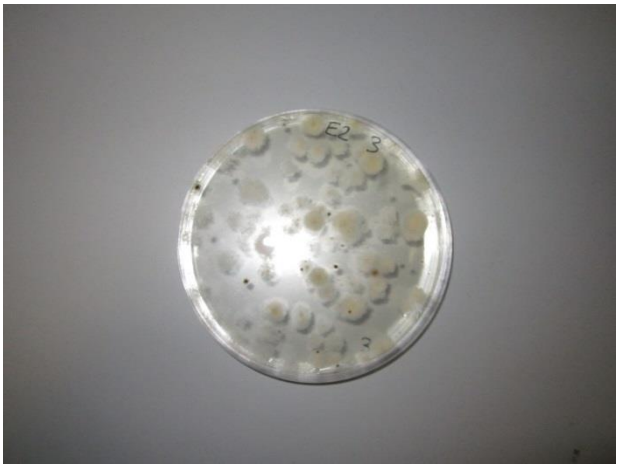


Plate 5.12 Round shaped creamy bacterial growth in digesta of broilers having 0% wDDGS and enzyme B

5.4 Discussion

The dietary composition of the experimental diets (Table 5.7 and 5.8) showed that the analysed concentrations of CP, EE, NDF, ADF, calcium and phosphorus were consistent across diets, that is not much variation in composition between the starter and finisher periods (Table 5.9). This indicates that the variation in batches and storage time did not affect the composition of the diets which is in line with the findings of Youssef *et al.* (2009) who reported the possibility of storing DDGS for a long time without deleterious effect. Numerically, most of the analysed dietary nutrients in the 6 diets were slightly lower than the calculated values perhaps due to the effect of fermentation and drying processes of the wDDGS as discussed by Widyaratne and Zijlstra (2007) as well as grinding of the ingredients.

Neither the starter nor the finisher phase of the broilers showed any noteworthy differences in growth performance (daily feed intake, live weight gain and FCR) when fed 0 or 15% of wDDGS with or without the inclusion of enzymes. In this regard, Thacker and Widyaratne (2007) reported similar results when they conducted experiments that observed the weight gain and feed intake of broilers on wDDGS diets. Additional studies conducted by Iji *et al.* (2003) and Sayyazadeh *et al.* (2006) also detected no positive effects of dietary enzyme supplementation for broilers. Similarly, these findings corroborate with the findings of Mahagna *et al.* (1995) who used microbial amylase and protease as a supplement in starter and finisher broilers and found no significant effect of enzyme supplementation at the starter period of broiler chickens. Production costs, meat quality, nutrient digestibility, FCR, feed intake and body weight have not displayed any negative reactions to the introduction of enzymes to the broiler diet, a finding supported by various research projects (Doskovic *et al.*, 2013). However, there is still a large amount of uncertainty regarding the economic benefits of use of exogenous enzymes.

The only notable difference in the experimental diet composition was that the 15% wDDGS diet contained higher levels of fibre. The water intake of broilers was unaffected by the high fibre content. The birds' water intake was not influenced by the enzyme supplementation either. Excreta and litter moisture contents are expected to increase when dietary modification results in the birds increasing their water intake (Eichner *et al.*, 2007). The 15% wDDGS had lower litter moisture content for the majority of the cases than the 0% wDDGS. This is in line with the findings of Hocking (2006) who found that the litter moisture of broilers decreased as the fibre content increased.

Results in chapter four stated that the protein digestibility was lower, and fibre digestibility higher, in diets containing 15% wDDGS than it was in 0% wDDGS; this was confirmed by the findings of this study. Steenfeldt *et al.* (2007) had attributed the increased fibre digestibility (NDF and ADF) to caecal fibre digestion. They also discovered that when layer hens were fed large quantities of roughage the fibre digestibility varied between 24-29% and Hetland *et al.*, (2004) found the excellent ability of the gizzard to grind structural components of the cell wall. One hypothesis suggests that the pH of the birds' guts may have a digestive effect, though it has not been measured in this study, as Svihus (2011) reported that a lower pH may lead to enhanced gastric digestion of the bird. It has also been reported (Salim *et al.* (2010)) that undigested fibre deriving from DDGS included in poultry diets is fermented by microbes in the large intestine. An improvement in digestibility of fibre fractions was expected, especially with addition of enzyme as seen in experiment 2 at 15% wDDGS, but this was not substantiated in this study. Hence, the anti-nutrients (arabinoxylan) in both wheat and wDDGS need to be treated by exogenous enzyme application to degrade the cell wall polysaccharides in order to increase the rate of digestion, absorption and metabolism of the complex feed nutrients (Douglas *et al.*, 2000).

The calculated levels of dietary AAs of the experimental diets were in agreement with the recommended level of NRC 1994 but 15% wDDGS diets had a lower lysine content which could most probably be due to lower lysine in wDDGS incorporated. The experimental diets were formulated on the basis of feed analysis data/calculations as such it is assumed that AAs are 80-90% available from the feed protein as reported by NRC (1994). Youssef *et al.* (2008) suggested that DDGS should not be used as a sole source of protein; as such it was used alongside other protein source, soybean meal in this study but still the lower lysine level was observed. However, despite the slightly lower content of methionine in 15% wDDGS diets, it was still within the range of the recommended level. It is important to note that the profile of an AA, its balance and accessibility in feed need to be considered in order to ascertain if it is appropriate for use as a source of protein. In addition, AA profiles vary greatly; something which is not beneficial in commercially prepared and processed animal feed. Due to the extremely high temperatures of around [~ 315^{o}C (600^{o}F)] which were used when drying DDGS it is common for lysine bioavailability and other AAs to be degraded (Lumpkins and Batal, 2005). It is due to this fact that the ease of digestion of lysine is an area of interest related to the use of wDDGS, principally because lysine is an important limiting amino acid in the diet of poultry. It appeared that the lower lysine content of the wDDGS diets

was consistent in the previous and present experiments which were a reflection and confirmation of the lower lysine content of the diets and their poor digestibility.

Meanwhile, the higher LWG and the reduced breast with bone weight of birds fed wDDGS diets observed in this experiment could be due to differences in requirement of lysine for body weight and that of breast meat as reported by Hickling *et al.* (1990) and Kerr *et al.* (1999) that abundant evidence exist suggesting the lysine requirement for feed efficiency is higher than body weight and that of meat yield is higher than that considered sufficient for feed efficiency. Further, the reduced breast with bone meat could be explained by the lysine inadequacy which has been shown to reduce breast meat yield compared with other muscles. Also diets containing low lysine can limit breast meat formation early in development by reducing protein build-up from protein synthesis (Tesseraud *et al.*, 1992; Tesseraud *et al.*, 1996a; Tesseraud *et al.*, 1996b; Dozier *et al.* (2008).

As enzyme addition only influenced higher digestibility of ether extract where digestibility was significantly better with enzyme B than enzyme A in AIA method. This is comparable to the results obtained in the total collection method herein. Conversely, enzyme B supplementation did not improve the digestibility of calcium and phosphorus as seen with the total collection method. The consistency in the pattern of digestibility results obtained by both total collection and AIA methods in the previous and present study, especially of protein, EE and NDF, reassures the feasibility of using the AIA method instead of total collection which is more expensive, time consuming and labour intensive to carry out.

The heightened fat digestibility by enzyme inclusion observed in this research had been attributed to the degradation effect of the enzyme on the cereal cell wall, as similar results have been found in other research (Bedford and Schulze, 1998). This effect is far more pronounced than other nutrients. As there are only minimal amounts of dietary fat in cereal endosperm cells, it is improbable that the dissolution of the cell walls plays a crucial role in the action or effect of these enzymes (Bedford and Schulze, 1998).

Chapter four also stated that, though enzyme A had only minimal effects on fibre digestibility for 15% wDDGS, it was enhanced for 30% wDDGS which was indicated in this research as well. An increased digestibility of phosphorus was observed in the previous experiment and calcium and phosphorus in the present study, which are in accordance with the findings of Kim *et al.* (2005) that the digestibility of phosphorus was increased by xylanase, presumably associated with the non-phytate phosphorus

being released from its encapsulated state in the arabinoxylan based fibrous material (Frolich and Asp, 1985).

The live weight gain was lower for 15% wDDGS than 0% wDDGS was reflected in the cold carcass weight (g) in both caged and deep litter birds. The supplementation of enzyme A used in this research increased the cold carcass weight of caged birds which is the primary concern in broiler production. Some digestive organs such as the empty crop, total gut, empty gizzard and liver may have had a higher weight due to the 15% wDDGS's higher fibre content but this has reduced economic value.

Almost all of the data obtained for the components of the carcass and digestive organs on caged and deep litter birds were numerically comparable except for the final live weight and cold carcass weight, where the former increased while the latter decreased. At the end of the trial in the cages the final live weight had considerably risen and the reason that the cold carcass weight was lower than that of the deep litter birds is not clearly understood. Increased thigh and wing weights were observed with 15% wDDGS inclusion however, a decreased breast weight was also exhibited which is an undesirable result. Wang *et al.* (2007) found that the inclusion of high percentages of DDGS also reduced the breast meat yield which they attributed to the overall reduced performance possibly due in part to a deficiency of some amino acids that were not typically accounted for in formulation and in part to reduced feed quality associated with higher inclusion of DDGS.

Consumers are concerned with the chemical make-up of broiler meat and usually favour low fat and high protein (Suchy *et al.*, 2002; Nkukwana *et al.*, 2014). 15% wDDGS resulted in a decreased protein percentange and an increased ether extract percentage which decreases the broilers meat quality. Various muscles in the broilers may have had a higher protein retention due to the higher protein digestilibity of 0% wDDGS. The quality of the meat was improved by the decreased ether extract content and higher protein content caused by the enzyme supplementation.

Consumers of quality meat are very concerned for the composition of fatty acids. Rymer and Givens (2005) stated that the saturation of fatty acids in edible meat may be affected by the make-up of experimental diets. The inclusion of wDDGS resulted in a decreased PUFA and increased MUFA composition, which suggests that there were some occasions in which the wDDGS level had a positive effect on the meat quality. MUFA does not affect blood cholesterol as SFA does and so it is a key component of fat (Schreiner *et al.*, 2005). The introduction of enzyme A increased the concentration of palmitic acid and enzyme B increased the concentration of arachidonic acid. As

arachidonic acid facilitates blood clotting and attaches to endothelial cells during wound healing, this conveys desirable characteristics to consumers (Rahnan *et al.*, 1995) and dietary palmitic acid has been shown to reduce serum cholesterol levels in human beings (Sundram *et al.*, 1994).

No effect on the bacterial count in digesta by the addition of the enzymes and wDDGS was observed, nor was there any interaction. There is a paucity of literature that correlates bacterial profile to the ingestion of wDDGS by broilers.

On the basis of the findings of this study, both enzymes gave improvement in some of the measured parameters, with no clear and consistent superiority of one over the other in terms of overall performance and digestibility of nutrients. EA supported better growth performance while EB supported better carcass weight, dressing percentage and breast with bone meat yield, which are the targets of the producer. This may be attributable to the finding that EB supported greater digestibility values when assessed by both total and AIA methods of measuring digestibility, especially ether extract, calcium and phosphorous digestibilities and also numerically better protein digestibility. Therefore, more research is needed to ascertain the most effective combination of enzymes and optimum inclusion in the diets to obtain uniform but maximum efficacy of the enzyme. Furthermore, despite the increased digestibility of some diet components at 15% wDDGS noted in chapter four, this study has concluded that the heightened quantities of enzyme A and enzyme B did not produce the desired effects on digestibility. The inconsistent results caused by these enzymes remain a great concern in this area of research. Future studies will need to continue to investigate this technology of enhancing the nutritive availability to broiler chicken through the development and use of specific enzymes for wDDGS.

CHAPTER 6:
GENERAL DISCUSSION

6.1 Introduction

Research into poultry nutrition is mostly aimed at producing high quality protein from poultry products at relatively low costs and consideration to the overall welfare of the birds, humans and the environment. The search for alternative sources of feedstuffs for the poultry industry has become imperative in order to reduce, or ultimately eliminate, the competition amongst humans, animals and industry for scarce and exorbitantly priced cereal grains and legumes. Thus, the current studies focused on evaluating the nutritional value of wDDGS in feeding broiler chickens.

Three experiments were conducted to evaluate the nutritional value of wDDGS by broiler chickens. In experiment 1, the optimum inclusion level of wDDGS in broiler diets was investigated. Experiment 2 studied the effect of supplementing graded levels of wDDGS-based diets with an exogenous enzyme. Experiment 3 evaluated the supplementation of wDDGS-based diets with an increased level of 2 separate enzyme mixtures on performance, nutrient digestibility, carcass characteristics and digestive organs as well as gut microbial load and fatty acids of ether extract of broiler breast meat.

6.2 The nature of the raw material

The proximate composition data of wDDGS reported in all the 3 chapters (experiments 1, 2 & 3) showed that there were no considerable differences among them and they are within the range reported in literature (NRC, 1984; NRC, 1994). Although wDDGS were found to contain high levels of protein, their high fibre (NSP) content limits their inclusion levels in poultry diets. The high fibre content is a reflection of the original feedstock. The low fat and starch contents of wDDGS make it a low energy feedstuff for poultry relative to more traditional ingredients. The protein quality of wDDGS was not measured in this study, but literature suggests that wDDGS have been characterized by poor/low lysine digestibility (Bandegan *et al.*, 2009) which most likely results from overheating during processing the wDDGS.

The low mortality observed in the 3 experiments indicates the safety of the ingredients/diets fed to the birds. This is in line with the findings of Hartini *et al.* (2002) who observed reduced mortality with inclusion of fibre in the diet when they compared a commercial laying hen diet based on wheat that contained 2.9% CF with diets that included a millrun from wheat as fibre source.

6.3 Comparative performance in the three experiments

Data from the control groups of all 3 experiments were taken to assess the variability and or compare the performance of birds among the 3 experiments (Table 6.1). Despite the fact that the same strain of broiler chicks as well as the same basal diets, housing and equipment were used in all the 3 experiments, feed intake of birds varied among the 3 experiments being lowest in the first experiment and highest in the last experiment (Table 6.1). This could probably be due to yearly changes in growth rate and genetic characteristics that might have been introduced by the breeding company during the development of these 3 strains of birds. Jaap (1970) reported that early growth in broilers is still increasing annually but at a slower rate than during the past decades. However, there is no evidence that the limit has been reached as more rapid growth rates are being reported annually. The wide variation in growth performance among the three experiments could be attributed to the differences in the initial age and weight of the birds as their feed intake increases with their age likewise their weight gain. Surprisingly, during experiment 2 birds consumed less feed but gained more weight and utilised feed more efficiently than birds in experiment 3. Of interest is the weight gain observed in birds raised in experiments 2 and 3, as compared with birds in experiment 1. It appears that weight gain in both experiments was related to their feed intake and feed conversion efficiency, though protein levels were similar for their diets. Meanwhile, cold carcass weight also followed a similar trend in the same way as above; the greater cold carcass weight of birds in experiments 2 and 3 could also be attributed to the differences in their killing weights. Birds in these experiments gained more weight compared to those in experiment 1. The substantial difference in cold carcass weight of birds recorded in experiment 1 relative to their killing weight could be explained by the fact that it excludes the weights of internal organs during processing, but still low relative to their lower killing weights as compared to those of experiments 2 and 3.

Table 6.1 Summary of the overall performance of the control (0% wDDGS) diet

	Experiments		
	1	**2**	**3**
Parameters			
Initial age of birds (days)	1	5	3
Mean initial weight of birds (g)	43	102	80
Killing age of birds (days)	42	46	45
Mean killing weight of birds (g)	1850	2437	3100
Feed intake (g/b/day)	85	112	124
Live weight gain (g/b/day)	43	73	72
FCR (g feed/g gain)	1.9	1.5	1.7
Cold carcass weight (g)	1382*	2357**	2848**

*FCR = Feed Conversion Ratio, * excluding GIT, ** including GIT*

Table 6.2 shows the values for digestibility of the key nutrients in each of the 3 experiments calculated by 2 different methods; i) by total collection (TC) in metabolism cages. This is considered as conventional but such a procedure involves a great deal of time and labour to measure nutrient digestibility by collection of samples, drying and subsequent nutrient analysis and calculation, which makes it expensive to carry out. ii) a marker acid insoluble ash (AIA) method which involved simply analysing small quantities of samples in the laboratory. A paired sample t test was used to generate the results in Table 6.2, comparing the results obtained using the same bird strain, same diet, and same origin of ingredients, as well as same housing and management, for the two different methods in three experiments.

Table 6.2 Comparison of nutrient digestibility of wheat-based control diets (g /kg) determined by TC and AIA methods (mean ± SE)

	Experiments								
	1			2			3		
Nutrient (g/kg)	**TC**	**AIA**	**P value**	**TC**	**AIA**	**P value**	**TC**	**AIA**	**P value**
Protein	525±30.8	481±30.8	0.052	528±21.9	499±30.6	0.473	556±11.7	463±20.7	0.798
Ether Extract	770±9.4	749±9.9	0.044	657±15.4	641±20.4	0.373	799±10.4	787±11.0	0.453
NDF	127±9.9	104±20.6	0.011	337±22.9	344±22.4	0.433	387±15.0	323±25.1	0.436
Calcium	239±19.0	262±28.9	0.233	263±12.1	259±17.1	0.007	297±9.3	287±23.0	0.818
Phosphorus	385±10.5	377±5.5	0.346	388±5.8	333±8.3	0.209	383±9.8	369±18.4	0.777

EE= Ether Extract, NDF = Neutral Detergent Fibre

Comparing values for the control diets, which had the same formulation in each of the experiments, there were slight differences between experiments but not in a systematic way. Thus, the different batches of raw materials used between experiments like wheat, wDDGS and SBM could explain some of this effect. This slight variation seems not be due to differences in the age of the birds used between experiments but is possibly due to differences in their feed intake. Despite the use of the same strain of birds, same diet, same origin of ingredients, same housing and management, the digestibility estimates obtained in the first experiment were found to be more different between the two methods than those obtained in the second and third experiments. As Table 6.2 shows, there is no significant difference in the estimates of digestibility between TC and AIA methods except for ether extract and NDF for experiment 1 and for calcium in experiment 2.

The digestibility experiments yielded greater digestibility values in most measures for TC method in all three experiments compared to AIA values except for calcium where AIA value is greater than TC value (262 vs. 239) for experiment 1, and for NDF (344 vs 337) for experiment 2; this is in agreement with the report of Moughan *et al*. (1999) who reported that total collection method gave higher apparent digestibility coefficients than chromic oxide based method. The lower digestibility values obtained through AIA in comparison to TC method in all the experiments except for calcium in experiment 1 and NDF in experiment 2 might probably be explained by some loss of excreta during collection or removal and transfer from trays to containers. This would affect estimates based on the TC calculation but not a marker method. Other influences on estimates derived from the TC method may be partly attributed to volatilization of some compounds after voiding of excreta but before collection, as it was reported by Bedford (1996) that many of the products of microbial fermentation present in the excreta are volatile, e.g. VFAs, which means that storage at warm temperatures may drive these compounds off. The marker method allows a single freshly voided sample to be taken and frozen, avoiding this problem.

The consistently greater numerical estimate digestibility of protein by TC method in all the experiments was in line with the findings of McCarthy *et al*. (1974) who reported significantly higher energy and nitrogen digestibility estimates from total collection than by either AIA or chromic oxide marker techniques. Conversely, this result disagrees with the report of Scott and Hall (1998) who reported prediction of AME and nitrogen retention using the AIA method was less accurate.

Table 6.2 also shows that protein, NDF and Ca digestibility followed a similar trend as feed intake and live weight gain of birds. The digestibilities increased across from experiment 1 to 3. The higher protein digestibility values observed in experiments 2 and 3 could be related to the age of the birds being older and thus able to digest and absorb dietary protein which is known to be influenced by the age of the bird. This was demonstrated by researchers like Batal and Parsons, (2002) who reported a significant increase of protein (amino acid) digestibility with increasing age of commercial broiler chickens. Generally, the greater digestibility values recorded in experiments 2 and 3 could also be explained by the fact that the birds were older as they reached 54 and 52 days of age respectively, compared to birds in experiment 1 (49 days) at the termination of excreta collection. Literature shows that the age of the birds affects the digestibility of nutrients. Huang *et al.* (2005) reported that digestibility increases with advancing age

of the birds. Thus, older birds are able to digest nutrients more, possibly due to larger mature gastrointestinal tract and microbial fermentation that occurs in the hindgut.

Steenfeldt *et al.* (2007) had attributed the increased fibre digestibility (NDF and ADF) to caecal fibre digestion, and possibly due to the excellent ability of the gizzard to grind structural components of the cell wall of cereals as reported by Hetland *et al.*, (2004) and the digestive capacity as well as the reduced intestinal viscosity of older birds (Bedford, 1996).

Ether extract and phosphorus digestibilities were not consistent among the three experiments. However, the higher EE digestibility observed in experiments 1 and 3 could be the result of increased lipid intake with a corresponding ability to digest and absorb lipids being fully developed in older birds. Krogdahl *et al*. (1985) and Sell, (1996) demonstrated that in older birds the lipid digestive processes are fully functional and increased concentration of some digestive enzymes such as lipase and others in the gland results in increased lipid digestion.

In all the 3 experiments, standard errors in most measures were smaller for values determined with TC method in comparison to the AIA method, indicating better accuracy of measurement.

Data from all three experiments were combined to determine the overall picture for comparison between the digestibility measurements from TC and AIA methods. A paired sample t-test was used to compare the values and results from the test are presented in Table 6.3.

Table 6.3 Overall mean values and differences in digestibility estimates (g/kg DM) of wheat-based control diets from 3 experiments comparing Total Collection and AIA methods by Paired t-test analysis

	Mean				
Nutrient	**TC**	**AIA**	**Difference**	**SED**	**P value**
Protein	546	534	12	17.7	0.514
Ether Extract	741	733	8	10.3	0.444
NDF	298	284	14	21.2	0.528
Calcium	254	221	33	21.8	0.161
Phosphorus	387	364	24	15.5	0.153

NDF = Neutral Detergent Fibre, SED = Standard Error of Difference

As shown in Table 6.3, there is no statistically significant difference between the averages at the 5% level for any parameter. This result agrees with the findings of Miraglia *et al.* (1999) who compared the two methods in light horses and found no statistical differences between the two methods. This shows the possibility of measuring digestibility of deep litter managed broilers using the AIA method, since this method

offers the possibility of obtaining representative sample from a single sample of feed and faeces from a flock of chickens raised on the floor, thereby eliminating the need of special equipment (e.g. cages to confine the birds) and longer experimental duration (i.e. an extended period of time for feeding or collection of faeces and an estimation of feed digestibility).

Furthermore, the AIA method offers some distinct benefits over the traditional total faecal collection method as demonstrated in previous studies by Tillman and Waldroup (1988), who reported that AIA may be a more acceptable method for AME because of its shorter collection period, compared to the total collection method which requires a lot more time and resources to continuously collect total faeces over a much longer time. Moreover, studies by Van Keulen and Young (1977) demonstrated that the cheapness, simplicity and convenience of the AIA method opens the possibility of its adoption by feed quality control laboratories responsible for evaluation of feedstuffs for animals.

On the basis of the findings of this study, while both methods supported the determination of nutrient digestibility, there was no significant difference between TC and AIA methods overall. Hence, the simpler AIA method can be used for digestibility trials in broiler chickens because of its advantages in terms of cheapness, feasibility and convenience for deep litter reared broiler chickens.

6.4 The effect of wDDGS inclusion level

The growth performance data reported in chapters 3, 4 and 5 showed that the performance of birds fed diets which contained 15% wDDGS during the starter period confirmed the loss in performance with this inclusion level. Similarly, during the finisher period the LWG of broilers fed 15% wDDGS were numerically lower than those fed 0% wDDGS in both experiments 2 & 3 though statistically similar in experiment 2. The higher fibre content of wDDGS would be expected to reduce energy availability, and although the diets were balanced to contain similar levels of protein, the lower LWG of birds fed wDDGS diets may also be due to the poor/low protein digestibility and absorption of nutrients by birds_are consequences of the presence of anti-nutrients especially NSP in wheat and wDDGS of the diets that depress protein digestibility and utilization and possibly increasing endogenous amino acid loss (Bryden and Li, 2010; Hughes and Choct, 1999).

Protein digestibility was reduced with wDDGS inclusion in this study which was probably due to poor protein quality/digestibility as a consequence of overheating during processing of wDDGS and anti-nutritional factors such as NSP (arabinoxylans)

that bind the protein, or to the slow fibre digestion rate and relatively short retention time in the GIT of broiler chickens. The high fibre levels in wDDGS generally have been of concern to nutritionists and producers, especially the negative effects of high intestinal digesta viscosity caused by viscous NSP on nutrient digestion. Digestibility was lowest in experiment 1 when the diets were not supplemented with enzymes and improved (in some cases) with addition of enzymes in subsequent experiments. NDF digestibility values, which increased consistently from experiment 1 – 3 (Table 6.1), indicated that the fibre in wDDGS was somehow slightly more digestible, perhaps due to the nature of the fermentation process during its production. The improvement in digestibility of NDF as well as that of calcium in experiment 3 which was best among the experiments may reflect differences in the wDDGS used as a consequence of variability of composition between batches, as demonstrated by (Belyea *et al.,* 2004). The variation is largely due to different cereal sources and processing procedures. The improvement in digestibility observed supports the better performance results observed in experiment 2.

6.5 The effect of enzyme supplementation

Inconsistencies were observed in the action of enzymes as seen in experiment 2, where an enzyme mixture (xylanase, amylase and protease) demonstrated some effects especially in NDF digestibility, but this was not substantiated in experiment 3 when an increased amount of the enzyme was added. This indicated that it is not the level of enzyme inclusion that limited efficacy in this study but perhaps the type of enzyme combination used. Similarly, both enzyme mixtures used in experiments 2 and 3 were not consistent in their actions. Meanwhile, the low LWG of birds which were fed diets with higher wDDGS inclusion are a reflection of poor digestibility and absorption of nutrients, indicating that further work to develop more suitable enzymes is required. Addition of exogenous enzymes to diets of chickens has been shown to reduce the viscosity of the intestinal contents and minimize susceptibility to infections.

The third objective of this study was to determine the carcass characteristics of broiler chicks fed wDDGS-based diets. As consumer preferences and acceptability are an important consideration in sustainable intensive broiler production, consumers' awareness about various aspects of broiler production is increasing nowadays. The high protein content of broiler breast meat and reduced fat content as well as increased PUFA and decreased SFA observed in experiment 2 as influenced by enzyme addition indicate the quality attributes desired by consumers, but this was not the case in experiment 3. Additionally, breast with bone yield, which is a consequence of protein digestibility as

breast meat yield (being impacted by intake and absorption of protein and specifically some amino acids), was not improved by protein digestibility either in this study. Hence, the findings of experiment 3 corroborate the findings of Heincinger *et al.* (2012) who found that inclusion of 15% wDDGS did not have any measureable effect on meat quality, chemical composition and malondialdehyde content of breast meat of turkeys.

6.6 Other considerations for DDGS use in livestock diets

The mass production of wDDGS globally is seen as a waste if not utilised; these materials can therefore be used to help with resource recovery and improved environment management as advocated by UNDP (2002). Several studies have reported the benefits of low levels of fibre inclusion on the growth performance of broilers that were related to improved nutrient digestibility (Mateos *et al.*, 2012). Poultry manure has become a concern due to its phosphorus content present which may contribute to environmental contamination (Kim *et al.*, 2006). Thus, efficient phosphorus utilization (i.e. reduced excretion) is of great concern. Dietary manipulations such as incorporation of increased levels of co-products such as wDDGS in the diets appeared to increase manure output and phosphorus excretion in poultry waste, and such formulations also reduce diet costs and the levels of non-renewable inorganic phosphorus required. However, the higher phosphorus digestibility observed in experiment 2 in this study compared to control diets suggests feeding wDDGS supplemented with a xylanase, amylase and protease mixture might improve phosphorus management in broiler diets and manure. Nitrogen excretion in excess from poultry remains another great concern for the public as high ammonia levels from excreta breakdown have been shown to reduce chicken growth and increase the risk of certain diseases such as dermatitis and air sac inflammation (Algers and Svedberg, 1989). However, supplementation of broiler diets with exogenous enzyme; improved amino acid digestibility as well as phase feeding has been established to reduce excess nitrogen excretion and air pollution (Powers and Angel, 2008; Carter and Kim, 2013; DoskoviĆ *et al.*, 2013). The single phase feeding system being practiced and high fibre diets used in all the experiments would not favour reduced nitrogen excretion. However, the exogenous enzymes application in this study coupled with the tendency for numeric increase of protein digestibility by enzyme B (xylanase and glucanase mixture) might have contributed to reduced nitrogen and phosphorus levels in excreta, and consequently very less air pollution as reported by Nahm and Carlson (1998) and Faria Filho *et al.* (2005). The fibre may also have provided energy to the bacteria in the gut, where the bacteria use nitrogen and in-turn produce short chain fatty acids which lower ammonia emission.

This reduction in ammonia emission would assist in alleviating common health problems in chickens such as gastro-intestinal tract irritation and respiratory diseases.

6.7 Conclusion and recommendation

Feeding wDDGS-based diets with up to 10% inclusion in broiler production resulted in no negative impacts to broiler performance or carcass characteristics. 15% wDDGS inclusion resulted in some reduction in performance, but increased protein content and reduced the ether extract content of breast meat. The lack of starch originally was thought to reduce the energy content of diets with distillers' grains. In this work, inclusion of 15% wDDGS was found to increase the digestibility of ether extract and NDF thereby providing energy to the birds with highly digestible fat and fibre but, due to the presence of anti-nutritional factors in wheat and wDDGS, the energy content was not fully available to the birds. In the current study, supplementation with exogenous enzyme mixtures such as xylanase, amylase and protease as well as xylanase and glucanase mixtures was found to improve (to some extent) the digestibility of the fibre fraction and minerals, though not in all cases. This did not translate into any performance benefits. Presently wDDGS is among the cheapest sources of protein available for animal feeds, as reported by researchers like Reveco and Drew (2012). This indicates its feasibility for reducing the diets costs when used as a broiler feed ingredient, and merits further investigation to fully exploit its rich nutrient content.

Further research is needed into the use of enzyme supplementation to diets with high levels of wDDGS. This should investigate, for wDDGS-based broiler diets, the optimum levels of enzyme to be applied as well as to develop a specific enzyme mix that would target/attack the various structures of cell wall NSP in wDDGS. There are currently no commercially prepared exogenous enzymes which target wDDGS NSP and these are needed to enhance the nutritional values of wDDGS, with a view to further improve the nutrient digestibility and performance of broiler chickens. To this end, special emphasis should also be given to the economic aspect, broiler welfare and environmental quality as well.

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