THE NUTRITIONAL VALUE FOR POULTRY AND PIGS OF BIOFUEL CO-PRODUCTS

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ABSTRACT

A total of five studies were conducted to determine the nutritional value of co-products of bioethanol production for poultry and pigs.

The objective in the first study was to evaluate the relationship between the chemical components of maize- and wheat distillers dried grains with solubles (DDGS) as well as develop prediction equations for indispensable amino acids (IAA), total indispensable amino acid (TIAA) and total amino acid (TAA) contents using nutrient composition data available in literature. The relationship between the chemical constituents of maize- and wheat-DDGS and associated probability values were determined by correlation analysis. Prediction models for determining the IAA, TIAA and TAA contents of maize- and wheat-DDGS from their crude protein (CP) and amino acids (AA) contents were developed using step-wise multiple regression analyses. Maximum improvement in adjusted r^2 (adj r^2) and reduction in Mallows Cp were the model selection criteria. The chemical composition of maize- and wheat-DDGS varied among sources with coefficient of variation (CV) ranging from 8.5% to 53.5% for total P and Ca respectively in maize-DDGS and 10.5% to 36.1% for CP and acid detergent fibre (ADF) in wheat-DDGS respectively. Of the IAA, Lys, Met and Trp were most variable in maize-DDGS with CV of 13.1%, 12.0%, 10.3%, respectively, whereas Lys, Phe and Met were the most variable IAA in wheat-DDGS with CV of 20.2%, 17.3%, and 16.9%, respectively. For maize-DDGS, there were positive correlations ($P < 0.05$) between CP and CF, NDF, Ca, ash (r ranged from 0.45 and 0.61). Adjusted r^2 ranged from 0.57 to 0.99 in the best models for predicting the IAA in maize- and wheat-DDGS from CP and AA. Except for Trp and Lys, the IAA contents of maize- and wheat-DDGS can be predicted from their CP content alone. The best models for predicting TIAA and TAA in maize-DDGS included Arg, His and Leu (adj r^2= 0.98) and His, Leu and Trp (adj r^2= 0.90) respectively, the regression equations being TIAA (% DM) = 0.77 + 1.36 (Arg) + 3.87 (His) + 1.99 (Val) and TAA = -3.03 + 14.1 (His) + 3.79 (Leu) + 23.4 (Trp) respectively. For wheat-DDGS, the best three variables for predicting TIAA were Arg, Leu and Val (adj r^2=0.99), the regression equation being TIAA (% DM) = -0.07 + 1.11 (Arg) + 0.99 (Leu) + 5.02 (Val). Predicted values were close to actual values in the prediction models for IAA, TIAA and TAA. It was concluded that the IAA, TIAA and TAA contents of both maize- and wheat-DDGS can be predicted from their CP contents with high accuracy.

In the second study, the nutritional value of wheat-DDGS without- or with exogenous enzymes for broiler was determined using three experiments. The N-corrected- and apparent metabolisable energy contents (AME_n and AME, respectively) without- or with added

admixture of xylanase, amylase and protease (XAP) was determined in experiment 1, true P digestibility without- or with supplemental phytase was determined in experiment 2, whereas the apparent- or standardised ileal digestibility (AID and SID, respectively) of AA without- or with added protease was determined in experiment 3. Birds were fed a nutrient adequate pre-experimental diet from d 1 to 14 post-hatch followed by the dietary treatments from d 14 to 21 in experiment 1 and 2, or from d 25 to 28 in experiment 3, respectively. Each of the 3 experiments was arranged as a randomised complete block design consisting of 7 replicate pens and 3 birds per pen. Six dietary treatments consisting of 3 levels of wheat-DDGS (0, 300 or 600 g/kg of diet) and 2 levels of XAP (0 or 0.25 g/kg) were used in experiment 1. Six diets consisting of 3 levels of wheat-DDGS (200, 400 or 600 g/kg of diet) and 2 levels of phytase (0 or 1000 FTU/kg) were used in experiment 2, whereas four treatments consisting of a nitrogen-free diet (NFD) and an assay diet, both diets without- or with supplemental protease were used in experiment 3. In experiment 1, increasing the level of wheat-DDGS in the basal diet decreased linearly ($P < 0.001$) dry matter (DM) and energy retention, AME and AME_n. Supplemental XAP tended to improve both the dietary AME ($P = 0.059$) and AME_n ($P = 0.085$) values of the diet. The AME value of wheat-DDGS without- or with supplemental XAP was determined to be 15.0 or 15.5 MJ/kg, respectively. Corresponding values for AME_n were 14.0 and 14.5 MJ/kg, respectively. Supplemental XAP did not improve the energy value of wheat-DDGS for broilers. In experiment 2, increasing the level of wheat-DDGS in the diet decreased linearly ($P < 0.05$) ileal DM digestibility, DM retention and apparent P retention but there was no difference in apparent ileal P digestibility. Except for Fe and Zn at the ileal, and Mn and Zn at the total tract level, increasing the level of wheat-DDGS in the diet increased linearly ($P < 0.05$) the flow of all other minerals. Flow of minerals at the ileal and total tract level were not different with phytase supplementation. True ileal P digestibility in the wheat-DDGS for broilers was 93.6 or 96% without- or with added phytase, respectively. Corresponding values at the total tract level were 92.4 and 93.5%, respectively. Phytase addition did not improve P utilisation at the ileal or total tract level. In experiment 3, AID ranged from 33% (Asp) to 75% (Pro) without added protease whereas the range was 31% (Asp) to 82% (Pro) with protease supplementation. The AID of Lys was nil regardless of protease supplementation. Supplemental protease improved ($P < 0.05$) the AID of Arg and Pro and tended to improve ($P < 0.10$) the AID of Met. Without protease supplementation, SID ranged from 43% (Asp) to 84% (Pro) whereas the range was from 54% (Asp) to 93% (Pro) with added protease. Supplemental protease improved ($P < 0.05$) the SID of Arg, Leu, Phe, Met, Val and Pro by 21, 14, 13, 26, 13 and 10 percentage points, respectively. It was concluded that wheat-DDGS is a good dietary source of metabolisable energy and P for

broilers. The ileal AA digestibility of wheat-DDGS for broilers is quite variable and generally low. Further, the ileal digestibility of some AA in the wheat-DDGS improved with protease supplementation.

Using three experiments the third study determined the metabolisable energy content, true P digestibility and retention and AIAAD and SIAAD of wheat-DDGS for turkey. The AME_n and AME content of wheat-DDGS without- or with XAP was determined in experiment 1, the true P digestibility and retention without- or with supplemental phytase was determined in experiment 2, whereas the AIAAD and SIAAD of wheat-DDGS without- or with a protease were determined in experiment 3. Experiment 1 and 2 lasted for 21 days whereas experiment 3 lasted for 28 days. Experimental diets were fed for 7, 5 or 3 d in experiment 1, 2 or 3, respectively. Each of the 3 experiments was arranged as a randomised complete block design consisting of 7 replicate pens and 3 birds per pen. Six dietary treatments consisting of 3 levels of wheat-DDGS (0, 300 or 600 g/kg of diet) and 2 levels of XAP (0 or 0.25 g/kg) were used in experiment 1. Six diets consisting of 3 levels of wheat-DDGS (200, 400 or 600 g/kg of diet) and 2 levels of phytase (0 or 1000 FTU/kg) were used in experiment 2, whereas four diets consisting of a NFD and an assay diet, both diets without- or with supplemental protease were used in experiment 3. In experiment 1, increasing the dietary inclusion of wheat-DDGS from 0 to 600 g/kg decreased linearly ($P < 0.05$) DM and energy retention. There was wheat-DDGS × XAP interaction ($P < 0.05$) for dietary AME and AME_n. Dietary AME and AME_n values decreased linearly ($P < 0.001$) as the level of wheat-DDGS increased in the diets without XAP, whereas there was no effect of increasing wheat-DDGS level on dietary AME or AME_n for the XAP-supplemented diets. From the regression of wheat-DDGS-associated energy intake (MJ) against wheat-DDGS intake (kg), the AME values (MJ/kg of DM) of wheat-DDGS without- or with supplemental XAP were determined to be 14 or 14.9, respectively. Corresponding AME_n values (MJ/kg of DM) were 13 and 13.8, respectively. Supplemental XAP did not improve the energy value of wheat-DDGS for turkey. In experiment 2, increasing the dietary inclusion level of wheat-DDGS decreased linearly ($P < 0.05$) DM intake, ileal DM digestibility and DM retention. Apparent ileal P digestibility and apparent P retention were not affected by either wheat-DDGS inclusion level or phytase supplementation. Except for Mn and Zn, flow of minerals at either the ileal or total tract level increased linearly ($P < 0.05$) with graded levels of wheat-DDGS in the diet. Flow of minerals (Cu, Fe, Mg, Mn, K, Na, Zn) at the ileal or total tract level (mg/kg of DM intake) were not different with phytase supplementation. True ileal P digestibility was determined to be 75.8% or 82.1% for wheat-DDGS without- or with supplemental phytase, respectively. Respective values at the total tract were 70.7% and 81.6%. In experiment 3, the ileal digestibility of Lys

was zero regardless of protease supplementation. Apparent ileal digestibility was lower than 50% for all AA except for Glu (70%) and Pro (81%) in the wheat-DDGS without supplemental protease. Also, SIAAD ranged from 41% (Thr) to 89% (Pro) without added protease whereas the range was from 56% (Arg) to 88% (Pro) with added protease. With the exception of Cys and Pro, supplemental protease increased ($P < 0.05$) the AIAAD and SIAAD of all other AA from between 5 to 19 percentage points. It was concluded that wheat-DDGS is a good source of metabolisable energy and P for turkey. The ileal digestibility of AA in wheat-DDGS is generally low. In addition, supplemental protease improved the ileal digestibility of majority of the AA in the wheat-DDGS for turkey.

The metabolisable energy, digestible AA and P values of wheat-DDGS determined and reported in the second study were used in a fourth study to formulate diets for broilers. These diets were used to determine the effect of XAP or phytase added individually or in combination on growth performance, jejunal morphology, intestinal pH and caecal volatile fatty acids (VFA) production in broilers receiving a wheat-SBM based diet containing wheat-DDGS. Two hundred and eighty-eight 1-d old broiler chicks were allocated to eight dietary treatments in a randomized complete block design consisting of 6 replicate pens and 6 birds per pen. The treatments were 1) a positive control (PC1); wheat-soyabean meal (wheat-SBM) diet and adequate in metabolisable energy (ME) and all nutrients, 2) a second positive control (PC2); wheat-SBM based diet containing wheat-DDGS and adequate in ME and all nutrients; 3) a negative control (NC1) marginal in ME (minus 0.63 MJ/kg), 4) NC1 plus XAP added to provide per kg of diet, 2000, 200 and 4000 U of xylanase, amylase and protease, respectively 5) a negative control (NC2) marginal in available P (minus 0.15%) 6) NC2 plus phytase added to provide 1000 FTU per kg of diet, 7) a negative control (NC3) that is low in ME and available P (minus 0.63 MJ/kg and 0.15%, respectively), 8) NC3 plus a combination of XAP and phytase at the rates in diets 4 and 6, respectively. Wheat-DDGS was included in the diet at the rate of 12, 22 or 25% at the starter (d 1 to 10), grower (d 11 to 24) or finisher (d 25 to 42) phases. Reducing the ME and non-phytate P in the NC diets depressed ($P < 0.05$) bodyweight gain (BWG), final bodyweight (FBW) and gain:feed (G:F) compared with the PC diets. From d 1 to 24, birds receiving the PC diet containing wheat-DDGS were heavier and consumed more ($P < 0.01$) compared with birds receiving the PC diet containing no wheat-DDGS. An admixture of XAP improved ($P \leq 0.05$) BWG and G:F above the NC1 diet from d 1 to 24 whereas supplemental phytase had no effect on growth performance. From d 25 to 42, BWG and FBW did not differ between the birds receiving the PC1 and PC2 diets, but G:F was superior ($P < 0.01$) for birds receiving the PC1 diet. From d 1 to 42, addition of XAP improved ($P < 0.05$) G:F and tended to improve ($P < 0.10$) BWG above the NC diet. Further,

performance responses did not differ between birds receiving the PC2 and XAP diet. Inclusion of wheat-DDGS in the diet reduced (P < 0.05) digesta pH at the caeca, but pH did not differ among treatments at the duodenum. Volatile fatty acids production in the caeca was not affected by either XAP or phytase supplementation, but wheat-DDGS reduced ($P < 0.05$) the production of n-butyric acid. Jejunal villi height was not different among the dietary treatments but XAP increased crypt depth. In conclusion, the addition of an admixture of XAP to a wheat-SBM based diet containing wheat-DDGS produced modest improvements in the growth performance of broilers whereas phytase had no effect.

There is substantial data about the nutritional value of maize- and wheat-DDGS for pigs but there is no information about the effect of dietary fibre type on nutrient digestibility due to differences in the chemical characteristics of the protein feedstuff used. The fifth study determined the effect of dietary fibre type and protein level on ileal amino acids digestibility for growing pigs. Twenty boars (Yorkshire × Landrace) with average initial bodyweight of 35 kg and fitted with a simple T-cannula at the terminal ileum were used in the current study. The dietary treatments were three fibre types (SBM, canola meal (CM) or maize-DDGS) and two levels of CP (adequate (18%) or reduced (14%)). In each period, two pigs with bodyweights closest to the mean bodyweight of the twenty pigs were offered a nitrogen free diet to determine basal endogenous ileal amino acid flow. The remaining eighteen pigs were allocated to the experimental diets using a replicated 6 × 2 Youden square design. Ileal digesta was collected for two days in each period after five days of adaptation to the diet. In comparison, AIAAD for the SBM diet were greater ($P < 0.05$) compared with the CM diet except for Met, Trp, Cys and Pro. Apparent ileal digestibility of DM, Gly and Asp were greater ($P < 0.05$) for the SBM diet compared with the maize-DDGS diet. The AID of the following AA were greater in the maize-DDGS diet compared with the CM diet: Ile, Leu, Phe, Val, Ala, Tyr and Asp. There was fibre type × protein level interaction ($P < 0.05$) for the AID of Lys because in the CP-adequate diets, the AID of Lys differed ($P < 0.05$) amongst the dietary fibre sources, whereas the AID of Lys was not different in low-CP diets. The SIAAD of the SBM diet was greater ($P < 0.05$) than those of the CM diet for all AA except for Trp and Pro, whereas Gly and Asp were more digestible ($P < 0.05$) in the SBM diet compared with the maize-DDGS diet. Standardised ileal digestibility of the following AA was greater in the maize-DDGS diet compared with the CM diet: Ile, Leu, Val, Ala, Tyr and Asp. Reducing dietary protein level by 4% did not affect DM utilisation or the AID or SID of N and AA in the current study. It was concluded that the choice of protein feed ingredient used in swine diets in relation to the fibre composition affects ileal amino acids digestibility. Furthermore,

AA digestibility is not affected by a 4% reduction in dietary crude protein level for growing pigs.

Collectively, it was concluded from these experiments that mathematical models are a useful tool to predict the amino acids content of maize- and wheat-DDGS. The ME in wheat-DDGS was comparable to those of wheat and maize grain for broilers and turkey, therefore, wheat-DDGS may be used as a substitute for wheat or maize in diets for broiler and turkey. The digestible P content in wheat-DDGS for broilers and turkey is greater than in most other major feedstuffs. The use of wheat-DDGS in poultry diet may therefore reduce the quantity of inorganic P compounds used, reduce P loss in manure and overall may reduce feed cost. Ileal AA digestibility in the wheat-DDGS for broilers and turkey was variable and generally low. It was recommended that the low digestibility of essential AA in wheat-DDGS should be accounted for when using wheat-DDGS as a feedstuff for poultry. Although maize-DDGS contain greater levels of fibre, ileal AA digestibility are similar to that of SBM for pigs but CM was inferior to the other two protein sources. The differences in fibre characteristics of protein feedstuffs affects ileal AA digestibility.

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Publications

Peer reviewed journal

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Popular press

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Other

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Dedication

This thesis is dedicated to my Wife and Son, Oluwatola and Adedunmola Adebiyi; you are the best. And to mum, Patience Adebiyi; the tuitions you paid made all the difference.

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Authors Declaration

This thesis has been written by the author and has not been presented in any previous application for a degree. The studies in this thesis were done by me, and all sources of information have been acknowledged using appropriate references.

ADEKUNLE OLALEKAN ADEBIYI

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List of Abbreviations

AA: amino acid

ADF: acid detergent fibre

ADL: acid detergent lignin

AID: apparent ileal digestibility

AIAAD: apparent ileal amino acids digestibility

AME: apparent metabolisable energy

AME_n: nitrogen-corrected apparent metabolisable energy

Ala: alanine

Arg: arginine

Asp: aspartic acid

BWG: bodyweight gain

Ca: calcium

CD: crypt depth

CDS: condensed distillers solubles

CF: crude fibre

CM: canola meal

CP: crude protein

Cr: chromium

Cu: copper

CV: coefficient of variance

Cys: cystine

DDGS: distillers dried grains with solubles

DM: dry matter

EAAF: endogenous amino acids flow

EE: ether extract

E-mill: enzymatic milling

EPL: endogenous phosphorus loss

FBW: final bodyweight

FCR: feed conversion ratio

Fe: iron

G:F: ratio of gain to feed

GE: gross energy

Glu: glutamic acid

Gly: glycine

His: histidine

HPmaize-DDGS: high-protein maize distillers dried grains with solubles

IAA: indispensable amino acids

Ile: isoleucine

K: potassium

Kcal: kilocalories

Kg: kilograms

Leu: leucine

Lys: lysine

ME: metabolisable energy

Met: methionine

Mg: magnesium

MJ: megajoules

Mn: manganese

N: nitrogen

Na: sodium

NDF: neutral detergent fibre

NFD: nitrogen free diet

NSP: non-starch polysaccharide

P: phosphorus

Phe: phenylalanine

Pro: proline

SBM: soyabean meal

Ser: serine

SIAAD: standardised ileal amino acids digestibility

SID: standardised ileal digestibility

TAA: total amino acid

Ti: titanium

TiO_2: titanium dioxide

TIAA: total indispensable amino acids

TIAAD: true ileal amino acids digestibility

Thr: threonine

TID: true ileal digestibility

TME_n: nitrogen-corrected true metabolisable energy

Trp: tryptophan

TPI: true phosphorus indigestibility

TPD: true phosphorus digestibility

TPR: true phosphorus retention

Tyr: tyrosine

U: unit

Val: valine

VFA: volatile fatty acids

VH: villi height

WDG: wet distiller's grains

XAP: mixture of xylanase, amylase and protease

Zn: zinc

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Biofuels are expected to replace up to 20% of the total gasoline used in the UK by 2020, and the vast majority of these are expected to be produced from wheat and oilseeds. Bioethanol production from wheat is currently on the increase in the UK and this industry is expected to expand rapidly. Bioethanol production from wheat will also result in an increase in the quantity of wheat Distillers Dried Grains with Solubles (wheat-DDGS) available as a feed ingredient for poultry and pigs. Although co-products of ethanol production from beverage-ethanol facilities have been available for decades, they have usually been used for feeding ruminants. However, with the anticipated increase in its availability, coupled with a high crude protein (CP) and bioavailable P content (Nyachoti *et al*., 2005; Thacker and Widyaratne, 2007), DDGS is a viable feedstuff for poultry and pigs. So far, the preponderance of published literature (mainly from the USA) has reported the nutritive value of maize-DDGS. On the other hand, there is very little information about the nutritive value of wheat-DDGS for poultry, and there is hardly any information for UK-produced wheat-DDGS. In view of the potential of using wheat-DDGS in poultry diets in the UK, data on its nutritional value for poultry, especially broilers and turkey is essential. In the case of pigs, the energy value and nutrient digestibility of maize- and wheat-DDGS has been described by Widyaratne and Zijlistra (2007) and Stein and Shurson (2009). However, the effects on nutrient digestibility when common protein sources such as soyabean meal (SBM) or canola meal (CM) are replaced with biofuel co-products (maize-DDGS) in pig diet are not known and require investigation.

There are two main methods for producing ethanol from cereal grains, namely; dry grind and wet milling process. The major co-product of ethanol by the dry grind process is DDGS, whereas gluten meal and gluten feed are the co-products in wet milling. An overview of the dry-grind process is presented in Figure 1-1. Briefly, the dry-grind process begins with the milling of the grain, mixing with water and cooking. Alpha-amylase enzymes are added to aid conversion of starch to sugars such as glucose, maltose and α-limit dextrins; yeast is employed to convert the resultant sugars to ethanol. Post-fermentation, the grain solid components (thick stillage) are separated from the liquid by centrifugation or by pressing, after which the ethanol is removed from the liquid component by distillation. The remaining liquid, known as condensed distillers soluble (CDS) are often mixed with the thick stillage also known as wet distillers grains (WDG) and dried to form DDGS. Alternatively, the grains may be dried without the CDS component as dried distillers grains or the CDS dried without the grains. During the fermentation process of bioethanol production, the starch fraction of the

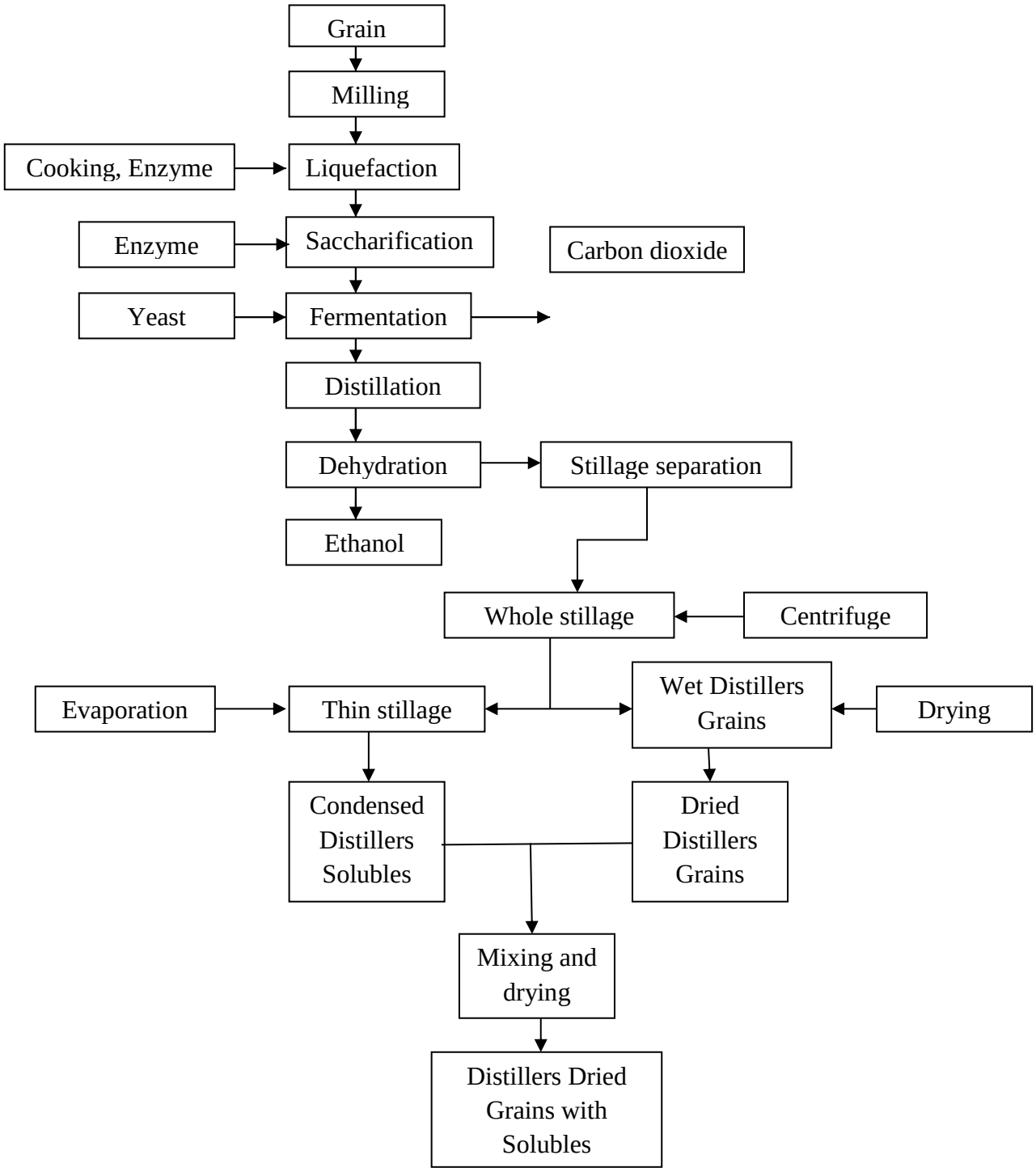


Figure 1-1. The dry-grind process of bioethanol production

grain is converted to ethanol, as such; other chemical components in the grain are concentrated approximately 3-fold in DDGS (Nyachoti *et al*., 2005). Typically, each unit mass of wheat grain produces approximately equal proportions of ethanol, carbon dioxide and wheat-DDGS.

The quantity of bioavailable P is also increased in DDGS because yeast phytase hydrolyses phytate P (the insoluble storage form of P in the grain) releasing available P in the process (Spiehs *et al.,* 2002). Essentially, the concentration of nutrients in DDGS and increased concentration of available P makes it a potential source of protein, amino acids (AA) and minerals for poultry and other livestock (pig, horse) feeds. However, the use of DDGS in poultry feed is currently limited because the physical, chemical and nutritional characteristics of DDGS vary widely among sources (Fastinger *et al*., 2006; Bandegan *et al*., 2009; Belyea *et al*., 2010).

The chemical properties of DDGS vary with the type of cereal grain from which it is produced (maize or wheat) (Nuez-Ortin and Yu, 2009), however, the processes (grinding, cooking, enzyme treatment, fermentation, distillation and drying) before the production of DDGS may be more culpable for the variations observed when the same type of grain is used (Belyea *et al*., 2004; Liu, 2011). The DDGS produced by beverage-ethanol producers may also be different to the increasingly available DDGS produced by fuel-ethanol facilities due to differences in processing techniques and efficiency of starch fermentation.

1.2 Effect Of Processing on DDGS Quality

A number of factors are responsible for the wide variability in the physical, chemical and nutritional characteristics of DDGS. These factors include, but are not limited to; variations in the chemical composition of the grain (maize or wheat), differences in processing techniques among bioethanol plants, and differences in analytical methods (Kim *et al*., 2008; Belyea *et al*., 2010; Liu, 2011). It is thought that variation in chemical composition of cereal grains due to differences in variety and growing conditions may affect the chemical characteristics of the DDGS (Stein *et al*. 2009). However, a lack of correlation between the chemical components in maize grain and corresponding maize-DDGS has been reported (Stein *et al*., 2009; Belyea *et al*., 2004). This may suggest that factors other than differences in the chemical composition of the grain are more important in causing variability to the physical and chemical properties of the DDGS.

Because the efficiency of fermentation, types of enzymes used, the ratio of CDS combined with WDG to form DDGS and temperature and duration of drying often vary among

bioethanol plants, the characteristics of DDGS produced among these sources also differ (Spiehs *et al*., 2002; Noll *et al*., 2007a; Nuez-Ortin and Yu, 2009). During bioethanol production ammonia, urea, sodium hydroxide and sulphuric acid are often added to the mash to control pH and to sanitize plant equipment (Liu, 2011). Differences in the quantity of these exogenous substances among bioethanol facilities may influence the chemical composition of the DDGS. High concentrations (more than 100-fold increase compared to maize grain) of Ca, S and Na in maize-DDGS that may be attributed to the addition of exogenous substances during the dry-grind process have been reported in literature (Oryschak *et al*., 2010; Liu and Han, 2011; Liu, 2011). In addition, whereas ethanol and DDGS are the primary end-product of the dry-grind process, other substances such as yeast protein, ethyl acetate, acetaldehyde and methanol may also be produced and remain in DDGS (Cozannet *et al*., 2010a) and these substances may also influence its chemical characteristics.

1.3 Physical Characteristics of DDGS

1.3.1 Colour

The colour of DDGS is often used as a measure of the intensity and duration of heat treatment (Fastinger *et al*., 2006). The colour of DDGS is particularly relevant because of the negative effect of heat treatment on the concentration and digestibility of AA such as Lys. Cromwell *et al*. (1993) observed that Lys concentrations were lower in darker coloured maize-DDGS samples compared with samples that were lighter in colour. Similarly, using the Hunterlab colour grading system (L*; lightness, a*; redness and b*; yellowness), Fastinger *et al*. (2006) reported that the concentration of Lys in maize-DDGS decreased as the L* value decreased. Because there was a high correlation between colour score and Lys content in maize-DDGS in the Fastinger *et al*. (2006) study, the authors suggested colour as a good predictor of AA concentration in maize-DDGS.

The digestibility of Lys in maize-DDGS has been widely reported to decrease in poultry as the colour of maize-DDGS becomes darker (Ergul *et al.*, 2003; Batal and Dale, 2006). Similarly, low concentrations and digestibility of Lys in dark coloured wheat-DDGS samples has been reported by Cozannet *et al*. (2010a; 2011) in broilers. Fastinger *et al*. (2006) and Noll *et al*. (2007b) investigated the relationship between the colour of maize-DDGS and nitrogen-corrected true metabolisable energy (TME_n) value whereas Cozannet *et al*. (2010b) investigated the same in wheat-DDGS. Based on the poor correlation and variations in data currently available in the literature, the relationships between colour of maize-/wheat-DDGS and TME_n values are not yet established. Because CDS is darker in colour compared to WDG,

the amount of CDS combined with WDG in DDGS may also influence the colour of DDGS (Noll *et al*., 2007a). Kingsly *et al*. (2010) noted that the L* colour value of maize-DDGS decreased as the proportion of CDS in maize-DDGS increased when drying was conducted at the same temperature and duration between treatments. As such, although colour may be used to rapidly predict the quality of DDGS, the effects of drying may not be the only factor affecting the colour of the final product.

1.4 Chemical Characteristics of DDGS

1.4.1 Energy Value

Because the starch fraction in the grain is converted to ethanol, the concentration of CP, AA, ether extract, crude fibre and minerals is two to three times greater in DDGS. However, because the fermentation process cannot effectively convert all the starch in the substrate (maize/wheat grain) into ethanol under normal processing conditions, there are usually residual starch and sugars in the co-product at variable quantities depending on the efficiency of fermentation (Vilarino *et al*., 2007). Thus, it is possible that the differences in the quantity of residual sugars in DDGS among sources are responsible, at least in part, for the variability in its gross energy (GE) value. However, although the GE value of DDGS varies among sources, the GE in DDGS is usually similar or greater than in the grain. Nyachoti *et al*. (2005) reported a higher GE in wheat-DDGS compared to wheat (20.5 vs 16.9 MJ/kg, respectively) and Cozannet *et al*. (2010b) similarly reported a higher GE value for wheat-DDGS compared with wheat (18.7 *vs*. 16.2 MJ/kg, respectively). The GE value of wheat-DDGS was reported to be higher than in wheat grain and soyabean meal (SBM) (19.8, 18.6 and 18.2 MJ/kg respectively) in a study by Thacker and Widyaratne (2007). The GE value of maize-DDGS ranged from 20.5 to 23.2 MJ/kg and averaged 22.1 MJ/kg in 5 different studies (Fastinger *et al*., 2006; Stein, 2007; Olukosi *et al*., 2010; Oryschak *et al*., 2010; Rochell *et al*., 2011); this value is greater than the average GE value of 18.9 MJ/kg for maize grain (Zhao *et al*., 2008). The higher GE value in maize-DDGS compared to maize may be due to the increased concentrations of CP and ether extract fractions in the DDGS which also negates the energy diluting effect of the increased fibre fractions. Futhermore, the GE value of maize-DDGS is higher compared with wheat-DDGS; and this may be due to the higher lipid (i.e. from ether extract) content in maize compared to wheat (Nuez Ortin and Yu, 2009).

1.4.2 Crude Protein and Amino Acid Composition

The CP and AA composition of DDGS varies widely among sources (Fastinger *et al*., 2006; Vilarino *et al*., 2007). However, there is usually a much wider variation in the concentrations

of AA compared to CP content in DDGS. Cozannet *et al*. (2011) observed more than three-fold difference in the concentrations of Lys in 19 wheat-DDGS samples obtained from 7 different plants around Europe, whereas less variation was observed for the CP levels. Similarly, Fastinger *et al*. (2006) reported the total Lys content in 5 maize-DDGS samples to vary between 0.48 and 0.76%, whereas the range of CP in their study was much narrower (27.0 – 29.8%). More than two-fold differences in the concentrations of AA such as Arg (Cromwell *et al*., 1993), Leu and Val (Vilarino *et al*., 2007) and Met (Spiehs *et al*., 2002) in maize-DDGS have also been reported previously.

A number of factors may cause variation in the CP and AA composition of DDGS among sources. They include; differences in the quantity of exogenous non-protein-nitrogen substances added during the bioethanol process, temperature and duration of drying DDGS, differences in the contribution of yeast AA to total amino acid (TAA) content in DDGS as well as differences in the analytical methods used for estimating the chemical components in DDGS (Kim *et al*., 2008).

Drying techniques (rotary kiln *vs* ring drying), drying temperatures, amount of solubles added in relation to duration of drying (Noll *et al*., 2007a), over-drying, inconsistent drying leading to localised burning or "hot spots", and pre-cooking of the grain during processing have all been implicated for the variability in the protein quality of DDGS (Kingsly *et al*., 2010; Liu, 2011). This is particularly important with respect to Lys digestibility which varies and decreases substantially due to its susceptibility to heat damage (Nyachoti *et al*., 2005). Excessive heat treatment of DDGS may cause the amino group on Lys to react with the carbonyl group on the reducing sugars in a Malliard reaction. Because poultry speciess lack the enzymes capable of breaking the bond between Lys and the sugar residue, the Malliard reaction product is generally not available for hydrolysis in the gastrointestinal tract and is excreted (Cromwell *et al*., 1993). However, the amount of free sugars would be expected to be lower in the DDGS, since fermentation should change them to alcohol. The reduction in sugars may not however reduce the potential for Maillard reactions, since the amount of free AA are thought to increase with fermentation (Vilarino *et al*., 2007).

The duration and temperature of drying influences the composition and digestibility of Lys in maize-DDGS (Cromwell *et al*., 1993; Stein *et al*., 2009) and wheat-DDGS (Cozannet *et al*., 2010a). Cromwell *et al*. (1993) observed that Lys concentrations were lowest in the darkest coloured, and highest in the lightest coloured maize-DDGS samples. Fastinger *et al*. (2006) also reported the lowest Lys content in the darkest of 5 maize-DDGS samples. However, Liu

(2011) observed that drying causes minimal changes to the CP and AA concentrations of maize-DDGS.

Ingledew (1999) reported that yeast may constitute up to 5.3% of the total protein in maize-DDGS. Belyea *et al*. (2004) noted that yeast protein contribute approximately 55% to the total protein content of maize-DDGS. However, the value by Belyea *et al*. (2004) may have overestimated the contribution of yeast protein to total protein in DDGS because they did not account for dispensable AA in their approach. Martinez-Amezcua (2005) reported that approximately 10% of the TAA in maize-DDGS is contributed by yeast. Belyea *et al*. (2004) also argued that AA, Lys that is found in low concentrations in maize grain (0.24g/100g) and in much higher concentrations in yeast (3.32g/100g) increased in maize-DDGS (0.77g/100g). In the Liu (2011) study, the authors found that post-fermentation of maize, there were rapid increases in the concentration of some AA, the concentration of some AA remained unchanged and other AA decreased in concentration.

1.4.3 Mineral Composition: Phosphorus and Other Minerals

The concentration of minerals in DDGS vary widely among sources (Spiehs *et al*., 2002; Batal and Dale, 2003; Martinez-Amezcua *et al*., 2004). Wide variability in the concentrations of minerals in maize-DDGS have been reported in literature; P (Martinez-Amezcua *et al*., 2004), Zn (Spiehs *et al*., 2002), Na (Belyea *et al*., 2004), and S (Liu and Han, 2011). Martinez-Amezcua *et al*. (2004) reported a P bioavailability of between 69 to 102% (relative to monopotassium phosphate) in 4 commercial maize-DDGS samples and noted that the highest bioavailability values were observed in the darkest coloured samples. Martinez-Amezcua and Parsons (2007) in another study demonstrated that increased heating of maize-DDGS for varying amounts of time (by autoclaving at 121°C and 124 KPa or by dry oven-heating at 55°C and 121°C) increased the bioavailability of P but reduced the digestibility of all AA with large negative effects on Lys digestibility (decreased from 68 to 8%) in broiler chicks. Lumpkins and Batal (2005) also reported the relative bioavailability of P in maize-DDGS to be 54 and 68% in 2 different experiments. The P content of maize-DDGS may also be affected by the fractionation method used during processing (Martinez-Amezcua *et al*., 2007; Kim *et al*., 2008). The P content of high-protein maize-DDGS (HPmaize-DDGS) was reported to be lower than that of maize-DDGS, although, there was no differences in relative P bioavailability of HPmaize-DDGS compared to maize-DDGS (Kim *et al*., 2008).

Variability in the concentration of minerals in DDGS may be due to a number of factors that include; differences in the ratio of WDG and CDS in DDGS, exogenous addition of mineral

compounds, and differences in processing techniques among bioethanol plants. Because WDG is composed mainly of the CP and crude fibre fractions of the grain, whereas CDS contains the soluble EE, ash and residual sugar fractions (Kim *et al*., 2008), the ratio of WDG and CDS combined influences the mineral characteristics of DDGS (Belyea *et al*. 2004). An unusual wide variation in sodium concentration among maize-DDGS samples from the same plant have been reported by Shurson *et al*. (2003) and among plants by Batal and Dale, (2003) and Noll *et al*. (2007b). High concentrations of calcium and sodium (more than 260-fold increase compared with maize) have also been reported for maize-DDGS (Noll *et al*., 2007b; Oryschak *et al*., 2010; Liu and Han, 2011).

Variable and high concentrations of sulphur in maize-DDGS samples have been reported by Nuez-Ortin and Yu (2009). High dietary contents of sulphur (through high dietary inclusions of maize-DDGS) may interfere with calcium and other trace-mineral absorption and consequently affect bone mineralisation and egg quality in poultry (Pineda, 2008). The wide variability and abnormal high concentrations of minerals in DDGS may be due to the addition of exogenous substances to control pH as well as sanitize plant equipment (Liu, 2011). The addition of exogenous sulphuric acid, ammonia and sodium hydroxide during bioethanol production has been documented in literature (Parsons *et al*. 2006; Kingsly *et al*. 2010; Liu and Han, 2011).

Distillers dried grains with solubles is considered to be an economic source of available P because the fermentation and drying processes increases the concentration of inorganic P by releasing some of the phytate-bound P in the grain (Martinez-Amezcua *et al*. 2004; Martinez-Amezcua and Parsons, 2007, Widyaratne and Zijlstra, 2007). Steiner *et al*. (2007) noted that about 67% of the total P in legume seeds, cereals and cereal by-products may be bound as phytate. Because the bulk of poultry feed is composed almost entirely of plant materials, the amount of phytate in livestock and poultry feeds may become high. Not only does phytate level differ between feedstuffs, its location also varies. The largest portion of phytate in maize is located within the germ (88%), whereas 87% of the phytate in wheat resides in the aleurone layer (Szczurek, 2009). This is significant because the germ is generally highly digestible whereas the contents within the aleurone remain mostly undisturbed because they are protected by a fibrous cell wall. Liu and Han (2011) assessed the concentrations of different forms of P (non-phytate bound P, phytate P, and total P) in different streams of the bioethanol production process and reported an increase in maize-DDGS over maize grain of 1.8 fold in phytate P and 10.8 fold in non-phytate bound P. The authors (Liu and Han, 2011) found that during the fermentation process, percent phytate P in total P decreased significantly whereas

percent non-phytate bound P in total P increased. These observations suggest that phytate underwent degradation through the actions of yeast phytase.

1.4.4 Non-Starch Polysaccharides

The fibre fraction in cereal grains are composed mainly of cellulose, hemicellulose and lignin. The non-cellulosic polysacharrides consist predominantly of arabinoxylans and β-glucans and other small fractions of arabinogalactans, galactans and pectic polysaccharides (Zijlstra *et al*., 2007). Cellulose is an un-branched linear molecule and is highly insoluble in water. Conversely, arabinoxylans and β-glucans are highly water-soluble and digestive and nutrient absorption processes in the gastrointestinal tract are compromised when they are ingested in excessive amounts (Choct *et al*., 2004). Water-soluble non starch polysaccharides (NSP) exert their anti-nutritive properties by their high affinity to water and formation of gel-like substances. The formation of the gel medium causes an increase in digesta viscosity, slower rate of digesta transit in the gastrointestinal tract and also a reduction in nutrient absorption by encapsulation of other nutrients and enzymes within the gel medium (Choct *et al*., 2004; Adeola and Cowieson, 2011). These effects have negative consequences on energy and nutrient utilisation. Carre *et al*. (2002) found a negative correlation between *in vitro* viscosity and metabolisable energy (ME) of wheat in broilers, Adeola and Bedford (2004) also reported that nutrient and energy digestibility decreased with increased digesta viscosity in wheat, Boros *et al*. (2002) reported a 9% reduction in feed conversion and fat digestion in broilers due to an increase in dietary concentration of soluble arabinoxylans from 3.4 to 7.7% whereas a negative correlation between digestible energy and total NSP, soluble NSP and xylose concentration were reported by Zijlstra *et al*. (1999) in growing pigs.

The anti-nutritive effects of NSP may limit the use of DDGS in practical poultry diets (Thacker and Widyaratne, 2007; Wang *et al*., 2008). Because wheat grain contains about 5-8% arabinoxylans, up to 1% β-glucans and 2-3% cellulose (Choct *et al*., 2004), NSP degrading enzymes are often used during bioethanol production to reduce the viscosity of the mash in view of improving the efficiency of starch fermentation. Although NSP degrading enzymes are used during bioethanol production, the concentration of NSP increases 3-fold in DDGS compared to the grain (Widyaratne and Zijlstra, 2007).

1.5 Biological Characteristics of DDGS

Mycotoxins such as aflatoxins, deoxynivalenol, fumonisms and zearalenone are secondary metabolites of fungi that are often found on plant materials and cereal grains. Ingestion of excessive amount of mycotoxins may cause illness and death of poultry and livestock animals

(Zhang *et al*., 2009). One potential problem with the transportation and storage of DDGS is mycotoxin contamination. Because fermentation of wheat concentrates the chemical components in DDGS, it is possible that the concentration of mycotoxins also increases in DDGS. Schaafsma *et al*. (2009) found the concentration of deoxynivalenol in CDS to be four times greater than in maize grain. Similarly, Rodrigues (2008) reported 99 percent of 103 samples of maize-DDGS tested contained at least one detectable mycotoxin. Zhang *et al*. (2009) found varying concentrations of aflatoxins, deoxynivalenol, fumonisms and zearalenone among 10 sources of maize-DDGS. On the other hand, Pineda (2008) found no detectable levels of aflatoxin, vomitoxin, zearalenol, zearalenone and T-2 toxin in maize-DDGS in their study. However, the reported mycotoxin concentrations in maize-DDGS are usually lower than recommended harmful concentrations (Rodrigues 2008; Schaafsma *et al*., 2009; Zhang *et al*., 2009).

1.6 Use of DDGS in Poultry Diets and Effect on Bird Performance

A few studies have argued that a correctly balanced feeding ration of DDGS may successfully replace some of the well characterized feedstuffs in poultry diets. Apart from the increased concentration of CP, AA, and minerals in DDGS, the crude fibre composition also increases 3-fold. Because poultry are not efficient at utilizing dietary fibre, it is essential to evaluate how much DDGS can be incorporated into poultry diets without compromising performance. The few studies that have evaluated the use of wheat-DDGS in monogastric diets have focused more on broilers, fewer still on pigs (Thacker, 2006; Widyaratne and Zijlstra, 2007; Lan *et al*., 2008) and it appears that the currently available studies with turkey have used only maize-DDGS. The review is thus concentrated on the use of maize-DDGS as a possible indication of opportunities and limitations of using wheat-DDGS in poultry. It is acknowledged that the characteristics of these two DDGS are different because wheat-DDGS contain greater levels of CP and fibre compared with maize-DDGS.

1.6.1 Effect on Growth Performance

The inclusion of maize-DDGS in poultry diets is not new (Matterson *et al*., 1966; Morrison, 1954; Waldroup *et al*., 1981; Parson *et al*., 1983), however, majority of the DDGS described in growth performance and digestibility studies in poultry and swine before the last decade were mostly from the brewing industry. The DDGS produced by the brewing industry may however be different in chemical composition to the DDGS produced by modern bioethanol facilities due to improved fermentation techniques. Morrison (1954) observed that up to 8% maize-DDGS may be included in practical broiler chick diet without detrimental effects on

body weight. In addition, the author also reported that including 10% maize-DDGS in layer hen diets did not cause a reduction in egg production. In another study, up-to 25% maize-DDGS was included in nutritionally-adequate broiler diets without causing a reduction in body weight or feed intake (Waldroup et al., 1981). Further studies using maize-DDGS by Parsons *et al*. (1983), proposed that up-to 40% of SBM protein can be replaced by maize-DDGS as long as the dietary Lys content is adequate.

There is a general trend that growth performance is depressed as the quantity of DDGS in the diet is increased. The decline in growth performance as the level of maize-DDGS in the diet is increased may be due to the inefficiency of poultry at utilising dietary fibre. Stated differently, along with other nutrients the crude fibre composition of DDGS is increased about 3-folds by fermentation. As such, high inclusion levels of DDGS may increase dietary fibre content which in turn may impede nutrient digestibility. Thacker and Widyaratne (2007) evaluated the inclusion of wheat-DDGS in broiler chick diets at a rate of 0, 5, 10, 15 and 20%. In that study, there were no differences in body weight gain (BWG), feed intake and feed conversion ratio (FCR) in all the dietary treatments compared with the controls. However, because there was a high mortality at 20% DDGS inclusion, the authors recommended that wheat-DDGS be incorporated at 15% provided that the low energy and Lys contents of wheat-DDGS are compensated for during diet formulation. Similarly, Loar *et al*. (2010) reported no differences in the final body weight (FBW), feed intake and FCR of broilers fed 0 or 8% maize-DDGS in their diets during the starter period.

Wang *et al*. (2007a) observed that broilers fed 15% maize-DDGS did not differ from control in body weight, feed intake, and FCR at 42 days of age; however, 30% inclusion of maize-DDGS reduced feed efficiency without any effect on feed intake or BWG. In another study by the same authors (Wang *et al*., 2007b) it was noted that 15 to 20% maize-DDGS may be incorporated into broiler diets formulated on digestible AA without detrimental effects on bird performance. Shim *et al*. (2011) observed greater BWG in the starter period of broilers fed 24% maize-DDGS against the control.

Lumpkins *et al*. (2004) recommended an optimum inclusion rate of maize-DDGS at 9% in broiler starter diets and 12 to 15% in the grower to finisher period because above these levels, maize-DDGS depressed growth performance. In Hoskova *et al*. (2010) study, including 0 or 25% wheat-DDGS in broiler diets from 12 to 35 days of age produced similar feed intake and FCR but the treatment containing 0% wheat-DDGS produced superior growth performance. Vilarino *et al*. (2007) observed improvement in FCR for broilers fed either 10 or 20% wheat-DDGS from day old to 10 days of age compared with controls (0% wheat-DDGS), but feed

intake and final body weight at 37 days of age reduced as the level of wheat-DDGS increased. Similarly, Richer *et al*. (2006) observed a reduction in BWG and feed intake of broilers at the finisher phase as the level of wheat-DDGS increased to 20% in the diet. Lukasiewicz *et al*. (2009) observed improvement in FCR for broilers receiving 7%, 9.5% and 9.5% wheat-DDGS during the starter, grower and finisher periods respectively, however mean BWG was consistently higher in the control groups (0% wheat-DDGS) and also in males fed wheat-DDGS compared to females. Lukasiewicz *et al*. (2009) further noted that the inclusion of wheat-DDGS in the diet for broilers increased the number of beneficial micro-organisms in the gut (there was a decline in caecal population of Enterobacteriaceae).

According to Potter (1966), if Lys and ME content of feed are appropriately balanced, up to 20% maize-DDGS could be fed to turkey without any detrimental effect on body weight or FCR. Roberson (2003) fed diets formulated on digestible AA basis and including between 0 and 27% maize-DDGS to female turkey, the authors observed a linear decrease in body weight as the level of maize-DDGS increased in the diet as well as a linear increase in FCR. Because there was an increase in the incidence of pendulous crop as the level of maize-DDGS increased (with incidence highest at 18 to 27% inclusion levels) in that study, the authors recommended that maize-DDGS be used at no more than 10% during the grower/finishing period in female turkey.

Extensive feeding trials by Noll *et al*. (2002; 2003ab) in turkey investigated the possibility of using maize-DDGS in grower/finishing diets of heavy toms receiving diets formulated on digestible AA basis. In those studies there were no differences observed in live performance of turkey relative to body weight and FCR at 10% inclusion rate of maize-DDGS. In another study, 15 and 20% levels of maize-DDGS in turkey diets resulted in performance similar to the control (Noll, 2004), however 20% inclusion of maize-DDGS depressed FBW at 19 weeks of age in a similar but subsequent study by the same authors (Noll *et al*., 2005). Further studies by Noll *et al*. (2009) showed that body weight was greater at 5 weeks of age in turkey fed on diets containing 10, 20 and 30% maize-DDGS compared with turkey fed diets with no maize-DDGS.

Because exogenous enzymes in poultry diets can help promote growth, efficiency of nutrient utilisation, and nutrient excretion, some authors have assessed their benefits in poultry diets containing DDGS. Slominski (2010) observed growth performance response of broilers to feeding a blend of maize-DDGS and wheat-DDGS with or without enzyme supplementation. The authors noted that 10% inclusion of the maize-/wheat-DDGS blend supported growth performance similar to the control diet (0% DDGS) in the absence of enzyme and that in the

presence of enzyme, 15% DDGS level supported growth similar to the control. In Olukosi *et al*. (2010) study, inclusion of 10% maize-DDGS in broiler diets supplemented with an admixture of phytase, xylanase, amylase and protease enzymes produced superior BWG, feed intake and feed efficiency at 3 weeks of age compared with diets without maize-DDGS or supplemental enzymes.

Due to the potential anti-nutritive effects of NSP in DDGS, some authors have studied the effect of processing techniques to reduce the NSP level. Oryschak *et al*. (2010) examined the use of extruded (physical disruption of cell wall and reduction in molecular weight of substrate) and non-extruded maize-DDGS and wheat-DDGS between 0 to 30% in broiler diet. The authors recommended an inclusion rate of no more than 10% for either maize-DDGS or wheat-DDGS with or without extrusion because above this level growth performance responses were depressed. Overall, it was generally consistent in the literature that increasing the inclusion level of maize- or wheat-DDGS in broiler and turkey diets compromised growth performance. It was also noted that the maize- or wheat-DDGS inclusion rates at which a decline in growth performance was noted also varied among studies. Although differences in the nutritional quality of the DDGS used may be responsible for the variations in growth performance response reported among studies, factors such as the chemical characteristics of the diet used, breed and age of bird and enviromental conditions may also affect bird performance. On the other hand, there is possibility that a diet containing DDGS that is formulated using digestible nutrient values will support growth performance and further benefits may be derived by supplementing such diet with exogenous enzymes.

1.6.2 Effect on Egg Production and Quality

Some authors have assessed the possibility of using DDGS in laying hens diets. Richter *et al*. (2006) fed laying hens diets containing up to 15% wheat-DDGS with or without an admixture of supplemental xylanase, amylase, glucanase, cellulase and protease enzymes between 20 to 64 weeks of age. In that study, egg number or quality was not affected by the inclusion of 15% wheat-DDGS. Lumpkins *et al*. (2003) fed laying hens 15% maize-DDGS from 21 to 43 weeks of age and observed no detrimental effect on egg production, quality or egg shell quality compared with the controls (0% maize-DDGS). Wu-Haan *et al*. (2010) observed that feeding laying hens diets containing up to 20% maize-DDGS had no effect on egg weight, egg production, feed intake or BWG between 21 to 26 weeks of age. Similarly, Scheideler *et al*. (2008) noted that egg production, feed intake, and BWG were not affected by dietary maize-DDGS inclusion up to 25% in laying hen diets.

Inclusion of 10% maize-DDGS in layer hen diets produced similar egg production, egg weight, feed intake or BWG compared with the controls (0% maize-DDGS) in a study by Roberts *et al*. (2007a). Jung and Batal (2009) demonstrated the effect of feeding up to 12% HPmaize-DDGS to laying hens and found that inclusion levels of 3 and 12% improved hen day egg production compared to those fed the control diet without HPmaize-DDGS, and also that, egg mass was significantly improved in hens fed the 3% HPmaize-DDGS diets.

Two experiments were conducted by Roberson *et al*. (2005) to evaluate the effect of maize-DDGS inclusion at a level increasing from 0 to 15% on the performance of laying hens and egg production indices. In the first experiment the authors observed a linear decrease in egg production as the level of maize-DDGS in the diets increased at 52 to 53 weeks of age. However no effect of maize-DDGS inclusion was observed at other periods (49, 51, 55 weeks) in the study. In the same experiment there was a decrease in egg mass at 51 and 53 weeks of age as the level of maize-DDGS increased in diet, but shell quality was not affected by dietary treatments. In the second experiment, although egg production and egg mass were not affected by 15% inclusion of maize-DDGS in the diets there was a linear decrease in egg weight while maize-DDGS inclusion at levels as low as 5% led to darker coloured yolks in both experiments. Maize-DDGS is a good source of the pigment (carotenoids) that produce the desirable dark-yellow coloured egg yolks in layers, however, the concentration of xanthophylls in maize-DDGS vary among sources due to its susceptibility to heat damage (Roberson *et al*., 2005).

The effect of feeding graded levels of maize-DDGS (0, 5, 10, 15, and 20%) in diets formulated to be isocaloric and isonitrogenous on laying hen performance and egg quality was investigated by Swiatkiewicz and Koreleski (2006). The authors observed no significant effect of maize-DDGS inclusion on all production indices during the first phase of the study (26 - 43 weeks); however a reduction in egg production and daily egg weight was reported in laying hens fed 20% maize-DDGS during the second phase (44 - 68 weeks). Supplementation of the diet with an NSP hydrolyzing enzyme mix (endo-1,4-β-xylanase, endo-1,3-β-glucanase, pentosanase, hemicellulase and pectinase) during the 44 to 68-week feed phase helped offset the drop in lay rate and daily egg mass in that study (Swiatkiwicz and Koreleski, 2006). Shurson (2003) evaluated the effect of feeding 10% maize-DDGS on egg production, egg quality and egg yolk colour in layer hens and observed no difference in average hen body weight compared to the control (0 % maize-DDGS) during the first 2 weeks. In that study, total egg production and body weight at 3 to 12 weeks of age was greater for hens fed 10%

maize-DDGS compared with the controls. Inclusion of maize-DDGS in the diet also resulted in darker coloured egg yolks.

Green *et al*. (2010) studied the effect of using high levels of maize-DDGS on the long term performance of laying hens; the authors observed a linear decrease in both feed intake and egg production as the level of maize-DDGS increased towards 50% in the diet. The authors attributed the decline in egg production to methionine deficiency in hens fed the maize-DDGS diets. In Pineda (2008) study, 0, 23, 46 or 69 % maize-DDGS was incorporated into layer hen diets and fed for 8 weeks. Although there was no significant differences among dietary treatments with regards to body weight, egg production decreased whereas egg weight increased linearly with increasing levels of maize-DDGS in the diets.

There is limited information on the use of DDGS in duck nutrition; however Huang *et al*. (2006) fed laying ducks from 14 to 50 weeks of age on dietary treatments of 0, 6, 12 and 18% maize-DDGS. The authors showed that inclusion of maize-DDGS at levels up to 18% had no effect on feed intake, FCR or egg quality, and egg production rate increased with increasing maize-DDGS inclusion during cold weather conditions. The differences in egg production and quality among studies may be due in part to differences in the nutritional quality of the maize-DDGS used, although it is noted that other factors such the age of the bird, inclusion level of maize-DDGS and chemical composition of the diet used may also be implicated.

1.6.3 Effect on Carcass Characteristics and Meat Quality

Many of the studies evaluating the use of bioethanol co-products in poultry diets have focused largely on increasing inclusion rates without compromising performance. However, the chemical profile of the feed or feedstuff (fatty acid and AA composition) may have an effect on the chemical properties and partitioning of nutrients in livestock tissues (Loar *et al*. 2010). Information in the literature regarding effects of feeding maize-DDGS on poultry meat quality is rare and is non-existent for wheat-DDGS. Corzo *et al*. (2009) using diets that were formulated to be both isocaloric and isonitrogenous studied the effect of feeding 0 or 8% maize-DDGS on meat quality in broilers. The authors reported the mean breast meat pH to be similar at 15 minutes and at 24 hours post-mortem between the two dietary treatments as well as colour, cooking loss percentage, tenderness and sensory analysis in the breast meat for both treatments. The effect of feeding up to 24% maize-DDGS on broiler meat quality was determined by Schillings *et al*. (2010). Although the authors noted increasing breast meat pH with increasing maize-DDGS inclusion in the diet (indicative of less acidity and hence better meat quality), the other meat quality indices were not affected by the treatments.

1.7 Nutrient Digestibility of DDGS for Poultry

1.7.1 Metabolisable Energy

Although performance data can give an indication of the potential value of a feedstuff, diet formulation requires more specific information on nutrient utilisation. In the case of DDGS, variable and generally lower digestibility of nutrients (compared to conventional feed ingredients) has been reported. Fastinger *et al*. (2006) evaluated the TME_n values of 5 samples of maize-DDGS using adult caecectomized roosters, the authors reported an average value of 2871 kcal/kg with large differences among the 5 samples (±563 kcal/kg). Such large variations in TME_n values were also reported by Parsons *et al*. (2006), in a study to determine the energy value of 20 maize-DDGS samples. Furthermore, Fastinger *et al*. (2006) reported the lowest TME_n value in the darkest of 5 maize-DDGS samples analysed (600 kcal/kg less TME_n in the darkest compared to lightest) and noted a colour threshold (L* between 28 and 34) where the AA and TME_n value in maize-DDGS is reduced. Also investigating the relationship between colour and energy value of DDGS, Noll *et al*. (2007a) reported a negative correlation (r = –0.98) between the L* value of maize-DDGS and the amount of solubles added back to DDG and further observed that darker coloured maize-DDGS samples have higher TME_n values. The observation that darker coloured DDGS contain greater ME in the study of Noll *et al*. (2007a) is direct opposite of the observations in the study of Fastinger *et al*. (2006). It is speculated that the dark colour of the maize-DDGS used in the Noll *et al*. (2007a) study may have been due to greater proportions of CDS in the DDGS rather than excessive heat treatment of the DDGS.

Lumpkins *et al*. (2004) analyzed the TME_n and true AA digestibility of maize-DDGS using cecectomized roosters, and reported an average TME_n value of 2905 kcal/kg. A study by Batal and Dale (2006) using maize-DDGS from 17 different samples and 6 different plants in the Midwest region of the USA reported a TME_n value ranging from 2490 to 3190 kcal/kg and a coefficient of variation of 6.4% in cecectomized roosters. The authors developed a prediction equation for TME_n based on fat, protein, fiber and ash content of maize-DDGS, but reported fat as the single best predictor of TME_n content, however the overall regression coefficient was also low (r^2 = 0.29). Using the precision-fed intact rooster assay, Jung and Batal (2009) analysed the TME_n value of 8 samples of HPmaize-DDGS for laying hens. The average TME_n value of the HPmaize-DDGS samples was 2851 kcal/kg with a range between 2667 and 3282 kcal/kg. In another study, Kim *et al*. (2008) determined the TME_n contents of conventionally processed maize-DDGS, HPmaize-DDG, and maize germ. The TME_n content of HPmaize-DDG and maize germ was 2694 and 4137 kcal/kg respectively, whereas the

TME_n value for maize-DDGS was 3266 kcal/kg. Kim *et al*. (2008) also showed that the HPmaize-DDG contained about 17% less TME_n than the maize-DDGS and the maize germ contained about 22% more TME_n than the maize-DDGS which was attributed to the differences in fat and protein contents between the co-products.

Cozannet *et al*. (2010a) measured the apparent metabolisable energy (AME) and nitrogen-corrected apparent metabolisable energy (AME_n) value of 10 wheat-DDGS samples in roosters, broilers, layers and growing turkey, although the AME_n:GE ratios in the study were generally low for all speciess, the values reported (10.3, 9.9, 9.6, and 9.9 MJ/kg of DM for roosters, broilers, layers and turkey respectively), showed that AME_n for wheat-DDGS differed between the poultry speciess. The authors further suggested that AME_n can be predicted from either the ADF content ($r^2 = 0.79$) or L* score ($r^2 = 0.77$) of wheat-DDGS. The AME_n value of two different batches of wheat-DDGS was measured in adult cockerels by Vilarino *et al*. (2007) using the difference method, the authors noted that the AME_n values differed between batches of wheat-DDGS (11.19 *vs* 10.57 MJ/kg) and also observed a low AME_n:GE for the two batches of wheat-DDGS (55.8 and 52.1%) compared to wheat (78%). However AME_n:GE values of wheat-DDGS were higher than those of rapeseed meal (34%) and similar to SBM (54%) in that study.

Adeola and Ilekeji (2009) compared practical and semi-purified diets in determination of the AME and AME_n value of maize-DDGS for broiler chickens using the regression method. The authors observed a linear decrease in energy retention as the level of maize-DDGS increased from 0 to 60 % in the practical diets (78.6 - 58.6%) and a rather lesser decrease when using the semi-purified diet (86.8 - 75.4%). Based on this result the authors (Adeola and Ilekeji, 2009) suggested that the ME value of maize-DDGS may be affected by the basal diet used in the bioassay and that the greater proportional dietary energy utilisation in the semi-purified nitrogen-free diet may be due to associative effects of dietary energy sources. In summary the wide differences in reported energy values of both maize-DDGS and wheat-DDGS shows the need to develop a standardised method for determining the energy value of DDGS. Although it is already known that DDGS nutrient contents vary among sources, the methodology, age and speciess of poultry used for estimating energy value are also potential sources of variation.

1.7.2 Amino Acid Digestibility

Crude protein and AA digestibility of maize- and wheat-DDGS have been reported to vary substantially in poultry (Batal and Dale, 2006; Fastinger *et al*., 2006; Cozannet *et al*., 2010a).

As mentioned earlier, a factor that may reduce as well as cause variability in the digestibility of CP and AA in DDGS for poultry is heat treatment. Excessive application of heat during drying reduces the digestibility of AA (especially Lys) in feedstuffs for monogastrics due to the formation of insoluble AA-carbohydrate compounds by the Malliard reaction. This may be exascerbated in DDGS because several steps in bioethanol production (jet cooking, liquefaction, saccharification, drying) involve heat application. Liu and Han (2011) noted that the formation of carbohydrate-AA complexes in maize-DDGS may not be solely limited to drying, because a proportion of Lys in WDG and CDS are already bound by the Malliard reaction before the drying process.

The TME_n and true ileal amino acid digestibility (TIAAD) value of eight maize-DDGS samples were analysed using precision-fed caecectomized rooster assay by Batal and Dale (2006). In that study, the effect of heat damage on Lys digestibility during processing was demonstrated; with the highest and lowest apparent ileal digestibility (AID) for Lys observed in the lightest- and darkest coloured maize-DDGS samples respectively. Furthermore, Batal and Dale (2006) also reported strong correlations between digestible Lys, Thr, Arg, His and Trp contents and L* values (r = 0.87, 0.53, 0.71, 0.84 and 0.72 respectively) and b* values (0.96, 0.76, 0.87, 0.88 and 0.77 respectively) of maize-DDGS. Correlations between L* values and standardised ileal digestibility (SID) of Lys (L* = 53.8; b* = 42.8 associated with 0.65 digestible Lys) was reported by Ergul *et al*. (2003) in 22 samples of maize-DDGS using caecectomized roosters. The authors also noted that except for Leu and Ser, the digestible AA content was highly variable among the 22 maize-DDGS sources investigated.

Fastinger *et al*. (2006) reported the AID and true ileal digestibility (TID) of AA to be 30 and 15 percentage units lower in the darkest coloured of five maize-DDGS samples assessed. The authors also noted that in addition to reducing AA digestibility, excessive heat treatment also reduced the total Lys content of maize-DDGS. The AID and SID of AA in maize-DDGS for broilers was assessed by Bandegan *et al*. (2009). In that study, the most variable and lowest AID estimates were observed for Lys (24 - 48%), Thr (48 - 61%) and His (57 - 69%) but the overall SID for CP was 69.1%. Furthermore, the lowest average SID was observed for Lys (40%) whereas Phe, Met and Leu were the most digestible AA (SID; 86.4, 75.7 and 75.6%, respectively).

Cozannet *et al*. (2010b) determined the standardised ileal amino acid digestibility (SIAAD) of 7 wheat-DDGS samples using caecectomized roosters and reported wide variability in the SID of Lys (49 - 71%). In the same study, the SID of CP ranged from 76 and 85%. Further studies by Cozannet *et al*. (2011) also reported a wide variability in SID of Lys (-0.04 – 0.71)

in 10 samples of wheat-DDGS assessed using caecectomized roosters. Furthermore, Cozannet *et al*. (2011) also observed a positive relationship between colour score (L* value) and the Lys content of wheat-DDGS (r = 0.63), as well as the SID value of Lys (r = 0.64) for broilers. In addition, the authors developed predictive models which indicated that the Lys content of wheat-DDGS can be determined from a quadratic (R^2 = 0.94) or a linear-plateau model (R^2 = 0.90; breakpoint for 1.9 g/100g Lys in CP and a 0.63 plateau SID value).

Nutrient digestibility increases with age and differs between poultry speciess and feed ingredients (Batal and Parsons, 2002). Adedokun *et al*. (2008) assessed the SIAAD of five plant-based feed ingredients (light and dark coloured maize-DDGS, canola meal, maize, and SBM) in 5 and 21 day-old broiler chicks and turkey poults. In that study, the SIAAD of the feed ingredients evaluated increased with age in all poultry speciess, except for the dark coloured maize-DDGS. The lack of improvement in the digestibility of the dark coloured maize-DDGS with age in the Adedokun *et al*. (2008) study may be due to the poor solubility of AA in the gastrointestinal tract as a consequence of their presence as Malliard complexes. Collectively, it was consistent in the literature that excessive heat treatment reduces protein and AA digestibility in DDGS for poultry and that darker coloured DDGS samples are less digestible compared with lighter coloured DDGS.

1.7.3 Nutrient Retention and Excretion

The excretion and volatilization of nutrients of dietary origin are responsible for a large part of the environmental issues associated with poultry production. Nitrogen and P from poultry litter have been reported to have potentially negative effects on air, soil and water quality in terms of eutrophic conditions that result from excess P in excreta in run-off water and acidification of the environment resulting from ammonia, S compounds, volatile organic compounds and nitrogen oxides (Roberts *et al*., 2007b; Pineda, 2008). A main disadvantage of using high levels of DDGS has been identified to be an increase in nutrient and dry matter (DM) excretion (Widyaratne and Zijlstra, 2007; Pineda, 2008). Because DDGS contains a higher level of CP and P compared to maize and SBM, high level of DDGS inclusion and its lower digestibility compared to maize or wheat may lead to an increase in excretion of nutrients (Leytem *et al*. 2008). Additionally, increased N excretion demands metabolic energy for N removal, consequently leaving less energy available for the animal for maintenance and productive purposes.

Widyaratne and Zijlstra (2007) studied the effect of feeding 40% wheat-DDGS in pigs and found N output to increase linearly with increasing inclusion rates. Similarly, Leytem *et al*.

(2008) demonstrated the effect of feeding 20% wheat-DDGS on nutrient excretion in broilers. The authors noted that the apparent retention of both N and P decreased linearly with increasing DDGS inclusion and led to an increase in N and total P excretion. The authors also observed an increase in excretion of phosphate P and a concomitant decrease in phytate-P. Pineda (2008) observed an increase in N retention in laying hens fed up to 69% maize-DDGS, however due to the excessive amount of CP and higher N consumption, there was also an increase in N excretion in their study. The nutritional value of HPmaize-DDGS for broilers and its effect on nutrient excretion was assessed by Applegate *et al*. (2009), the authors found that the dietary treatments satisfactorily optimized BWG and carcass yields, but there was also 21.9 and 31.8% more manure DM and N, respectively in birds fed the 50% HPmaize-DDGS compared to the control maize-SBM diet.

Although increased ammonia emission from the manure will presumably be associated with increased N excretion, dietary DDGS appear to have an attenuating effect on ammonia emissions (Roberts *et al*., 2007b). Crude fibre is not digested by the birds, and some of the fibre is fermented by microbes in the large intestines, producing short chain fatty acids, which in turn lowers the manure pH. The lowered pH is thought to result in a shift in the NH_3 equilibrium toward the less volatile ammonium ion ($NH_3 + H^+ \leftrightarrow NH4^+$). Therefore, birds receiving DDGS may excrete more N, but the N does not volatilize. This effect of dietary fibre on manure acidification and NH_3 emission has been demonstrated in laying hens using diets containing maize-DDGS. Roberts *et al*. (2007b) fed laying hens 10% maize-DDGS and crude fibre from wheat middlings and soyabean hulls between 28 and 58 weeks of age and then determined their effect on NH_3 emissions. In that study, there were differences in N excretion between treatments fed 10% maize-DDGS and the control (maize-SBM diet). Wu-Haan *et al*. (2010) fed laying hens up to 20% maize-DDGS and reported a reduction in the mass of NH_3 emissions as the level of maize-DDGS increased in the diet (daily emissions were 105.4, 91.7 and 80.2 mg/g of N consumed for 0, 10 and 20% maize-DDGS respectively). Overall the authors indicated that the inclusion of maize-DDGS at 20% in diets of laying hens can reduce NH_3 emissions by approximately 24%. In the same study a 58% reduction in H_2S emissions was also observed when laying hens were fed 20% maize-DDGS.

1.8 Dietary Fibre Type and Crude Protein Level

The digestible energy and nutrient digestibility in maize- and wheat-DDGS are reasonably well defined for pigs and will not be reviewed in the current report. However, it is not known whether the choice of protein feedstuff in relation to its fibre characteristics affects nutrient digestibility. It is intuitive that a diet formulated for pigs using maize-DDGS as protein source

will contain greater levels of soluble fibre compared with a diet formulated using SBM as protein source. Dietary fibre is found in different forms and quantities in feed ingredients that are used in diets for pigs. Although, the NSP found in cereals exert greater anti-nutritive effects compared with legumes and oil seeds, the contributions of NSP by legumes cannot be underestimated because legumes often constitute a large proportion of the diet. A plethora of studies have reported a decrease in energy utilisation and/or, protein and AA digestibility with increased dietary fibre in pigs (Sauer *et al*., 1991; Wang *et al*., 2006; Wilfart *et al*., 2006).

Due to the environmental implications, there is an increased need to mitigate excess N excretion from pigs. One of the means of reducing N excretion by pigs is by reducing dietary CP intake. Decreasing the CP content of the diet by 2 to 4% resulted in an average of 20% decrease in N excretion by finishing pigs (Lee *et al*., 2001). In addition, it appears that pigs are able to compensate for the reduction in CP intake by increasing the efficiency of nutrient utilisation. Christensen (1984) noted that animals may respond to restricted protein intake by increasing intestinal amino acid (AA) absorption. Otto *et al*. (2003) reported an increase in ileal AA digestibility for growing pigs by decreasing dietary CP concentration from 15 to 6%. In most cases, dietary CP level is reduced by replacing SBM with a feed ingredient with low CP content relative to SBM. Usually such feed ingredient also contains greater levels of fibre. This is important because the physical and chemical properties of dietary fibre may affect nutrient utilisation (Sauer *et al*., 1991). Because feed ingredients contain different types and levels of fibre, it is important to investigate whether the type of feed ingredient used as protein source has an effect on the digestibility of AA or the ability of growing pigs to cope with a reduction in dietary CP level.

1.9 Improving DDGS Nutritional Quality

The main anti-nutritive factors in DDGS as a feed ingredient for poultry are the high crude fibre content and the low digestibility of CP and AA. These factors are known to play a major negative role in feed utilisation and nutrient excretion in poultry (Widyaratne and Zijlstra, 2007; Pineda, 2008). Altogether, based on present knowledge, there may be a potential for enhancing DDGS digestibility for poultry. Methods such as enzyme supplementation and fractionation (removal of the fibre fraction) have been demonstrated by a few studies as having the potential of improving the value and encourage the use of DDGS at higher inclusion rates (Swiatkiewicz and Koreleski, 2006; Kim *et al*., 2010; Olukosi *et al*., 2010).

1.9.1 Exogenous Enzymes in Poultry Diets and Potential Value for DDGS

The use of exogenous enzymes in poultry nutrition is well documented in scientific literature and has been reviewed (Cowieson *et al*., 2006; Slominski, 2010; Adeola and Cowieson, 2011). Generally, the main objectives of supplementing enzymes in poultry diets are to break down the anti-nutritive factors in feed ingredients, to enhance the overall digestibility of the feed, to make certain nutrients biologically more available, and to reduce environmental pollution from animal excreta by reducing nutrient and DM excretion. Other benefits, such as the use of exogenous enzymes in improving nutrient digestion in young poultry at early ages due to a lack or insufficiency of digestive enzymes have also been reported in literature (Slominski, 2010).

Exogenous enzymes such as carbohydrases, phytases and proteases or a combination of these enzymes are often incorporated into poultry diets but there is a dearth of information on the efficacy of these enzymes in poultry diets containing varying levels of DDGS. In addition to improving digestibility, supplementing diets containing DDGS with exogenous enzymes may reduce variability in the nutritive value of the product, as well as ameliorate the adverse effects of the anti-nutritional factors. Reduction in the variability in nutrient quality of feed ingredients with the use of exogenous enzymes has been reported in literature (Bedford and Schulze, 1998; Bedford, 2000) and improvement in growth performance and nutrient utilisation have also been observed to be greater for poor quality raw materials (Classen *et al*., 1995; Bedford and Schulze, 1998).

1.9.1.1 Carbohydrases

Carbohydrases are enzymes that hydrolyse NSP into oligosaccharides and monosaccharides. The nutritive benefits of supplemental carbohydrases in diets includes reduced digesta viscosity in the gastrointestinal tract due to the hydrolysis of soluble arabinoxylans and β-glucans, the release of nutrients encapsulated in the NSP structure and gel matrix and a greater exposure of substrates to digestive enzymes (Bedford, 2000). The types and concentrations of NSP vary among feedstuffs. For example, wheat, maize, triticale and rye contain predominantly arabinoxylans, whereas barley and oats are rich in β-glucans. Therefore the types of carbohydrases that are supplemented to diets vary according to the dietary NSP composition. Hence, xylanases are supplemented to wheat, maize, triticale and rye-based diets, whereas β-glucanases are more effective in barley and oat-based diets.

Xylanases are hydrolases depolymerising the plant cell wall component xylan. The depolymerisation action of endo-xylanase results in the conversion of the polymeric substance into xylooligosaccharides and xylose. The complex structure of xylan requires different

enzymes for its complete hydrolysis. Endo-1, 4- β-xylanases depolymerise xylan by the random hydrolysis of xylan backbone and 1, 4- β-D-xylosidases split off small oligosaccharides. The side groups present in xylan are liberated by α-L-arabinofuranosidase, α-D-glucuronidase, galactosidase and acetyl xylan esterase. Diverse forms of these enzymes exist, displaying varying folds, mechanisms of action, substrate specificities, hydrolytic activities (yields, rates and products) and physicochemical characteristics (Adeola and Cowieson, 2011). Research has focused mainly on only two of the xylanase containing glycoside hydrolase families, namely families 10 and 11, yet enzymes with xylanase activity belonging to families 5, 7, 8 and 43 have also been identified and studied, although to a lesser extent (Collins *et al*., 2005).

The plant cell wall is a composite material in which cellulose, hemicellulose (mainly xylan) and lignin are closely associated. Wheat contains 5 to 8% arabinoxylans (pentosans consisting of the monosaccharides; arabinose and xylose linked in β-1-4 linkages), up to 1% β-glucans and 2-3% cellulose (Choct *et al*. 2004). Supplementation of wheat-based diets with exogenous xylanase has been documented to be effective at ameliorating the negative effects of NSP in poultry diet (Choct *et al*. 2004; Adeola and Cowieson, 2011).

Improvements in growth responses and nutrient digestibility with the supplementation of xylanase in wheat-based poultry diets have been widely reported in literature. Olukosi *et al*. (2007) reported improvement in BWG, feed intake and feed efficiency in broilers using low and high levels of supplemental xylanase. Improvement in FCR in broilers fed either ground or whole wheat supplemented with a xylanase was reported by Wu *et al*. (2004). Amerah and Ravindran (2009) observed an increase in feed intake and BWG in broiler starters fed xylanase supplemented soft wheat-based diet; xylanase supplementation of high-viscosity wheat-based diet improved weight gain and feed efficiency by 13 and 12%, respectively, and true metabolisable energy in ducks (Adeola and Bedford 2004).

Veldman and Vahl (1994) reported an improvement in FCR and BWG in broilers fed xylanase supplemented diets related to lowering of digesta viscosity, Nian *et al*. (2011) reported an improvement in FCR, diet AME and ileal digestibility of hemicellulose in 4 week old broilers. An increase in ileal digestibility of insoluble NSP in low-ME wheat was reported by Choct *et al*. (2004). Improvement in the AID of 17 AA (average of 4.8%) was observed by Selle *et al*. (2009) in xylanase supplemented diets for broilers whereas supplementation of xylanase and β-glucanase enzymes yielded modest improvements in FCR of turkey receiving wheat-, barley- or wheat-based diets (Mathlouthi *et al*., 2003). The use of xylanases in wheat-based diets reduced the viscosity of digesta by 30% and 50% in studies by Wu *et al*. (2004)

and Steenfeldt *et al*. (1998), respectively. Reducing digesta viscosity for poultry using carbohydrases is important because in a recent review, Adeola and Cowieson (2011) noted that the benefits derived from a reduction in digesta viscosity are often greater than improvement in energy utilisation and the effect of digesta viscosity are usually more pronounced in poultry compared to most other monogastrics.

The few studies examining the efficacy of exogenous enzymes for improving the nutritive value of DDGS have focused entirely on maize-DDGS, whereas wheat is expected to contain higher levels of arabinoxylans than maize. In a study investigating the effect of supplemental xylanase on growth performance and nutrient digestibility in broilers, Liu *et al*. (2011) reported an increase of 20% and 620 MJ/kg in the dissapearance of hemicelluloses and ME respectively in maize-DDGS diets with supplemental xylanase. Addition of an NSP hydrolysing enzyme to a 20% maize-DDGS diet for broilers increased the SID of all AA and AME of ME-deficient diets in a study by Lee *et al*. (2010). Similarly, Emiola *et al*. (2009) observed improvement in AA digestibility in pigs using a multi-carbohydrase supplemented DDGS diet. Overall, it appears that supplementing a diet containing DDGS with an enzyme mixture containing carbohydrases and protease may help mitigate the negative effects of fibre in DDGS, thus improve its nutritional value and encourage its use for poultry.

1.9.1.2 Phytases

Phytase (*myo*-inositol hexakisphosphate phosphohydrolases) catalyzes the hydrolysis of phytate into myo-inositol and free phosphates. Phytases can be classified into 3-phytase (EC 3.1.3.8), 5-phytase or 6-phytase (EC 3.1.3.26) depending on where dephosphorylation begins (Selle and Ravindran, 2007), but the two most common in nature are the 3- and 6-phytases. A 3-phytase initiates catalysis by removing the phosphate in the carbon 3 position, whereas a 6-phytase begins with the phosphate in the carbon 6 position. Both types of phytase are effective at removing the phosphate groups, with the exception of the axial phosphate in the second position. The use of phytases in animal diets is not new; a plethora of studies have documented the value of exogenous phytases in releasing phytate bound P and improving P utilisation for both poultry and swine. Generally, most of these studies focused more on determining the ability of phytase to replace inorganic P supplementation. Responses of interest have included BWG, feed intake, feed efficiency (Ravindran *et al*., 2001; Selle *et al*., 2007; Letourneau-Montminy *et al*., 2008; Olukosi *et al*., 2008a) tibia, femur or toe breaking strength and ash content (Ravindran *et al*., 2001; Martinez-Amezcua *et al*., 2006), and egg production in layers (Wu *et al*., 2006). Phytase activity is defined as fytase units (FTU),

where 1 fytase unit is defined as the quantity of enzyme required to liberate 1 μmol inorganic orthophosphate/min at pH 5.5 from an excess of 15 μ_M-sodium phytate at 37 ^oC.

Phytate is thought to negatively affect the digestibility of energy and AA. Selle and Ravindran (2008), proposed three possible mechanisms by which these negative effects may occur. First, phytate may form a binary protein-phytate complex, which would cause protein to be excreted along with the phytate bound P. Second, phytate may increase endogenous AA flows, which will reduce the AID of AA. Third, intestinal absorption of AA may be compromised by the presence of phytate in the gut lumen because AA may bind to the phytate molecule, which cannot be absorbed. However, the response in AA or protein digestibility with phytase supplementation is not consistent. Some studies have reported improvements in CP and AA to phytase supplementation of diets for poultry (Yi *et al*., 1996; Rutherford *et al*., 2002; Martinez-Amezcua *et al*., 2006; Ravindran *et al*., 2006; Selle *et al*., 2006, 2007), whereas others have found no benefits (Biehl and Baker, 1997; Ravindran *et al*., 1999; Augspurger and Baker, 2004). Further importance of phytase in improving DM and energy utilisation for poultry has also been evaluated. Ravindran *et al*. (2001) demonstrated that increasing the level of supplemental phytase to Lys deficient diets increased AME in addition to improving the digestibility of CP and AA in broilers. The authors further calculated that phytase supplementation in that experiment was the equivalent to adding 0.074% Lys to the diet.

There are only few studies that have assessed the use of microbial phytase in diets containing DDGS, more focus have been on multi-enzyme complexes (combination of xylanase, amylase, protease and phytase). Although available P in DDGS is greater than in many other plant feedstuffs, more than 25% of the P is still thought to be non-bioavailable (Martinez-Amezcua *et al*., 2004). Martinez-Amezcua *et al*. (2006) conducted 3 experiments to determine the effects of phytase and citric acid in releasing phytate-P in diets containing maize-DDGS, and on AME_n and AA digestibility in New Hampshire x Columbian chicks. In the first experiment, phytase increased tibia ash but had no effect on $AME_{n,}$ phytase supplementation increased AID of AA in the second experiment, whereas a combination of phytase and citric acid increased tibia ash in the third experiment. Improvements in the coefficient of apparent ileal dry matter digestibility was reported by Olukosi *et al*. (2010) in broilers fed diets containing 10% maize-DDGS supplemented with a phytase. There are few studies in the literature that have determined the benefits of supplemental phytase in diets containing DDGS. Although the levels of phytate-bound P in DDGS have been reported to be low due to phytate hydrolysis by yeast phytase during the fermentation process (Liu, 2011), birds may benefit from "extra-phosphoric" effects of supplemental phytase. Apart from releasing phytate

bound P, phytase have also been shown to improve AME, AA and mineral digestibility in the diet for poultry (Ravindran *et al*., 2001). Nonetheless, the extra-phosphoric effects of phytase are generally inconsistent in the literature and require further investigation.

1.9.1.3 Proteases

Protease refers to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins. Over the years, the use of proteases as feed additives has been a regular practice in cereal-based diets usually as an integral part of enzyme admixtures. A number of potential modes of action have been suggested two of which are: 1) proteases may supplement endogenous peptidase production, reducing the requirement for AA and energy and 2) proteases may hydrolyse protein-based anti-nutrients such as lectins or trypsin inhibitors, improving the efficiency by which the bird utilizes AA and reducing protein turnover (Adeola and Cowieson, 2011).

The benefits of supplementing proteases in diets containing DDGS may be particularly significant in view of variability in the quality and quantity of the CP and AA components in such diets. However, reports of using proteases alone are scarce as they are more often incorporated as a part of a mix of enzymes. Odetallah *et al*. (2003) determining the efficacy of a broad-spectrum protease enzyme (keratinase) in maize-SBM broiler starter diet reported improved growth performance, BWG and FCR in broiler chicks fed either low or high amounts of protein. Brenes *et al*. (1993) similarly observed improved FCR and AME in a protease-based enzyme preparation fed to chicks and laying hens.

1.9.1.4 Enzyme Combinations

It is reasonable to assume that if carbohydrases can breakdown NSP and elicits beneficial improvements, a combination with other enzymes (proteases and phytases) exhibiting different catalytic activities and producing positive effects might improve the scale and consistency of response. Several studies have reported beneficial effects of supplemental enzyme admixtures in wheat-, maize- and barley-based poultry diets (Cowieson and Adeola, 2005; Thacker, 2005; Francesch and Geraert, 2009; Olukosi *et al*., 2007; Olukosi *et al*., 2008b; Selle *et al*., 2009) Therefore, the use of a multi-enzyme complex in diets containing DDGS may help improve the nutritive worth for poultry and swine. Masa'deh *et al*. (2010) reported no negative effect of feeding 30% maize-DDGS in diets of broilers supplemented with an admixture of enzymes containing phytase, protease, pentosanase, pectinase, cellulase, beta-glucanase and amylase activities. Jung *et al*. (2010) evaluated the importance of a supplemental enzyme admixture (hemicell and Avizyme) on carcass characteristics and

performance of broilers fed diets containing 12% maize-DDGS and observed improvements in BWG and FCR. In Olukosi *et al*. (2010) study, the use of phytase alone improved nutrient utilisation in broilers fed 10% maize-DDGS in their diets but a combination of enzymes (phytase, carbohydrases and protease) did not produce greater benefits than using the phytase alone.

1.9.1.5 Effect of Diet and Exogenous Enzymes on Gut Morphology

The efficiency of digestion and absorption of dietary nutrients by poultry is affected by the development and health of the gastrointestinal tract. The physical and chemical characteristics of the diet have been reported to have an effect on the morphology of the small intestinal absorptive structure (Smits and Annison, 1996). The changes to the morphology of the gastrointestinal tract are often due to the presence of toxins or the anti-nutritive effects of dietary fibre. (Smits and Annison, 1996). Short villi are an indication of less surface area for nutrient absorption. The crypt depth may give an indication of the rate of cell proliferation and an increase in crypt depth is an indication of faster cell turnover and greater metabolic cost for cell replacement (Yang *et al*., 2008; Rebole *et al*. 2010). Exogenous enzymes may be used in diets containing wheat-DDGS for poultry to ameliorate the anti-nutritive effects of dietary fibre which may lead to improvements in the small intestinal absorptive structure. However, the effect of exogenous enzymes on gastrointestinal tract morphology of broilers has not been consistent. Mathlouthi *et al*. (2002) reported improvements in the gut morphology of broilers with xylanase supplementation of a rye-based diet whereas Yang *et al*. (2008) noted that supplemental xylanase did not affect jejunal villi height but reduced crypt depth of broilers receiving a wheat-SBM based diet at seven days of age. Using supplemental phytase, Wu *et al*. (2004) noted an increase in duodenal villi height but no effect on crypt depth in broilers at 21 days of age. In the study of Iji *et al*. (2001) there was no effect of xylanase on gut morphology of broilers receiving a wheat-based diet in their study. The responses noted in the studies described above indicate that the effect of exogenous enzymes on cellular development of the gastrointestibnal tract structure is inconsistent and requires further investigation.

1.9.2 Fractionation

Fractionation is designed to separate or reduce the CF fraction in DDGS consequently reducing NSP content, increasing the concentration of energy and other nutrients in the product. Based on a study by Kim *et al*. (2010), the CF fraction of DDGS can be successfully removed before fermentation or in the finished product by two newly developed processing

technologies namely; the enzymatic milling (E-mill) or the elusieve process. The E-mill process uses proteases and starch-degrading enzymes to separate the non-fermentable fibre and germ before fermentation which consequently creates a modified DDGS low in fibre and increased in protein content. On the other hand, the elusieve method involves the recovery of the fibre fraction from DDGS by sieving into different size categories. This process is usually performed by elutriating fibre components via different air velocities and as expected both methods produced DDGS that was higher in CP and lower in CF content in this study (Kim *et al*., 2010). Digestibility studies using precision-fed caecectomized roosters showed an increase in the TME_n and AA digestibility for both methods compared to conventional DDGS, although digestibility was greater with the E-mill method (Kim *et al*., 2010).

Extrusion is another processing technology that could increase the nutritional value of DDGS by physical disruption of cell walls and cleavage of NSP into smaller fragments as demonstrated by Oryschak *et al.* (2010). This process involves the combined treatments of moisture, pressure, temperature and mechanical shear using a single or twin screw extruder. In the study by Oryschak *et al*. (2010), extrusion of maize-DDGS significantly increased the AID of all AA with Lys, Thr, Val and Arg increased by 31, 26, 23 and 21% respectively at 15% inclusion rate in broiler diets. A similar trend in improvement in nutrient digestibility was also recorded at 30% dietary inclusion of extruded wheat-DDGS. Parsons *et al*. (2006) compared the nutritional value of maize-DDGS as affected by different processing techniques reported in literature. The processes evaluated were namely; the modified dry grind process, the quick germ-quick fibre process (these two processes are slight modifications of the E-mill process earlier described) and the elusieve process. Although the authors observed higher CP and lower CF contents in maize-DDGS from all methods of processing compared to conventional maize-DDGS, digestibility results using caecectomized roosters assay indicated that processing method had little or no effect on the digestibility of AA in the processed maize-DDGS samples. It was generally reported in the literature that removing the fibre fraction in DDGS increases its nutritional value for poultry. However, the fractionation processes currently available are costly and may increase the cost of DDGS which will defeat the purpose of using DDGS as a low cost alternative for wheat or maize.

1.10 Knowledge Gaps

The use of maize-/wheat-DDGS for poultry is not new, but a rapid increase in bioethanol production for fuel is expected in the future and this will increase the quantity of DDGS available as a feed ingredient for poultry. Because wheat is more available in the UK compared with other cereal grains, it is the quantity of wheat-DDGS that is projected to

increase. However, in comparison to maize-DDGS, the nutritional value of wheat-DDGS is poorly described for poultry. A review of the literature indicated that the chemical composition of maize- and wheat-DDGS varies widely among sources and the energy value and nutrient digestibility also varies among poultry speciess (broilers, turkey, laying hens and ducks). In addition, only a few studies have assessed the growth and nutrient utilisation responses of broilers and turkey to enzyme-supplemented diets containing wheat-DDGS. To facilitate the use of wheat-DDGS as a feed ingredient for poultry, it is essential to determine its nutritional value for broilers and turkey. Whilst the use of DDGS for pigs is becoming more popular, the effect of dietary fibre in protein feedstuffs on nutrient digestibility is not known. Because maize-DDGS will normally contain greater levels of soluble and insoluble fibre compared with SBM, it is important to evaluate the effect of changes in dietary fibre on nutrient digestibility for pigs.

1.11 STUDY OBJECTIVES

1. Develop prediction models for nutrients, particularly amino acids (AA), for maize- and wheat-DDGS

2. Evaluate the energy value and apparent, true or standardized digestibility of AA and P of wheat-DDGS with and without added enzymes for broilers and turkey

3. Evaluate the growth performance and gastrointestinal tract characteristics of broilers in response to receiving wheat-DDGS and exogenous enzymes in their diet

4. Determine the effect of dietary fibre type and CP level on ileal amino acids digestibility for growing pigs.

CHAPTER 2

CHEMICAL COMPOSITIONS AND PREDICTION OF AMINO ACID CONTENT OF MAIZE- AND WHEAT DISTILLER'S DRIED GRAINS WITH SOLUBLES

2.1 INTRODUCTION

The main co-product of producing ethanol from cereal grains by the dry-grind process is Distillers Dried Grains with Solubles (DDGS). Because the starch fraction of the grain is converted to ethanol by fermentation, other chemical components in the grain are concentrated approximately 3-fold in DDGS. The increased concentration of protein, amino acid (AA) and phosphorus in DDGS is desirable for poultry; however, the use of DDGS as feedstuff for poultry is limited because its chemical composition varies widely among sources. Whilst the chemical composition of DDGS is generally characteristic of the grain (maize, wheat, triticale, sorghum or combination of these) from which it is produced (Zijlstra *et al*., 2007), variability in the AA (Fastinger *et al*., 2006), P (Belyea *et al*., 2004) and gross energy (Cozannet *et al*., 2010a) contents of DDGS when the same type of grain is used have been reported.

A number of factors that include variations in the chemical composition of the grain, differences in processing techniques among bioethanol plants, and differences in analytical methods may act singly or in combination to cause variability in the chemical composition of DDGS. In addition, the DDGS produced by newly constructed fuel-ethanol plants may differ in chemical composition to the DDGS produced by beverage-alcohol manufacturers due to differences and improvements in the technique and efficiency of fermentation. However, although the variability in the chemical composition of maize- and wheat-DDGS amongst sources is well documented in literature (Cozannet *et al*., 2010b; Fastinger *et al*., 2006; Belyea *et al*., 2004), majority of these descriptions are often based on data collected from relatively few (less than 10) and closely related bioethanol plants.

For the purpose of rapid assessment of the nutritive value of DDGS, it is necessary to develop reliable predictions of the individual or total amino acids from the chemical composition of the DDGS, such prediction equations are lacking in the literature. Prediction models have been employed to determine the indispensable amino acids (IAA) (Fiene *et al*., 2006) and total amino acids (TAA) (Roush and Cravener, 1997) contents in feedstuffs based on their chemical compositions. Therefore, the objective of this study was to evaluate the relationship between the chemical components of maize- and wheat-DDGS and to develop prediction equations for individual IAA, total indispensable amino acid (TIAA) and TAA contents using their nutrient composition data.

2.2 MATERIALS AND METHODS

2.2.1 Data Collection and Statistical Analyses

Nutrient composition data of maize- and wheat-DDGS were compiled from 52 recently published literatures and summarised. All data analyses were done using Genstat 11 (VSN International, 2008). Correlations among chemical components and the associated probability values for maize- and wheat-DDGS were determined. Prediction models for determining the IAA, TIAA and TAA contents of maize- and wheat-DDGS from their crude protein (CP) and AA contents were developed using stepwise multiple regression analysis. Maximum improvement in adjusted r^2 (adj r^2) and reduction in Mallows Cp were the model selection criteria. The best model subset for each response variable was identified using a balance between maximum improvement in adj r^2, lowest Cp value and lowest number of explanatory variables possible.

The adj r^2 indicates the best of a number of models based on the largest variance explained and unlike r^2, adj r^2 increases only if the addition of an extra predictor variable improves the model more than would be expected by chance. Mallows Cp is a useful tool for selecting among many alternative subset regressions by comparing the error sums of squares. This criterion helps eliminate the effect of multicollinearity among predictor variables and over-fitting of the regression model. Within each combination of subsets, the model with the least Cp value (or ideally with Cp equal to or less than the number of explanatory variables) is considered the best and least biased.

For a linear regression model fitted to a data set with relatively small number of observations, the presence of an outlier may cause severe distortion to the fitted regression line and may improperly suggest a lack of fit. Therefore, in this study, data points with high standardised residuals (outliers) and high leverage (influential points) were removed and the data re-analysed. The difference between the estimated regression coefficients based on all data points and the regression coefficients when outlying points are removed, denoted as DFBETAS, was used to measure the influence of the outlying cases on the fit of the regression line. Generally, a large value of DFBETAS (greater than 1) is indicative of a large influence. In cases where the distribution of error terms was unequal, transformation of the dependent variable (using the square root in the regression equation) was used to normalise the error variances. Model validation was performed using information from a data set that was not used in developing the models.

2.3 RESULTS

The range, mean, SD and CV (%) of the chemical and amino acids compositions of maize- and wheat-DDGS are presented in Tables 2-1 and 2-2, respectively. For maize-DDGS, ADF and Ca were the most variable (CV; 24.2 and 53.5% respectively) in maize-DDGS whereas CP was the least variable (CV = 8.51%). The order of variability for the chemical components in maize-DDGS from highest to lowest was; Ca > ADF > EE > NDF > crude fibre (CF) > Ash > total P > CP. Amongst the chemical components in wheat-DDGS (Table 2-2), CP was the least variable (CV = 10.8) whereas ADF and Ca were the most variable (CV = 37.8 and 31.5% respectively). For wheat-DDGS, the components varied from highest to lowest in the following order; ADF > Ca > NDF > EE > Total P > Ash > CF > CP.

Of the AA in maize-DDGS, Glu, Lys and Met were most variable, whereas, Thr, Leu and Val were the least variable (Table 2-2). In line with the CV values, the AA in wheat-DDGS that were most variable are Lys, Cys and Phe, whereas, Trp, Thr and Asp were the least variable (Table 2-2). The TIAA contents (% DM) in maize- and wheat-DDGS were 11.63 and 12.56, respectively, corresponding TAA contents (% DM) for maize and wheat-DDGS were 24.47 and 32.94, respectively (data not shown). In addition, on the average (% DM), maize-DDGS contained more Leu and Lys whereas mean values for all remaining IAA were higher in wheat-DDGS. The correlation coefficients for the chemical compositions in wheat-DDGS are presented in Table 2-3. There were positive correlations ($P < 0.05$) between CP and CF ($r = 0.46$), CP and NDF ($r = 0.61$), CP and Ca ($r = 0.56$), and CP and ash ($r = 0.45$) contents of maize-DDGS (data not shown). Also in maize-DDGS, there was a positive correlation ($P < 0.01$) between ash and CF, the r being 0.59 (data not shown). There were trends for negative correlations between EE and CP as well as ADF ($P < 0.10$), with EE explaining 49 and 60% of the variations in CP and ADF respectively. The correlation matrix for correlations between CP, IAA, TIAA and TAA of maize-DDGS is presented in Tables 2-4. Except for Arg, Ile, Lys and Trp, there were positive correlations ($P < 0.05$) between CP and other IAA. In addition, there were positive correlations ($P < 0.01$) between all IAA (except Trp) and TIAA as well as TAA in maize-DDGS with r ranging from 0.58 to 0.96. The correlation matrix for correlations between CP, IAA and TIAA in wheat-DDGS is presented in Tables 2-5.

Table 2-1. Chemical compositions (%) of maize- and wheat-distillers dried grains with solubles[1,2]

	Maize-DDGS						Wheat-DDGS					
	n	Max	Min	Mean	SD	CV	n	Max	Min	Mean	SD	CV
CF	33	11.3	6.2	7.4	1.1	15.1	12	8.6	6.1	7.7	0.9	11.0
NDF	17	51.0	27.7	36.6	5.8	15.7	11	46.8	21.8	32.6	7.5	23.1
ADF	19	18.5	8.6	13.6	3.3	24.2	10	22.3	7.4	14.0	5.3	37.8
EE	37	17.7	3.2	10.8	2.4	22.0	15	7.0	2.9	5.4	1.1	20.0
Ash	36	5.9	3.1	4.5	0.6	13.6	15	6.6	4.6	5.3	0.6	11.9
Total P	25	0.98	0.69	0.80	0.07	8.8	11	1.11	0.65	0.92	0.14	14.8
Ca	21	0.08	0.02	0.04	0.02	53.5	9	0.24	0.1	0.16	0.05	31.5
CP	44	34.7	23.3	27.9	2.4	8.5	18	46.3	32.1	38.0	4.09	10.8

[1]Values are expressed in or have been converted to % dry matter basis; n – sample number; Max – maximum; Min – minimum; SD - Standard deviation; DM- dry matter; NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – ether extract; Total P – total phosphorus; CP – crude protein; CV – coefficient of variation (%); Data are averages of values reported by the following sources; Spiehs *et al*. (2002); Belyea *et al*. (2004); Nyachoti *et al*. (2005); Robertson *et al*. (2005); Arvalis (2006); Batal and Dale (2006); Fastinger *et al*. (2006); Kleinschmit *et al*. (2006); Shurson and Noll (2006); Noll *et al*. (2007a); Stein (2007); Thacker and Widyaratne (2007); Vilarino *et al*. (2007); Widyaratne and Zijlistra (2007); Janicek *et al*. (2008); Kim *et al*. (2008); Lan *et al*. (2008); McKinnon and Walker (2008); Pineda (2008); Bandegan *et al*. (2009); Szczurek (2009); Youssef *et al*. (2009); Avelar *et al*. (2010); Cozannet *et al*. (2010b); Han and Liu (2010); Kluth and Ruderhutscord (2010); Kong and Adeola (2010); Mjoun *et al*. (2010); Olukosi *et al*. (2010); Oryschak *et al*. (2010); Randall and Drew (2010); Szczurek (2010); ADM (2011); Chrenkova *et al*. (2011); Cromwell *et al*. (2011); Olukosi (2011); Rochell *et al*. (2011); UOM (2011); Own data (2 wheat-DDGS samples).

Table 2-2. Amino acid compositions (%) of maize- and wheat-distillers dried grains with solubles[1].

	Maize-DDGS						Wheat-DDGS					
	n	Max	Min	Mean	SD	CV	n	Max	Min	Mean	SD	CV
Arg	26	1.46	1.06	1.22	0.1	8.0	16	2.01	1.18	1.54	0.20	13.0
His	24	0.91	0.65	0.74	0.07	9.4	13	1.02	0.66	0.82	0.12	14.2
Ile	27	1.25	0.96	1.07	0.07	6.7	16	1.66	1.09	1.33	0.17	12.9
Leu	24	3.62	2.89	3.21	0.21	6.6	16	3.13	2.09	2.55	0.33	12.8
Lys	28	1.11	0.62	0.90	0.12	13.1	16	1.17	0.60	0.77	0.15	20.6
Phe	24	1.51	1.09	1.29	0.12	9.6	16	2.22	1.11	1.70	0.30	17.3
Thr	28	1.16	0.93	1.03	0.07	6.5	16	1.4	0.99	1.17	0.11	9.6
Val	26	1.61	1.30	1.42	0.09	6.7	16	2.09	1.37	1.64	0.21	12.6
Met	28	0.72	0.44	0.52	0.06	12.0	14	0.71	0.42	0.55	0.09	16.9
Trp	27	0.26	0.16	0.22	0.02	10.3	9	0.44	0.36	0.39	0.03	7.0
Ala	21	2.1	1.56	1.83	0.14	7.6	13	1.77	1.19	1.38	0.15	10.8
Cys	26	0.7	0.41	0.51	0.06	11.1	14	1.0	0.57	0.73	0.13	18.3
Glu	21	5.48	2.93	3.61	0.62	17.1	13	12	8.17	9.79	1.29	13.2
Gly	21	1.24	0.95	1.08	0.07	6.8	13	1.92	1.28	1.51	0.17	11.1
Pro	21	2.21	1.66	1.93	0.17	8.7	8	4.11	2.63	3.33	0.50	15.1
Ser	22	1.45	1.01	1.17	0.11	9.1	13	2.08	1.45	1.68	0.17	10.2
Tyr	22	1.2	0.91	1.01	0.07	7.2	8	1.35	0.9	1.07	0.15	13.6
Asp	21	1.97	1.49	1.73	0.13	7.6	13	2.25	1.6	1.85	0.19	10.2

[1] Values are expressed in or have been converted to % dry matter basis; n – sample number; SD - standard deviation; CV – coefficient of variation (%); Data are averages of values reported by the following sources; Spiehs *et al*. (2002); Nyachoti *et al*. (2005); Arvalis (2006); Batal and Dale (2006); Fastinger *et al*. (2006); Shurson and Noll (2006); Noll *et al*. (2007a); Stein (2007); Thacker and Widyaratne (2007); Vilarino *et al*. (2007); Widyaratne and Zijlistra (2007); Kim *et al*. (2008); Lan *et al*. (2008); Pineda (2008); Bandegan *et al*. (2009); Avelar *et al*. (2010); Han and Liu (2010); Kluth and Ruderhutscord (2010); Kong and Adeola (2010); Olukosi *et al*. (2010); Oryschak *et al*. (2010); Szczurek (2010); ADM (2011); Cromwell *et al*. (2011); Olukosi (2011); UOM (2011); Own data (2 wheat-DDGS samples).

Table 2-3. Correlation matrices for chemical components in wheat-distillers dried grains with solubles

Relationship between CP, ADF and NDF fractions (n = 11)			
	CP	ADF	NDF
CP	-	0.38	0.28
ADF	-	-	0.21
P-values	-	0.24	0.40
			0.53
Relationship between EE, CP and Ash fractions (n = 14)			
	EE	CP	Ash
EE	-	-0.49	0.39
CP	-	-	0.22
P-values			
		0.07	0.17
			0.45
Relationship between NDF, ADF and EE fractions (n = 10)			
	NDF	ADF	EE
NDF	-	0.16	0.41
ADF	-	-	-0.60
P-values			
	-	0.66	0.24
	-	-	0.07

n – Sample number; CP – crude protein; ADF – acid detergent fibre; NDF – neutral detergent fibre; EE – ether extract.

Table 2-4. Correlation matrix of crude protein and amino acids of maize-distillers dried grains with solubles

	TAA	TIAA	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
CP	0.79**	0.65**	0.44	0.68**	0.26	0.49*	0.22	0.73**	0.81**	0.59**	0.33	0.61**
TAA		0.91**	0.76**	0.87**	0.58**	0.70**	0.58**	0.87**	0.80**	0.81**	0.31	0.83**
TIAA			0.84**	0.87**	0.75**	0.85**	0.74**	0.88**	0.67**	0.86**	0.07	0.96**
Arg				0.89**	0.43	0.49*	0.76**	0.77**	0.45*	0.81**	0.14	0.76**
His					0.44	0.52*	0.68**	0.89**	0.65**	0.75**	0.17	0.84**
Ile						0.85**	0.54*	0.47*	0.32	0.52*	0.02	0.81**
Leu							0.49*	0.65**	0.53*	0.72**	-0.14	0.83**
Lys								0.58**	0.22	0.59**	-0.10	0.68**
Met									0.69**	0.79**	0.09	0.84**
Phe										0.46*	0.16	0.59**
Thr											0.19	0.75**
Trp												0.03
Val												-

Number of samples = 20, CP – crude protein, TIAA – total indispensable amino acids, TAA – total amino acid, * $P < 0.05$, ** $P < 0.01$

Table 2-5. Correlation matrix of crude protein and amino acids of wheat-distillers dried grains with solubles

	TIAA	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
CP	0.96**	0.86**	0.89**	0.87**	0.95**	0.50	0.81**	0.97**	0.91**	0.52	0.93**
TIAA		0.86**	0.89**	0.96**	0.95**	0.65*	0.88**	0.97**	0.91**	0.45	0.99**
Arg			0.60	0.74**	0.78**	0.65*	0.88**	0.79**	0.66*	0.44	0.81**
His				0.86**	0.88**	0.41	0.64*	0.96**	0.89**	0.55	0.90**
Ile					0.90**	0.66*	0.85**	0.92**	0.90**	0.36	0.98**
Leu						0.44	0.86**	0.94**	0.87**	0.33	0.92**
Lys							0.57	0.50	0.55	0.17	0.68*
Met								0.79**	0.64*	0.31	0.83**
Phe									0.92**	0.56	0.96**
Thr										0.44	0.93**
Trp											0.44
Val											-

Number of samples = 11, CP – crude protein, TIAA – total indispensable amino acid, * $P < 0.05$, ** $P < 0.01$.

There were positive correlations ($P < 0.05$) between CP and all IAA (except Lys and Trp) in wheat-DDGS. In addition, there were positive correlations ($P < 0.05$) between all IAA (except Trp) and TIAA in wheat-DDGS. Regression equations for predicting the IAA contents of maize- and wheat-DDGS from their CP composition are presented in Table 2-6. For maize-DDGS, r^2 ranged from 0.02 to 0.64, and was only greater than 0.50 in the models for predicting TAA (0.61), Met (0.51) and Phe (0.64). Crude protein did not explain any of the variation in the Lys content of maize-DDGS; as such, prediction model using CP alone was not developed. In the case of wheat-DDGS, except for Lys and Trp where r^2 were 0.17 and 0.19 respectively, CP explained more than 60% of the variation in the TIAA and all other IAA.

Regression equations for predicting the composition of individual IAA from CP and IAA in maize- and wheat-DDGS are presented in Table 2-7. For both maize- and wheat-DDGS, adj r^2 ranged from 0.57 to 0.99 for the best models for predicting the IAA. For maize-DDGS, the best models for predicting Phe and Lys contained one variable; both Val and Met are best predicted using two variables in the model, whereas Arg, His, Ile and Leu are best predicted with 3 variables in the model. Except for His and Val in wheat-DDGS that are best predicted with 2 variables, the best models for predicting all other IAA incorporated 3 or more variables in the model. The best regression equations for predicting Lys and Thr in maize-DDGS were -0.11 + 0.83 (Arg) (adj $r^2 = 0.57$) and 0.25 + 0.20 (Arg) – 0.51 (Ile) + 0.43 (Leu) – 0.23 (Phe) (adj $r^2 = 0.86$), respectively. Similarly, the best equations for predicting Lys and Thr in wheat-DDGS were -0.77 – 1.15 (Ile) – 1.44 (Phe) + 3.34 (Val) (adj $r^2 = 0.77$) and 0.32 – 0.02 (CP) + 0.46 (Ile) + 0.65 (Met) (adj $r^2 = 0.95$), respectively.

The best model subsets with increasing number of independent variables (IAA and CP) for predicting the TIAA and TAA contents in maize-DDGS and TIAA content in wheat-DDGS are shown in Table 2-8. The adj r^2 increased as the number of variables in the models increased; however, there were generally no substantial improvements in the fit of the regression models when more than 3 predictor variables were included in the models. Therefore, the two best models within each subset of variables from the 1- to 4-variable models are presented in Table 2-8. For maize-DDGS, His and Leu were common as the best 2 variables for predicting both its TAA and TIAA contents. In addition, both His and Leu were also among the best 3-variables for predicting the TIAA and TAA contents of maize- DDGS, with Arg and Trp being the third variables in the best 3-variable models for TIAA and TAA respectively. Also, Val was common to both maize- and wheat-DDGS as the best 1-variable predictor of their TIAA contents and in the case of wheat-DDGS, Val was common to the best

2- (the other being CP) and 3-variable (others being Arg and Leu) models for predicting its TIAA content.

The intercept and slope of the best models for predicting TIAA and TAA in maize- and wheat-DDGS are presented in Table 2-9. Val was common to both maize- and wheat-DDGS as the best 1-variable predictor of TIAA content, with Met being the second best variable for maize-DDGS and Phe being the second best for wheat-DDGS. The best 2-variable model for predicting the TIAA and TAA contents in maize-DDGS consisted of His and Leu in both cases, respectively (adj r^2 = 0.97 and 0.83; Cp = 74.97 and 28.53 respectively), whereas CP and Val (adj r^2 = 0.99; Cp = 9.47) were the best 2 variables for predicting the TIAA content in wheat-DDGS. The three AA included in the best 3-variable models for predicting the TIAA and TAA in maize-DDGS were Arg, His and Leu (adj r^2= 0.98; Cp = 40.01) and His, Leu and Trp (adj r^2= 0.90; Cp = 12.40), respectively, the regression equations are TIAA = 0.77 + 1.36 (Arg) + 3.87 (His) + 1.99 (Leu) and TAA (% DM) = -3.03 + 14.1 (His) + 3.79 (Leu) + 23.4 (Trp) respectively. Although the 2-variable model consisting of Val and CP explained 99% of the variation in TIAA content of wheat-DDGS, there was a substantial decrease in the sum of squares error (9.47 vs. 1.14) with the inclusion of an additional variable in the model. Therefore, the best 3-variable model for predicting TIAA content in wheat-DDGS utilised Arg, Leu and Val (adj r^2=0.99; Cp = 1.14) in the model, the regression equation being TIAA = -0.07 + 1.11 (Arg) + 0.99 (Leu) + 5.02 (Val).

The prediction equations for IAA were validated by comparing the predicted with actual data from an independent dataset. The predicted and actual values of the prediction models developed from the CP compositions of maize- and wheat-DDGS for their IAA, TIAA and TAA contents are shown in Table 2-10. In maize-DDGS, the predicted values were close to actual values for TIAA, TAA and all IAA (except Leu). Similarly, except for Met and Lys in wheat-DDGS, the predicted values were close to actual values for TIAA and all other IAA. For both types of DDGS, the predicted- and actual values were closest in the models for TIAA, Arg, Thr, Trp and Val contents. The predicted and actual values for the best models for predicting the IAA, TIAA, TAA contents of maize-DDGS are presented in Table 2-11. Corresponding values for wheat-DDGS are presented in Table 2-12. Predicted values for Met and Ile were farthest from the actual values amongst all the IAA in wheat-DDGS, however, there was a good agreement between the predicted and actual values for all other IAA in wheat- and all IAA in maize-DDGS. The accuracy of the prediction models for TIAA and TAA contents for both maize- and wheat-DDGS improved as the number of independent

variables in the models increased up to 3; there were no further improvements with addition of a fourth predictor variable in the models.

Table 2-6. Prediction models for the amino acids contents of maize- and wheat-distillers dried grains with solubles (% DM)

Amino acid	r^2	Equations
Maize-DDGS		
TIAA	0.39	2.72 + 0.34 (CP)
TAA	0.61	-1.77 + 0.99 (CP)
Arg	0.15	0.27 + 0.04 (CP)
His	0.43	-0.24 + 0.037 (CP)
Ile	0.02	0.76 + 0.01 (CP)
Leu	0.20	1.38 + 0.07 (CP)
Lys	0.00	
Met	0.51	-0.26 + 0.03 (CP)
Phe	0.64	-0.42 + 0.06 (CP)
Thr	0.31	0.31 + 0.03 (CP)
Trp	0.06	0.09 + 0.01 (CP)
Val	0.34	0.32 + 0.04 (CP)
Wheat-DDGS		
TIAA	0.92	0.30 + 0.003 (CP)
Arg	0.71	0.13 + 0.04 (CP)
His	0.77	-0.03 + 0.02 (CP)
Ile	0.72	0.09 + 0.03 (CP)
Leu	0.89	-0.10 + 0.07 (CP)
Lys	0.17	0.11 + 0.02 (CP)
Met	0.62	-0.07 + 0.02 (CP)
Phe	0.94	-0.40 + 0.06 (CP)
Thr	0.81	0.41 + 0.02 (CP)
Trp	0.19	0.30 + 0.003 (CP)
Val	0.86	0.12 + 0.04 (CP)

CP – crude protein, TIAA – total indispensable amino acid, TAA – total amino acids

Table 2-7. Best prediction models for indispensable amino acids in maize- and wheat-distillers dried grains with solubles (% DM)

Amino acid	Adj r^2	Equations
Maize-DDGS		
Arg	0.84	0.35 – 0.029 (CP) + 1.30 (His) + 0.69 (Thr)
His	0.93	-0.03 + 0.11 (Lys) + 0.60 (Met) + 0.25 (Val
Ile	0.84	0.03 - 1.15 (His) + 0.18 (Lys) + 1.22 (Val)
Leu	0.95	-0.34 + 1.19 (Ile) + 0.65 (Phe) + 1.36 (Thr)
Lys	0.57	-0.11 + 0.83 (Arg)
Met	0.85	-0.12 + 0.01 (CP) + 0.64 (His)
Phe	0.74	-0.42 + 0.06 (CP)
Thr	0.86	0.25 + 0.20 (Arg) – 0.51 (Ile) + 0.43 (Leu) – 0.23 (Phe)
Trp	0.59	-0.24 + 0.01 (CP) + 0.51 (Ile) – 0.18 (Leu) + 0.26 (Thr)
Val	0.95	0.10 + 0.99 (His) + 0.55 (Ile)
Wheat-DDGS		
Arg	0.95	0.58 + 0.06 (CP) – 1.43 (His) – 1.52 (Thr) + 1.03 (Val)
His	0.97	0.22 – 0.23 (Arg) + 0.56 (Phe)
Ile	0.99	0.03 – 0.37 (Arg) – 0.36 (His) + 0.61 (Met) + 1.11 (Val)
Leu	0.96	0.15 – 0.88 (Arg) + 0.07 (CP) + 1.94 (Met)
Lys	0.77	-0.77 – 1.15 (Ile) – 1.44 (Phe) + 3.34 (Val)
Met	0.90	0.34 – 1.15 (Thr) + 0.02 (CP) + 0.60 (Ile)
Phe	0.99	-0.40 + 0.03 (CP) + 0.83 (His) + 0.24 (Ile)
Thr	0.95	0.32 + 0.02 (CP) + 0.46 (Ile) – 0.65 (Met)
Trp	0.19	0.30 + 0.003 (CP)
Val	0.98	0.05 + 0.02 (CP) + 0.77 (Ile)

CP – crude protein; Adj r^2 – adjusted r^2

Table 2-8. Best model subsets for the total indispensable amino acids and total amino acids of maize- and wheat-distillers dried grains with solubles

No of variables	Adj r^2	Mallows' Cp	Variables used
Maize-DDGS			
TIAA (% DM)			
1	0.91	301.96	Val
1	0.77	814.48	Met
2	0.97	74.93	His, Leu
2	0.96	127.94	Thr, Val
3	0.98	40.01	Arg, His, Leu
3	0.98	43.55	His, Leu, Lys
4	0.99	24.20	His, Leu, Lys, Trp
4	0.99	30.97	His, Leu, Lys, Arg
TAA (% DM)			
1	0.75	49.12	His
1	0.74	52.10	Met
2	0.83	28.53	His, Leu
2	0.82	30.84	His, CP
3	0.90	12.40	His, Leu, Trp
3	0.87	17.82	CP, his, Ile
4	0.92	8.22	His, Leu, Trp, Val
4	0.91	10.03	CP, His, Leu, Trp
Wheat-DDGS			
TIAA (% DM)			
1	0.98	26.47	Val
1	0.94	89.75	Phe
2 (√ of TIAA)	0.99	9.47	CP, Val
2	0.99	9.82	CP, Ile
3	0.99	1.14	Arg, Leu, Val
3	0.99	5.09	Arg, CP, Ile

TIAA - indispensable amino acid; TAA – total amino acid; CP – crude protein; Adj r^2 – adjusted r^2; Mallow's Cp- tool for selecting among many alternative subset regressions by comparing the error sums of squares

Table 2-9. Prediction models for total indispensable amino acid and total amino acids content of maize- and wheat-distillers dried grains with solubles

No of variables	Adj r^2	Intercept	Variable(s)	Slope(s)
Maize-DDGS				
TIAA (% DM)				
1	0.91	1.23	Val	7.33
2	0.97	1.02	His	5.72
			Leu	2.01
3	0.98	0.77	Arg	1.36
			His	3.87
			Leu	1.99
4	0.99	0.24	His	4.55
			Leu	2.01
			Lys	1.11
			Trp	2.97
TAA (% DM)				
1	0.75	9.59	His	20.18
2	0.83	2.95	His	16.19
			Leu	3.03
3	0.90	-3.03	His	14.14
			Leu	3.79
			Trp	23.41
4	0.92	-2.53	His	20.98
			Leu	6.35
			Trp	24.03
			Val	-9.71
Wheat-DDGS				
TIAA (% DM)				
1	0.98	0.13	Val	7.46
2	0.99	1.77	Val	0.73
			CP	0.01
3	0.99	-0.07	Arg	1.11
			Leu	0.99
			Val	5.02

TIAA - indispensable amino acid; TAA – total amino acid; CP – crude protein; Adj r^2 – adjusted r^2; DM – dry matter

Table 2-10. Predicted- and actual amino acids values for prediction models developed from the crude protein content of maize- and wheat-distillers dried grains with solubles[1]

Amino acid	Actual	Predicted
Maize-DDGS[2]		
TIAA	14.1	13.35
TAA	30.29	29.48
Arg	1.47	1.44
His	0.83	0.92
Ile	1.24	1.14
Leu	3.94	3.50
Lys	1.15	-
Met	0.73	0.66
Phe	1.65	1.58
Thr	1.22	1.17
Trp	0.24	0.25
Val	1.63	1.62
Wheat-DDGS[3]		
TIAA	13.40	13.39
Arg	1.60	1.64
His	0.79	0.91
Ile	1.21	1.43
Leu	2.66	2.75
Lys	1.00	0.81
Met	0.79	0.58
Phe	1.79	1.88
Thr	1.27	1.22
Trp	0.43	0.42
Val	1.86	1.79

[1]Values are expressed in or have been converted to % dry matter basis; [2] actual data from Soares *et al*. (2011); [3] actual data from Slominski *et al*. (2010)

Table 2-11. Predicted- and actual amino acids values for prediction models developed from the crude protein and individual amino acids content of maize-distillers dried grains with solubles[1]

Amino acid		Actual	Predicted
Maize-DDGS[2]			
Arg		1.47	1.35
His		0.83	0.93
Ile		1.24	1.26
Leu		3.94	3.87
Lys		1.15	1.11
Met		0.73	0.70
Phe		1.65	1.58
Thr		1.22	1.24
Val		1.63	1.60
Trp		0.24	0.65
TIAA	1-variable model	14.1	13.17
	2-variable model		13.69
	3-variable model		13.83
TAA	1-variable model	30.29	26.34
	2-variable model		28.33
	3-variable model		29.26

[1]Values are expressed in or have been converted to % dry matter basis; [2] actual data from Soares *et al*. (2011)

Table 2-12. Predicted- and actual amino acids values for prediction models developed from the crude protein and individual amino acids content of wheat-distillers dried grains with solubles[1]

Amino acid		Actual	Predicted
Wheat-DDGS[2]			
Arg		1.60	1.65
His		0.79	0.88
Ile		1.21	1.43
Leu		2.66	2.73
Lys		1.00	1.05
Met		0.79	0.60
Phe		1.79	1.88
Thr		1.27	1.21
Val		1.86	1.98
Trp		0.43	0.42
TIAA	1-variable model	13.40	14.00
	2-variable model		13.74
	3-variable model		13.68

[1]Values are expressed in or have been converted to % dry matter basis; [2]actual data from Slominski *et al*. (2010)

2.4 DISCUSSION

The objectives of this study were to examine the variability in the chemical components of maize- and wheat-DDGS, evaluate the relationships between the chemical components in maize- and wheat-DDGS as well as develop prediction equations for IAA, TIAA and TAA contents from their CP alone or CP and IAA contents. It was anticipated that the use of data from a wide range of sources would provide an adequate representation of the variability in the chemical composition of both maize- and wheat-DDGS.

A number of factors that may affect the chemical composition of DDGS have been identified and they include; variations in the chemical composition of the grain, differences in processing techniques, and differences in analytical methodologies (Belyea *et al*., 2004; Kim *et al.*, 2008; Kingsly *et al*., 2010). In addition, heterogeneity in the efficiency of fermentation, types of enzyme used, the ratio of wet distillers grains (WDG) to condensed distillers soluble (CDS) combined to produce DDGS and differences in temperature and duration of drying have all been reported to influence the chemical characteristics of the final product (Cromwell *et al*., 1993; Kingsy *et al*., 2010; Liu 2011). However, among these factors, the ratio of WDG and CDS combined to form DDGS may be the most important. This is because WDG is composed mostly of CP and CF whereas CDS contains mainly the EE, ash and residual sugar fractions (Kim *et al*., 2008).

Although the variations in chemical composition of the grain among sources are usually low, it is possible that the concentration of nutrients in DDGS post-fermentation exaggerates the differences. However, Belyea *et al*. (2004) reported no correlation (r ranged from -0.21 to 0.16) between the chemical components (EE, CP, residual starch, CF and ADF) of maize grain and maize-DDGS using data collected in one bioethanol plant over a three-year period. Therefore, it is possible that the variability in the chemical composition of DDGS is caused by external factors apart from the chemical composition of the grain.

In the current study, there was wide variability in the concentrations of CP and AA of the DDGS samples, but the variability in CP was narrower compared with that of AA in both maize- and wheat-DDGS. The variability in CP and AA compositions of DDGS may be due to several factors that include; differences in non-protein-nitrogen content among samples, temperature and duration of drying, and the contribution of yeast AA to TAA in DDGS (Kim *et al*., 2008; Liu, 2011). In ascending order of variability, Met, Lys and Glu were the most variable AA in maize-DDGS whereas Phe, Cys and Lys were the most variable AA in wheat-DDGS in the current study. On the other hand, the least variable AA were Thr and Leu for

maize-DDGS and Trp and Thr for wheat-DDGS. Although there does not appear to be a obvious explanation for the wide variability observed for Glu, Met and Phe composition of maize- or wheat-DDGS compared with other AA, it is possible that these particular AA are more affected by factors (or their combination) causing variability in the chemical composition of the DDGS. However, formation of insoluble Lys-carbohydrate moieties and partial destruction of Cys in oilseeds, animal protein meals and plant-based feedstuffs due to excessive heat treatment has been reported in literature (Cromwell *et al*., 1993; Cozannet *et al*., 2010b).

During bioethanol production, non-protein nitrogenous compounds such as ammonia and urea are added to the mash to control the pH as well as serve as sources of N for the yeast (Liu, 2011). Variability in the quantity of exogenous nitrogenous substances added to the mash among bioethanol facilities may cause variability in the N value and consequently the CP value of the co-product. Since the CP and AA profile of DDGS are derived from the grain and yeast, the proportional contribution of either source will confer considerable variability in DDGS. Ingledew (1999) observed that yeast contributes approximately 5.3% of the total protein in maize-DDGS.

Belyea *et al*. (2004) observed that yeast protein constitutes up to 55% to the total protein content in maize-DDGS. However, the value by Belyea *et al*. (2004) may have overestimated the contribution of yeast protein to total protein in maize-DDGS because the approach used by the authors did not account for dispensable AA. However, Belyea *et al*. (2004) showed that AA such as Lys, that is typically low in maize grain (0.24g/100g) and higher in yeast protein (3.32g/100g), increased substantially in maize-DDGS after yeast fermentation (0.77g/100g). Liu (2011) similarly observed that after fermentation of maize grain by yeast, there were rapid increases in the concentration of some AA, the concentration of some AA remained unchanged and other AA decreased in concentration. On the other hand, Martinez-Amezcua (2005) reported that only about 10% of the TAA in maize-DDGS is contributed by the yeast AA.

In the current study, the compositions of P and Ca also varied. Wide variability in the concentrations of other minerals such as Zn, Na, and S in maize-DDGS has been reported (Spiehs *et al*., 2002; Belyea *et al*., 2006; Liu and Han, 2011). The variability in the concentrations of minerals in DDGS may be due to the differences in the ratio of WDG and CDS combined among DDGS sources.

Apart from the correlations between the fiber fractions (CF, NDF and ADF) in maize-DDGS, there were significant positive correlations between CP and the fiber fractions (CF, NDF, and ADF), CP and ash and CP and Ca. As earlier mentioned, DDGS is composed of the WDG and CDS fractions, and these fractions differ in chemical characteristics. Kingly *et al*. (2010) noted a strong positive correlation between CP and CF in maize-DDGS, as well as negative correlations between CP and CF and the chemical components (EE, ash, and residual sugars) of CDS. This is in line with the negative correlations between the EE and CP (-0.49) and EE and NDF (-0.60) of wheat-DDGS observed in the current study. On the contrary, there were significant positive correlations between CP and ash (0.45) and also CP and Ca (0.56) contents of maize-DDGS in the current study. The positive correlation between CP and ash in the current study may be due to the much wider variability in the nutrient composition data of maize-DDGS used, compared with Kingsly *et al*. (2010) study where the negative correlations were reported.

In the current study, there were significant positive correlations amongst all IAA (except Trp) and TIAA and TAA for both maize- and wheat-DDGS. Although CP, His and Phe explained greater than 50% of the variability in Trp content of wheat-DDGS in the current study, these correlations were not significant ($P > 0.05$). However, the relationship between Trp and the chemical components of maize-DDGS was generally poor. Weak correlations between Trp and the proximate fractions, as well as other IAA in maize-DDGS have been reported previously (Fiene *et al*., 2006).

The reasons for the poor correlation between Trp and other IAA as well as TIAA and TAA in maize- and wheat-DDGS are not clear. However, it is possible that differences in analytical techniques and errors during Trp determination may cause inconsistencies in the ratio of Trp to other chemical components in DDGS leading to the weak relationships observed (Nurit *et al*., 2009). The effect of variable or incomplete extraction of Trp during AA analysis of DDGS may also be of significance considering the fact that the concentration of Trp is low compared with that other IAA in DDGS.

It is desirable to be able to predict the chemical composition of DDGS from other chemical components. But more importantly, it is important to be able to predict individual AA from the CP level of DDGS or to predict some essential AA from other AA with high accuracy. Consequently, in the current study prediction models for essential AA using CP contents of maize- and wheat-DDGS were developed. For maize-DDGS, r^2 for predicting individual AA from CP content was generally low and was greater than 0.50 only for TAA, Met and Phe. On

the other hand, r^2 for predicting individual AA from wheat-DDGS CP content was greater than 0.60 (except for Lys and Trp).

In a study by Fiene *et al*. (2006), the authors noted the possibility of predicting the IAA contents of maize-DDGS from the analysed values of CP, EE and CF. In that study, r^2 ranged from 0.31 to 0.86 for the regression models and were lowest for Trp and Lys. Whereas the reasons for the poor r^2 observed for Trp in the current study and that of Fiene *et al*. (2006) are not clear, in the case of Lys, it is possible that the low r^2 is a result of the wide variability in Lys concentration caused by the formation of insoluble carbohydrate-Lys compounds during the drying process.

In the current study, the best candidate models for predicting the TIAA and TAA contents of both maize- and wheat-DDGS were selected using adj r^2 and Mallows Cp as criteria. It was noted that models containing 3 independent variables were optimal for predicting TIAA and TAA in either maize- or wheat-DDGS because there were minimal improvements in the adj r^2 and no further substantial reductions in the sum of square errors (Mallows Cp) with the inclusion of a fourth variable. Valine was common to both maize- and wheat-DDGS as the best 1-variable predictor of TIAA contents. Although the adj r^2 explained majority of the variation in the TIAA contents of maize- and wheat-DDGS in the 1-variable model consisting of Val (adj r^2 = 0.91 and 0.98, respectively), addition of a second predictor variable (and up-to the third variable) into the models increased their accuracy because there were substantial reductions in the sum of square error of the models.

The validity of the prediction models developed in the current study was tested by comparing predicted with actual values from an independent data set. The accuracy of the prediction models for TIAA and TAA in maize-DDGS and TIAA in wheat-DDGS increased as the number of predictor variables (up to 3 variables) in the models increased. Although, it did not appear that His, Leu, Arg and Val were different from the other IAA in the current study, it was noted that these IAA combined, were the most important for predicting the TIAA and TAA contents of maize- and wheat-DDGS. It is however surprising that despite the poor correlation between Trp and TAA in maize-DDGS (r = 0.31), Trp was amongst the best 3- and 4-variables for predicting the TAA composition of maize-DDGS. The authors are not aware of reported prediction models for TIAA and TAA in maize- or wheat-DDGS, therefore it is not possible to compare our findings with any other.

In conclusion, the results of this study showed that there is wide variability in the chemical compositions of maize- and wheat-DDGS from the different sources. The study also

established that the indispensable amino acids and total amino acids contents of maize- and wheat-DDGS can be predicted from their crude protein and/or amino acids contents with reasonable accuracy.

CHAPTER 3

METABOLISABLE ENERGY CONTENT AND STANDARDISED OR TRUE DIGESTIBILITY OF AMINO ACIDS AND PHOSPHORUS OF WHEAT DISTILLERS' DRIED GRAINS WITH SOLUBLES WITHOUT- OR WITH EXOGENOUS ENZYMES FOR BROILERS

3.1 INTRODUCTION

Wheat Distillers' Dried Grains with Solubles (wheat-DDGS) is the main co-product of bioethanol produced from wheat by the dry-grind process. Recently, the quantity of wheat-DDGS available in the UK has increased because wheat is increasingly being used as feedstock for bioethanol production. It is possible to use wheat-DDGS in the diets of poultry because the conversion of starch in the wheat into ethanol increases the concentration of crude protein (CP), amino acids (AA) and P approximately 3-fold in the wheat-DDGS. The majority of the wheat-DDGS described previously in the literature as a feed ingredient for poultry originated from beverage-alcohol production. On the other hand, there is limited information about the nutritive value of wheat-DDGS derived from bioethanol production. It is important to determine the nutritive value of wheat-DDGS from bioethanol plants for broilers because their chemical characteristics may differ from those produced by beverage-alcohol producers (Liu, 2011).

Wheat grain is commonly used as a source of ME and nutrients for poultry and it is likely that wheat-DDGS will also be a good source of ME and nutrients for poultry. The apparent metabolizable energy (AME), nitrogen-corrected AME (AME_n) contents and amino acids (AA) digestibility of maize-DDGS have been determined for broilers (Batal and Dale, 2006; Adeola and Ilekeji, 2009) and the inclusion of maize-DDGS in diets for broilers have been reported to support growth performance (Thacker and Widyaratne, 2007; Loar et al. 2010). Compared with maize-DDGS, there is a dearth of information about the nutritive value of wheat-DDGS for broilers. In addition, the use of wheat-DDGS for poultry may reduce competition between wheat demand for poultry and bioethanol production. In view of the possibility of using wheat-DDGS as a feedstuff for broilers, it is essential to determine its nutritional value.

Exogenous enzymes such as carbohydrases, proteases and phytases or a combination of these are often incorporated into poultry diets. These enzymes have the ability to enhance the overall digestibility of feed or feedstuffs (Selle *et al*., 2009) and reduce environmental pollution from poultry (Adeola and Cowieson, 2011). In addition, exogenous enzymes are effective at ameliorating the negative effects of non-starch polysaccharide and phytate, in wheat-based diets for poultry (Choct *et al*. 2004; Adeola and Cowieson, 2011). Data about the use of exogenous enzymes to improve nutrient digestibility of wheat-DDGS for broilers are scanty. Such data are important to inform nutritional-adequate feed formulations without excessive surfeit.

It was hypothesized that wheat-DDGS will be a valuable dietary source of metabolisable energy (ME), AA and P for broilers. The overall objective of the current study was to provide data on ME value, AA digestibility and P utilisation of wheat-DDGS for broilers. The specific objectives were to: 1) determine the AME and AME_n contents of wheat-DDGS without- or with an admixture of xylanase, amylase and protease (XAP) for broilers by the regression method, 2) evaluate the true ileal digestibility or total tract P retention of wheat-DDGS without- or with phytase for broilers and 3) determine apparent or standardised ileal AA digestibility (AIAAD or SIAAD, respectively) of wheat-DDGS without- or with protease for broilers.

3.2 MATERIALS AND METHODS

3.2.1 Animals and Management

The Animal Experimentation Committee of the Scotland's Rural College approved all bird handling and sample collection procedures.

A total of 336 male broiler chicks (Ross 308) were brooded together and fed a nutrient adequate pre-experimental starter diet (Table 3-1) from d 0 to 14 in experiment 1 and 2 or from d 0 to 24 in experiment 3. On d 14, birds were weighed individually and divided into 3 groups of similar bodyweight consisting of 126, 126 or 84 birds for experiment 1, 2 or 3, respectively. In each experiment, birds were allocated to one of the experimental diets in a randomized complete block design using d 14 bodyweight as blocking criterion and transferred to metabolism cages on d 14. Each treatment had seven replicate cages and three birds per replicate cage. Birds were weighed individually on d 14 and at the end of the experimental period (d21 or 28). In experiment 2 and 3, birds were euthanized by cervical dislocation on d 28 to allow collection of ileal digesta samples. Birds were provided *ad libitum* access to the experimental diets and water throughout the pre- and experimental periods. The birds were reared in a house with facilities to control temperature, light, and humidity. Room temperature was maintained at 35^{o}C, 32^{o}C, 27^{o}C and 23^{o}C for day 1 to 7, 8 to 14, 15 to 21 and 22 to 28, respectively. Titanium dioxide (TiO_2) was added to the diets (3 g/kg of diet) as an indigestible marker to enable determination of ME content and P and AA utilisation by the index method.

3.2.2 Dietary Treatments and Sample Collection

Experiment 1

The chemical composition of the wheat-DDGS used in the current study is presented in Table 3-2. The energy value of wheat-DDGS for broilers was determined using a total of six diets in experiment 1. Wheat-DDGS was incorporated in a wheat-soybean meal diet at 3 levels (0, 300, or 600 g/kg) without- or with added XAP (0 or 0.25 g/kg) to make six diets in total. At a rate of 0.25 g/kg, the XAP (Danisco Animal Nutrition, Marlborough, UK) supplied 2000, 200 and 4000 U of xylanase, amylase and protease, respectively per kg of diet. The xylanase is a Endo-1,4-beta-xylanase produced by a *Trichoderma longibrachiatum* and expressed in the same organism. The amylase was produced by *Bacillus amyloliquifaciens* and expressed in *Bacillus subtilis*. The subtilisin (protease) was derived from *Bacillus subtilis*. These 3 enzymes were produced separately and later blended to produce the xylanase-amylase-protease (XAP) admixture. One unit (U) of xylanase was defined as the quantity of the enzyme that liberates one mmol of xylose equivalent per minute. One unit of amylase was defined as the amount of the enzyme catalysing the hydrolysis of one mmol glucosidic linkage per minute and one protease unit was defined as the quantity of the enzyme that solubilised one mg of azo-casein per minute.

Because maintaining a similar ratio of energy-yielding components is important when using the regression method, energy-yielding ingredients such as wheat, soybean meal (SBM), gluten meal and soy oil were substituted with wheat-DDGS in a way that their ratios were the same across all the experimental diets. These ratios were 1.93, 11.1, 10.4, 5.37, 5.76, and 1.07 for wheat:SBM, wheat:gluten meal, wheat:soyoil, SBM:soyoil, SBM:gluten meal, and soyoil:gluten meal, respectively for the experimental diets presented in Table 3-3.

Experimental diets were fed from d 15 to 21. Excreta was collected daily from each cage for three days (d 18 to 20), dried and pooled for each cage for the analysis of gross energy (GE), dry matter (DM), N and Ti to determine AME and AME_n.

Table 3-1. Ingredient and nutrient composition of pre-experimental standard diet.

Ingredients, g/kg	
Maize	538.8
Soybean meal -48%	370
Soybean oil	50
Limestone (38% Ca)	10
Dicalcium phosphate[1]	19
Common salt	3.25
Vitamin/mineral premix[2]	4
DL-Methionine	2.8
L-Lysine HCl	1.6
Threonine	0.6
Calculated nutrient composition	
Protein, g/kg	230
ME, MJ/kg	12.7
Calcium, g/kg	11.5
Total phosphorus, g/kg	6.8
Non-phytate P, g/kg	4.3
Ca:P	1.7
Indispensable amino acids, g/kg	
Arg	14.5
His	5.1
Ile	9.4
Lys	9.4
Met	12.8
Phe	10.0
Thr	2.8
Trp	13.4
Val	5.3

[1]Contain 21.3% Ca and 18.7% P.

[2]Vitamin/mineral premix supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.

Table 3-2. Analysed nutrient composition of wheat Distillers' Dried Grains with Solubles (as-is basis)

Item	g/kg
Dry matter	858
Crude protein	326
Gross energy (MJ/kg)	18.5
Crude fibre	80.0
Ether extract	72.5
NDF	389
ADF	223
Ash	46.0
Calcium	1.10
Phosphorus	6.50
Potassium	11.3
Sodium	5.20
Amino acids	
Ala	14.0
Arg	11.8
Asp	18.3
Cys	5.90
Glu	84.9
Gly	14.9
His	8.30
Ile	13.7
Leu	22.6
Lys	7.70
Met	4.50
Phe	15.8
Pro	30.2
Ser	17.0
Thr	11.5
Tyr	10.2
Trp	3.80
Val	16.2

Experiment 2

A total of six diets were used in experiment 2. Three levels of wheat-DDGS (200, 400 or 600 g/kg) was incorporated in a corn-starch based diet (wheat-DDGS being the only source of P) without- or with added phytase. The phytase (Danisco Animal Nutrition, Marlborough, UK) was added to the diet to supply 1000 FTU/kg. The phytase was derived from *Escherichia coli* and expressed in *Schizosaccharomyces pombe*. One phytase unit was defined as the quantity of enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5 from an excess of 15 µM sodium phytate at 37^{o}C. The ingredient and analysed chemical compositions of the experimental diets are presented in Table 3-4. Diets were fed from d 15 to 21. Excreta samples were collected daily for 3 days (d 18 to 20) for the determination of total tract P utilisation. On d 21, all birds were euthanized by cervical dislocation and ileal digesta samples were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water. Ileal digesta samples were pooled per cage and stored frozen (-20^{o}C) pending chemical analysis.

Table 3-3. Ingredient and analysed nutrient composition of experimental diets to determine metabolisable energy value of wheat-DDGS for broilers with- or without added xylanase, amylase and protease.

	Level of dietary wheat distillers dried grains with solubles, g/kg					
	Without XAP			With added XAP		
Item	0	300	600	0	300	600
Ingredients, g/kg						
Wheat, White	561	385.2	209.2	561	385.2	209.2
Soybean meal -48%	291.2	199.9	108.6	291.2	199.9	108.6
Soybean oil	54.2	37.2	20.2	54.2	37.2	20.2
Gluten meal	38.6	22.7	7.0	31.6	15.7	0
DDGS	0	300	600	0	300	600
XAP premix[1]	0	0	0	7.0	7.0	7.0
Others[2]	55.0	55.0	55.0	55.0	55.0	55.0
Analysed energy and nutrient composition[3]						
Dry matter, g/kg	880	875	870	880	880	870
Gross energy, MJ/kg	17.4	17.6	17.8	17.3	17.8	17.8
CP (N x 6.25), g/kg	223	252	275	226	256	276
Xylanase activity, U/kg	-	-	-	1423	1399	1442
Amylase activity, U/kg	-	-	-	262	262	262
Protease activity, U/kg	<100	<100	<100	3064	3064	3064

[1]XAP premix made with gluten meal as carrier; formulated to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease.

[2]Others consists of 18.5 g/kg of Limestone (38% Ca); 14 g/kg of Dicalcium phosphate (Contain 21.3% Ca and 18.7% P); 1 g/kg of Common salt; 3 g/kg of Vitamin/mineral premix (vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 1 g/kg of DL-Metionine; 2.5 g/kg of L-Lysine HCl; 15 g/kg of marker premix (prepared as 1 g of titanium dioxide added to 4 g of gluten meal).

[3]Values are means of duplicate analyses.

Table 3-4. Ingredient and chemical composition of experimental diets to determine phosphorus utilisation of wheat-DDGS for broilers.

	Inclusion level of dietary wheat distillers' dried grains with solubles, g/kg					
	Without Phytase			With added Phytase		
Item	200	400	600	200	400	600
Ingredients, g/kg						
Corn starch	526	303.5	87	516	293.5	77
DDGS	200	400	600	200	400	600
Soybean oil	18	36	48	18	36	48
Limestone	4.5	9	13.5	4.5	9	13.5
Others[1]	251.5	251.5	251.5	251.5	251.5	251.5
Phytase premix[2]	0	0	0	10	10	10
Analysed composition[3], g/kg						
Dry matter	880	890	880	880	890	885
Phosphorus	2.3	3.3	4.0	2.0	2.9	4.2
Calcium	3.6	5.4	6.3	3.5	4.7	6.9
Copper	0.011	0.012	0.013	0.010	0.010	0.015
Iron	0.512	0.129	0.138	0.087	0.117	0.147
Magnesium	0.8	1.2	1.4	0.7	1.1	1.6
Sodium	3.3	4.2	4.6	3.2	3.8	5.0
Phytase activity, FTU/kg	<50	<50	<50	962	767	822

[1]Others consist of: 100 g/kg of dextrose; 130 g/kg of sucrose; 2.5 g/kg of vitamin/mineral premix (supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.2 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 4 g/kg of common salt; 15 g/kg of marker premix (prepared as 1 g Titanium dioxide added to 4 g of gluten meal).

[2]Phytase premix made with cornstarch as carrier; Formulated to supply 1000 FTU/kg. [3]Values are means of duplicate analyses

Experiment 3

Four dietary treatments were used to determine ileal AA digestibility of wheat-DDGS in experiment 3. The dietary treatments were 2 nitrogen-free diets (NFD) (without- or with protease) and 2 semi-purified diets in which wheat-DDGS was the only source of AA (without- or with protease). The protease was added to provide 4000 U per kg of diet (Danisco Animal Nutrition, Marlborough, UK). One protease unit was defined as the quantity of the enzyme that solubilises one mg of azo-casein per minute. Basal ileal endogenous AA flow from birds fed a NFD without- or with protease was used to correct AIAAD values to SIAAD. The ingredient compositions of the experimental diets are presented in Table 3-5. The analysed CP and AA compositions for the diets are presented in Table 3-6. The assay diets were formulated to contain 22% CP and balanced for mineral and vitamins to meet breeder nutrient specifications. Experimental diets were fed from d 25 to 28. Because of health and welfare issues associated with feeding birds NFD, experimental diets were fed for three days in experiment 3. A 3-d feeding period is optimal to fulfil the objectives of the experiment (Kluth and Rudehurscord, 2010). On d 28, ileal digesta samples were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water. Ileal digesta samples were pooled for each cage and stored frozen (-20 ^{o}C) prior to chemical analysis.

3.2.3 Chemical Analysis

Where necessary, diet, wheat-DDGS, ileal digesta and excreta samples were analysed for GE, DM, Ti, N, AA and P. Except for the ileal digesta samples for AA analysis that were lyophilized, all other samples were oven dried and ground to pass through a 0.5 mm screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis. For DM determination, samples were dried at 105 ^{o}C for 24 hours in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England. UK) (AOAC International 2006, method 934.01). Gross energy was determined in an adiabatic oxygen bomb calorimeter using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Nitrogen was determined by combustion method (AOAC International 2006, method 968.06). For AA analysis, samples were hydrolysed for 24 hours in 6 N hydrochloric acid at 110 ^{o}C under an atmosphere of N. For Met and Cys, performic acid oxidation was carried out before acid hydrolysis. The AA in the hydrolysate were determined by HPLC after post-column derivatization [(AOAC International 2000, method 982.30E (a, b, c)]. Analysis for Ti was performed as described by Short *et al*. (1996). Mineral concentrations in the samples were determined using inductively coupled plasma

spectrophotometry (ICP) according to the procedures of Olsen and Sommers (1982). Xylanase activity in diets was measured using a kit (Megazyme International Ireland Ltd., Bray, Ireland) using the method of McCleary (1991). Amylase activity in the diet was measured using Phadebas (Megazyme International Ireland Ltd.) tablets using the method described by McCleary and Sheehan (1989). Protease activity was analysed using the modified method of Lynn and Clevette-Radford (1984) with azocasein used as substrate. Phytase activity in the diets was analysed using the AOAC official method (2000.12, AOAC, 2000).

Table 3-5. Ingredient composition of experimental diets to determine ileal amino acids digestibility of wheat-DDGS for broilers.

	Without protease		With protease	
Item	NFD[1]	W-DDGS[2]	NFD[1]	W-DDGS[2]
Ingredients, g/kg				
DDGS	0	675	0	675
Corn starch	566	10	556	0
Dextrose	200	200	200	200
Vitacell[3]	85	0	85	0
Soybean oil	50	50	50	50
Vitamin-mineral premix[4]	5	5	5	5
Dicalcium phosphate[5]	31	31	31	31
$NaHCO_3$	20	0	20	0
KCl	12	0	12	0
MgO	2	0	2	0
Choline chloride	3	3	3	3
Limestone (38% Ca)	9	9	9	9
Salt	2	2	2	2
Marker premix[6]	15	15	15	15
Protease premix[7]	0	0	10	10

[1]N-free diet
[2]Wheat distillers dried grains with solubles
[3]Vitacell: Purified cellulose
[4]Vitamin/mineral premix supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.
[5]Contain 21.3% Ca and 18.7% P.
[6]Prepared as 1 g titanium dioxide oxide added to 4 g of cornstarch.
[7]Protease (4000 U/kg of diet) premix made with cornstarch as carrier.

Table 3-6. Analysed chemical composition of experimental diets to determine ileal amino acids digestibility of wheat-DDGS for broilers.

	Without protease		With protease	
Item	NFD[1]	DDGS[2]	NFD[1]	DDGS[2]
Dry matter, g/kg	830	860	865	870
Crude protein (N x 6.25), g/kg	<3.13	23.6	<3.13	24.7
Protease activity, U/kg	<100	<100	3177	3459
ME, MJ/kg (calculated)	13.7	12.2	13.7	12.2
Indispensable amino acids, g/kg				
Arg	1.2	9.6	1.4	10
His	2.8	6.4	3.1	5.8
Ile	2	9.5	2.1	9.1
Leu	5.7	17.1	5.7	16.9
Lys	1.9	5.2	1.9	5.3
Phe	3.6	12.3	2.9	12.2
Thr	2.6	7.5	2.7	7.6
Met	1.3	3.6	1	3.5
Val	3	12.2	2.8	12.2
Dispensable amino acids, g/kg				
Ala	4.2	8.9	3.9	8.7
Cys	0.7	3.3	0.9	3.4
Glu	9	66.9	8.1	67.4
Gly	3.7	9.8	3.5	9.3
Pro	5.3	24.7	4.4	25.6
Ser	5.8	11.7	6.1	10.1
Tyr	3.2	8	3.1	8
Asp	4.3	12.8	4.1	12.4

[1]N-free diet
[2]Wheat distillers dried grains with solubles

3.2.4 Calculations and Statistical Analysis

All statistical analyses were performed using GenStat program (VSN International, 2011). Statistical significance was set at $P < 0.05$ and tendency at $0.05 < P < 0.1$ for all mean comparisons.

Experiment 1

Energy utilisation coefficient was calculated using the following equation:

1. $\mathrm{MEc} = [1 - \left(\frac{\mathrm{Ti}}{\mathrm{To}}\right) \mathrm{x} \left(\frac{\mathrm{Eo}}{\mathrm{Ei}}\right)]$

where MEc is energy utilisation coefficient, T_i is the concentration of titanium in diet (mg/kg), T_o is the concentration of titanium in excreta (mg/kg), E_o and E_i are the GE in excreta and diet, respectively (MJ/kg).

Apparent metabolisable energy was calculated using the following equation:

2. $\mathrm{AME} = \mathrm{MEc} \times \mathrm{GEdiet}$

where AME is apparent metabolisable energy (MJ/kg), ME_c is the energy utilisation coefficient and GE_{diet} is the GE (MJ/kg) in the diet.

Nitrogen-corrected AME was calculated using the following equation:

3. $\mathrm{AMEn} = \mathrm{AME} - (8.73 \times \mathrm{N\ gain})$

where AME_n is nitrogen-corrected apparent metabolisable energy (MJ/kg), N gain is nitrogen retained (g/kg of DM intake) and 8.73 is the caloric correction factor for retained nitrogen (Titus, 1956).

Nitrogen gain was calculated using the following equation:

4. $\mathrm{N\ gain} = \mathrm{Ndiet} - (\mathrm{Nexcreta} \times \mathrm{Ti/To})$

where N_{diet} and $N_{excreta}$ are the nitrogen in diet and excreta, respectively (g/kg of DM), T_i and T_o are the concentration of titanium (mg/kg) in the diet and excreta, respectively.

Wheat-DDGS-associated AME intake was calculated as illustrated by Adeola *et al*. (2010) using the following equations:

If the coefficients of AME for the assay diet, basal diet and test ingredient (wheat-DDGS) are represented by Cad, Cbd and Cti, respectively. Assuming additivity in diet formulation, the

proportional contribution of energy by the basal (Pbd) and test ingredients (Pti) to the assay diet will be equal to 1. Mathematically; Pbd + Pti = 1 or Pbd = 1 – Pti.

Therefore;

5. $\mathrm{Cad} = (\mathrm{Cbd\ x\ Pbd}) + (\mathrm{Cti\ x\ Pti})$

By solving for Cti,

6. $\mathrm{Cti} = [\mathrm{Cad} - (\mathrm{Cbd\ x\ Pbd})]/\mathrm{Pti}$

Substituting 1 – Pti for Pbd;

7. $\mathrm{Cti} = \{\mathrm{Cbd} + \left[\frac{\mathrm{Cad-Cbd}}{\mathrm{Pti}}\right]\}$

The product of Cti at each level of wheat-DDGS substitution rate (300 or 600 g/kg), the GE of wheat-DDGS, and wheat-DDGS intake in kg is the wheat-DDGS-associated AME intake in MJ.

Energy utilisation data were analysed as a randomised complete block design of 3 levels of wheat-DDGS (0, 300 and 600 g/kg) and 2 levels of enzyme supplementation (not added or added). In the 7 blocks, each consisting of 3 cages containing one of 0, 300, or 600g of wheat-DDGS per kg of diet without- or with added XAP, AME or AME_n intake (MJ) was regressed against wheat-DDGS intake (kg) for each block to generate intercepts and slopes for each of the 7 blocks per XAP (not added or added). The intercept and slope data were analysed as a one-way analysis of variance in a completely randomized design using intercept or slope as the dependent variable and XAP (not added or added) as the independent variable. The additional energy provided by the XAP was determined using ANOVA procedures as the difference between the slopes of dietary treatments without and those with supplemental XAP. Orthogonal contrast was used to determine the differences in metabolisable energy between the dietary treatments with different inclusion levels of wheat-DDGS and those without- or with added XAP.

Experiment 2

Apparent ileal P digestibility or apparent P retention was calculated using the following equation:

8. $\mathrm{APD/APR} = \left[1 - \left(\frac{\mathrm{Ti}}{\mathrm{To}}\right) \mathrm{x}\ \left(\frac{\mathrm{Po}}{\mathrm{Pi}}\right)\right] \mathrm{x}\ 100$

where APD/APR is apparent P digestibility (%) or apparent P retention (%); T_i and T_o are the concentrations (mg/kg) of titanium in diet and ileal digesta or excreta, respectively. P_o is the phosphorus in the ileal digesta or excreta (g/kg of DM output) and P_i is the phosphorus in the diet (g/kg of DM).

Mineral flow at the ileum or total tract was calculated using the following equation:

9. $\text{MO-dmi} = \text{MO-dmo x } (\frac{\text{Ti}}{\text{To}})$

where MO-dmi and MO-dmo are mineral output (ileal or total tract) on DM intake and DM output basis, respectively (mg/kg); T_i and T_o are the concentrations of titanium (mg/kg) in the diet and digesta or excreta, respectively.

True P digestibility or retention was determined from regressing P output (ileal or total tract) against dietary P intake per block of 3 treatments within each block (one block without-, the other with added phytase) using the following model;

10. $\text{PO-dmi} = (\text{TPI x Pi}) + \text{EPL}$

where PO-dmi is phosphorus output (mg/kg) on DM intake basis (dependent variable); TPI is the slope of the model or true P indigestibility; P_i is the phosphorus in the diet (g/kg of DM intake) (independent variable) and EPL is the intercept of the model or mean endogenous phosphorus loss (DM intake basis).

True P digestibility or retention was calculated from the measure of P indigestibility using the following equation:

11. $\text{TPD/TPR} = 100 - (\text{TPI x } 100)$

where TPD or TPR are true P digestibility or true P retention and TPI is true P indigestibility (%), respectively.

Experiment 3

Basal ileal AA flow was calculated using the following equation:

12. $\text{EAAF} = [\text{AAo x } (\frac{\text{Ti}}{\text{To}})]$

where EAAF is endogenous ileal AA flow (mg/kg of DM intake); AAo is the AA in ileal digesta (mg/kg of DM); T_i and T_o are the concentrations of titanium (mg/kg) in diet and ileal digesta, respectively.

Apparent ileal AA digestibility was calculated using the following equation:

$$\text{AIAAD} = \left[1 - \left(\frac{\text{Ti}}{\text{To}}\right) \text{x} \left(\frac{\text{AAo}}{\text{AAi}}\right)\right] \text{x } 100$$

where AIAAD is apparent ileal amino acid digestibility (%); T_i and T_o are the concentrations (mg/kg) of titanium in diet and ileal digesta, respectively; AA_o is the amino acid in the digesta (g/kg of DM) and AA_i is the amino acid in the diet (g/kg of DM).

Standardised ileal AA digestibility was calculated using the following equation:

13. $$\text{SIAAD} = \text{AIAAD} + \left[\left(\frac{\text{EAAF}}{\text{AAi}}\right) \text{x } 100\right]$$

where SIAAD is standardized ileal AA digestibility (%); AIAAD is apparent ileal AA digestibility (%); EAAF is the endogenous basal ileal AA flow (g/kg of DM intake) and AA_i is the amino acid in the diet (g/kg of DM).

Data for the AIAAD and SIAAD without- or with supplemental protease were subjected to a one-way analysis of variance to determine differences.

3.3 RESULTS

3.3.1 Metabolisable Energy Value of Wheat Distillers Dried Grains with Solubles without- or with an Admixture of Xylanase, Amylase and Protease for Broilers

The wheat-DDGS used in the current study contained by analysis 18.5 MJ/kg of GE, 326 g/kg of CP, 6.5 g/kg of P, 80 g/kg of crude fibre and 858 g/kg of DM (Table 3-2). The ingredient and analysed nutrient composition of the experimental diets are presented in Table 3-3. The average xylanase activity in the diets containing 0, 300 or 600 g/kg of wheat-DDGS with supplemental XAP was 1421 U/kg and this value is lower than the formulated value of 2000 U/kg. On the average, amylase and protease activity in these diets were 262 and 3064 U/kg, respectively compared to the formulated value of 200 and 4000 U/kg, respectively. Enzyme activity in the diets without supplemental XAP was generally below the detectable limit.

The effects of wheat-DDGS inclusion level and XAP supplementation on growth performance responses are presented in Table 3-7. Increasing the inclusion level of wheat-DDGS from 0 to 600 g/kg in the diet decreased body weight gain (BWG) and gain to feed ratio (G:F) in a quadratic ($P < 0.001$) manner. Final bodyweight (FBW) at d 21 and feed intake (FI) decreased in a quadratic manner ($P < 0.05$) as the dietary inclusion of wheat-DDGS increased to 600

g/kg. Weight gain, FI, G:F, and FBW were greatest ($P < 0.001$) for birds fed diets containing 300 g/kg of wheat-DDGS, these responses were lowest ($P < 0.001$) for the birds fed the diets containing 600 g/kg of wheat-DDGS whereas these responses were intermediate for the birds fed the reference diet without wheat-DDGS. Addition of XAP to the diets did not improve the BWG, G:F or FBW of the birds.

Dry matter retention and energy utilisation for broilers fed graded levels of wheat-DDGS without- or with added XAP are shown in Table 3-8. Increasing the inclusion level of wheat-DDGS from 0 to 600 g/kg in the diet decreased linearly ($P < 0.001$) DM and energy retention. Dry matter retention decreased ($P < 0.001$) by 11% when wheat-DDGS was increased from 0 to 300 g/kg in the reference diet. The decrease was 16% when the level of wheat-DDGS was increased from 0 to 600 g/kg. Similarly, energy retention decreased ($P < 0.001$) by 9% when wheat-DDGS was increased from 0 to 300 g/kg in the reference diet whereas the decrease was 13% when the dietary inclusion of wheat-DDGS was increased to 600 g/kg. Increasing the level of wheat-DDGS from 0 to 600 g/kg in the reference diet decreased linearly ($P < 0.001$) dietary AME whereas the decrease was quadratic in manner ($P < 0.05$) for dietary AME_n. Substitution of 300 g/kg of wheat-DDGS in the reference diet decreased ($P < 0.001$) both the AME and AME_n (MJ/kg) by 8%. The decrease ($P < 0.001$) in dietary AME and AME_n (MJ/kg) was 10% when the inclusion level of wheat-DDGS was increased to 600 g/kg. Supplemental XAP tended to improve ($P < 0.1$) dietary AME and AME_n.

The AME and AME_n values of wheat-DDGS without- or with added XAP determined from regressing wheat-DDGS-associated energy intake against wheat-DDGS intake are presented in Table 3-9. The slope for the regression analyses are shown in Figures 3-1. From the slope of the linear regression, the AME values (MJ/kg DM) of wheat-DDGS for broilers without- or with supplemental XAP were determined to be 15 or 15.5, respectively. Corresponding AME_n (MJ/kg DM) were 14 or 14.5, respectively. Comparison using ANOVA procedures indicated that the slope when XAP was added was not greater than when XAP was not added. Numerical increases in AME and AME_n (MJ/kg DM) values of wheat-DDGS with supplemental XAP were 0.47 and 0.43, respectively.

Table 3-7. Growth performance of broilers fed graded levels of wheat-DDGS without or with an admixture of xylanase, amylase and protease[1,2]

Measurement	Gain, g/bird	FI, g/bird	G:F, g:kg	Initial weight, g	Final weight, g
Diet effect					
0 g/kg of diet (A)	263	470	558	396	659
300 g/kg of diet (B)	313	511	611	394	707
600 g/kg of diet (C)	205	441	465	396	602
s.e.d	15.4	23.2	11.2	-	31.0
P values for main effects of DDGS inclusion	<0.001	0.016	<0.001	-	0.006
Enzyme effect					
Without XAP	262	475	547	396	658
With XAP	259	473	542	395	654
s.e.d	12.6	19.0	9.12	-	25.3
P values for main effects of XAP supplementation	0.813	0.905	0.585	-	0.874
DDGS × XAP interaction	0.998	0.898	0.432	-	0.996
P values for contrasts					
Diet (linear)	<0.001	0.214	<0.001	-	0.072
Diet (quadratic)	<0.001	0.009	<0.001	-	0.007
A vs. B	0.003	0.086	<0.001	-	0.131
A vs. C	<0.001	0.214	<0.001	-	0.072

[1]Data are means of 7 replicate cages; Experimental diets fed from d 15 to 21 posthatch.
[2]Enzyme admixture added to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease
s.e.d - standard error of difference

Table 3-8. Dry matter and energy utilisation for broilers fed diets containing graded levels of wheat-DDGS without or with an admixture of xylanase, amylase and protease[1,2]

Measurement	DM retention, %	Energy retention, %	AME, MJ/kg	AME_n, MJ/kg
Diet effect				
0 g/kg of diet (A)	72.7	76.3	15.1	14.2
300 g/kg of diet (B)	65.1	69.1	13.9	13.1
600 g/kg of diet (C)	60.9	66.5	13.6	12.8
s.e.d	1.32	1.18	0.24	0.21
P values for main effects of DDGS inclusion	<0.001	<0.001	<0.001	<0.001
Enzyme effect				
Without XAP	65.2	69.7	14.0	13.2
With XAP	67.2	71.5	14.4	13.5
s.e.d	1.08	0.97	0.20	0.17
P values for main effects of XAP supplementation	0.062	0.073	0.063	0.057
DDGS × XAP interaction	0.920	0.931	0.976	0.982
P values for contrasts				
Diet (linear)	<0.001	<0.001	<0.001	<0.001
Diet (quadratic)	0.142	0.031	0.059	0.038
A vs. B	<0.001	<0.001	<0.001	<0.001
A vs. C	<0.001	<0.001	<0.001	<0.001

[1]Data are means of 7 replicate cages; Dietary treatments fed from d 15 to 21 posthatch.
[2]Enzyme admixture added to supply 2000 U/kg of xylanase, 200 U/kg of amylase and 4000 U/kg of protease
s.e.d - standard error of difference

Table 3-9. Linear terms showing the apparent metabolisable energy content of wheat-DDGS without or with added admixture of xylanase, amylase and protease for broilers[1,2]

Measurements	Regression equation	s.e.d intercept	s.e.d slope	s.e.d model	r^2	P-value
AME, MJ/kg						
No added XAP	Y = 15.0X + 0.013	0.078	0.246	0.222	0.995	<0.001
Added XAP[3]	Y = 15.5X – 0.01	0.117	0.366	0.337	0.989	<0.001
AME_n, MJ/kg						
No added XAP	Y = 14.0X + 0.021	0.069	0.219	0.197	0.995	<0.001
Added XAP[3]	Y = 14.5X – 0.005	0.103	0.323	0.297	0.990	<0.001

[1]AME and AME_n values of wheat-DDGS determined from regressing wheat-DDGS-associated AME or AME_n against wheat-DDGS intake; Y is in MJ, intercept is in MJ, and slope is in MJ/kg of DM.
[2]Addition of XAP did not improve ($P > 0.05$) the AME or AME_n values of the wheat-DDGS for broilers
[3]Enzyme admixture added to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease
s.e.d - standard error of difference

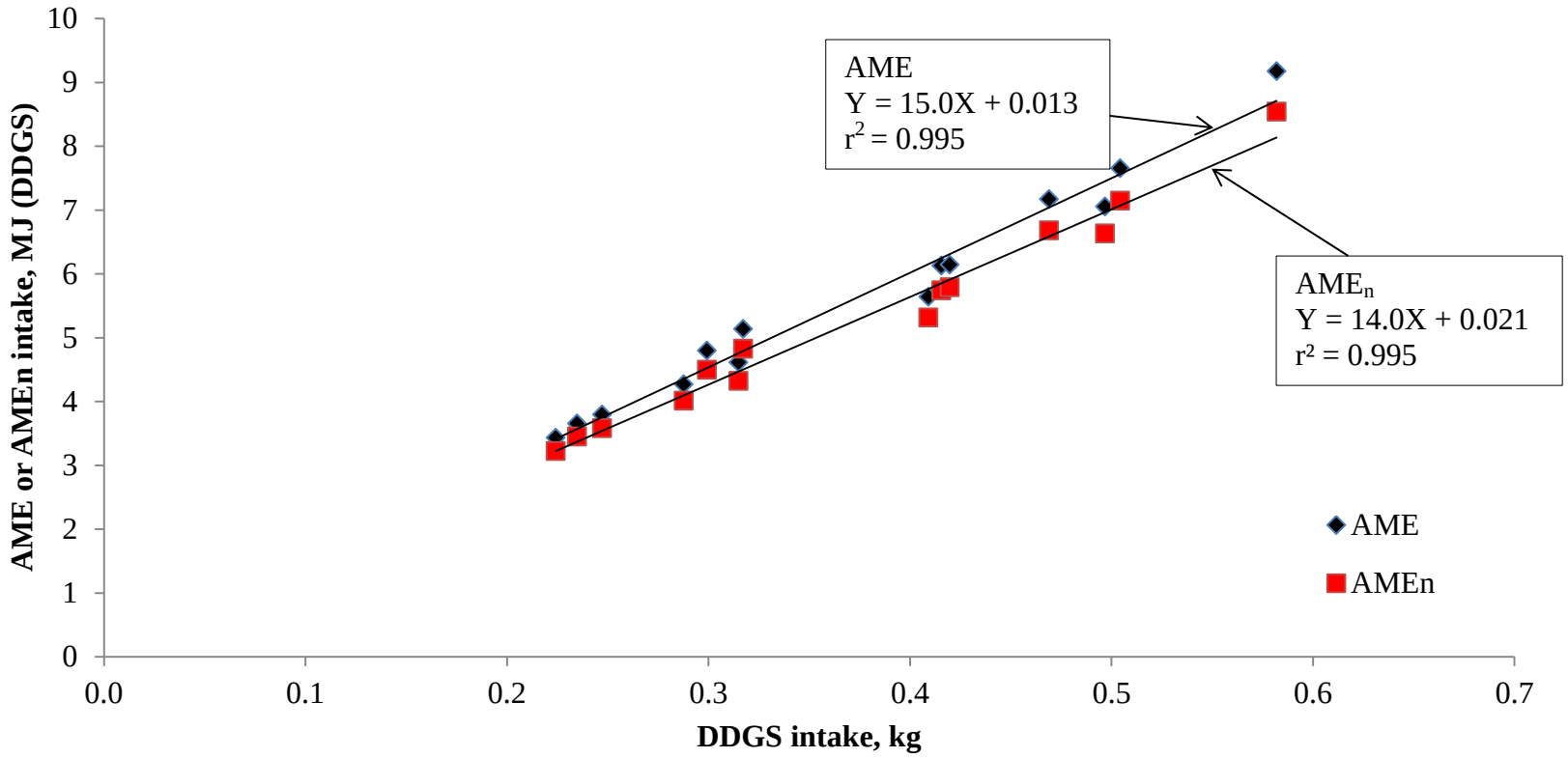


Figure 3-1. Regression line showing the AME and AMEn values of wheat-DDGS for broilers.

3.3.2 True Phosphorus Digestibility and Retention of Wheat Distillers Dried Grains with Solubles without- or with Supplemental Phytase for Broilers

The ingredient and analysed chemical compositions for the six dietary treatments in the current study are presented in Table 3-4. Analysed phytase activities were 962, 767 or 822 FTU/kg in the phytase-supplemented diets containing 200, 400 or 600 g/kg of wheat-DDGS, respectively, but these values are marginally lower than the formulated value of 1000 FTU/kg. Diets not supplemented with phytase contained less than 50 FTU/kg of diet. The utilisation of DM and P at the ileal and total tract for broilers fed graded levels of wheat-DDGS without- or with supplemental phytase are presented in Table 3-10. Increasing the dietary inclusion of wheat-DDGS from 200 to 600 g/kg did not affect dry matter intake (DMI), but decreased ileal DM digestibility and retention in a linear manner ($P < 0.001$). Further, increasing the level of wheat-DDGS from 200 to 600 g/kg in the diet did not affect apparent ileal P digestibility but decreased apparent P retention in a linear manner ($P < 0.05$).

The true ileal digestibility and total tract retention of P in wheat-DDGS for broilers are presented in Table 3-11. From the regression of P output (mg/kg of DMI) at the ileal level against dietary P intake (mg/kg of DM), true P digestibility of wheat-DDGS without- or with supplemental phytase were determined to be 93.6 or 96.0%, respectively. Corresponding values at the total tract level were 92.4 and 93.5%, respectively. The regression lines showing the TPI of wheat-DDGS at the ileal and total tract level for broilers are shown in Figure 3-2. True P digestibility or retention was not different between the treatments without- and with phytase. The true digestible P and true retainable P contents of the wheat-DDGS were calculated as the coefficient of TPD/TPR multiplied by the analysed P composition (%) of the wheat-DDGS. The true digestible P (%) in the wheat-DDGS for broilers without- or with added phytase was 0.60 or 0.62, respectively. Respective values for true retainable P (%) were 0.60 or 0.61.

Flow of minerals at the ileal level is presented in Table 3-12 and those at the total tract level in Table 3-13. Increasing the dietary inclusion of wheat-DDGS from 200 to 600 g/kg increased linearly ($P < 0.05$) the flow of Cu, Mg, Mn, K, and Na but not those of Fe or Zn at the ileal level. Increasing the dietary inclusion of wheat-DDGS from 200 to 600 g/kg linearly increased ($P < 0.05$) the flow of Cu, Fe, Mg, K, and Na but did not affect the flow of Mn and Zn at the total tract level. Phytase supplementation did not affect ($P > 0.05$) the flow of any of the minerals at the ileal or total tract levels.

Table 3-10. Dry matter and dietary P utilisation by broiler chicks fed graded levels of wheat-distillers dried grains with solubles without or with a phytase[1].

Measurement	DM intake, g per chick	Ileal DM digestibility, %	DM retention, %	Apparent Ileal P digestibility, %	Apparent P retention, %
Diet effect					
0 g/kg of diet (A)	297	78.7	79.1	63.2	59.8
300 g/kg of diet (B)	282	70.5	73.3	56.9	54.4
600 g/kg of diet (C)	270	65.1	68.2	60.5	46.3
s.e.d	15.6	1.59	0.969	3.20	4.26
P values for main effects of DDGS inclusion	0.237	<0.001	<0.001	0.154	0.011
Enzyme effect					
Without phytase	284	72.6	74.2	59.5	54.7
With phytase	282	70.2	72.9	60.9	52.4
s.e.d	12.8	1.30	0.791	2.61	3.48
P values for main effects of phytase supplementation	0.872	0.068	0.11	0.609	0.511
DDGS × Phytase interaction	0.771	0.969	0.660	0.493	0.574
P values for contrasts					
Diet (linear)	0.093	<0.001	<0.001	0.392	0.003
Diet (quadratic)	0.909	0.299	0.676	0.082	0.708
A vs. B	0.342	<0.001	<0.001	0.053	0.214
A vs. C	0.093	<0.001	<0.001	0.387	0.003

[1]Data are means of 7 replicate cages; Dietary treatments fed from d 15 to 21 posthatch.
DMI is dry matter intake; s.e.d - standard error of difference

Table 3-11. True phosphorus digestibility and retention determined from regressing ileal or total tract P output (mg/kg of DM intake) against dietary P intake (mg/kg of DM) for broilers fed wheat-DDGS supplemented without or with phytase.

	Regression equation[1]	r^2	SE slope[2]	SE intercept[2]	Endogenous P loss, mg/kg of DMI	TPD/ TPR[3], %	TDP/TRP of wheat-DDGS[4], %	P-value
Ileal								
Without phytase	Y= 0.064X - 476	0.661	0.010	320	-476 ± 320	93.6	0.60	< 0.001
With phytase	Y = 0.040X + 174	0.725	0.005	164	174 ± 164	95.9	0.62	< 0.001
Total tract								
Without phytase	Y = 0.063X - 625	0.534	0.016	487	-625 ± 487	92.4	0.60	< 0.001
With phytase	Y = 0.065X - 201	0.689	0.010	297	-201 ± 297	93.5	0.61	< 0.001

[1]Ileal or excreta P output (mg/kg of DM intake) regressed against dietary P intake (mg/kg of DM). The intercept of the regression term represents the endogenous P loss (mg/kg of DMI) whereas the slope represents the true P indigestibility.
[2]Standard error of regression components for 42 observations
[3]TPD or TPR is true P digestibility or true P retention, calculated as 100 x (1 - true P indigestibility); TPD and TPR were not improved by phytase
[4]TDP and TRP are true digestible P and true retainable P contents of wheat-DDGS, respectively. Calculated as (true P utilisation (%) /100) multiplied by analysed P composition of wheat-DDGS (%).

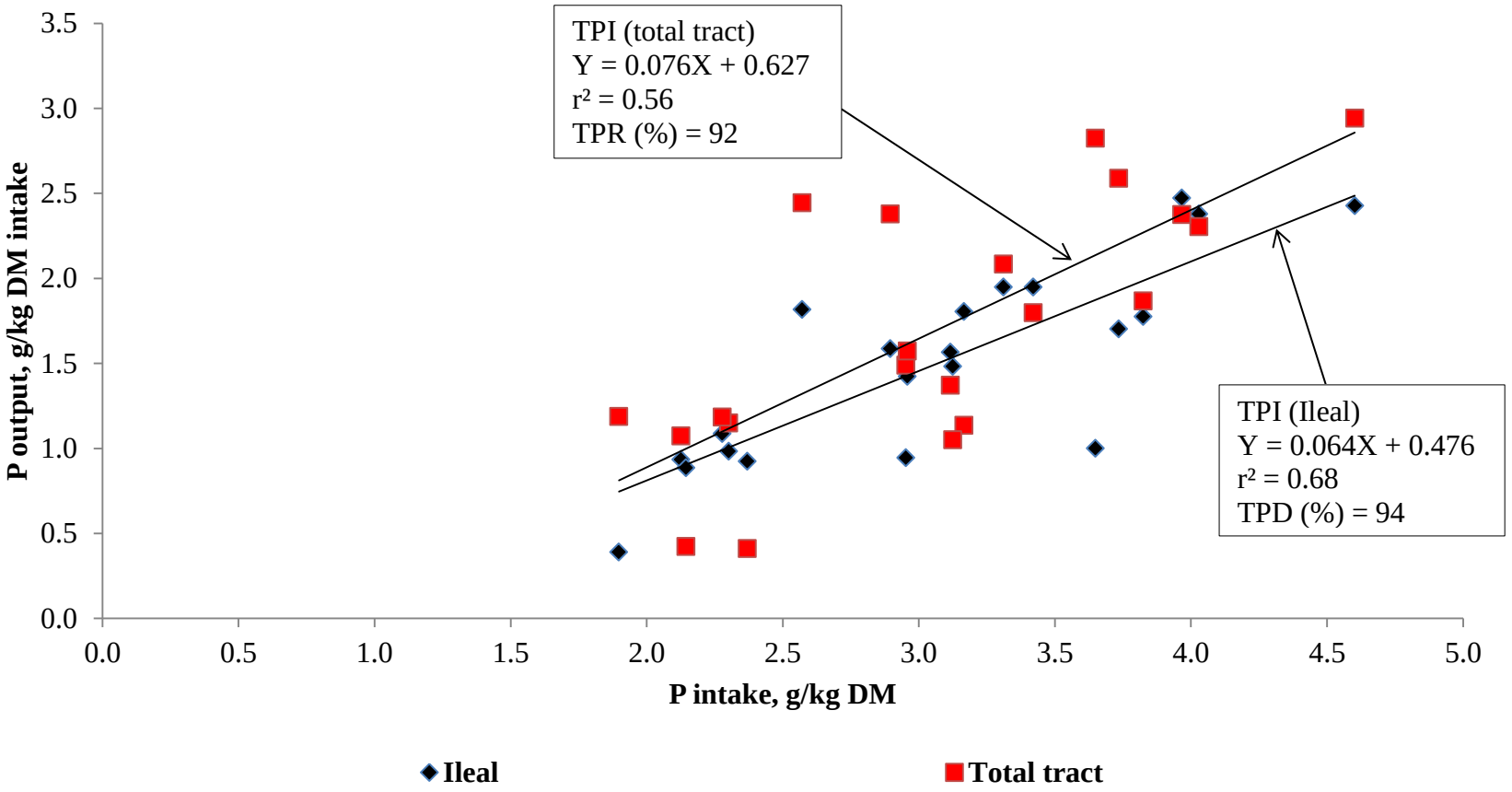


Figure 3-2. True phosphorus indigestibility (TPI) of wheat-DDGS at the ileal and total tract level for broilers. True P digestibility (TPD) or true P retention (TPR) calculated as 100 - (TPI × 100)).

Table 3-12. Flow of minerals at the ileal level (mg/kg of DM intake) for broilers fed graded levels of wheat-DDGS without or with supplemental phytase[1].

Measurement	Cu	Fe	Mg	Mn	K	Na	Zn
Diet effect							
0 g/kg of diet (A)	11.1	179	566	86.9	1372	2528	136
300 g/kg of diet (B)	13.5	203	852	97.9	2185	3024	146
600 g/kg of diet (C)	14.2	186	1177	107.6	2666	3763	174
s.e.d	1.25	39.5	53.4	5.92	174	309	35.0
P values for main effects of DDGS inclusion	0.049	0.817	<0.001	0.005	<0.001	0.001	0.534
Enzyme effect							
Without phytase	12.4	172	867	98	1945	2900	126
With phytase	13.5	206	863	96.9	2204	3310	178
s.e.d	1.02	32.2	43.6	4.83	143	253	28.6
P values for main effects of phytase supplementation	0.277	0.297	0.915	0.828	0.077	0.113	0.074
DDGS × Phytase interaction	0.103	0.086	0.927	0.906	0.601	0.964	0.852
P values for contrasts							
Diet (linear)	0.02	0.862	<0.001	0.001	<0.001	<0.001	0.281
Diet (quadratic)	0.442	0.544	0.669	0.892	0.281	0.653	0.782
A vs. B	0.067	0.541	<0.001	0.067	<0.001	0.124	0.769
A vs. C	0.02	0.862	<0.001	0.001	<0.001	<0.001	0.295

[1]Mineral flow calculated as mineral output at the ileal level multiplied by the ratio of marker (titanium intake/output)
s.e.d - standard error of difference

Table 3-13. Flow of minerals at the total tract (mg/kg of DMI) for chicks fed graded levels of wheat-DDGS without or with supplemental phytase[1].

Measurement	Cu	Fe	Mg	Mn	K	Na	Zn
Diet effect							
0 g/kg of diet (A)	9.99	66.3	724	97.4	3835	2955	105
300 g/kg of diet (B)	11.2	85.0	991	105	5127	3714	112
600 g/kg of diet (C)	12.1	115	1205	106	6183	4288	117
s.e.d	0.529	8.43	43.7	4.59	216	168	5.75
P values for main effects of DDGS inclusion	0.001	<0.001	<0.001	0.126	<0.001	<0.001	0.106
Enzyme effect							
Without phytase	10.9	85.7	958	103	4936	3597	113
With phytase	11.4	91.8	989	103	5161	3708	110
s.e.d	0.43	6.88	35.7	3.75	176	137	4.69
P values for main effects of phytase supplementation	0.242	0.375	0.393	0.886	0.21	0.423	0.496
DDGS × Phytase interaction	0.476	0.955	0.864	0.529	0.507	0.203	0.430
P values for contrasts							
Diet (linear)	<0.001	<0.001	<0.001	0.068	<0.001	<0.001	0.037
Diet (quadratic)	0.675	0.443	0.489	0.361	0.531	0.53	0.932
A vs. B	0.024	0.032	<0.001	0.086	<0.001	<0.001	0.251
A vs. C	<0.001	<0.001	<0.001	0.064	<0.001	<0.001	0.035

[1]Mineral flow calculated as mineral output at the ileum multiplied by the ratio of marker (titanium intake/output)
s.e.d - standard error of difference

3.3.3 Apparent- and Standardised Ileal Amino Acids Digestibility of Wheat Distillers Dried Grains with Solubles without- or with Protease for Broilers

The analysed chemical composition and protease activities for the four experimental diets used in the current study are presented in Table 3-6. The analysed nutrient composition for the four diets was in good agreement with the expected values. Average protease activity in the experimental diets supplemented with protease was 3318 U/kg. The analysed protease activity is lower than the expected value of 4000 U/kg.

The AIAAD and SIAAD of wheat-DDGS without- or with supplemental protease for broilers are presented in Table 3-14. Apparent ileal digestibility (AID) of N in wheat-DDGS without- or with added protease for broilers was 49.3 or 60.2%, respectively. Respective standardised ileal digestibility (SID) values were 51.3 and 62.8%. Protease supplementation increased ($P < 0.05$) the AID or SID of N by 10.9 or 11.5 percentage units, respectively. The AID of Lys in the wheat-DDGS for broilers was zero. The lowest AID were observed for Asp (34%) and Ala (35%), whereas, Pro (75%), Glu (75%) and Phe (56%) were the most digestible AA in the wheat-DDGS. Apparent ileal AA digestibility ranged from 35% (Ala) to 75% (Pro) in the dietary treatments without added protease whereas the range was 42% (Thr) to 82% (Pro) in the protease supplemented diets. Of the indispensable AA, the highest and lowest AID was observed for Phe (56%) and Met (37%), respectively, for the diets without supplemental protease. Protease improved ($P < 0.05$) the AID of Arg and Pro and tended to improve ($P < 0.10$) the AID of Met.

The mean SID for Lys in the wheat-DDGS without- or with supplemental protease for broilers was 2 or 43%, respectively. A large increase in the SID of Lys was noted with the addition of protease (41%). The lowest and highest SID values (excluding Lys) were observed for Asp (43%) and Pro (84%), respectively. This range was from 54% (Asp) to 93% (Pro), respectively with the addition of protease. Histidine (72%) and Phe (71%) were the most digestible (SID) amongst the indispensable AA in the wheat-DDGS. Protease addition improved ($P < 0.05$) the SID of Arg, Leu, Phe, Met, Val and Pro by 21, 14, 13, 26, 13 and 10 percentage points, respectively.

Table 3-14. Apparent- and standardised ileal amino acids digestibility of wheat-DDGS without or with supplemental protease for broilers[1]

	Apparent, %			Standardised, %			Protease effect[3]	
Item	No protease	With protease[2]	s.e.d	No protease	With protease[2]	s.e.d	Apparent	Standardised
Nitrogen	49.3	60.2	3.96	51.3	62.8	3.96	0.017	0.013
Indispensable amino acids								
Arg	37.5	52.5	5.93	53.9	75.2	5.93	0.026	0.004
His	52.2	56.4	6.39	71.8	79	6.39	0.524	0.286
Ile	43.7	52.7	6.34	57.4	70.6	6.34	0.182	0.059
Leu	49.6	59.1	5.58	64.4	78.2	5.58	0.115	0.029
Lys	-0.28	0.05	15.1	2.2	43.9	15.1	0.049	0.017
Phe	55.6	65.1	5.52	70.2	82.7	5.52	0.11	0.043
Thr	37.1	41.8	6.44	52.4	66.3	7.24	0.478	0.081
Met	37.4	49.4	6.62	58.4	74.4	6.62	0.094	0.032
Val	43.9	53.5	5.47	59.1	72.6	5.47	0.106	0.029
Dispensable amino acids								
Ala	35.2	45.1	7.19	50.9	65.4	7.19	0.194	0.067
Cys	47.1	53.4	6.73	63.1	70.4	6.73	0.371	0.303
Glu	74.9	78.9	2.75	81.8	87.5	2.75	0.175	0.062
Gly	49.4	48.3	6.27	65.8	67.9	6.27	0.869	0.75
Pro	75.2	82.3	3.12	83.7	93.3	3.12	0.041	0.01
Ser	54.3	56	8.38	70.9	75	8.38	0.843	0.633
Tyr	44.5	54.4	6.98	64.2	78.9	6.98	0.182	0.057
Asp	33.7	30.7	6.32	43.8	53.6	7.15	0.644	0.197

[1]Data are means of 7 replicates
[2]Protease added to supply 4000 U/kg
[3]*P* values for comparison between diets without- and with protease
s.e.d - standard error of difference

3.4 DISCUSSION

Metabolisable energy content of wheat-DDGS without- or with a combination of xylanase, amylase and protease enzymes for broilers

Because of the increased availability of wheat-DDGS, it is now possible to substitute wheat with wheat-DDGS as a dietary source of energy for broilers. The current study determined therefore, the AME and AME_n value of wheat-DDGS for broilers as well as the improvements to the energy value of wheat-DDGS by supplementation of a combination of exogenous xylanase, amylase and protease. The hypotheses were that wheat-DDGS is a good dietary source of energy for broilers and that XAP will increase the energy value of wheat-DDGS for broilers.

The wheat-DDGS used in the current study was produced and acquired from a new fuel bioethanol production facility in the UK. The wheat-DDGS contained by analysis, 858 g/kg of DM, 326 g/kg of CP, 18 MJ/kg of GE, 80 g/kg of CF, 73 g/kg of ether extract (EE), 389 g/kg of neutral detergent fibre (NDF), 223 g/kg of acid detergent fibre (ADF) and 46 g/kg of ash. In comparison, the chemical characteristics of this wheat-DDGS is close to those used in the study of Bolarinwa and Adeola (2012) as well as the mean values of 930 g/kg of DM, 38 g/kg of CP, 20 MJ/kg of GE, 77 g/kg of CF, 54 g/kg of EE, 344 g/kg of NDF, 139 g/kg of ADF and 53 g/kg of ash from 11 sources of wheat-DDGS (Chapter 2).

A limitation with using wheat-DDGS as a feed ingredient for poultry is the variation in its chemical composition among sources (Fastinger *et al*., 2006). The NDF and ADF contents for the wheat-DDGS used in the current study was slightly greater compared with those reported by Nyachoti *et al*. (2005) or Bolarinwa and Adeola, (2012). The greater levels of NDF and ADF observed for the wheat-DDGS used in the current study compared with those of Nyachoti *et al*. (2005) and Bolarinwa and Adeola, (2012) may be due to a high level of NDF and ADF fractions in the wheat used to produce the DDGS or a greater efficiency in the conversion of starch into ethanol leading to a much larger concentration of the fibre fractions in the wheat-DDGS due to a lower dilution from residual starch. Another notable practice that may cause variability in wheat-DDGS composition is the amount of condensed solubles added back to distillers grains, and this is because the fibre composition of these two products differ significantly (Liu 2011).

Increasing the level of wheat-DDGS in the reference diet decreased linearly DM retention, AME and AME_n, regardless of XAP supplementation. Inclusion of 30% wheat-DDGS to the

reference wheat-SBM diet reduced DM retention by 11% and energy utilisation by 8% whereas the reductions were 16 and 11%, respectively when wheat-DDGS inclusion was increased to 60%. It is common knowledge that dietary fibre reduces DM retention in broilers due to its low digestibility (Adeola *et al*. 2010). The increase in dietary fibre associated with increasing wheat-DDGS levels may explain the reductions in DM retention and energy utilisation observed in the current study. Bolarinwa and Adeola (2012) noted a linear reduction in DM and energy utilisation of the reference diet when wheat-DDGS was incorporated at an inclusion level of 20%. Similarly, Adeola *et al*. (2010) reported an average reduction in AME and AME_n of 23% when using maize-DDGS at 600 g/kg in a maize-SBM reference diet. Also, Adeola and Ileleji (2009) noted a linear decrease from 79 to 59% in energy retention as the level of maize-DDGS increased from 0 to 60% in the reference maize-SBM diet.

Despite the fact that non starch polysaccharide (NSP) degrading enzymes are used during bioethanol production to reduce mash viscosity, the concentration of NSP in maize-DDGS have been reported to still increase substantially (Widyaratne and Zijlstra, 2007). The anti-nutritional effects of NSP for poultry are well described in literature (Adeola and Bedford 2004; Choct *et al*., 2004). Carbohydrases are able to hydrolyse NSP into sugars that can be utilised by the bird (Bedford, 2000) whereas proteases help to improve protein utilisation (Adeola and Cowieson, 2011). The wheat-DDGS used in the current study contained 389 g/kg of NDF that could be substrates for carbohydrase enzymes. These enzymes have been shown to be effective in improving energy value and nutrient digestibility of wheat-based diets for poultry (Choct *et al*. 2004; Adeola and Cowieson, 2011). Therefore, it was expected that an enzyme admixture containing xylanase, amylase and protease activities will increase the nutritive value of the diet for broilers by improving energy and protein utilisation. Indeed, XAP increased the dietary AME and AME_n contents of the wheat-DDGS for broilers in the current study; however the improvements in dietary energy utilisation noted were not statistically significant. Previously, Liu *et al*. (2011) reported a 20% reduction in hemicellulose levels and a 2.59 MJ/kg increase in AME in diets containing maize-DDGS when investigating the effect of supplemental xylanase on growth performance and nutrient digestibility in broilers. Also, addition of an NSP hydrolysing enzyme to a diet containing 20% of maize-DDGS increased significantly the dietary AME for broilers in a study by Lee *et al*. (2010). In the current study, the improvements noted in the energy value of the wheat-DDGS due to XAP supplementation were marginal and were not statistically significant. The lack of XAP effect in the current study is least expected because feed ingredients or diets that contain substantial concentrations of fibre respond to a greater extent to carbohydrase

supplementation (Bedford, 2000). The effect of XAP to improve the ME value of wheat-DDGS for broilers may require further investigation.

The AME value of wheat-DDGS for broilers was determined to be 15.01 MJ/kg of DM in the current study. This value is greater compared with the 11.1 or 9.27 MJ/kg of DM for 2 wheat-DDGS samples, reported in the Bolarinwa and Adeola (2012) study, as well as the range of 8.97 to 12 MJ/kg of DM for 10 samples of wheat-DDGS noted in the study of Cozannet *et al*. (2010a). The AME derived for wheat-DDGS in the current study was at the least 4.81 MJ/kg of DM greater compared with average AME values noted by Bolarinwa and Adeola (2012) and Cozannet *et al*. (2010a). It is common practice to correct the AME value of feed ingredients for nitrogen retention in order to account for variability in energy utilisation that may occur due to differences in age and species of the animal as well as the protein quality of a diet. Correction for N retention resulted in a 6.4% reduction in the AME value of the wheat-DDGS in the current study which is similar to the 6 to 7% reduction reported by Bolarinwa and Adeola (2012). The AME_n value of wheat-DDGS was determined to be 14.04 MJ/kg of DM in the current study. Similarly, the AME_n value determined in the current study was greater compared with the mean values of 9.53, 9.93 and 10.9 MJ/kg of DM reported by Bolarinwa and Adeola (2012), Cozannet *et al*. (2010a) and Vilarino *et al*. (2007), respectively.

The reason/s for the greater energy value for wheat-DDGS noted in the current study compared with other studies (Cozannet *et al*; 2010a; Bolarinwa and Adeola, 2012) may be due to the differences in the characteristics of the co-product. Under normal circumstances, the fermentation process cannot effectively convert all the starch in the grain into ethanol. Thus, there are usually residual starch and sugars in the co-product at variable quantities depending on the efficiency of fermentation (Vilarino *et al*., 2007). This may explain some of the differences observed in the GE values of the DDGS among sources. Because sugars and starch are more readily utilised in the gut, it is possible that differences in the quantity of residual sugars and starch in the DDGS among sources may also affect its AME value. The GE in the wheat-DDGS used in the current study was greater compared with the average of those used in the study of Bolarinwa and Adeola (2012) (21.6 *vs* 18.9 MJ/kg DM, respectively). Nonetheless, energy metabolisability in the wheat-DDGS in the current study was 68% and was close to the 63% reported by Bolarinwa and Adeola (2012). It therefore appears that the GE of the wheat-DDGS is vital to determining its metabolisable energy content for broilers.

The efficacy of exogenous enzymes to improve the nutritive value of bioethanol co-products has been determined predominantly for maize-DDGS (Adeola and Ileleji, 2009; Liu *et al*., 2011). Greater benefits may be derived from using exogenous enzymes in diets containing wheat-DDGS because wheat contains higher levels of NSP than maize. An admixture of XAP did not improve the AME or AME_n of the wheat-DDGS for broilers in the current study. It is not clear why the analysed xylanase and protease activities were about 20% lower than the expected values; nonetheless, the disparity should have little effect on the outcomes given that the analysed enzyme activities were within the range where an improvement in the energy value of the wheat-DDGS could still be expected. In a broiler study using a mixture of xylanase and amylase enzymes, Adeola *et al*. (2010) reported a 5.7 or 6.2% improvement in AME or AME_n values, respectively for maize-DDGS. More studies are needed to examine the benefits of supplemental XAP on the energy value of wheat-DDGS for broilers considering that wheat-DDGS contains a greater quantity of dietary fibre compared with maize-DDGS.

In conclusion, the AME and AME_n values of wheat-DDGS are 15.01 and 14.04 MJ/kg of DM, respectively for broilers. There is possibility that the gross energy value of wheat-DDGS may define its metabolisable energy content for broilers and this may explain some of the differences in the AME values noted among sources. A combination of xylanase, amylase and protease marginally increased the metabolisable energy content in the wheat-DDGS for broilers in the current study.

True P digestibility and retention of wheat-DDGS without- or with supplemental phytase for broilers

Excessive P in poultry manure is harmful to the environment whereas below optimal levels of dietary P reduces animal productivity. Therefore, evaluating the digestible P for feed ingredients used for broilers is essential to avoid oversupply or under provision of P in the diet. The objective of the current study was to determine the digestible P in wheat-DDGS without- or with exogenous phytase for broilers. It was hypothesized that wheat-DDGS is a good source of digestible P for broilers and that supplemental phytase will increase P in wheat-DDGS.

During bioethanol production, the concentration of P is increased 3-fold in the wheat-DDGS after the removal of starch from the wheat by fermentation, but what is more important is that a large proportion of the phytate-bound P in the wheat are dissociated from phytate by yeast phytase. For this reason, DDGS is generally considered a valuable source of digestible P for

monogastrics (Spiehs *et al.*, 2002). The wheat-DDGS used in the current study contained 7.6 g/kg DM of total P which is lower compared with the 12.3 g/kg DM reported by Thacker and Widyaratne (2007) or the 9.4 g/kg DM noted by Nyachoti *et al*. (2005). The differences in the P content of wheat-DDGS highlights the variability in its chemical composition among sources. The variability in the total P concentration of wheat-DDGS among sources is likely due to differences in the P composition of the wheat used to produce the DDGS.

Increasing the dietary inclusion level of wheat-DDGS decreased apparent P retention in a linear manner, whereas, apparent ileal P digestibility did not differ among all treatments. Because the diets were formulated to contain total P at levels lower than are required by the bird so as to achieve a linear response in P utilisation, it is likely that the increase in dietary fibre level as wheat-DDGS replaced the more readily digestible corn starch impaired nutrient digestibility as explained by the reduction in dietary ileal DM digestibility and total tract retention. Thacker and Widyaratne (2007) reported a reduction in apparent P retention when using graded levels of wheat-DDGS in a practical wheat-SBM diet for broilers. Dilger and Adeola (2006) on the other hand reported a linear increase in diet apparent ileal P digestibility and total tract retention when determining the true P digestibility and retention of SBM for broiler chicks. The difference between the observations made in the study of Dilger and Adeola, 2006 and the current may be partly explained by the lower levels of dietary insoluble fibre levels in SBM compared with the wheat-DDGS being tested in the current study.

Supplemental phytase did not improve dietary ileal P digestibility or total tract P retention in the current study. The efficacy of phytase to hydrolyse phytate P into non phytate bound P have been extensively described and reviewed (Selle and Ravindran, 2007; Woyengo and Nyachoti, 2011). Liu and Han (2011) assessed the concentrations of different forms of P (non phytate-P, phytate-bound P, and total P) in different streams of the bioethanol production process and reported an increase in maize-DDGS over maize grain of 1.8 fold in phytate-P and 10.8 fold in non-phytate P. The authors found that during the fermentation process, percentage phytate-P in total P decreased significantly whereas percentage non phytate-P in total P increased. These observations suggest that phytate underwent degradation through the actions of yeast phytase. It is acceptable to speculate that the lack of improvement in P digestibility and retention noted in the current study may be because the majority of the phytate bound P in the wheat are already hydrolysed during the production of wheat-DDGS; thus leaving little or no substrate for phytase to hydrolyse.

The regression method utilises the relationship between undigested P and dietary P intake to simultaneously determine the true P digestibility or retention and basal endogenous P loss. In the current study, there was a strong relationship between undigested P and dietary P intake; a relationship that is pre-requisite for the use of the regression technique. The linear regression method has been used previously to determine true P retention of feed ingredients for broilers (Dilger and Adeola, 2006) and swine (Akinmusire and Adeola, 2009) as well as for determination of true ileal AA digestibility of feed ingredients for broilers (Kong and Adeola, 2011). True P digestibility or retention of wheat-DDGS for broilers was greater than 90% in the current study. This observation suggests that majority of the P in wheat-DDGS may have been present in the form that is readily utilisable for the bird (Liu and Han, 2011).

Martinez-Amezcua et al. (2004) observed that more than 25% of the total P may be bound to phytate in maize-DDGS, and this is a reason why it is necessary to determine the efficacy of supplemental phytase in improving P utilisation for DDGS. Phytate may increase endogenous mineral losses by increasing secretion of mucin (Cowieson *et al*., 2004), forming complexes with cations and making them unavailable for absorption or bonding with endogenous enzymes and as a result reducing their efficacy (Dilworth *et al*., 2005), or cause a modification to the gastrointestinal electrolyte balance leading to less efficient mineral utilisation (Ravindran *et al*., 2008). On the other hand, phytase improves the utilisation of minerals by counteracting the anti-nutritional effects of phytate (Cowieson *et al*., 2004; Liu and Ru, 2010). Except for Fe and Zn at the ileal, and Mn and Zn at the total tract level, increasing the dietary inclusion of wheat-DDGS increased the flow of all other minerals in the current study. Because the current study was designed primarily to determine the TPD or TPR of wheat-DDGS for broilers, the dietary treatments were formulated in such a way that P was the only mineral that was limiting. It is therefore not surprising that increasing the inclusion level of wheat-DDGS resulted in an increase in the flow of majority of the minerals at the ileal and total tract as increasing the level of wheat-DDGS would have caused a further increase in the dietary intake of minerals beyond the levels required by the birds.

Supplemental phytase did not affect the flow of minerals at either the ileal or total tract level in the current study. Compared with other studies (Cowieson *et al*., 2004; Liu and Ru, 2010) where exogenous sodium phytate was used to increase the levels of phytate in the diet; wheat-DDGS was the only possible source of phytate in the current study. It is speculated that the level of phytate in the wheat-DDGS may have been low as evidenced by the high P digestibility and retention values noted, therefore, there would have been a low levels of

substrate (phytate) for the supplemental phytase to hydrolyse which may be a possible reason for the lack of phytase effect on mineral flow.

In conclusion, the results from the current study indicate that wheat-DDGS is a good source of digestible P for broilers; therefore the inclusion of wheat-DDGS in the diet will reduce the use of inorganic P sources in the diet. The true ileal digestibility of wheat-DDGS for broilers is 96% and true P retention is 93% at the total tract level. Supplemental phytase did not improve the P digestibility or retention in the wheat-DDGS for broilers.

Apparent- and standardised ileal amino acid digestibility of wheat-DDGS without- or with supplemental protease for broilers

An important chemical property of wheat-DDGS is its similar CP and AA content with other conventional protein feed ingredients such as canola meal. Compared with maize-DDGS, there is limited data about the digestibility of AA in wheat-DDGS for broilers and no information about the efficacy of exogenous protease to improve AA digestibility. Because information about the profile, balance and utilisation of AA in feed ingredients are essential prerequisites in diet formulations for broilers, the objective of the current study was to determine the AIAAD and SIAAD of wheat-DDGS without- or with a protease. The AID of CP was determined to be 49.3% in the current study indicating that only half of the total protein in the wheat-DDGS was utilised by the birds. In a similar experiment, Bandegan *et al*. (2009) found the average AID of CP for 5 samples of wheat-DDGS to be 67% for broilers. In addition, the SID for wheat-DDGS in the current study was lower than those reported by Bandegan *et al*. (2009) (69%) as well as that of Kluth and Rudehutscord (2010) (64%). Cozannet *et al*. (2010b) also reported the mean SID of CP in 7 wheat-DDGS samples to be 80% in caecectomised roosters.

Apparent ileal amino acids digestibility of wheat-DDGS for broilers was generally low in the current study. The least digestible AA in wheat-DDGS were Lys and Asp. In fact, the AID and SID values recorded for Lys were zero. Similar zero digestibility for Lys in wheat-DDGS have been reported by Cozannet *et al*. (2011) whereas Kluth and Rodehuscord (2010) have also noted the AID of Lys and Asp to be the lowest in wheat-DDGS for broilers. The observation in the current study that Lys is the least digestible AA in wheat-DDGS is also consistent with those of Bandegan *et al*. (2009) and Cozannet *et al*. (2011) as well as in studies using maize-DDGS for broilers (Lumpkins *et al*., 2004; Batal and Dale, 2006). Except for His, Phe, Glu, Ser and Pro, AID was lower than 50% for all other AA with the mean

AIAAD (with Lys excluded) being 49%. Further, the SIAAD of wheat-DDGS in the current study ranged from 51% (Ala) to 84%. The range for SIAAD of wheat-DDGS for broilers in the current study is similar to those of Bandegan *et al*. (2009) and Cozannet *et al*. (2011). Of the indispensable AA in the current study, the SID of Phe was the greatest, an observation that is consistent with those of Bandegan *et al*. (2010) in broilers and Lan *et al*. (2008) in finishing pigs. Kluth and Rudehuscord (2010) used a regression method to determine SIAAD of wheat-DDGS for broilers. The SIAAD in the study of Kluth and Rudehuscord (2010) are generally greater than the SIAAD for wheat-DDGS recorded in the current study and in fact they reported an SID of 72% for Lys compared with the zero SID noted in the current study.

Crude protein and AA digestibility of maize- and wheat-DDGS have been reported to vary substantially in poultry (Batal and Dale, 2006; Fastinger *et al*., 2006; Cozannet *et al*., 2010b). Heat treatment during the production of wheat-DDGS has been widely implicated to reduce as well as cause variability to the digestibility of CP and AA in DDGS for poultry (Fastinger *et al*., 2006; Cozannet *et al*., 2010b). Possibly, this may be the reason for the mean AIAAD of wheat-DDGS being lower in the current study compared with those reported in the study of Bandegan *et al*. (2009) (49 *vs.* 67%). Excessive application of heat during drying reduces the digestibility of AA in feed ingredients for poultry due to the formation of insoluble AA-carbohydrate compounds by Malliard reaction. This may be exacerbated in DDGS because a number of steps in the bioethanol production (jet cooking, liquefaction, saccharification, drying) involve heat application. Indeed, Liu and Han (2011) noted that the formation of carbohydrate-AA complexes in maize-DDGS was not solely limited to the final drying step of the product, because a proportion of Lys in wet distillers grains and condensed solubles (the two products combined to form DDGS before drying) were already bound to carbohydrates before the final drying process.

The colour of the DDGS is a tool that may be used to determine the intensity of heat treatment (Fastinger *et al*., 2006). A picture of the wheat-DDGS used in the current study is shown in Figure 3-3. Although colorimetric measurement was not used to grade the colour of the wheat-DDGS used in the current study, comparisons using a maize-DDGS colour score chart showed that the wheat-DDGS was dark in colour to a level 5 (Figure 3-3). However, it is noteworthy that the colour of maize-DDGS may vary slightly from that of wheat-DDGS. Light coloured maize-DDGS samples have been reported to have greater AA digestibility than their darker coloured counterparts for broilers (Ergul *et al*., 2003; Batal and Dale, 2006) and caecectomized roosters (Fastinger *et al*., 2006; Cozannet *et al*., 2011). It is speculated that the dark colour of the wheat-DDGS used in the current study may have been due to excessive

heat treatment and may be responsible for the low AID and SID observed for Lys. However, it is noteworthy that whilst the colour of the DDGS is mainly affected by the intensity of heat treatment, a combination of other factors such as the amount of condensed distillers solubles added back to the distillers grains, the colour of the grain used, storage conditions and presence of toxins may play a part in defining the colour of the DDGS (Liu, 2011; Shurson, 2011).

Protease either alone or as a part of an admixture of enzymes is often supplemented in the diet to increase protein and/or AA digestibility for poultry. It was therefore hypothesized in the current study that supplemental protease will improve AA digestibility in wheat-DDGS for broilers. Indeed, addition of protease increased the ileal digestibility of N and AA in the wheat-DDGS for broilers by 10 percentage points. The improvement in N and AA digestibility in the wheat-DDGS noted in the current study may be due to one or a combination of the following. Supplemental protease may supplement endogenous peptidase production, reducing the requirement for AA and energy and/or help hydrolyse protein-based anti-nutrients such as lectins or trypsin inhibitors, improving the efficiency by which the bird utilises AA and reducing protein turnover (Adeola and Cowieson, 2011). Proteases are more often supplemented to the diet as a part of an admixture of xylanase, amylase and protease; as such, improvement in AA digestibility of feed ingredients due to supplemental protease alone is not common. Nonetheless, Jung *et al*. (2010), Masa'deh *et al*. (2010) and Olukosi *et al*. (2010) have all reported improvements in either nutrient utilisation in the diet or the growth performance of broilers when diets containing maize-DDGS and an admixture of enzymes containing protease are fed. In conclusion, the ileal digestibility of AA in wheat-DDGS for broilers is quite variable and low. The digestibility of Lys is zero and is most likely due to excessive heat treatment of the wheat-DDGS during production. Therefore, the variable and low digestibility of wheat-DDGS needs to be accounted for in feed formulations. On the average, protease improved the digestibility of N and AA in the wheat-DDGS for broilers by 10 percentage units.

Collectively, it was concluded that wheat-DDGS is a valuable dietary source of energy and non-phytate P for broilers, but care needs to be taken to balance for digestible AA (especially Lys) due to its variable and generally low digestible AA content.

Figure 3-3. An image of the wheat distillers' dried grains with solubles used in the current study (above) and a maize distillers' dried grains with solubles colour score chart (below) (Source: Shurson, 2011).

CHAPTER 4

METABOLISABLE ENERGY CONTENT, TRUE PHOSPHORUS DIGESTIBILITY AND ILEAL DIGESTIBILITY OF AMINO ACIDS IN WHEAT DISTILLERS' DRIED GRAINS WITH SOLUBLES WITHOUT OR WITH EXOGENOUS ENZYMES FOR TURKEY

4.1 INTRODUCTION

The nutritional value of wheat Distillers Dried Grains with Solubles (wheat-DDGS) for broilers was determined and reported in Chapter 3 of this thesis. Because wheat-DDGS can also be used for turkey and the metabolisable energy and digestible nutrient values in feedstuffs for turkey and broilers are different, it is also important to determine the nutritional value of wheat-DDGS for turkey. Because the objectives in the current chapter are similar to Chapter 3, the Materials and Methods section is similar to the previous chapter. However, the discussion section in the current chapter detailed the differences between the nutritive value of wheat-DDGS for turkey and broilers.

Bioethanol production from wheat is currently on the increase in the UK and this industry is expected to expand rapidly. In fact, biofuels are expected to replace up to 20% of the total gasoline used in the UK by 2020 and the vast majority of this are expected to be produced from wheat and oilseeds. Bioethanol production from wheat will also result in an increase in the quantity of wheat Distillers Dried Grains with Solubles (wheat-DDGS) available as a feed ingredient for poultry. During bioethanol production, the conversion of starch in the wheat by fermentation increases the concentrations of crude protein (CP), gross energy (GE) and total P in wheat-DDGS approximately 3-fold (Nyachoti *et al*., 2005). It is possible to use wheat-DDGS as a cheaper and alternative source of energy and amino acids (AA) (partially replacing wheat and soybean meal) for poultry, especially where the use of wheat for ethanol production may result in a reduction in the quantity available for use in poultry diets. In view of the potential of using wheat-DDGS in turkey diets, it is essential that accurate nutrient values be assigned to the product.

Exogenous enzymes are capable of ameliorating the anti-nutritive effects of non starch polysaccharides (NSP) and phytate, and hence enhance the digestibility of feed ingredients and reduce nutrient excretion to the environment by poultry (Adeola and Cowieson, 2011; Woyengo and Nyachoti, 2011). Information about the value of exogenous enzymes on energy utilisation, AA and P digestibility of wheat-DDGS for turkey is currently lacking in the literature. Development of nutrient matrix values for exogenous enzymes in wheat-DDGS will help in designing a more accurate diet formulation when using enzymes in diets containing wheat-DDGS.

The overall objective of the current study was to provide data on energy and nutrient values of wheat-DDGS for turkey. Specific objectives were to: 1) determine the apparent metabolisable

energy (AME) and nitrogen-corrected apparent metabolisable energy (AME_n) of wheat-DDGS without or with an admixture of xylanase, amylase and protease (XAP) for turkey using a multiple linear regression method, 2) evaluate the true P digestibility and retention of wheat-DDGS with or without a phytase for turkey and 3) evaluate the ileal AA digestibility (apparent and standardised) of wheat-DDGS supplemented without- or with a protease for turkey. It was hypothesized that wheat-DDGS will be a valuable dietary source of energy, AA and P for turkey.

4.2 MATERIALS AND METHODS

4.2.1 Animals and Management

The Scotland's Rural College Animal Experimentation Committee approved all bird handling and sample collection procedures.

A total of 336 male BUT 10 turkey poults were raised together and offered a pre-experimental diet formulated to meet energy and nutrient requirements (Table 4-1). On d 14, birds were weighed individually and divided into 3 groups of similar bodyweight consisting of 126, 126 or 84 birds for experiment 1, 2 or 3, respectively. In each experiment, birds were allocated to one of the experimental diets in a randomized complete block design using d 14 bodyweight as blocking criterion and transferred to metabolism cages on d 14. Each treatment had seven replicate cages and three birds per replicate cage. Birds were weighed individually on d 14 and at the end of the experimental period (d21 or 28). In experiment 2 and 3, birds were euthanized by cervical dislocation on d 28 to allow collection of ileal digesta samples. Birds were provided *ad libitum* access to the experimental diets and water throughout the pre- and experimental periods. The birds were reared in a house with facilities to control temperature, light, and humidity. Room temperature was maintained at 35^{o}C, 32^{o}C, 27^{o}C and 23^{o}C for day 1 to 7, 8 to 14, 15 to 21 and 22 to 28, respectively. Titanium dioxide (TiO_2) was added to the diets (3 g/kg of diet) as an indigestible marker to enable determination of ME content and P and AA utilisation by the index method.

4.2.2 Diets and Sample Collection

Experiment 1

The chemical composition of the wheat-DDGS used in the current study is presented in Table 4-2.

In experiment 1, the metabolisable energy content of wheat-DDGS for turkey was determined using a total of six diets. Wheat-DDGS was incorporated in a wheat-soybean meal diet at 3 levels (0, 300, or 600 g/kg) without- or with added XAP (0 or 0.25 g/kg). At a rate of 0.25 g/kg, the XAP (Danisco Animal Nutrition, Marlborough, UK) supplied 2000, 200 and 4000 U of xylanase, amylase and protease, respectively per kg of diet. The xylanase was a Endo-1,4-beta-xylanase produced by a *Trichoderma longibrachiatum* and expressed in the same organism. The amylase was produced by *Bacillus amyloliquifaciens* and expressed in *Bacillus subtilis*. The subtilisin (protease) was derived from *Bacillus subtilis*. These 3 enzymes were produced separately and later blended to produce the xylanase-amylase-protease (XAP) admixture. One unit (U) of xylanase was defined as the quantity of the enzyme that liberates one mmol of xylose equivalent per minute. One unit of amylase was defined as the amount of the enzyme catalysing the hydrolysis of one mmol glucosidic linkage per minute and one protease unit was defined as the quantity of the enzyme that solubilised one mg of azo-casein per minute. Energy-yielding ingredients such as wheat, soybean meal (SBM), gluten meal and soy oil were substituted with wheat-DDGS in a way that their ratios were the same across all the experimental diets to allow the use of the regression method. These ratios were 1.43, 6.06, 16.2, 11.3, 4.25, and 0.38 for wheat:SBM, wheat:gluten meal, wheat:soyoil, SBM:soyoil, SBM:gluten meal, and soyoil:gluten meal, respectively. The ingredient and nutrient composition for the experimental diets are presented in Table 4-3. Experimental diets were fed from d 15 to 21. Excreta was collected daily from each cage for 3 days (d 18 to 20), dried and pooled for each cage for the analysis of GE, dry matter (DM), N and Ti to determine AME and AME_n.

Table 4-1. Ingredient and nutrient composition of pre-experimental standard diet.

Ingredients, g/kg	
Maize	538.8
Soybean meal -48%	370
Soybean oil	50
Limestone (38% Ca)	10
Dicalcium phosphate[1]	19
Common salt	3.25
Vitamin/mineral premix[2]	4
DL-Methionine	2.8
L-Lysine HCl	1.6
Threonine	0.6
Calculated component composition	
Protein, g/kg	230
ME, MJ/kg	12.7
Calcium, g/kg	11.5
Total phosphorus, g/kg	6.8
Non-phytate P, g/kg	4.3
Ca:P	1.7
Indispensable amino acids, g/kg	
Arg	14.5
His	5.1
Ile	9.4
Lys	9.4
Met	12.8
Phe	10.0
Thr	2.8
Trp	13.4
Val	5.3

[1]Contain 21.3% Ca and 18.7% P.

[2]Vitamin/mineral premix supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.

Table 4-2. Analysed nutrient composition of wheat Distillers' Dried Grains with Solubles (as-is basis)

Item	g/kg
Dry matter	858
Crude protein	326
Gross energy (MJ/kg)	18.5
Crude fibre	80.0
Ether extract	72.5
NDF	389
ADF	223
Ash	46.0
Calcium	1.10
Phosphorus	6.50
Potassium	11.3
Sodium	5.20
Amino acids	
Ala	14.0
Arg	11.8
Asp	18.3
Cys	5.90
Glu	84.9
Gly	14.9
His	8.30
Ile	13.7
Leu	22.6
Lys	7.70
Met	4.50
Phe	15.8
Pro	30.2
Ser	17.0
Thr	11.5
Tyr	10.2
Trp	3.80
Val	16.2

Experiment 2

A total of six dietary treatments were used to determine the true ileal digestibility and total tract retention of P of wheat-DDGS for turkey in experiment 2. The dietary treatments consisted of 3 levels of wheat-DDGS (200, 400 or 600 g/kg) without- or with added phytase (0 or 1000 FTU/kg). Wheat-DDGS was the only source of P in these diets. The phytase (Danisco Animal Nutrition, Marlborough, UK) was derived from Escherichia *coli* and expressed in Schizosaccharomyces *pombe*. One phytase unit (FTU) was defined as the quantity of enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5 from an excess of 15 µM sodium phytate at 37^{o}C. The ingredient and analysed chemical compositions of the experimental diets are shown in Table 4-4. Experimental diets were fed for 5 days (d 17 to 21). Excreta samples were collected daily for 3 days (d 18 to 20) for the determination of P retention. On d 21, ileal digesta samples were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water. Ileal digesta samples were pooled per cage and stored frozen (-20^{o}C) pending chemical analysis.

Table 4-3. Ingredient and analysed nutrient composition of experimental diets to determine metabolisable energy value of wheat-DDGS for turkey with- or without added xylanase, amylase and protease.

	Level of dietary wheat distillers dried grains with solubles, g/kg					
	Without XAP			With added XAP		
Item	0	300	600	0	300	600
Ingredients, g/kg						
Wheat, White	484.5	328.9	173.5	484.5	328.9	173.5
Soybean meal -48%	340	230.9	121.7	340	230.9	121.7
Soybean oil	30	20.4	10.7	30	20.4	10.7
Gluten meal	68	42.3	16.6	58	32.3	6.6
DDGS	0	300	600	0	300	600
XAP premix[1]	0	0	0	10	10	10
Others[2]	77.5	77.5	77.5	77.5	77.5	77.5
Analysed composition[5]						
Dry matter, g/kg	879	879	879	883	883	874
Gross energy, MJ/kg	16.8	17.0	17.4	16.7	17.1	17.6
CP (N x 6.25), g/kg	24.8	27.5	29.4	25.8	27.7	29.3
Xylanase activity, U/kg	99	106	118	1442	1220	1770
Amylase activity, U/kg	-	-	-	262	262	262
Protease activity, U/kg	543	<100	<100	3064	3064	3064

[1]XAP premix made with gluten meal as carrier; formulated to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease.
[2]Others consists of 13 g/kg of Limestone (38% Ca); 35 g/kg of Dicalcium phosphate (Contain 21.3% Ca and 18.7% P); 3 g/kg of Common salt; 4 g/kg of Vitamin/mineral premix (vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 1.5 g/kg of DL-Methionine; 6 g/kg of L-Lysine HCl; 15 g/kg of marker premix (prepared as 1 g of titanium dioxide added to 4 g of gluten meal).
[3]Values are means of duplicate analyses

Table 4-4. Ingredient and chemical composition of experimental diets to determine P utilisation of wheat-DDGS for turkey.

	Inclusion level of dietary wheat distillers' dried grains with solubles, g/kg					
	Without Phytase			With added Phytase		
Item	200	400	600	200	400	600
Ingredients, g/kg						
Corn starch	526	303.5	87	516	293.5	77
DDGS	200	400	600	200	400	600
Soybean oil	18	36	48	18	36	48
Limestone	4.5	9	13.5	4.5	9	13.5
Others[1]	251.5	251.5	251.5	251.5	251.5	251.5
Phytase premix[2]	0	0	0	10	10	10
Analysed composition						
Dry matter, g/kg	880	890	880	880	890	885
Phosphorus, g/kg	2.3	3.3	4.0	2.0	2.9	4.2
Calcium, g/kg	3.6	5.4	6.3	3.5	4.7	6.9
Copper, mg/kg	10	11	12	10	10	13
Iron, mg/kg	64	81	126	116	106	121
Magnesium, g/kg	0.6	1.0	1.4	0.6	1.2	1.7
Manganese, mg/kg	72	79	99	72	82	96
Potassium, g/kg	2.7	4.4	6.5	2.6	4.2	5.7
Sodium, g/kg	2.6	3.5	4.5	2.5	4.3	5.6
Zinc, mg/kg	62	105	80	64	70	76
Phytase activity, FTU/kg	<50	<50	<50	853	810	933

[1]Others consist of: 100 g/kg of dextrose; 130 g/kg of sucrose; 2.5 g/kg of vitamin/mineral premix (supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 4 g/kg of common salt; 15 g/kg of marker premix (prepared as 1 g Titanium dioxide added to 4 g of gluten meal).

[2]Phytase premix made with cornstarch as carrier; Formulated to supply 1000 FTU/kg.

Experiment 3

Four dietary treatments were used in experiment 3. The diets were 2 nitrogen-free diets (without- or with protease) and 2 semi-purified diets in which wheat-DDGS was the only source of AA (without- or with protease). Protease was added at a rate of 4000 units per kg of diet. Standardised ileal amino acid digestibility (SIAAD) were obtained by correction of apparent ileal amino acid digestibility values (AIAAD) with basal ileal endogenous AA flow determined from birds fed a nitrogen free diet (NFD) without- or with protease. The protease was supplied by Danisco Animal Nutrition, Marlborough, UK. One protease unit was defined as the quantity of the enzyme that solubilises one mg of azo-casein per minute. The assay diets contained at the least 24% CP and were balanced for minerals and vitamins to meet breeder nutrient specifications (Table 4-5). Because of health and welfare issues associated with feeding birds NFD, experimental diets were fed for three days in experiment 3. A 3-d feeding period is optimal to fulfil the objectives of the experiment (Kluth and Rudehurscord, 2010). Table 4-6 shows the analysed CP and AA compositions of the experimental diets. On d 28, birds were euthanized by cervical dislocation and ileal digesta samples were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water. Digesta samples were pooled for each cage and stored frozen (-20 ^{o}C) prior to chemical analysis.

Table 4-5. Ingredient composition of experimental diets to determine ileal amino acids digestibility of wheat-DDGS for turkey.

	Without protease		With protease	
Item	NFD[1]	W-DDGS[2]	NFD[1]	W-DDGS[2]
Ingredients, g/kg				
DDGS	0	743	0	743
Corn starch	451	10	441	0
Dextrose	200	132	200	132
Vitacell[3]	200	0	200	0
Soybean oil	50	50	50	50
Vitamin-mineral premix[4]	5	5	5	5
Dicalcium phosphate[5]	31	31	31	31
$NaHCO_3$	20	0	20	0
KCl	12	0	12	0
MgO	2	0	2	0
Choline chloride	3	3	3	3
Limestone (38% Ca)	9	9	9	9
Salt	2	2	2	2
Marker premix[6]	15	15	15	15
Protease premix[7]	0	0	10	10

[1]N-free diet
[2]Wheat distillers dried grains with solubles
[3]Vitacell: Purified cellulose
[4]Vitamin/mineral premix supply per kilogram of diet: vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.
[5]Contain 21.3% Ca and 18.7% P.
[6]Prepared as 1 g titanium dioxide oxide added to 4 g of cornstarch.
[7]Protease (4000 U/kg of diet) premix made with cornstarch as carrier.

Table 4-6. Analysed chemical composition of treatment diets to determine ileal amino acids digestibility of wheat-DDGS for turkey.

	Without protease		With protease	
Item	NFD[1]	W-DDGS[2]	NFD[1]	W-DDGS[2]
Dry matter, g/kg	868.5	851.2	869.3	860.5
Protease activity, U/kg	<100	<100	3418	3291
ME, MJ/kg (calculated)	12	11.9	12	11.9
Indispensable amino acids, g/kg				
Arg	0.9	9.1	0.9	9.8
His	1.1	5.2	1.2	5.6
Ile	0.7	9.1	0.8	9.7
Leu	2.3	16.8	2.4	17.6
Lys	0.8	4.9	0.8	5.4
Phe	1.4	11.2	1.8	12.3
Thr	1	7.1	1.1	7.9
Met	0.5	3.5	0.6	3.8
Val	1.3	11.6	1.4	12.1
Dispensable amino acids, g/kg				
Ala	1.7	9	1.4	10
Cys	0.2	2.9	0.2	3.2
Glu	3.6	62.4	4.1	66
Gly	1.7	9.9	1.2	11.2
Pro	2.2	30.8	2.4	23.6
Ser	2.2	10	2	11.2
Tyr	1.5	8	1.7	8.5
Asp	1.6	12.1	1.4	13.1

[1]N-free diet
[2]Wheat distillers dried grains with solubles

4.2.3 Chemical Analysis

Where required, diets, wheat-DDGS, ileal digesta and excreta samples were analysed for GE, DM, Ti, N, AA and P. Except for the ileal digesta samples used for AA analysis that were lyophilized, all other samples were oven dried and ground to pass through a 0.5 mm screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis. For DM determination, samples were dried at 105 ^{o}C for 24 hours in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England. UK) (AOAC International 2006, method 934.01). Gross energy was determined in an adiabatic oxygen bomb calorimeter using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Nitrogen was determined by combustion method (AOAC International 2006, method 968.06). For AA analysis, samples were hydrolysed for 24 hours in 6 N hydrochloric acid at 110 ^{o}C under an atmosphere of N. For Met and Cys, performic acid oxidation was carried out before acid hydrolysis. The AA in the hydrolysate were determined by HPLC after post-column derivatization [(AOAC International 2000, method 982.30E (a, b, c)]. Analysis for Ti was performed as described by Short *et al*. (1996). Mineral concentrations in the samples were determined using inductively coupled plasma spectrophotometry according to the procedures of Olsen and Sommers (1982). Xylanase activity in diets was measured using a kit (Megazyme International Ireland Ltd., Bray, Ireland) based on the method by McCleary (1991). Amylase activity in feed was measured using Phadebas (Megazyme International Ireland Ltd.) tablets according to the method described by McCleary and Sheehan (1989). Protease activity was analysed using the modified method of Lynn and Clevette-Radford (1984) with azocasein used as substrate. Phytase activity in the diets was analysed using the AOAC official method (2000.12, AOAC, 2000).

4.2.4 Calculations and Statistical Analysis

All statistical analyses were performed using GenStat program (VSN International, 2011). Statistical significance was set at $P < 0.05$ and tendency at $0.05 < P < 0.1$ for all mean comparisons.

Experiment 1

Energy utilisation coefficient was calculated using the following equation:

1. $\mathrm{MEc} = [1 - \left(\frac{\mathrm{Ti}}{\mathrm{To}}\right) \mathrm{x} \left(\frac{\mathrm{Eo}}{\mathrm{Ei}}\right)]$

where MEc is energy utilisation coefficient, T_i is the concentration of titanium in diet (mg/kg), T_o is the concentration of titanium in excreta (mg/kg), E_o and E_i are the GE in excreta and diet, respectively (MJ/kg).

Apparent metabolisable energy was calculated using the following equation:

2. $\text{AME} = \text{MEc x GEdiet}$

where AME is apparent metabolisable energy (MJ/kg), ME_c is the energy utilisation coefficient and GE_{diet} is the GE (MJ/kg) in the diet.

Nitrogen-corrected AME was calculated using the following equation:

3. $\text{AMEn} = \text{AME} - (8.73 \text{ x N gain})$

where AME_n is nitrogen-corrected apparent metabolisable energy (MJ/kg), N gain is nitrogen retained (g/kg of DM intake) and 8.73 is the caloric correction factor for retained nitrogen (Titus, 1956).

Nitrogen gain was calculated using the following equation:

4. $\text{N gain} = \text{Ndiet} - (\text{Nexcreta x } \left(\frac{\text{Ti}}{\text{To}}\right))$

where N_{diet} and $N_{excreta}$ are the nitrogen in diet and excreta, respectively (g/kg of DM), T_i and T_o are the concentration of titanium (mg/kg) in the diet and excreta, respectively.

Wheat-DDGS-associated AME intake was calculated as illustrated previously by Adeola *et al*. (2010) using the following equations:

If the coefficients of AME for the assay diet, basal diet and test ingredient (wheat-DDGS) are represented by Cad, Cbd and Cti, respectively. Assuming additivity in diet formulation, the proportional contribution of energy by the basal (Pbd) and test ingredients (Pti) to the assay diet will be equal to 1. Mathematically; Pbd + Pti = 1 or Pbd = 1 – Pti.

Therefore;

5. $\text{Cad} = (\text{Cbd x Pbd}) + (\text{Cti x Pti})$

By solving for Cti,

6. $\text{Cti} = [\text{Cad} - (\text{Cbd x Pbd})]/\text{Pti}$

Substituting 1 – Pti for Pbd;

7. $\mathrm{Cti} = \{\mathrm{Cbd} + \left[\frac{\mathrm{Cad}-\mathrm{Cbd}}{\mathrm{Pti}}\right]\}$

The product of Cti at each level of wheat-DDGS substitution rate (300 or 600 g/kg), the GE of wheat-DDGS, and wheat-DDGS intake in kg is the wheat-DDGS-associated AME intake in MJ.

Energy utilisation data were analysed as a randomised complete block design of 3 levels of wheat-DDGS (0, 300 and 600 g/kg) and 2 levels of enzyme supplementation (not added or added). In the 7 blocks, each consisting of 3 cages containing one of 0, 300, or 600g of wheat-DDGS per kg of diet without or with added XAP, AME or AME_n intake (MJ) was regressed against wheat-DDGS intake (kg) for each block to generate intercepts and slopes for each of the 7 blocks per XAP (not added or added). The intercept and slope data were analysed as a one-way analysis of variance in a completely randomised design using intercept or slope as the dependent variable and XAP (not added or added) as the independent variable. The additional energy provided by the XAP was determined using ANOVA procedures as the difference between the slopes of dietary treatments without and those with supplemental XAP. Orthogonal contrast was used to determine the differences in metabolisable energy between the dietary treatments with different inclusion levels of wheat-DDGS and those without or with added XAP.

Experiment 2

Apparent ileal P digestibility or apparent P retention was calculated using the following equation:

1. $\mathrm{APD/APR} = \left[1 - \left(\frac{\mathrm{Ti}}{\mathrm{To}}\right) \mathrm{x} \left(\frac{\mathrm{Po}}{\mathrm{Pi}}\right)\right] \mathrm{x}\ 100$

where APD/APR is apparent P digestibility (%) or apparent P retention (%); T_i and T_o are the concentrations (mg/kg) of titanium in diet and ileal digesta or excreta, respectively. P_o is the phosphorus in the ileal digesta or excreta (g/kg of DM output) and P_i is the phosphorus in the diet (g/kg of DM).

Mineral flow at the ileum or total tract was calculated using the following equation:

2. $\mathrm{MO\text{-}dmi} = \mathrm{MO\text{-}dmo}\ \mathrm{x}\ (\frac{\mathrm{Ti}}{\mathrm{To}})$

where MO-dmi and MO-dmo are mineral output (ileal or total tract) on DM intake and DM output basis, respectively (mg/kg); T_i and T_o are the concentrations of titanium (mg/kg) in the diet and digesta or excreta, respectively.

True P digestibility or retention was determined from regressing P output (ileal or total tract) against dietary P intake per block of 3 treatments within each block (one block without-, the other with added phytase) using the following model;

3. $\text{PO-dmi} = (\text{TPI x Pi}) + \text{EPL}$

where PO-dmi is phosphorus output (mg/kg) on DM intake basis (dependent variable); TPI is the slope of the model or true P indigestibility; P_i is the phosphorus in the diet (g/kg of DM intake) (independent variable) and EPL is the intercept of the model or mean endogenous phosphorus loss (DM intake basis).

True P digestibility or retention was calculated from the measure of P indigestibility using the following equation:

4. $\text{TPD/TPR} = 100 - (\text{TPI x } 100)$

where TPD or TPR are true P digestibility or true P retention and TPI is true P indigestibility (%), respectively.

Experiment 3

Basal endogenous ileal AA flow was calculated using the following equation:

8. $\text{EAAF} = [\text{AAo x } \left(\frac{\text{Ti}}{\text{To}}\right)]$

where EAAF is endogenous ileal AA flow (mg/kg of DM intake); AAo is the AA in ileal digesta (mg/kg of DM); T_i and T_o are the concentrations of titanium (mg/kg) in diet and ileal digesta, respectively.

Apparent ileal AA digestibility was calculated using the following equation:

$$\text{AIAAD} = \left[1 - \left(\frac{\text{Ti}}{\text{To}}\right) \text{x } \left(\frac{\text{AAo}}{\text{AAi}}\right)\right] \text{x } 100$$

where AIAAD is apparent ileal amino acid digestibility (%); T_i and T_o are the concentrations (mg/kg) of titanium in diet and ileal digesta, respectively; AA_o is the amino acid in the digesta (g/kg of DM) and AA_i is the amino acid in the diet (g/kg of DM).

Standardised ileal AA digestibility was calculated using the following equation:

9. $\text{SIAAD} = \text{AIAAD} + [\left(\frac{\text{EAAF}}{\text{AAi}}\right) \text{x } 100]$

where SIAAD is standardized ileal AA digestibility (%); AIAAD is apparent ileal AA digestibility (%); EAAF is the endogenous basal ileal AA flow (g/kg of DM intake) and AA_i is the amino acid content of the diet (g/kg of DM).

Data for the AIAAD and SIAAD without- or with supplemental protease were subjected to a one-way analysis of variance to determine differences.

4.3 RESULTS

4.3.1 Metabolisable energy content of wheat Distillers Dried Grains with Solubles without- or with an Admixture of Xylanase, Amylase and Protease for Turkey

The wheat-DDGS used in the current study contained by analysis 18.5 MJ/kg of GE, 326 g/kg of CP, 6.5 g/kg of P, 80 g/kg of crude fibre and 858 g/kg of DM (Table 4-2). The ingredient and analysed nutrient composition of the experimental diets are presented in Table 4-3. The average xylanase activity in the diets containing 0, 300 or 600 g/kg of wheat-DDGS with supplemental XAP was1477 U/kg and this value is lower than the formulated value of 2000 U/kg. On the average, amylase and protease activity in these diets were 262 and 3064 U/kg, respectively compared to the formulated value of 200 and 4000 U/kg, respectively. Enzyme activities in the experimental diets not supplemented with XAP were generally low.

Table 4-7 shows the growth performance responses for turkey fed graded levels of wheat-DDGS without- or with supplemental XAP. There were quadratic decreases ($P < 0.05$) in body weight gain (BWG) and gain:feed (G:F) and a linear decrease ($P < 0.05$) in final body weight (FBW) as the level of wheat-DDGS increased from 0 to 600 g/kg in the diet. Supplemental XAP did not improve the growth performance responses of the turkey. Dry matter and energy utilisation for turkey fed graded levels of wheat-DDGS without- or with XAP is presented in Table 4-8. Increasing the dietary inclusion of wheat-DDGS from 0 to 600 g/kg of the diet decreased linearly ($P < 0.05$) DM and energy retention, irrespective of XAP. There were wheat-DDGS × XAP interactions ($P < 0.05$) for dietary AME and $AME_{n.}$ For the dietary treatments without supplemental XAP, increasing the level of wheat-DDGS in the reference diet decreased linearly ($P < 0.05$) the AME and $AME_{n.}$ On the other hand, there was no effect of increasing wheat-DDGS level on dietary AME or AME_n in the treatments supplemented with XAP. For the diets that were not supplemented with XAP, inclusion of 300 g/kg of wheat-DDGS in the reference diet decreased ($P < 0.001$) the AME by 0.49 MJ/kg. Corresponding decrease ($P < 0.001$) in AME_n was 0.60 MJ/kg. Further, the decrease ($P < 0.001$) in dietary AME was 1.76 MJ/kg when the inclusion level of wheat-DDGS was

increased to 600 g/kg. Corresponding decrease ($P < 0.001$) in AME_n was 1.81 MJ/kg. Supplemental XAP did not improve either the AME or AME_n of the diet for turkey.

The AME and AME_n values of wheat-DDGS without- or with added XAP determined from the regression of wheat-DDGS-associated energy intake (MJ) against wheat-DDGS intake (kg) is presented in Table 4-9. The line of the regression equations for AME and AME_n content of wheat-DDGS are shown in Figure 4-1. From the slopes of the linear regression equations, the AME values (MJ/kg DM) of wheat-DDGS without- and with supplemental XAP were determined to be 14 and 14.9, respectively. Corresponding AME_n values (MJ/kg DM) were 13 and 13.8, respectively. Numerical increases in AME and AME_n (MJ/kg DM) values of wheat-DDGS with supplemental XAP were 0.85 and 0.77, respectively.

Table 4-7. Growth performance responses of turkey fed graded levels of wheat-DDGS without or with an admixture of xylanase, amylase and protease[1]

Measurement	Gain, g/bird	FI, g/bird	G:F, g:kg	Initial weight,g	Final weight, g
Diet effect					
0 g/kg of diet (A)	246	402	693	257	503
300 g/kg of diet (B)	231	392	669	257	488
600 g/kg of diet (C)	176	352	568	256	432
Pooled s.e.d	11.2	16.8	11.6	-	22.2
P values for main effects of DDGS inclusion	<0.001	0.014	<0.001	-	0.006
Enzyme effect					
Without XAP	219	381	651	255	474
With XAP	216	383	636	258	474
s.e.d	9.13	13.8	9.45	-	18.1
P values for main effects of XAP supplementation	0.708	0.328	0.111	-	0.997
DDGS × XAP interaction	0.864	0.871	0.508	-	0.906
P values for contrasts					
Diet (linear)	<0.001	0.006	<0.001	-	0.003
Diet (quadratic)	0.038	0.328	<0.001	-	0.278
A vs. B	0.211	0.545	0.045	-	0.518
A vs. C	<0.001	0.006	<0.001	-	0.003

[1]Data are means of 7 replicate cages; Experimental diets fed from d 15 to 21 posthatch.
[2]Enzyme admixture added to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease

Table 4-8. Dry matter and energy utilisation for turkey fed diets containing graded levels of wheat-DDGS without or with an admixture of xylanase, amylase and protease[1,2]

Measurement	DM retention, %	Energy retention, %	AME, MJ/kg	AME_n, MJ/kg
Diets				
Without added XAP				
0 g/kg of diet (A)	67.3	72.1	13.8	12.9
300 g/kg of diet (B)	63.7	68.6	13.3	12.3
600 g/kg of diet (C)	54.3	60.7	12.0	11.1
With added XAP				
0 g/kg of diet (A)	64.4	69.1	13.1	12.2
300 g/kg of diet (B)	62.9	67.7	13.1	12.1
600 g/kg of diet (C)	56.7	62.9	12.6	11.7
Pooled s.e.d	1.39	1.29	0.261	0.234
P values for main effects and interaction				
DDGS inclusion	<0.001	<0.001	<0.001	<0.001
XAP supplementation	0.699	0.596	0.681	0.622
DDGS × XAP interaction	0.17	0.125	0.038	0.015
P values for contrasts				
Without added XAP				
Diet (linear)	<0.001	<0.001	<0.001	<0.001
Diet (quadratic)	0.056	0.125	0.163	0.203
With added XAP				
Diet (linear)	0.002	0.005	0.234	0.153
Diet (quadratic)	0.216	0.337	0.534	0.571
A vs. B	0.079	0.063	0.321	0.149
A vs. C	<0.001	<0.001	<0.001	<0.001

[1]Data are means of 7 replicate cages; Experimental diets fed from d 15 to 21 posthatch.
[2]Enzyme admixture added to supply 2000 U/kg of xylanase, 200 U/kg of amylase and 4000 U/kg of protease
s.e.d - standard error of difference

Table 4-9. Linear terms for the metabolisable energy value of wheat-DDGS without or with added admixture of xylanase, amylase and protease for turkey[1,2]

Measurements	Regression equation	s.e.d intercept	s.e.d slope	s.e.d model	r^2	P-value
AME, MJ/kg						
No added XAP	Y = 14X + 0.201	0.159	0.382	0.452	0.985	<0.001
Added XAP[3]	Y = 14.9X + 0.034	0.138	0.323	0.401	0.991	<0.001
AME_n, MJ/kg						
No added XAP	Y = 13X + 0.184	0.143	0.342	0.406	0.986	<0.001
Added XAP[3]	Y = 13.8X + 0.04	0.122	0.285	0.354	0.992	<0.001

[1]AME and AME_n values of wheat-DDGS determined from regressing wheat-DDGS-associated AME or AME_n against wheat-DDGS intake; Y is in MJ, intercept is in MJ, and slope is in MJ/kg of DM.
[2]Addition of XAP did not improve ($P > 0.05$) the AME or AME_n values of the wheat-DDGS for turkey
[3]Enzyme admixture added to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease
s.e.d - standard error of difference

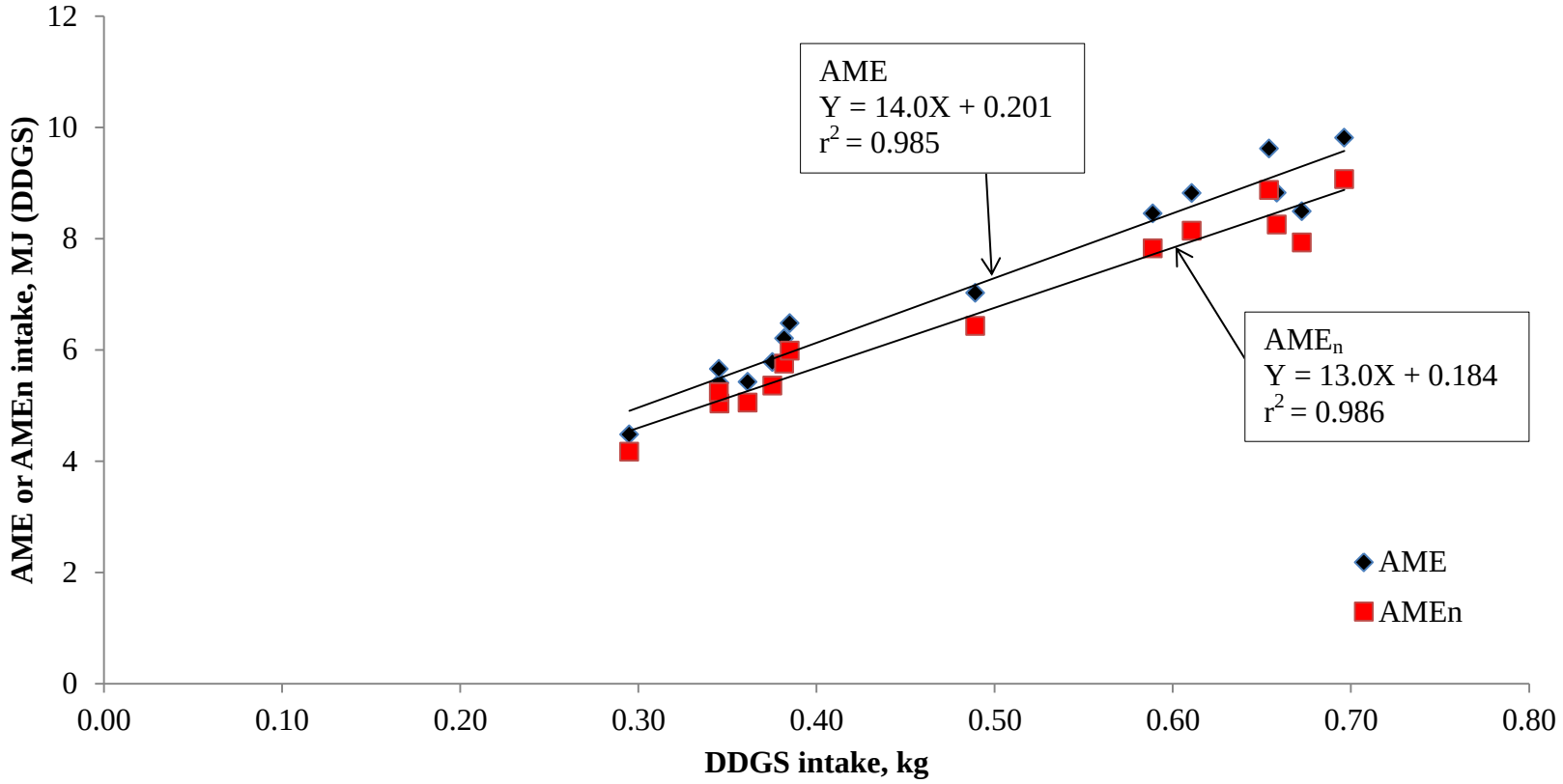


Figure 4-1. Regression line showing the AME or AMEn value of wheat-DDGS for turkey

4.3.2 True Phosphorus Digestibility or Retention of Wheat Distillers Dried Grains with Solubles without- or with Supplemental Phytase for Turkey

The analysed nutrient composition and phytase activity of the dietary treatments are presented in Table 4-4. Analysed phytase activity in the dietary treatments supplemented with phytase was 853, 810 or 933 FTU/kg for diets containing 200, 400 or 600 g/kg of wheat-DDGS, respectively. The phytase activity noted in these diets is lower than the expected value of 1000 FTU/kg. Phytase activity was less than 50 FTU/kg in the dietary treatments without supplemental phytase. Dry matter utilisation and P digestibility or retention for birds fed graded levels of wheat-DDGS without- or with supplemental phytase are presented in Table 4-10. Increasing the dietary inclusion level of wheat-DDGS decreased linearly ($P < 0.01$) DM intake, ileal DM digestibility and DM retention. Increasing the inclusion level of wheat-DDGS from 200 to 600 g/kg of the diet did not affect apparent ileal P digestibility or apparent P retention.

True P digestibility and retention (%) of wheat-DDGS for turkey without or with supplemental phytase is presented in Table 4-11. From the regression of P output (mg/kg of DMI) at the ileal level against dietary P intake (mg/kg of DM), the digestibility of wheat-DDGS without- or with supplemental phytase was 75.8 and 82.1%, respectively. Respective values at the total tract level were 70.7 and 81.6%. True P digestibility and retention was not different between the treatments without- and those with phytase. The regression lines showing the TPI of wheat-DDGS at the ileal and total tract level for turkey are shown in Figure 4-2. The true digestible P and true retainable P contents of the wheat-DDGS were calculated as the coefficient of TPD or TPR multiplied by the analysed P composition (%) of the wheat-DDGS. The true digestible P (%) in the wheat-DDGS for turkey without- or with added phytase was 0.49 or 0.53, respectively. Corresponding values for true retainable P (%) were 0.46 or 0.53, respectively. Flow of minerals at the ileal level is presented in Table 4-12 and those at the total tract in Table 4-13. With the exception of Zn at the total tract level, increasing the level of wheat-DDGS in the diet increased linearly ($P < 0.05$) the flow of all minerals at either the ileal or total tract level regardless of phytase supplementation. Phytase supplementation did not have an effect ($P > 0.05$) on mineral flow at either the ileal or total tract.

Table 4-10. Dry matter and dietary P utilisation for turkey fed graded levels of wheat-distillers dried grains with solubles.

Measurement	DM intake, g per chick	Ileal DM digestibility, %	DM retention, %	Apparent ileal P digestibility, %	Apparent P retention, %
Diet effect					
0 g/kg of diet (A)	125	74.5	75.4	44.5	19.1
300 g/kg of diet (B)	115	60.9	67.6	38.0	24.8
600 g/kg of diet (C)	104	51.2	60.8	35.0	19.9
s.e.d	5.51	2.36	1.28	5.14	4.77
P values for main effects of DDGS inclusion	0.002	<0.001	<0.001	0.181	0.442
Enzyme effect					
Without phytase	114	60.7	67.1	35.3	19.0
With phytase	115	63.6	68.8	42.9	23.5
s.e.d	4.5	1.92	1.05	4.19	3.89
P values for main effects of phytase supplementation	0.764	0.137	0.104	0.078	0.257
DDGS × Phytase interaction	0.692	0.865	0.917	0.346	0.474
P values for contrasts					
Diet (linear)	<0.001	<0.001	<0.001	0.072	0.860
Diet (quadratic)	0.978	0.344	0.672	0.697	0.209
A vs. B	0.067	<0.001	<0.001	0.206	0.257
A vs. C	<0.001	<0.001	<0.001	0.064	0.922

[1]Data are means of 7 replicate cages; Dietary treatments fed for five days.
s.e.d - standard error of difference

Table 4-11. True P digestibility or retention determined from regressing ileal or total tract P output (mg/kg of DM intake) against dietary P intake (mg/kg of DM) for turkey fed wheat-DDGS supplemented with or without phytase.

	Regression equation[1]	r^2	SE slope[2]	SE intercept[2]	Endogenous P loss, mg/kg of DMI	TPD/ TPR[3], %	TDP/TRP of wheat-DDGS[4], %	P-value
Ileal								
Without phytase	Y = 0.242X - 430	0.65	0.039	436	430	75.8	0.49	<0.001
With phytase	Y = 0.179X - 98	0.422	0.047	512	98	82.1	0.53	<0.001
Total tract								
Without phytase	Y = 0.294X - 293	0.612	0.056	570	293	70.7	0.46	<0.001
With phytase	Y = 0.184X + 451	0.375	0.054	594	451	81.6	0.53	<0.001

[1]Ileal or excreta P output (mg/kg of DM intake) regressed against dietary P intake (mg/kg of DM). The intercept of the regression term represents the endogenous P loss (mg/kg of DMI) whereas the slope represents the true P indigestibility.
[2]Standard error of regression components for 42 observations
[3]TPD or TPR is true P digestibility or retention; calculated as 100 x (1 - true P indigestibility); True P digestibility and retention were not improved by phytase
[4]TDP and TRP are true digestible P and true retainable P contents of wheat-DDGS, respectively. Calculated as (true P digestibility or retention (%) /100) multiplied by analysed P composition of wheat-DDGS (%).

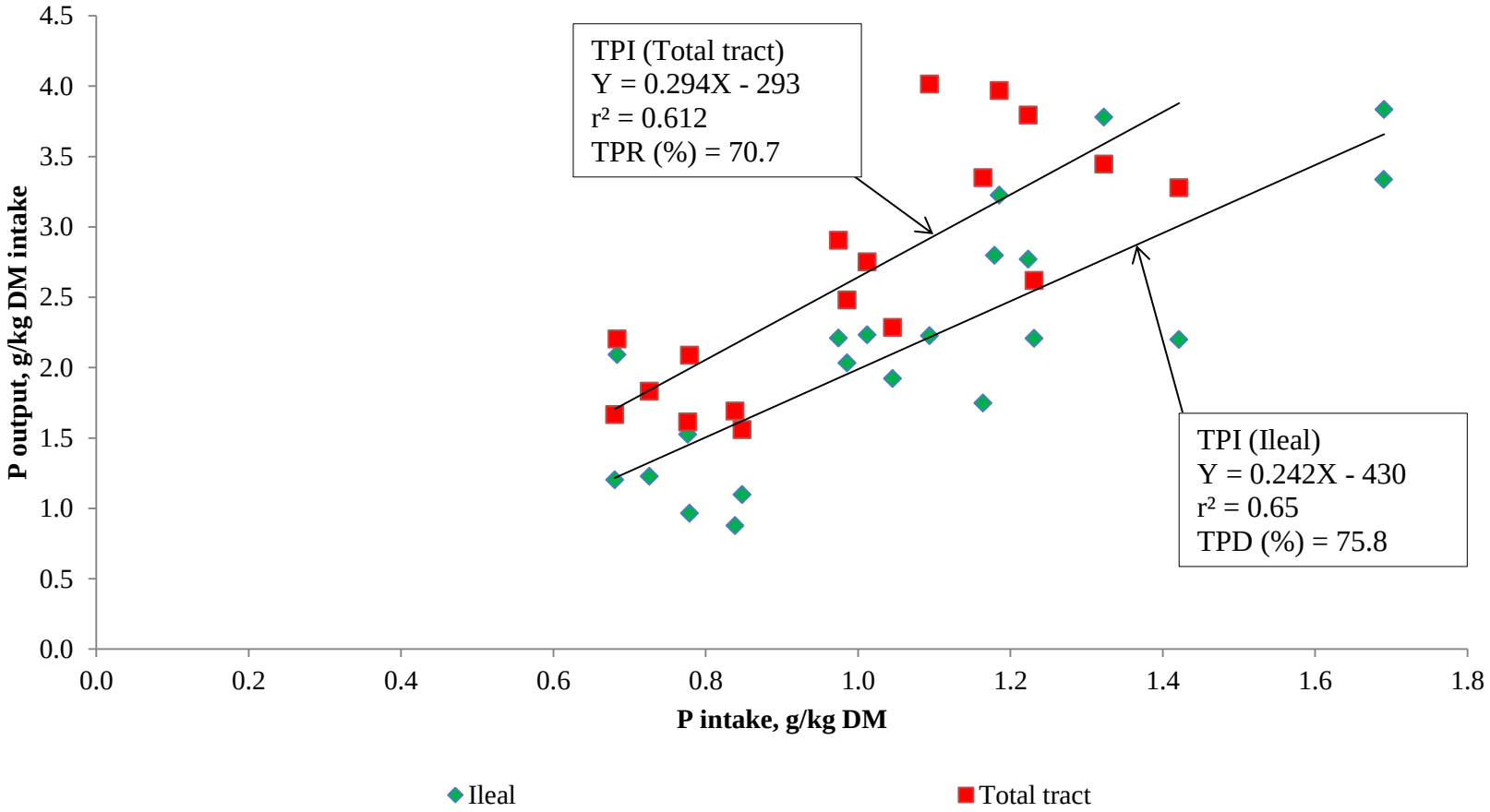


Figure 4-2. True phosphorus indigestibility (TPI) of wheat-DDGS at the ileal and total tract level for turkey. True P digestibility (TPD) or true P retention (TPR) calculated as 100 - (TPI × 100)).

4.3.3 Apparent- and Standardised Ileal Amino Acids Digestibility of Wheat-Distillers Dried Grains with Solubles without- or with Supplemental Protease for Turkey

The analysed chemical compositions and protease activity for the 4 experimental diets used in the current study are presented in Table 4-6. On the average, analysed protease activity in the diets supplemented with protease was 3350 U/kg however this value is lower than the formulated value of 4000 U/kg.

The AIAAD and SIAAD of wheat-DDGS without- or with supplemental protease for turkey are presented in Table 4-14. Irrespective of protease addition, the lowest and highest AIAAD and SIAAD values were observed for Lys and Pro, respectively. The apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of Lys in the wheat-DDGS for turkey was zero and that of Asp was the second lowest. The AIAAD of wheat-DDGS for turkey was lower than 50% for all AA except for Glu (70%) and Pro (81%) without protease supplementation. On the other hand, the range was from 35% (Thr) to 80% (Pro) in the diets supplemented with protease. Of the indispensable AA, the highest and lowest AID was noted for Phe (47%) and Thr (19%), respectively.

Standardised ileal amino acid digestibility ranged from 41% (Thr) to 89% (Pro) in the diets without added protease whereas the range was from 56% (Arg) to 88% (Pro) with protease addition. Except for Cys and Pro, supplemental protease tended to improve ($P < 0.10$) the AID and SID of Arg and Leu and improved ($P < 0.05$) the AID and SID of all other AA. On the average, protease increased the AID or SID of all AA in the wheat-DDGS by 10.5 percentage units.

Table 4-12. Flow of minerals at the ileal level (mg/kg of DM intake) for turkey fed graded levels of wheat-DDGS without or with supplemental phytase[1].

Measurement	Cu	Fe	Mg	Mn	K	Na	Zn
Diet effect							
0 g/kg of diet (A)	7.67	57.9	527	65.2	2181	2524	90.8
300 g/kg of diet (B)	9.55	109	846	97.6	3466	3723	94.7
600 g/kg of diet (C)	14.8	163	1277	118.3	4342	5284	135.2
s.e.d	2.19	17.9	128	15.4	597	500	16.0
P values for main effects of DDGS inclusion	0.007	<0.001	<0.001	0.005	0.003	<0.001	0.015
Enzyme effect							
Without XAP	10.1	110	942	89.8	3440	4054	105
With XAP	11.3	110	825	97.6	3219	3634	109
s.e.d	1.79	14.6	105	12.6	487	408	16.0
P values for main effects of phytase supplementation	0.513	0.988	<0.001	0.537	0.652	0.31	0.777
DDGS × Phytase interaction	0.224	0.172	0.834	0.888	0.88	0.957	0.398
P values for contrasts							
Diet (linear)	0.002	<0.001	<0.001	0.001	<0.001	<0.001	0.009
Diet (quadratic)	0.383	0.93	0.617	0.665	0.694	0.678	0.196
A vs. B	0.392	0.006	0.018	0.04	0.036	0.021	0.804
A vs. C	0.002	<0.001	<0.001	0.001	<0.001	<0.001	0.008

[1]Mineral flow calculated as mineral output at the ileal level multiplied by the ratio of marker (titanium intake/output)
s.e.d - standard error of difference

Table 4-13. Flow of minerals at the total tract (mg/kg of DM intake) for turkey fed graded levels of wheat-DDGS without or with supplemental phytase[1].

Measurement	Cu	Fe	Mg	Mn	K	Na	Zn
Diet effect							
0 g/kg of diet (A)	8.45	83.1	0.07	91.3	0.35	0.18	127.8
300 g/kg of diet (B)	10.56	111.6	0.11	100	0.54	0.29	123.6
600 g/kg of diet (C)	11.34	135.2	0.13	106.7	0.71	0.40	136.3
s.e.d	0.58	8.88	0.005	5.08	0.03	0.02	10.16
P values for main effects of DDGS inclusion	<0.001	<0.001	<0.001	0.016	<0.001	<0.001	0.454
Enzyme effect							
Without XAP	9.97	110.7	0.105	99.6	0.55	0.29	129.9
With XAP	10.27	109.2	0.100	99.1	0.52	0.29	128.6
s.e.d	0.47	7.25	0.004	4.15	0.02	0.01	8.3
P values for main effects of phytase supplementation	0.525	0.844	0.198	0.916	0.332	0.527	0.879
DDGS × Phytase interaction	0.086	0.636	0.313	0.594	0.801	0.533	0.471
P values for contrasts							
Diet (linear)	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.411
Diet (quadratic)	0.187	0.754	0.211	0.813	0.737	0.681	0.344
A vs. B	<0.001	0.003	<0.001	0.094	<0.001	<0.001	0.681
A vs. C	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.411

[1]Mineral flow calculated as mineral output at the total tract multiplied by the ratio of marker (titanium intake/output)
s.e.d - standard error of difference

Table 4-14. Apparent- and standardised ileal amino acids digestibility of wheat-DDGS without or with supplemental protease for turkey[1]

	Apparent, %			Standardised, %			Protease effect[3]	
Item	No protease	With protease[2]	s.e.d	No protease	With protease[2]	s.e.d	Apparent	Standardised
Indispensable amino acids								
Arg	30.0	39.7	4.41	45.8	55.5	4.41	0.055	0.055
His	33.1	44.4	4.67	55.0	66.3	4.67	0.039	0.039
Ile	35.0	45.9	4.17	50.4	61.3	4.17	0.028	0.028
Leu	40.5	48.9	3.97	55.2	63.7	3.97	0.062	0.062
Lys	-44.1	-15.5	8.94	-0.1	0.2	8.94	0.011	0.011
Phe	47.1	56.7	3.32	61.6	71.2	3.32	0.018	0.018
Thr	18.5	35.4	4.75	41.0	57.8	4.75	0.006	0.006
Met	24.4	41.2	4.97	46.5	63.3	5.00	0.008	0.008
Val	33.4	42.6	3.97	50.9	60.0	3.97	0.047	0.047
Dispensable amino acids								
Ala	23.6	40.5	4.12	44.0	60.9	4.12	0.003	0.003
Cys	31.3	43.5	6.94	45.0	56.8	6.94	0.112	0.112
Glu	69.9	74.8	1.88	77.0	81.9	1.89	0.029	0.029
Gly	32.1	47.8	4.32	52.5	68.1	4.33	0.006	0.006
Pro	80.7	80.0	2.01	88.9	88.1	2.01	0.713	0.713
Ser	33.9	50.3	5.24	58.4	74.8	5.24	0.012	0.012
Tyr	40.3	51.3	4.82	61.2	72.2	4.82	0.049	0.049
Asp	3.60	22.4	5.67	26.1	45.0	5.67	0.009	0.009

[1]Data are means of 7 replicates
[2]Protease added to supply 4000 U/kg
[3]*P* values for comparison between diets without- and with protease
s.e.d - standard error of difference

4.4 DISCUSSION

Metabolisable energy content of wheat-DDGS without- or with an admixture of xylanase, amylase and protease for turkey

Wheat and maize are the most popular sources of dietary energy for turkey; however it is possible to replace some of these ingredients with readily available and low cost alternatives. More often than not, the prospects of using alternative dietary energy sources are hampered by a lack of information about their ME contents. One of such dietary energy sources is wheat-DDGS. The current study therefore determined the AME and AME_n value of wheat-DDGS for turkey as well as quantified the improvements to the energy value of wheat-DDGS by supplementation of a combination of exogenous xylanase, amylase and protease enzymes. The hypotheses were; 1) wheat-DDGS is a good source of dietary energy for turkey and 2) XAP supplementation will increase the energy value for turkey.

The chemical characteristic of the wheat-DDGS used in the current study is close to those reported in the study of Bolarinwa and Adeola (2012). There is wide variability in the chemical characteristics of wheat-DDGS judging by the variability in the chemical compositions of 10 wheat-DDGS samples reported in the study of Cozannet *et al*. (2010a) and those for 11 sources of wheat-DDGS from bioethanol plants from the USA and Europe reported in Chapter 2 in this thesis. For example, the neutral detergent fibre and acid detergent fibre contents in the wheat-DDGS used in the current study are greater that those used in the study of Nyachoti *et al*. (2005). Factors such as differences in the chemical composition of the grain, processing method and efficiency, temperature and duration at drying, as well as the amount of condensed solubles added back to distillers grains have been implicated to cause variability to the chemical characteristics of wheat-DDGS among sources (Liu, 2011). Furthermore, it is noteworthy that fibre degrading enzymes are often used during bioethanol production to improve ethanol throughput, nonetheless, the concentrations of non starch polysaccharides (NSP) in the wheat-DDGS have been reported to increase 3-fold compared with wheat (Widyaratne and Zijlstra, 2007).

There was a 15 and 13% reduction in dietary DM and energy utilisation, respectively as the level of wheat-DDGS increased from 0 to 60% in the reference diet. The reduction in dietary energy utilisation as the level of wheat-DDGS increased to 60% in the current study is similar to what was observed with broilers (Chapter 3). Water-soluble NSP, as may be in wheat-DDGS, exert their anti-nutritive properties by their high affinity to water and formation of a

gel medium. The formation of the gel medium causes an increase in digesta viscosity, slower rate of digesta transit in the gastrointestinal tract and also a reduction in nutrient absorption by encapsulation of other nutrients and enzymes within (Choct *et al*., 2004; Adeola and Cowieson, 2011). As mentioned earlier, dietary fibre reduces DM retention for poultry due to its low digestibility (Adeola *et al*. 2010), and this may explain the linear decrease in dietary DM retention and energy utilisation observed for birds in the current study.

The AME and AME_n value of wheat-DDGS was determined to be 14 and 13 MJ/kg DM, respectively in the current study. Cozannet *et al*. (2010a) used the difference method in their study and determined the AME value of 10 samples of wheat-DDGS to range from 7.7 to 11.5 MJ/kg DM, with a mean value of 9.9 MJ/kg DM. Further, they reported the AME_n values to range from 7.4 to 10.7 MJ/kg with a mean of 9.3 MJ/kg DM. It is common knowledge that the chemical properties of DDGS differ significantly among sources (Fastinger *et al*., 2006). The GE content and the concentration and/or type of dietary fibre are important factors that may define the metabolisable energy content of the feed ingredient. In particular, the AME value of wheat-DDGS for turkey derived in the current study was 3.8 MJ/kg of DM greater compared with the average AME value of 9.9 MJ/kg DM noted by Cozannet *et al*. (2010a). Although the GE content of the wheat-DDGS used in the current study and those of Cozannet *et al*. (2010a) were similar (21.6 vs. 20.8 MJ/kg DM, respectively), energy metabolisability in the wheat-DDGS was greater in the current study (65 vs. 47%, respectively). It therefore appears that factors other than the GE content of the wheat-DDGS confer differences in its AME contents among sources. These factors include differences in the assay used, environmental conditions and species and age of birds used.

It is also noted that the AME and AME_n values of wheat-DDGS was greater for broilers compared with turkey. In Chapter 3 of this thesis, the AME and AME_n values of the wheat-DDGS for broilers were determined to be 15 and 14 MJ/kg, respectively. These values are 1 MJ/kg greater than the AME or AME_n values for turkey determined in the current study. Similarly, Cozannet *et al*. (2010a) observed that the average AME and AME_n for 10 samples of wheat-DDGS were (0.53 and 0.87 MJ/kg DM, respectively) greater for broilers at 21 days old compared with turkey at 13 weeks of age. It is speculated that the energy value of the wheat-DDGS was greater for broilers at 21 days of age possibly because the broilers were physiologically more mature than the turkey at 21 days old, hence broilers were able to utilise dietary nutrients more efficiently. At 21 days in the current study, broilers were at the grower phase whereas the turkey were at the starter phase. However, this speculation is hardly supported by the similarity between the observations noted in the current study and the study

of Cozannet et al. (2010) where the AME of wheat-DDGS for turkey was determined at 13 wks of age. On the other hand, it is possible that the greater AME and AME_n for wheat-DDGS noted in the current study for turkey compared with the study of Cozannet et al. (2010) are due to differences in the chemical characteristics of wheat-DDGS used.

The differences in age of physiological age between the broiler and turkey may also be explained by the differences in growth performance response during the 7-d experimental period in the current study. For turkey, increasing the inclusion level of wheat-DDGS from 0 to 60% linearly decreased weight gain and feed efficiency whereas broilers performed best when fed 300 g/kg of wheat-DDGS in their diet. The linear reduction in growth performance observed for turkey was likely due to a lesser ability to cope with the increase in dietary fibre compared with broilers as the inclusion level of wheat-DDGS increased in the diet.

The use of exogenous enzymes to improve nutrient utilisation in feedstuffs for poultry has been widely investigated. In particular, XAP has been shown to be effective at improving nutrient utilisation in the diet and growth performance for poultry (Adeola and Coweison, 2011). The mechanisms through which XAP may improve the nutritive value of a feedstuff or the diet include 1) hydrolysis of arabinoxylans and β-glucans into oligosaccharides and monosaccharides by xylanase and amylase, as a result reducing digesta viscosity, 2) release of encapsulated nutrients in the cell wall or gel matrix thereby making the available for absorption, 3) protease may supplement and at the same time reduce the energy required for endogenous peptidase production, 4) protease may hydrolyse protein-based anti-nutrients such as lectins or trypsin inhibitors therefore improving the efficiency of AA utilisation. In the current study, XAP increased the AME and AME_n value of the wheat-DDGS for turkey by 0.85 and 0.77 MJ/kg DM, respectively but these increases were not statistically significant. The reasons for the lack of improvement with the addition of XAP are not clear considering that wheat-DDGS was expected to contain high levels of NSP that are substrates for the carbohydrases in the enzyme admixture.

In Adeola et al. (2010) study, a cocktail of xylanase and amylase increased the AME and AME_n of corn distillers grains by 5.7% and 6.2%, respectively. In the current study, the increases noted in the energy value of the wheat-DDGS due to XAP supplementation were marginal and were not statistically significant. The lack of XAP effect in the current study is least expected because feed ingredients or diets that contain substantial concentrations of fiber respond to a greater extent to carbohydrase supplementation (Bedford, 2000). Adeola and Cowieson (2011) noted a trend that indicated that the effects of carbohydrase supplementation

are repressed when the energy value of the feed ingredient or diet being treated is high. The AME value of wheat-DDGS noted in the current study for broilers or turkey were greater compared with other reported values in the literature (Cozannet et al. 2010; Bolarinwa and Adeola, 2012) and was also greater than the AME content of wheat grain. Perhaps, the greater ME content in the wheat-DDGS used in the current study was partly responsible for the marginal effect of XAP. Also, analyzed xylanase and protease activities were approximately 20% lower than was expected in the XAP-supplemented diets for broilers and turkey in the current study, and may be partly responsible for the marginal increment in AME in the wheat-DDGS noted. Nevertheless, considering that the wheat-DDGS contain substantial levels of soluble fiber, it is unlikely that a combination of carbohydrases and proteases will not significantly improve its utilizable energy for broilers and turkey. It is therefore recommended that further studies be conducted to evaluate the efficacy of carbohydrases to improve the energy value of wheat-DDGS for broilers and turkey.

In conclusion, the AME and AME_n values of wheat-DDGS for turkey were determined to be 14 and 14.9 MJ/kg of DM, respectively. Supplemental XAP marginally increased the metabolisable energy content in wheat-DDGS for turkey. Increasing the inclusion level of wheat-DDGS in the diet reduced dry matter and energy utilisation of the diet for turkey most likely due to the increase in dietary fibre composition.

True phosphorus digestibility and retention of wheat-DDGS without- or with supplemental phytase for turkey

The current study determined the digestible P content of wheat-DDGS without- or with a phytase for 21 d old turkey using a linear regression method. We hypothesized that wheat-DDGS is a good source of digestible P for turkey and that supplemental phytase will release phytate bound P in the wheat-DDGS, thus increasing P utilisation in the feed ingredient. The wheat-DDGS used in the current study contained by analysis, 7.6 g/kg DM of P. Thacker and Widyaratne (2007) reported the total P content in wheat-DDGS to be 12.3 g/kg DM whilst Nyachoti *et al*. (2005) reported a value of 9.4 g/kg DM. The differences in the total P content of wheat-DDGS among sources further testifies to the variability that exist in its chemical composition among sources.

Increasing the inclusion level of wheat-DDGS in the dietary treatments reduced the utilisation of DM at the ileal and total tract level in the current study. The reduction in DM utilisation noted may be due to the increased level of dietary fibre associated with increasing the level of

wheat-DDGS in the diets. High levels of dietary fibre have been reported to reduce DM and nutrient utilisation in poultry (Choct *et al*., 2004). Also, supplementing the diets with phytase did not improve P utilisation at either the ileal or total tract level. The lack of response to phytase may have been due to the low level of phytate bound P in the wheat-DDGS because it is known that some hydrolysis of phytate occurs during the fermentation and drying steps of bioethanol production (Martinez-Amezcua *et al*. 2004; Liu and Han, 2011; Liu, 2011). For example, Liu and Han (2011) observed that during the fermentation process, the ratio of phytate P to non-phytate bound P decreased significantly; this is an indication that a large proportion of phytate are degraded by yeast phytase during fermentation. Nevertheless, Martinez-Amezcua *et al*. (2004) observed that the hydrolysis of phytate in the DDGS during fermentation may be incomplete, and that heat treatment during the drying step is also important in defining the non-phytate bound P content in the DDGS. Information about the phytate P content or temperature used to dry the DDGS used in the current study was not available, but because the DDGS was dark in colour it is speculated that the DDGS may have been substantially heat-treated.

Using the regression method, it is possible to extrapolate true P digestibility or retention and basal endogenous P loss from the linear relationship between undigested P and dietary P intake. In the current study, we observed a strong relationship between undigested P and dietary P intake, which is important pre-requisite for the use of the regression technique. The linear regression method has been used to determine true P retention of feed ingredients for broilers (Dilger and Adeola, 2006) and swine (Akinmusire and Adeola, 2008) as well as for determination of true ileal AA digestibility of feed ingredients for broilers (Kong and Adeola, 2011). True ileal P digestibility of wheat-DDGS for turkey was determined to be 75% whereas the true P retention was 71%. The TPD and TPR noted for wheat-DDGS indicates that majority of the P in wheat-DDGS was present in the form that is readily utilisable for the bird. The results in the current study are similar to those reported in Chapter 3 although broilers were able to utilise more of the P in the wheat-DDGS compared with turkey (90 *vs.* 70%, respectively). The difference in P digestibility between broilers and turkey in the current study is probably due to differences in physiological maturity between the two speciess at 21 d of age. The TPD and TPR of wheat-DDGS noted for broilers and turkey in the current study indicated that the majority of P in wheat-DDGS was digestible. Supplemental phytase did not affect P digestibility or retention for broilers and turkey in the current study. The high TPD and TPR of wheat-DDGS in the current study is an indication that the level of phytate in the wheat-DDGS could have been low and may possibly explain the lack of phytase effect.

Phytate may increase endogenous mineral losses by increasing secretion of mucin (Cowieson *et al*., 2004), forming complexes with cations making them unavailable for absorption or bonding with endogenous enzymes and as a result reducing their efficacy (Dilworth *et al*., 2005), or causing a modification to the gastrointestinal electrolyte balance leading to less efficient mineral utilisation (Ravindran *et al*., 2008). Phytase may improve the utilisation of minerals by counteracting the anti-nutritional effects of phytate (Cowieson *et al*., 2004; Liu and Ru, 2010). Except for Zn at the total tract level, increasing the inclusion level of wheat-DDGS in the diets increased the flow of all minerals in a linear manner at both the ileal and total tract. The current study was designed specifically to determine the true P digestibility and retention of wheat-DDGS for turkey, and as such the dietary treatments were formulated in such a way that P was the only mineral that was limiting. Because the dietary treatments were formulated to be adequate in all minerals except P, increasing the inclusion level of wheat-DDGS would have resulted in an increase in the dietary intake of other minerals beyond the levels required by the birds. This may be the reason for the increase in the flow of majority of the minerals at the ileal and total tract as the dietary inclusion level of wheat-DDGS increased in the current study. Except for Mg at the ileal level, phytase did not affect the flow of other minerals at either ileal or total tract level in the current study. The lack of phytase effect may have been due to the oversupply of the minerals with increasing levels of wheat-DDGS in the diet or low levels of phytate-bound cations in the gut.

In conclusion, the true ileal digestibility and retention of P in wheat-DDGS is about 70% for turkey. Supplemental phytase did not improve the ileal digestibility or retention of P in the wheat-DDGS for turkey.

Apparent- and standardised-ileal amino acid digestibility of wheat-DDGS without or with supplemental protease for turkey

Wheat-DDGS is increasingly being used as an alternative protein source to SBM in broiler and pig diets; however, there is currently no information in literature about the digestible AA content of wheat-DDGS for turkey. Because information about the digestible AA profile of feed ingredients is essential in diet formulations, the objective of the current study was to determine the AIAAD and SIAAD of wheat-DDGS without- or with a protease for turkey.

The AIAAD of wheat-DDGS for turkey were generally low in the current study. In fact, except for Glu and Pro, AIAAD was lower than 50% for all other AA and was zero for Lys. A number of studies (Lumpkins *et al*., 2004; Bandegan *et al*., 2009; Cozannet *et al*., 2011) using

either maize- or wheat-DDGS for broilers have also noted the lowest AID and SID values for Lys. The SIAAD of wheat-DDGS in the current study ranged from 41 to 81% with Thr being the least digestible and Pro being the most digestible AA, respectively. Overall, it was noted that Lys and Asp were the least digestible AA in wheat-DDGS for turkey. A possible explanation for the generally low AIAAD and SIAAD for wheat-DDGS observed in the current study may relate to changes caused to the nutritive value of the DDGS by heat treatment. Excessive heat application during the drying step of bioethanol production has been reported to reduce the digestibility of Lys in maize-DDGS (Fastinger *et al*., 2006). Heat treatment causes the bonding of Lys to carbohydrate moieties by Maillard reaction to form insoluble complexes that cannot be utilised by the bird. It is possible that the generally low and in particular, the zero AID and SID for Lys recorded in the current study was due to excessive heat treatment during the production of the DDGS, and this may also explain its dark colour.

The colour of the DDGS is sometimes used as an indication of the intensity of heat treatment during the drying process (Fastinger *et al*., 2006). Tools such as the Hunterlab colour grading system are often used to measure the degree of lightness (L*); redness (a*), and yellowness (b*) of a product. Although such tools were not used in the current study, a picture of the wheat-DDGS used in the current study indicated that the colour was very dark and closest to a level 5 on a maize-DDGS colour chart (Figure 3-3). There is an appreciation for the fact that the colour of maize-DDGS may differ slightly from that of wheat-DDGS when making this colour comparison. Light coloured maize-DDGS have been reported to have greater AA digestibility than dark coloured counterparts for broilers (Ergul *et al*., 2003; Batal and Dale, 2006) and caecectomized roosters (Fastinger *et al*., 2006; Cozannet *et al*., 2011).

Apart from the low AIAAD for wheat-DDGS noted in the current study, there was also wide variability in AA digestibility. Disregarding Lys and Asp whose digestibility were zero or very low, there was greater than 60 percentage unit difference between the AID of Pro and that of Thr. After the AIAAD values were corrected for endogenous AA losses, the variability in AA digestibility reduced; notably due to the large increase in the digestibility of Thr. Hence, it appears that the variability in the AID of wheat-DDGS may be partly explained by the differences in AA of endogenous origin. It was observed that the largest contributors to basal endogenous AA flow were Thr, Met, Asp, Ala and Ser and this is consistent with the fact that mucin proteins are high in Thr and Ser (Adedokun *et al*., 2007). Moughan and Schuttert (1991) observed that free AA and small peptides are more readily reabsorbed in pigs compared to mucin proteins due to their resistance to enzymatic hydrolysis. If the same is true

for turkey, it is possible that the high proportion of Thr, Met, Asp, Ala and Ser of endogenous origin is a result of slow rate of reabsorption of these AA as compared with other AA. This may partly explain the variability in the AA digestibility of wheat-DDGS for turkey and may be even more important because wheat-DDGS may increase mucin production in the gut due to its high fibre content.

The AIAAD and SIAAD of wheat-DDGS for broilers are reported in chapter 3. In general, the AIAAD and SIAAD of wheat-DDGS were greater for broilers compared with turkey. In both studies (broilers vs. turkey), it was consistent that the ileal digestibility of Lys was zero and that Pro was the most digestible AA in the wheat-DDGS. On the average, the AIAAD and SIAAD of the wheat-DDGS were 13 and 10 percentage units, respectively greater for broilers compared with turkey and the largest differences in AA digestibility were observed for His, Thr, Cys, Ser, Gly and Asp. Uni *et al*. (1995; 1999) observed that the post hatch development of the small intestine for turkey poults is slower compared with that of the broiler chick. It is speculated that broilers were physiologically more mature and were able to utilise the AA in the wheat-DDGS more efficiently compared with turkey.

The main benefits of using supplemental enzymes in poultry diets are to increase the nutritional value of the diet or feed ingredients and also reduce the variation in the nutrient quality of feed ingredients whilst at the same time reducing nutrient losses in manure (Bedford 2000). The main idea for supplementing protease in the diet is to increase protein and AA digestibility and so we hypothesized in the current study that protease will improve the AIAAD and SIAAD of wheat-DDGS for turkey. There are a number of ways through which exogenous protease may help improve AA digestibility in wheat-DDGS. Supplemental proteases may supplement endogenous peptidase production, reducing the requirement for AA and energy and/or help hydrolyse protein-based anti-nutrients such as lectins or trypsin inhibitors, improving the efficiency by which the bird utilizes AA and reducing protein turnover (Adeola and Cowieson, 2011). Indeed, except for Cys and Pro, protease increased the ileal digestibility of all other AA in the wheat-DDGS for turkey from between 5 to 19 percentage points in the current study.

In conclusion, the AIAAD and SIAAD of wheat-DDGS for turkey are quite low and variable. The digestibility of Lys and Thr in wheat-DDGS is very low and needs to be accounted for in diet formulations. Proline and Glu are the most digestible AA in wheat-DDGS for turkey. The digestibility of AA in wheat-DDGS for turkey is enhanced by addition of a protease from between 5 to 19 percentage units.

Collectively, it is concluded that wheat-DDGS is a useful dietary source of energy and P for turkey, however, the low Lys and Thr digestibility needs to be accounted when formulating wheat-DDGS in the diet.

CHAPTER 5

GROWTH PERFORMANCE AND GASTROINTESTINAL TRACT CHARACTERISTICS OF BROILERS RECEIVING A DIET CONTAINING WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES SUPPLEMENTED WITH AN ADMIXTURE OF XYLANASE, AMYLASE AND PROTEASE OR PHYTASE INDIVIDUALLY OR IN COMBINATION

5.1 INTRODUCTION

The energy value, amino acids digestibility and P utilisation in wheat distillers dried grains with solubles (wheat-DDGS) were determined and reported in Chapter 3 of this thesis. The results in Chapter 3 indicated that the metabolisable energy (ME) and digestible P content of wheat-DDGS are comparable to those of wheat and soyabean meal (SBM) for broilers. The comparable ME and digestible P contents of wheat-DDGS to wheat and SBM suggests that wheat-DDGS may be used to substitute these feedstuffs in broiler diets. Therefore, the ME and digestible nutrient values of wheat-DDGS determined in Chapter 3, and digestible nutrient values for other major feedstuffs derived from the literature were used to formulate diets for broilers to assess the performance of broilers fed enzyme-supplemented diets containing wheat-DDGS.

The growth performance responses of broilers receiving diets containing maize-DDGS are widely reported in the literature. Loar *et al*. (2010) and Shim *et al*. (2011) reported that including up to 24% maize-DDGS in a maize-SBM based diet improved broiler performance above feeding a maize-SBM based diet containing no DDGS. On the other hand, others have reported that bird performance depreciates at inclusion levels of 9% (Lumpkins *et al*., 2004) or 15% (Wang *et al*., 2007a) of maize-DDGS in a maize-SBM based diet. There are comparatively no data available in the literature for wheat-DDGS, however, it is speculated that the use of energy and digestible nutrient values to formulate a wheat-SBM based diet containing wheat-DDGS for broilers will support growth performance.

Supplemental carbohydrases, protease or phytase or a combination of these enzymes are often used in poultry diets. Carbohydrases may help promote growth, efficiency of nutrient utilisation, and reduce nutrient excretion by degradation of non starch polysaccharides (NSP) in the cell wall matrix causing a reduction in digesta viscosity thereby improving contact between digesta, digestive enzymes and the absorptive surface (Bedford and Schulze, 1998). Phytase may release P bound to phytate, whereas protease may help supplement endogenous peptidase production, thus reducing the requirement for amino acids (AA) and energy (Adeola and Cowieson, 2011). The efficacy of phytase and an enzyme mixture containing xylanase, amylase and protease activities have been investigated in a maize-SBM based diet containing maize-DDGS for broilers (Olukosi *et al*., 2010), but greater benefits can be derived from using these enzymes in a diet containing wheat-DDGS because of the greater concentration of dietary fibre in the latter (Vilarino *et al*., 2007).

The morphology of the small intestinal surface may be used as a measure of gut health and efficiency of nutrient absorption. An increased villus height to crypt depth ratio is an indication of lower energy and nutrient requirement by the bird for gut turnover and increased nutrient utilisation efficiency (Rebole *et al*., 2010). It is possible that improvements in growth performance where supplemental enzymes are used in broiler diets are linked to improvements in the structure of the jejunal absorptive surface. Further, the gastrointestinal tract of the bird comprises a wide variety of microorganisms and the chemical composition of diet may cause changes to the intestinal microbiota balance by selective stimulation of the growth of some bacteria. Proliferation of beneficial bacteria in the gut is often accompanied by a low digesta pH and an increase in the production of short chain fatty acids (Rebole *et al*., 2010). The effects of supplemental XAP or phytase on the health of the gastrointestinal tract of broilers receiving a wheat-SBM based diet containing up to 25% wheat-DDGS are yet to be determined.

The current study therefore examined the growth performance, jejunal morphology as a measure of cellular absorptive structure development, intestinal pH as a measure of gut health and caecal volatile fatty acids (VFA) production as a measure of microbial activity of broilers receiving a wheat-SBM based diet containing wheat-DDGS supplemented with an admixture of XAP or phytase added individually or in combination.

5.2 MATERIALS AND METHODS

5.2.1 Animals and Management

The Animal Experimentation Committee of the Scotland's Rural College approved all bird handling and sample collection procedures. A total of 288 male Ross 308 broiler chicks were used in the current 42-d study. On d 1, the birds were weighed and allocated to 8 dietary treatments in 48 floor pens in a randomised complete block design. Each treatment was replicated 6 times and there were 6 birds in each pen. Diets were randomly assigned to pens in each block. The experimental diets were formulated for the 3 growth periods consisting of the starter (d 1 to 10), grower (d 11 to 24), and finishing (d 25 to 42), respectively in order to account for the changing nutrient requirements of the bird. The experimental diets were formulated using metabolisable energy (ME) and digestible amino acid values of wheat, SBM and wheat-DDGS. In the case of wheat-DDGS, the metabolisable energy and standardised ileal amino acids digestibility (SIAAD) values determined previously and reported in Chapter

3 of this thesis were used. Birds had *ad libitum* access to the experimental diets and water throughout the study. The diets were provided in mash form.

5.2.2 Dietary Treatments

A total of 8 experimental diets were used in the current study. The diets were 1) a positive control (PC1); wheat-soyabean meal (wheat-SBM) diet and adequate in metabolisable energy (ME) and all nutrients, 2) a second positive control (PC2); wheat-SBM based diet containing wheat-DDGS and adequate in ME and all nutrients; 3) a negative control (NC1) marginal in ME (minus 0.63 MJ/kg), 4) NC1 plus XAP (Danisco Animal Nutrition, Marlborough, UK) added to provide per kg of diet, 2000, 200 and 4000 U of xylanase, amylase and protease, respectively 5) a negative control (NC2) marginal in available P (minus 0.15%) 6) NC2 plus phytase (Danisco Animal Nutrition, Marlborough, UK) added to provide 1000 FTU per kg of diet, 7) a negative control (NC3) that is low in ME and available P (minus 0.63 MJ/kg and 0.15%, respectively), 8) NC3 plus a combination of XAP and phytase at the rates in diets 4 and 6, respectively. Wheat-DDGS was included in the diet at the rate of 12, 22 or 25% at the starter, grower or finisher phases. The xylanase was a endo-1,4-β-xylanase produced by a *Trichoderma longibrachiatum* and expressed in the same organism. The amylase was produced by *Bacillus amyloliquifaciens* and expressed in *Bacillus subtilis*. The subtilisin (protease) was derived from *Bacillus subtilis*. The three enzymes described above were produced separately and later blended to produce the xylanase-amylase-protease (XAP) admixture. One unit of xylanase was defined as the quantity of the enzyme that liberates one mmol of xylose equivalent per minute. One unit of amylase was defined as the amount of the enzyme catalysing the hydrolysis of one mmol glucosidic linkage per minute and one protease unit was defined as the quantity of the enzyme that solubilised one mg of azo-casein per minute. The phytase (Danisco Animal Nutrition, Marlborough, UK) was derived from *Escherichia coli* and expressed in *Schizosaccharomyces pombe*. One phytase unit was defined as the quantity of enzyme required to liberate 1 µmol of inorganic P per minute, at pH 5.5 from an excess of 15 µM sodium phytate at 37^{o}C. The ingredient and chemical composition of the PC and NC diets are presented in Tables 5-1, 5-2 and 5-3 for the starter, grower and finishing periods, respectively.

Table 5-1. Ingredient and chemical composition (g/kg) of the positive and negative control diets for the starter period.

		Diets[1]			
Ingredients	PC1	PC2	NC1	NC2	NC3
Wheat, White	585	558	590	575	597
Soybean meal	325	250	244	247	245
Soybean oil	44.0	26.0	0.0	20.0	0.0
DDGS	0.0	120	120	120	120
Limestone (38% Ca)	16.0	17.0	17.0	17.0	17.0
Dicalcium phosphate[1]	17.0	15.5	15.5	7.50	7.50
Others[2]	13.5	13.5	13.5	13.5	13.5
XAP premix[3]	-	-	±	-	±
Phytase premix[4]	-	-	-	±	±
Nutrients and energy					
Crude protein (analysed)	213	208	213	218	213
ME, MJ/kg	12.7	12.7	12.1	12.7	12.2
Calcium (analysed)	13.6	15.0	12.0	9.4	10.4
Total phosphorus (analysed)	6.80	6.80	6.30	5.00	4.90
Non-phytate P	4.50	4.50	4.50	3.00	3.00
Ca:P	2.00	2.20	1.90	1.90	2.10
Sodium (analysed)	1.00	1.60	1.60	1.40	2.00
Chloride (analysed)	3.00	3.20	3.00	2.70	2.50
Iron (analysed)	0.09	0.13	0.13	0.11	0.10
Magnesium (analysed)	1.50	1.40	1.50	1.60	1.40
Manganese (analysed)	0.10	0.12	0.11	0.09	0.11
Potassium (analysed)	10.1	9.30	9.90	10.0	8.30
Lys	13.8	12.7	12.7	12.7	12.7
Met	4.80	4.90	4.90	4.90	4.90
Thr	8.30	8.40	8.40	8.40	8.40
Trp	2.50	2.50	2.50	2.50	2.50

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in metabolisable energy and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

[2]Others; 2 g/kg of Common salt; 3 g/kg of Vitamin/mineral premix (vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.222 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 2 g/kg of DL-Methionine; 5 g/kg of L-Lysine HCl; and 1.5 g/kg of Threonine.

[3]XAP premix made with wheat as carrier; formulated to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease.

[4]Phytase premix made with wheat as carrier; Formulated to supply 1000 FTU/kg.

Table 5-2. Ingredient and chemical composition (g/kg) of the positive and negative control diets for the grower phase.

	Diets[1]				
Ingredients	PC1	PC2	NC1	NC2	NC3
Wheat, White	592	547	585	566	596
Soybean meal	308	165	155	160	152
Soybean oil	62.0	28.0	0.0	22.0	0.0
DDGS	0.0	220	220	220	220
Limestone (38% Ca)	12.0	14.0	14.0	14.0	14.0
Dicalcium phosphate	17.0	14.5	14.5	6.50	6.50
L-Lysine HCl	2.00	5.00	5.00	5.00	5.00
Others[2]	7.00	7.00	7.00	7.00	7.00
XAP premix[3]	-	-	±	-	±
Phytase premix[4]	-	-	-	±	±
Nutrients and energy					
Crude protein (analysed)	217	195	216	203	201
ME, MJ/kg	13.2	13.2	12.6	13.2	12.7
Calcium (analysed)	8.60	10.5	8.50	7.60	9.80
Total phosphorus (analysed)	5.00	6.20	5.80	4.60	5.40
Non-phytate P	4.50	4.50	4.50	3.00	3.00
Ca:P	1.70	1.70	1.50	1.70	1.80
Sodium (analysed)	0.70	1.90	1.70	1.70	1.60
Chloride (analysed)	2.20	3.00	2.40	2.40	3.10
Iron (analysed)	0.07	0.11	0.10	0.10	0.11
Magnesium (analysed)	1.10	1.50	1.50	1.30	1.50
Manganese (analysed)	0.08	0.10	0.09	0.09	0.11
Potassium (analysed)	7.70	8.70	8.70	7.80	9.60
Arg	12.6	10.5	10.3	10.4	10.3
His	5.00	5.10	5.00	5.00	5.00
Lys	11.2	11.3	11.1	11.2	11.1
Met	4.30	4.30	4.40	4.30	4.40
Thr	7.00	7.20	7.10	7.20	7.10
Trp	2.40	2.40	2.40	2.40	2.40

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in metabolisable energy and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

2 Others; 2 g/kg of Common salt; 3 g/kg of Vitamin/mineral premix (vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.2 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg); 1.5 g/kg of DL-Methionine; and 0.5 g/kg of Threonine.

[3]XAP premix made with wheat as carrier; formulated to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease.

[4]Phytase premix made with wheat as carrier; Formulated to supply 1000 FTU/kg.

Table 5-3. Ingredient and chemical composition (g/kg) of the positive and negative control diets for the finishing period.

	Diets[1]				
Ingredients	PC1	PC2	NC1	NC2	NC3
Wheat, White	645	596	631	619	630
Soybean meal	255	90.0	82.0	84.0	82.0
Soybean oil	65.0	27.0	0.0	19.0	0.0
DDGS	0.0	250	250	250	250
Limestone (38% Ca)	11.0	13.0	13.0	13.0	13.0
Dicalcium phosphate[1]	16.0	13.0	13.0	4.0	4.0
L-Lysine HCl	2.00	5.00	5.00	5.00	5.00
Others[2]	6.20	6.20	6.20	6.20	6.20
XAP premix[3]	-	-	±	-	±
Phytase premix[4]	-	-	-	±	±
Vitacell[5]	0	0	0	0	9.50
Nutrients and energy					
Crude protein (analysed)	195	187	186	193	189
ME, kcal/kg	13.5	13.5	12.9	13.4	12.9
Calcium (analysed)	9.00	9.40	9.00	7.40	7.50
Total phosphorus (analysed)	5.70	5.70	5.30	4.20	4.30
Non-phytate P	4.20	4.20	4.20	2.60	2.60
Ca:P	1.58	1.65	1.70	1.76	1.74
Sodium (analysed)	0.70	2.10	2.00	2.00	2.10
Chloride (analysed)	1.90	2.90	3.10	2.90	2.80
Iron (analysed)	0.08	0.11	0.11	0.10	0.11
Magnesium (analysed)	1.30	1.40	1.20	1.40	1.40
Potassium (analysed)	8.50	7.30	6.50	7.20	7.20
Arg	11.2	8.70	8.60	8.60	8.60
Lys	9.90	9.70	9.60	9.60	9.60
Met	3.80	3.80	3.80	3.80	3.80
Thr	5.80	6.00	6.00	6.00	6.00
Trp	2.20	2.20	2.20	2.20	2.20

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in metabolisable energy and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

[2] Others; 2 g/kg of Common salt; 3 g/kg of Vitamin/mineral premix (vitamin A, 16,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin B1, 3 mg; vitamin B2, 10 mg; vitamin B6, 3 mg; vitamin B12, 15 µg; hetra, 5 mg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; Biotin, 125 µg; choline chloride, 25 mg; iron, 20 mg; copper, 10 mg; manganese, 100 mg; cobalt, 1.0 mg; zinc, 82.2 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg) and 1.2 g/kg of DL-Methionine.

[3]XAP premix made with wheat as carrier; formulated to supply 2000U/kg of xylanase, 200U/kg of amylase and 4000U/kg of protease.

[4]Phytase premix made with wheat as carrier; Formulated to supply 1000 FTU/kg.

[5]Vitacell: Purified cellulose

5.2.3 Growth Performance and Gut Profiling

Birds were weighed individually on d 1, 24, and 42, whereas feed intake was determined on pen basis on d 1, 24 and 42. On day 42, two birds in each of the 48 pens with bodyweights closest to the mean of the pen were euthanized by cervical dislocation. Duplicate readings of digesta pH at the caeca and duodenum of the two birds were taken using a sterile glass pH electrode (HI 99163, HANNA Instruments, Romania). Caecal contents were collected from the two birds to analyse for VFA concentrations. The caecal digesta was snap frozen in liquid N immediately after collection and stored at -20^{o}C pending chemical analysis. Tissue from the mid-section of the jejunum approximately 6 cm in length was collected from one bird. The tissue sections were flushed clean of digesta with phosphate buffer saline (pH 7.2), mounted and stapled on cardboards and stored fully immersed in 10% formalin solution. These sections were later dehydrated in series of ethyl alcohols of increasing concentrations (70, 90, and 100%), cleared with xylene, and embedded in polyfin embedded wax in a Shandon Excelsior Tissue Processor (Thermo Fisher Scientific, Cheshire, UK). They were cut into 2 µm by a Finesse Rotary Microtome (Thermo Shandon Inc, Pittsburgh, PA), placed on glass slides, and stained with haematoxylin (Gill no. 2, Sigma, St. Louis, MO) and eosin (Sigma). Images of the villus and crypts were captured using a Leica DM4000 B Digital Microscope (Leica Microsystems Imaging Solutions Ltd., Milton Keynes, UK) fitted with a Leica DC480 digital camera. Measurements of the villus and crypt lengths were done using the Image J software. Villus height was defined as the length from the villus-crypt junction to the tip of the villus. Crypt depth was described as the depth of the invagination between adjacent villi.

5.2.4 Chemical Analysis

The experimental diets were analysed for gross energy, N, minerals and enzyme activity. For DM determination, samples were dried at 105^{o}C for 24 hours in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England. UK) (AOAC International 2006, method 934.01). Gross energy was determined in an adiabatic oxygen bomb calorimeter using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, Illinois, USA). Nitrogen was determined by combustion method (AOAC International 2006, method 968.06). Mineral concentrations in the samples were determined using inductively coupled plasma spectrophotometry (ICP) according to the procedures of Olsen and Sommers (1982). Xylanase activity in diets was measured using a kit (Megazyme International Ireland Ltd., Bray, Ireland) using the method of McCleary (1991). Amylase activity in feed was measured using Phadebas (Megazyme International Ireland Ltd.) tablets using the method described by

McCleary and Sheehan (1989). Protease activity was analysed using the modified method of Lynn and Clevette-Radford (1984) with azocasein used as substrate. Phytase activity in the diets was analysed using the AOAC official method (2000.12, AOAC, 2000).

The caecal digesta samples were analysed for VFA using gas chromatography. Briefly, about 1 g of thawed digesta was mixed with 0.2 mL of 24% metaphosphoric acid solution, diluted with deionised water (4 ml), and centrifuged at 25,000 x g for 20 min at 4^{o}C. The supernatant was analysed for VFA using a gas chromatograph equipped with a column and flame ionisation detector.

5.2.5 Statistical Analysis

Bodyweight, feed intake, feed efficiency, digesta pH and jejunal dimensions data in response to the dietary treatments were analysed using the Genstat Statistical Package (11th edition, VSN International, 2008). Additivity of the effects of XAP and phytase for a particular response was determined as follows. Individual enzyme effect was determined as the difference between the treatments supplemented with either XAP or phytase and their corresponding NC diets. Combined enzyme effect was determined as the difference between the treatment supplemented with both XAP and phytase and the corresponding NC diet. If there was addititvity in the effect of XAP and phytase, the sum of their individual effects would not be different from the effect noted for their combination. Orthogonal contrast was used for mean comparisons and check for additivity in the effect of XAP and phytase. Statistical significance was set at $P \leq 0.05$ and tendency at $0.05 < P < 0.10$.

5.3 RESULTS

5.3.1 Diets

The ingredient and chemical compositions of the experimental diets used in the current study are presented in Tables 5-1, 5-2 and 5-3. Analysed xylanase activities were 1786, 1888 and 1528 U/kg in the NC1 diet with added XAP for the starter, grower and finishing diets, respectively. Corresponding phytase activities were 987, 1263 and 1415 FTU/kg in the NC2 diet with added phytase. For NC3 diet with added XAP and phytase, the values were 1498, 1335 and 1787 for xylanase and 1267, 1232 and 1318 FTU/kg for phytase, respectively. The analysed xylanase activities were generally lower than the expected value of 2000 U/kg. Xylanase and phytase activities in the experimental diets without added XAP or phytase were negligible. Analyses for amylase and protease activities were not done in the current study.

5.3.2 Growth Performance

Growth performance responses of broilers receiving wheat-DDGS, XAP and/or phytase from d 1 to 24 are presented in Table 5-4. Body weight gain, FBW and feed intake were greater ($P < 0.001$) for birds offered the PC diet containing wheat-DDGS compared with those offered the PC diet without wheat-DDGS. On the other hand, the birds receiving the PC2 diet had greater ($P < 0.01$) G:F compared with birds receiving the PC1 diet. An admixture of XAP alone improved ($P \leq 0.05$) BWG and FBW compared with birds offered the NC1 diet. However, the XAP-induced improvement in BWG did not ($P < 0.01$) restore performance to the level of birds receiving the PC2 diet. Phytase alone or combined with XAP did not improve any of the growth performance responses from d 1 to 24. In addition, growth performance was superior ($P < 0.01$) for the birds receiving the PC2 diet compared with those receiving the NC2 plus phytase or NC3 plus XAP and phytase. There was no additivity in the effect of XAP and phytase on any of the growth responses from d 1 to 24.

The performance of broilers in response to wheat-DDGS and XAP and/or phytase from d 25 to 42 is presented in Table 5-5. Bodyweight gain and FBW were similar for birds receiving the PC1 and PC2 diets. On the other hand, G:F was superior for birds receiving the PC1 diet ($P < 0.001$) whilst birds on the PC2 diet consumed more feed ($P < 0.001$). Growth responses did not differ between birds receiving the NC1 plus XAP diet and the PC2 diet from d 25 to 42. Phytase alone or in combination with XAP did not improve any of the growth responses from d 25 to 42. Birds receiving the PC2 diet were heavier and consumed more feed ($P < 0.01$) compared with those receiving the NC2 plus phytase or NC3 plus XAP and phytase diets from d 25 to 42. The effects of XAP and phytase were not additive for any of the growth responses from d 25 to 42.

The growth performance of broilers receiving wheat-DDGS and XAP and/or phytase from d 1 to 42 is presented in Table 5-6. Bodyweight gain and FBW were similar for birds receiving the PC1 and PC2 diets, but G:F was superior for birds receiving the PC1 diet ($P < 0.001$) whereas the birds receiving the PC2 diet consumed more ($P < 0.001$). An admixture of XAP improved G:F ($P < 0.05$) and tended to improve BWG and FBW ($P < 0.1$) of birds above those receiving the NC1 diet. Overall, growth performance was similar for birds receiving the PC2 diet and those receiving the NC1 plus XAP diet. Phytase alone or a combination of phytase and XAP did not improve growth performance of birds above those receiving the NC diets. Birds receiving the PC2 diet were heavier and consumed more feed ($P < 0.001$) compared with those receiving the NC2 plus phytase, but G:F was similar between the two

dietary treatments. In addition, BWG, FBW and G:F were superior ($P < 0.01$) and feed intake was greater ($P < 0.01$) for the birds receiving the PC2 diet compared with those receiving NC3 and a combination of XAP and phytase. There was no additivity in the effect of XAP and phytase on any of the growth responses from d 1 to 42.

Table 5-4. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both from 1 to 24 days of age[1].

Diets[1]	Weight gain[2], g	Final weight, g	Gain:Feed, g/kg	Feed intake, g
PC1	693.4	735.4	676.8	1027
PC2	944.1	985.8	629.6	1500
NC1	765.4	807.4	554.1	1383
NC1 plus XAP (1)	840.4	882.4	593.1	1417
NC2	665.5	707.6	540.2	1230
NC2 plus phytase (2)	705.3	747.8	547.0	1290
NC3	595.7	638.6	511.5	1168
NC3 plus XAP and phytase (3)	637.9	678.9	512.6	1245
s.e.d	41.4	41.0	20.6	67.3
P-values for main effect of diet	<0.001	<0.001	<0.001	<0.001
P-values for contrast				
PC1 *vs*. PC2	<0.001	<0.001	0.019	<0.001
PC2 *vs*. NC1 plus XAP	0.008	0.008	0.054	0.176
PC2 *vs*. NC2 plus phytase	<0.001	<0.001	<0.001	0.001
PC2 vs. NC3 plus XAP and phytase	<0.001	<0.001	<0.001	<0.001
NC1 *vs*. NC1 plus XAP	0.050	0.048	0.041	0.572
NC2 *vs*. NC2 plus phytase	0.289	0.279	0.717	0.326
NC3 *vs*. NC3 plus XAP and phytase	0.316	0.332	0.959	0.259
1 *vs*. 2	0.710	0.714	0.400	0.844
1 *vs*. 3	0.679	0.670	0.289	0.774
2 *vs*. 3	0.935	0.921	0.752	0.912
1 + 2 *vs*. 3	0.432	0.424	0.226	0.900

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

[2]Average initial bodyweight was 42g. s.e.d: standard error of difference

Table 5-5. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both from 25 to 42 days of age.

Diets[1]	Weight gain, g	Final weight, g	Gain:Feed, g/kg	Feed intake, g
PC1	1599	2343	569.2	2809
PC2	1542	2528	445.1	3464
NC1	1331	2140	418.7	3180
NC1 plus XAP (1)	1463	2347	446.6	3275
NC2	1225	1933	449.3	2727
NC2 plus phytase (2)	1275	2023	446.4	2857
NC3	1103	1742	430.3	2564
NC3 plus XAP and phytase (3)	1160	1800	437.7	2649
s.e.d	92.6	122	16.4	167
P-values for main effect of diet	<0.001	<0.001	<0.001	<0.001
P-values for contrast				
PC1 *vs*. PC2	0.541	0.110	<0.001	<0.001
PC2 *vs*. NC1 plus XAP	0.378	0.083	0.983	0.242
PC2 *vs*. NC2 plus phytase	0.005	<0.001	0.966	<0.001
PC2 *vs*. NC3 plus XAP and phytase	<0.001	<0.001	0.532	<0.001
NC1 *vs*. NC1 plus XAP	0.145	0.082	0.101	0.552
NC2 *vs*. NC2 plus phytase	0.574	0.441	0.810	0.418
NC3 *vs*. NC3 plus XAP and phytase	0.527	0.611	0.695	0.594
1 *vs*. 2	0.570	0.600	0.093	0.909
1 *vs*. 3	0.599	0.508	0.251	0.974
2 *vs*. 3	0.965	0.889	0.565	0.883
1 + 2 *vs*. 3	0.383	0.290	0.349	0.646

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg. s.e.d: standard error of difference

Table 5-6. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both from 1 to 42 days of age.

Diets[1]	Weight gain[2], g	Final weight, g	Gain:Feed, g/kg	Feed intake, g
PC1	2301	2343	598.5	3845
PC2	2486	2528	500.8	4965
NC1	2098	2140	459.7	4564
NC1 plus XAP (1)	2305	2347	490.9	4694
NC2	1891	1933	477.8	3957
NC2 plus phytase (2)	1981	2023	477.6	4147
NC3	1699	1742	455.2	3732
NC3 plus XAP and phytase (3)	1759	1800	439.2	4004
s.e.d	118.0	117.9	15.3	210.2
P-values for main effect of diet	<0.001	<0.001	<0.001	<0.001
P-values for contrast				
PC1 *vs*. PC2	0.112	0.122	<0.001	<0.001
PC2 *vs*. NC1 plus XAP	0.142	0.144	0.539	0.185
PC2 *vs*. NC2 plus phytase	<0.001	<0.001	0.146	<0.001
PC2 *vs*. NC3 plus XAP and phytase	<0.001	<0.001	<0.001	<0.001
NC1 *vs*. NC1 plus XAP	0.075	0.075	0.049	0.520
NC2 *vs*. NC2 plus phytase	0.428	0.426	0.985	0.349
NC3 *vs*. NC3 plus XAP and phytase	0.604	0.607	0.247	0.183
1 *vs*. 2	0.599	0.602	0.163	0.884
1 *vs*. 3	0.506	0.506	0.032	0.729
2 *vs*. 3	0.887	0.884	0.401	0.841
1 + 2 *vs*. 3	0.288	0.288	0.031	0.907

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

[2]Average initial bodyweight was 42 g. s.e.d: standard error of difference

5.3.3 Gastrointestinal Tract Characteristics

Digesta pH at the duodenum and caecum of broilers in response to the dietary treatments are presented in Table 5-7. Digesta pH averaged 6.0 at the duodenum and was similar amongst all the dietary treatments. At the caeca, inclusion of wheat-DDGS in the diet reduced ($P < 0.05$) digesta pH compared with the PC without wheat-DDGS. Further, digesta pH was lower ($P < 0.05$) at the caeca for birds receiving diet supplemented with XAP alone compared with those receiving the NC1 diet. Phytase alone or in combination with XAP did not affect digesta pH compared with the birds receiving the NC diets. Caecal digesta pH tended to be lower ($P < 0.1$) in birds receiving the NC1 plus XAP diet compared with birds receiving the PC2 diet. Digesta pH at the duodenum and caecum was not different between the birds receiving the PC2 diet and those receiving the NC2 plus phytase or NC3 plus XAP and phytase. The prominent VFA produced in the caeca of broilers in response to the dietary treatments in the current study are presented in Table 5-8. The VFA produced in lesser quantities in the caeca of broilers in the current study are presented in Table 5-9. Inclusion of wheat-DDGS in the PC2 diet reduced ($P < 0.05$) n-butyric acid production compared with birds receiving the PC diet containing no wheat-DDGS. Caecal VFA production was not affected by XAP or phytase alone but a combination of XAP and phytase tended to increase propionic acid production. Compared with birds receiving the PC2 diet, XAP tended to increase n-butyric production but supplemental phytase or the combination with XAP did not affect any of the VFA.

The morphometry of the jejunum of broilers in response to a diet cointaining wheat-DDGS and supplemental XAP or phytase are presented in Table 5-10. The micrographs of the villi and crypt of broilers receiving the dietary treatments in the current study are shown in Figure 5-1. Jejunal villi height (VH) was not affected by wheat-DDGS inclusion or supplemental XAP or phytase, but XAP alone increased crypt depth (CD). Dietary treatments did not affect VH:CD ratio. The jejunal villi and crypt architecture indicate that the villi were elongated, the crypt depth was moderate and there was no marked difference in the villi and crypt among the dietary treatments. The mean VH:CD was 3.65.

Table 5-7. Digesta pH at the duodenum and caecum of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both.

Diets[1]	Duodenum	Caeca
PC1	6.05	6.01
PC2	6.03	5.57
NC1	6.03	5.65
NC1 plus XAP	6.15	5.23
NC2	6.11	5.82
NC2 plus phytase	6.04	5.56
NC3	5.99	5.54
NC3 plus XAP and phytase	5.90	5.81
s.e.d	0.10	0.17
P-values for main effect of diet	0.408	0.002
P-values for contrast		
PC1 *vs*. PC2	0.820	0.012
PC2 *vs*. NC1 plus XAP	0.242	0.051
PC2 *vs*. NC2 plus phytase	0.890	0.953
PC2 *vs*. NC3 plus XAP and phytase	0.232	0.151
NC1 *vs*. NC1 plus XAP	0.261	0.018
NC2 *vs*. NC2 plus phytase	0.480	0.131
NC3 *vs*. NC3 plus XAP and phytase	0.390	0.115

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg
s.e.d: standard error of difference

Table 5-8. Volatile fatty acids production (mg/kg) at the caecum of broiler receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both.

Diets[1]	Acetic acid	Ethanol	Propionic acid	iso-Butyric acid	n-Butyric acid
PC1	6963	168	496	154	3043
PC2	5744	258	492	118	2168
NC1	5505	251	339	118	2653
NC1 plus XAP	6083	328	352	134	2880
NC2	6581	288	445	89.0	2564
NC2 plus phytase	6449	295	423	107	2837
NC3	5290	315	327	115	2194
NC3 plus XAP and phytase	4713	243	580	140	1687
s.e.d	760	67.7	132.3	45.3	399
P-values for main effect of diet	0.092	0.373	0.505	0.898	0.027
P-values for contrast					
PC1 *vs*. PC2	0.117	0.192	0.981	0.427	0.035
PC2 *vs*. NC1 plus XAP	0.658	0.307	0.294	0.734	0.083
PC2 *vs*. NC2 plus phytase	0.360	0.591	0.603	0.812	0.102
PC2 *vs*. NC3 plus XAP and phytase	0.183	0.828	0.512	0.635	0.235
NC1 *vs*. NC1 plus XAP	0.451	0.263	0.922	0.734	0.573
NC2 *vs*. NC2 plus phytase	0.863	0.926	0.868	0.696	0.499
NC3 *vs*. NC3 plus XAP and phytase	0.453	0.296	0.063	0.587	0.212

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg
s.e.d: standard error of difference

Table 5-9. Volatile fatty acids production (mg/kg) at the caecum of broiler receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both.

Diets[1]	Heptanoic acid	Hexanoic acid	iso-valeric acid	Propane-1,2-	n-Valeric acid
PC1	35.3	57.2	134	25.0	220
PC2	61.8	71.7	110	60.7	194
NC1	50.5	68.5	112	52.3	194
NC1 plus XAP	63.5	81.8	121	64.7	212
NC2	26.3	30.0	73.0	43.5	154
NC2 plus phytase	35.3	51.7	88.0	33.0	178
NC3	77.8	89.8	107	71.2	180
NC3 plus XAP and phytase	41.7	61.8	115	41.7	231
s.e.d	28.4	35.8	41.7	31.4	61.7
P-values for main effect of diet	0.623	0.798	0.885	0.812	0.936
P-values for contrast					
PC1 *vs*. PC2	0.357	0.687	0.552	0.262	0.684
PC2 *vs*. NC1 plus XAP	0.954	0.778	0.790	0.899	0.778
PC2 *vs*. NC2 plus phytase	0.357	0.579	0.612	0.383	0.795
PC2 *vs*. NC3 plus XAP and phytase	0.482	0.785	0.896	0.548	0.556
NC1 *vs*. NC1 plus XAP	0.650	0.711	0.839	0.696	0.780
NC2 *vs*. NC2 plus phytase	0.753	0.548	0.718	0.740	0.693
NC3 *vs*. NC3 plus XAP and phytase	0.211	0.438	0.849	0.353	0.416

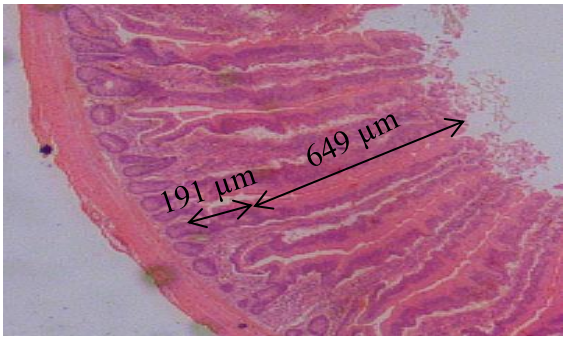
[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg. s.e.d: standard error of difference

Table 5-10. Jejunal morphology of broilers receiving receiving a wheat-soyabean meal based diet containing wheat-distillers dried grains with solubles supplemented with a enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both.

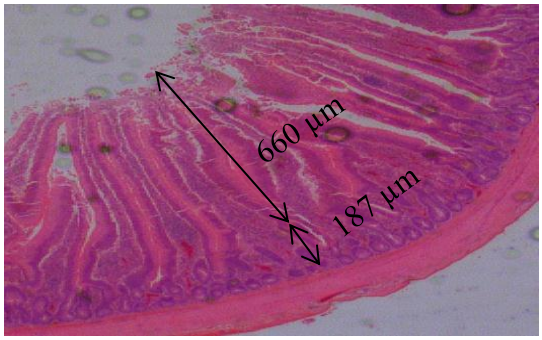
Diets[1]	VH, µm	CD, µm	VH:CD
PC1.	649	191	3.49
PC2	660	187	3.61
NC1	606	144	4.21
NC1 plus XAP	731	212	3.62
NC2	564	168	3.4
NC2 plus phytase	648	207	3.26
NC3	692	194	3.79
NC3 plus XAP and phytase	634	174	3.81
s.e.d	96.9	30.0	0.58
P-values for main effect of diet	0.793	0.387	0.816
P-values for contrast			
PC1 *vs*. PC2	0.909	0.903	0.829
PC2 *vs*. NC1 plus XAP	0.466	0.407	0.987
PC2 *vs*. NC2 plus phytase	0.899	0.506	0.542
PC2 *vs*. NC3 plus XAP and phytase	0.794	0.663	0.727
NC1 *vs*. NC1 plus XAP	0.203	0.029	0.314
NC2 *vs*. NC2 plus phytase	0.396	0.192	0.800
NC3 *vs*. NC3 plus XAP and phytase	0.552	0.513	0.963

[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - wheat-SBM-wheat-DDGS based diet marginal in ME and P (-0.63 MJ/kg and -0.15% non phytate P, respectively); XAP added to provide 2000, 200 and 400 U/kg of xylanase, amylase and protease, respectively; Phytase added to provide 1000 FTU/kg

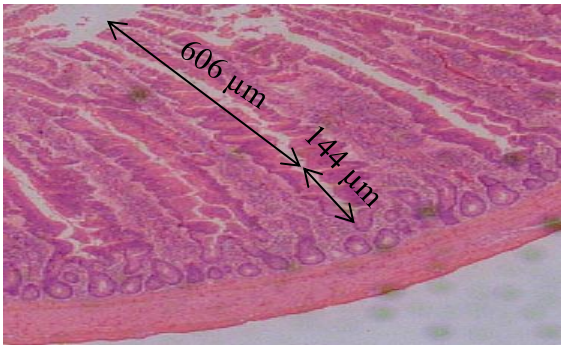
VH: villi length; CD: crypt depth; s.e.d: standard error of difference



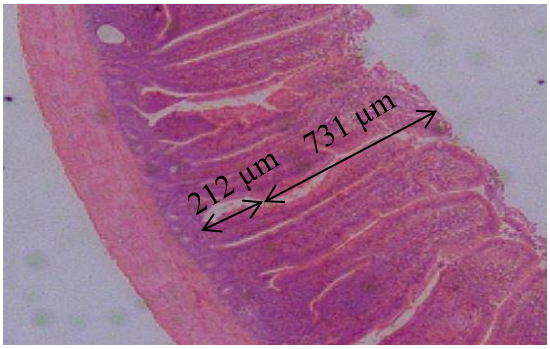
PC1



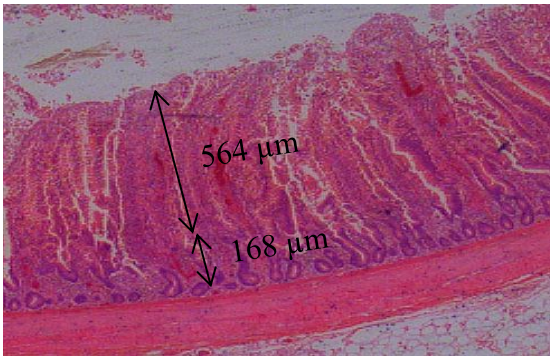
PC2



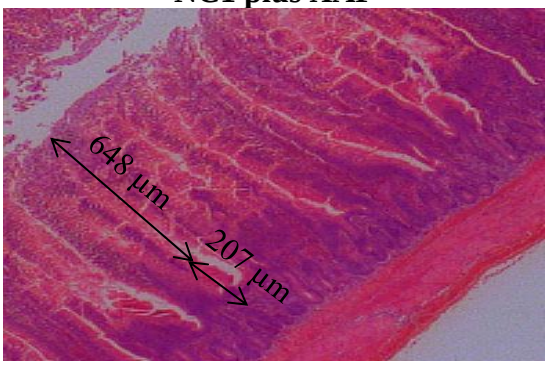
NC1



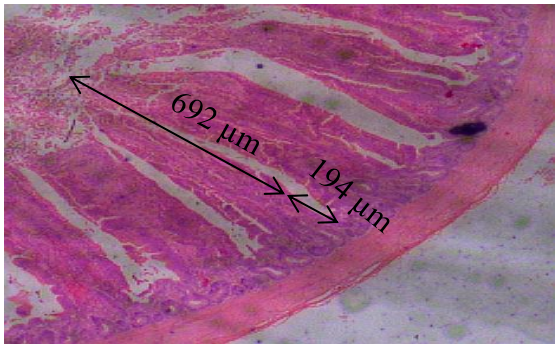
NC1 plus XAP



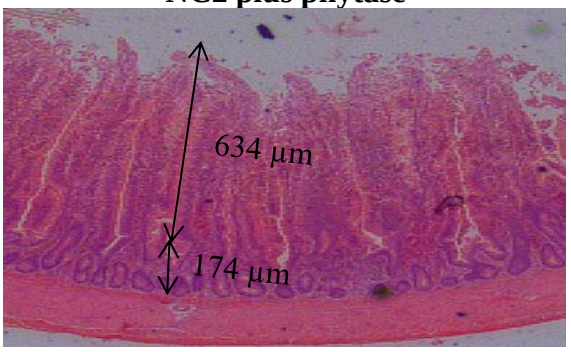
NC2



NC2 plus phytase



NC3



NC3 plus XAP and phytase

Figure 5-1. Micrographs of the jejunal villi height and crypt depth for broilers receiving the experimental diets in the current study.

5.4 DISCUSSION

The objective of the current study was to determine the effect of supplementing a wheat-SBM based diet containing wheat-DDGS with XAP and phytase individually and in combination on growth performance and gastrointestinal characteristics of broilers. The diets were formulated to be marginal in ME and/or available P to enable determination of the effects of XAP and phytase. In the current study, wheat-DDGS was included in a wheat-SBM based diet at the rate of 12, 22 or 25% at the starter, grower and finishing periods, respectively to ensure that the effect of DDGS addition was marked. It is important to use wheat-DDGS with moderation to avoid compromising growth performance due to increased dietary fibre content.

Thacker and Widyaratne (2007) observed that birds receiving a wheat-SBM based diet containing up to 15% wheat-DDGS performed similar to birds receiving a wheat-SBM based diet containing no wheat-DDGS. On the other hand, Richter *et al*. (2006), Vilarino *et al*. (2007) and Lukasiewicz *et al*. (2009) reported a decrease in the FBW of broilers receiving wheat-DDGS in their diets compared with those receiving a diet not containing wheat-DDGS. In studies using maize-DDGS, Shim *et al*. (2011) noted that broilers receiving a maize-SBM based diet containing 24% maize-DDGS were heavier compared with birds receiving no maize-DDGS from d 1 to 18. Similarly, Olukosi *et al*. (2010) reported greater BWG and G:F for broilers receiving a diet containing 10% maize-DDGS compared with birds receiving no maize-DDGS at 21 days of age. In the current study, it was noted that birds receiving the PC diet containing wheat-DDGS were heavier compared with birds receiving the PC diet without wheat-DDGS from d 1 to 24, whereas BWG was similar between these treatments from d 25 to 42 and from d 1 to 42.

The PC diet containing wheat-DDGS and the other not containing wheat-DDGS were formulated using the metabolisable energy and digestible nutrient values of all ingredients and these diets contained similar levels of ME and nutrients. For this reason, it may be expected that the growth performance of bird receiving the PC diet containing wheat-DDGS will be similar to those of birds receiving the PC diet without wheat-DDGS. Taking together the observation in the current study and those by Olukosi *et al*. (2010) and Shim *et al*. (2011), it appears that birds may derive greater benefits from the inclusion of DDGS in their diets at a younger age. The reasons why the inclusion of wheat-DDGS in a wheat-SBM based diet would produce superior growth performance in birds above feeding a wheat-SBM diet are not clear considering that DDGS inclusion would be expected to increase dietary fibre levels, but it is speculated that the wheat-DDGS used may have contained some residual starch and

sugars which are more readily utilisable for the bird. It is noted that under normal processing conditions, the fermentation process does not effectively convert all the starch in the maize or wheat grain into ethanol, and as a result some level of residual starch and sugars are found in the DDGS (Vilarino *et al*., 2007).

Wheat and SBM were the main feed ingredients in the experimental diets used in the current study. Wheat is known to contain substantial quantities of water-soluble carbohydrates (Bedford and Classen, 1992) which are substrates for carbohydrases. Depending on inclusion rate, the addition of wheat-DDGS to a wheat-based diet may increase the levels of NSP (Thacker and Widyaratne, 2007). Non starch polysaccharides increase the viscosity of digesta in the gastrointestinal tract causing a decrease in nutrient utilisation which has negative consequences on bird performance (Edward *et al*., 1988; Carre *et al*., 2002). The ability of xylanase to improve nutrient utilisation of wheat-based diets by reducing digesta viscosity and transformation of the improvement in nutrient utilisation to performance has been reported for poultry (Adeola and Bedford, 2004). Phytase on the other hand dephosphorylates phytate, releasing P and other nutrients that may have complexed with phytate in the process (Adeola and Cowieson, 2011). A reduction in digesta viscosity by XAP may complement phytase activity by increasing access to phytate molecules encapsulated in NSP. There are extensive reports in the literature about improvements in the growth performance of broilers using supplemental XAP or phytase or a combination of both (Cowieson and Adeola, 2005; Ravindran *et al*., 2001; Olukosi *et al*., 2007; Amerah and Ravindran 2009).

Supplementing P-marginal diets with phytase have been reported to improve BWG and G:F of broilers (Wu *et al*., 2004; Cowieson and Adeola, 2005) and phytase and an admixture of XAP may act synergistically to improve growth performance of broilers receiving a maize-SBM based diet (Cowieson and Adeola, 2005). A cocktail of XAP modestly improved the overall BWG and feed efficiency of broilers above the NC1 diet in the current study. But in the case of phytase, there was generally no effect on growth performance. Nitsan *et al*. (1991) observed that digestive enzyme production increases with age in broiler chicks, thus nutrient utilisation may be limiting in the first few days posthatch due to low levels of digestive enzymes. In the current study, supplemental XAP may have complemented endogenous amylase and protease activities which may have produced the modest improvement in BWG from d 1 to 24. The overall modest improvement in BWG and G:F of the broilers from d 1 to 42 is a likely indication that supplemental XAP was able to, among other possible mechanisms, release more dietary energy by breaking down structural carbohydrates or supplement endogenous protease.

During the fermentation process of bioethanol production, a large proportion of the phytate in the wheat is hydrolysed by yeast phytase, and as a result, wheat-DDGS may contain low levels of phytate (Liu, 2011). In Chapter 3 of this thesis, it was noted that apart from supplemental phytase not improving the digestible P content in wheat-DDGS, the true digestible P and true retainable P levels in the wheat-DDGS were above 90%. This is a likely indication that the wheat-DDGS contained low levels of phytate-bound P. Therefore, it appears that the substitution of wheat and SBM with wheat-DDGS would have reduced the level of phytate in the diet which may explain the lack of effect of phytase supplementation on growth performance in the current study.

There was no additivity in the effects of phytase and an admixture of xylanase, amylase and protease on the growth performance of broilers in the current study. The overall (d 1 to 42) improvement in BWG and G:F above the NC1 diet were 9.2% and 6.3%, respectively when XAP was used alone. Phytase alone on the other hand, increased BWG by 4.5% above the NC2 diet but did not increase G:F. Whereas, a combination of XAP and phytase increased BWG by 3.4% but did not increase G:F. These results indicate that a combination of XAP and phytase produced lesser improvement in BWG compared with either of the enzymes individually. It is possible that the improvement noted in growth performance when XAP was used alone were not observed when XAP was used in combination with phytase because the NC3 diet was also marginal in available P more so that phytase did not significantly improve growth performance in the current study. In other words, the birds may have been limited in their ability to benefit from the improvement produced by XAP because the diet was limiting in available P.

The inclusion of moderate levels of fibre in the diet may improve digestive organ development (Gonzalez-Alvarado *et al*., 2007) and stimulate digestive enzyme secretion (Svihus, 2011), as a result, improve nutrient digestibility (Amerah *et al*., 2009), growth performance (Gonzalez-Alvarado *et al*., 2010), gastrointestinal tract health (Perez *et al*., 2011) or enhance the proliferation of beneficial bacteria in the gut (Mateos *et al*., 2012). Wheat-DDGS contain substantial quantities of soluble fibre which may stimulate the aforementioned effects. Indeed, Lukasiewicz *et al*. (2009) noted that the inclusion of wheat-DDGS in the diet for broilers increased the population of beneficial micro-organisms of the *Enterobacteriaceae* family in the caecum. In the current study, inclusion of wheat-DDGS in the PC diet decreased digesta pH at the caecum but not at the duodenum. The decrease in caecal digesta pH with the inclusion of wheat-DDGS in the diet could be due to changes in VFA concentrations due to an increase in caecal fermentation as a result of increased dietary fibre intake.

The mechanisms through which XAP or phytase may reduce digesta pH in the small intestine of broilers are not clear, but it is suggested that xylanase may indirectly decrease digesta pH in the small intestine of broilers by reducing digesta viscosity and as a result increase digesta transit time which then reduces the time available for unfavourable micro-organisms to proliferate. On the other hand, supplemental phytase may accelerate the hydrolysis of phytate bound P and as a consequence reduce the quantity of P that is available to intestinal microorganisms. Also, supplemental xylanase may improve gut health by hydrolysis of NSP thereby aiding the colonisation of the gut with Lactobacilli (Vahjen *et al*., 1998). Proliferation of Lactobacilli is often associated with low digesta pH which may inhibit the growth of coliforms such as E. *coli* and as a result improve gut health (Pluske *et al*., 2001). Engberg *et al*. (2004) reported that supplemental xylanase reduced digesta pH in the gizzard and caecum of broilers and stimulated the growth of lactic acid bacteria in the small intestine of broilers receiving a wheat-based diet at 42 days of age. On the other hand, Rebole *et al*. (2010) and Jozefiak *et al*. (2007) reported that carbohydrase supplementation of a wheat-based diet had no effect on caecal digesta pH. In the current study, neither XAP nor phytase had an effect on digesta pH. It is possible that the difference in the effects of exogenous enzymes on digesta pH noted in the current study and that of Engberg *et al*. (2004) are due to differences in diet composition, enzyme type or activities or animals used. Nonetheless, there is need for more studies to understand more clearly the mechanisms by which exogenous enzymes may improve gut health of poultry.

There was largely no effect of wheat-DDGS inclusion or XAP or phytase on VFA concentrations in the current study except that, wheat-DDGS altered the fermentation pattern by reducing the concentration of n-butyric acid. In addition, the reduction in caecal digesta pH noted with the inclusion of wheat-DDGS was not complemented by a difference in caecal VFA concentrations between the birds receiving the diet not containing- or containing wheat-DDGS. The lack of a substantial effect of wheat-DDGS inclusion on VFA can hardly be expected as wheat-DDGS would have significantly increased dietary fibre intake. However, analysis for lactic acid concentration were not done in the current study; therefore, it is possible that the reduction in digesta pH noted in the caecum of birds receiving wheat-DDGS in their diet was due to an increase in the production of lactic acid. Wheat-DDGS was included in the finishing diets at the rate of 25% in the current study. At this inclusion level, there would have been an increase in the quantity of undigested soluble fibre reaching the caecum and an inherent increase the proliferation of fibre degrading microbes. It is possible that the lack of XAP effect on caecal VFA production in the current study was due to the high levels of highly fermentable fibre in the wheat-DDGS.

The jejunum is the major site of nutrient absorption in the small intestine of broilers; therefore the morphology of the jejunal absorptive surface may inform the efficiency of nutrient absorption. An increase in the ratio of villi height to crypt depth is an indication of an increase in jejunal absorptive surface or a reduction in cell turnover which corresponds with less energy used for gastrointestinal tract maintenance (Rebole *et al*. 2010). Phytate and NSP may cause atrophy of the villi or an increase in the size of the gastrointestinal tract (Jaroni *et al*. 1999) whereas phytase and XAP used individually or in combination may improve the jejunal absorptive surface by counteracting the antinutritional effects of phytate and NSP. There were no marked effects of wheat-DDGS or supplemental enzymes on the jejunal morphology of broilers in the current study. This suggests that the epithelial cells on the villi surface did not alter their capacity to assimilate nutrients to a change in diet composition or to the addition of exogenous XAP or phytase. Although supplemental XAP increased crypt depth, this observation is counter-intuitive because a decrease in crypt depth would have complemented the improvements in growth performance noted with XAP supplementation. Therefore, the trend for improvement in BWG and FBW and the improvement in G:F of the birds observed for supplemented XAP were not related to an improvement in the jejunal villi and crypt architecture.

The lack of significant effect of XAP or phytase supplementation on jejunal morphology in the current study may be due to the diets not containing sufficient levels of phytate or NSP to cause a significant negative effect to the jejunal absorptive structure. Previously, Mathlouthi *et al*. (2002) reported improvements in the gut morphology of broilers with xylanase supplementation of a rye-based diet. Unlike the current study where a wheat-based diet was used, the rye-based diet used in the Mathlouthi *et al*. (2002) study contained greater levels of soluble fibre which would have caused greater antinutritive effects. In other studies that used a wheat-based diet, the effect of supplemental xylanase or phytase on the gut morphology of broilers were variable. Yang *et al*. (2008) observed that supplemental xylanase did not affect jejunal villi height but reduced crypt depth of broilers receiving a wheat-SBM based diet at seven days of age whereas Wu *et al*. (2004) noted an increase in duodenal villi height but no effect on crypt depth in broilers at 21 days of age using supplemental phytase. Supplemental xylanase had no effect on gut morphology of broilers receiving a wheat-based diet in the study of Iji *et al*. (2001).

It is concluded that the addition of an admixture of XAP to a wheat-SBM based diet containing wheat-DDGS produced modest improvements in the growth performance of broilers, but phytase had no effect possibly because the diet contained more soluble fibre and

less phytate. The inclusion of wheat-DDGS in a wheat-SBM based diet for broilers has no negative effect on the jejunal absorptive structure but reduces digesta pH in the caecum.

CHAPTER 6

APPARENT- OR STANDARDISED ILEAL AMINO ACID DIGESTIBILITY RESPONSE TO DIETARY FIBRE TYPE AND CRUDE PROTEIN LEVEL FOR GROWING PIGS

6.1 INTRODUCTION

The nutritive value of wheat distillers dried grains with solubles (wheat-DDGS) for broilers and turkey were determined in Chapters 3, 4 and 5 of this thesis. In the case of pigs, the energy value and nutrient digestibility of maize- and wheat-DDGS has been described by Widyaratne and Ziljistra (2007) and Stein and Shurson (2009). However, the effects on ileal amino acids digestibility when common dietary protein sources such as soyabean meal (SBM) or canola meal (CM) are replaced with biofuel co-products such as maize- or wheat-DDGS is not known and is therefore addressed in the current chapter. Maize-DDGS was used in the current study because the study was conducted in the USA where maize-DDGS is more popular. A similar study using wheat-DDGS instead of maize-DDGS will be applicable to the UK and such studies may need to be conducted in the future. Nevertheless, the effects observed for maize-DDGS in the current study may be used as a possible indication of opportunities and limitations of using wheat-DDGS.

Fibre is found in different forms and quantities in feed ingredients. The most important fibre type is the non-cellulosic polysacharrides consisting of arabinoxylans and β-glucans that exert their anti-nutritive properties by increasing digesta viscosity. Insoluble dietary fibre such as lignin may act as a nutrient diluent, increase sloughing of intestinal surface or increase mucin production (Schneeman *et al*., 1982). Although, the non starch polysaccharides (NSP) found in cereals exert greater anti-nutritive effects compared with legumes and oil seeds, the contributions of NSP by legumes cannot be underestimated because the pig's diet may contain up to 50% legumes. The choice of feed ingredients used in non-ruminant animal diet is often driven by availability and cost. In cases where novel feed ingredients are being considered, the greater emphasis is also often placed on the protein and energy values of these ingredients and less on the impact that their associated fibre composition may have on nutrient digestibility. In the current study, SBM, CM and maize-DDGS were selected to determine the effect of protein-source-associated dietary fibre on ileal AA digestibility because these feed ingredients are currently the most popular protein sources used in the pigs diet and more importantly because their fibre characteristics are different.

Solvent extracted SBM may contain up to 48% crude protein (CP) and it is the most popular feed ingredient used as a source of protein in pig's diet. The average total dietary fibre content in SBM is 16.7% whereas the neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents are 8.2, 5.3 and 1.1%, respectively (NRC, 2012). It is very common to use CM in variable quantities as substitute to SBM in pig's diet. Canola meal

may contain up to 35% CP, but the average total dietary fibre (25.8%), NDF (23.8%), ADF (17.6%) and ADL (7.3%) contents in CM are greater than are present in SBM (NRC, 2012). Maize-DDGS is the co-product of bioethanol produced from maize grain and may contain up to 27% CP (Chapter 2). The use of maize-DDGS in pig diet is becoming more popular due to its increased availability and lower cost compared with SBM. The average total dietary fibre, NDF and ADF contents in maize-DDGS are 31.4, 32.5 and 11.8%, respectively (NRC, 2012) and these values are two to ten times greater than are present in SBM. Comparing the chemical composition of the three protein sources selected in the current study, it is obvious that SBM contain lower levels of both the soluble and insoluble fibre types, maize-DDGS contain greater levels of soluble fibre whereas CM contain greater levels of insoluble fibre compared with either SBM or maize-DDGS.

Excessive N excretion by pigs may be mitigated by reducing the protein content of the diet. A 2 to 4% reduction in dietary CP content reduced N excretion by 20% for finishing pigs (Lee *et al*., 2001). On the other hand, it also appears that pigs are able to compensate for the reduction in CP intake by increasing the efficiency of nutrient utilisation. Otto *et al*. (2003) reported an increase in ileal AA digestibility for growing pigs by decreasing the CP content in a practical maize-SBM diet from 15 to 6%. Reducing dietary CP is often done by wholly replacing or partially substituting SBM with feed ingredients that contain lower CP content. In most cases, the fibre content in such feed ingredients is greater and the types of the fibre they contain are also different. Therefore, the objective of the current study was to determine the effect of dietary fibre type and protein level on the apparent- or standardised ileal AA digestibility (AIAAD or SIAAD) for growing pigs. Interactions between dietary fibre type and CP level to affect AIAAD or SIAAD for growing pigs was also investigated.

6.2 MATERIALS AND METHODS

6.2.1 Animals and Management

All animal handling procedures were approved by the Purdue University Animal Care and Use Committee and the Animal Experimentation Committee of the Scotland's Rural College.

Twenty male pigs were obtained from the Animal Sciences Research and Education Centre of Purdue University each weighing approximately 25 kg. Pigs were fasted for 12 hours prior to the surgical procedure of fitting a T-cannula to the distal end of the ileum. The T-cannulas had an internal diameter of 1.3 cm, the wings were 2.5 cm wide and were 5 cm in length. The cannulation procedure was done under general anaesthesia. Comprehensive description of the

surgical procedure and post-operative care was as described by Dilger *et al*. (2004). All the pigs were conscious within a short time after the surgery and were allowed a 14 d recovery period.

6.2.2 Experimental Design, Dietary Treatments and Sample Collection

Twenty boars (Yorkshire × Landrace) with average initial bodyweight of 35 kg were used in the current study. The dietary treatments were three fibre types (SBM, CM or maize-DDGS) and two levels of protein (18 or 14%, respectively). In each period, two pigs having the bodyweights closest to the mean bodyweight of the twenty pigs were offered a nitrogen free diet to determine basal endogenous ileal amino acid flow. The remaining eighteen pigs were allocated to the experimental diets using a replicated 6 × 2 Youden square design. Chromic oxide was added to the diets at the rate of 5 g/kg to enable determination of AA digestibility by the index method. Daily feed allowance was divided into two equal portions and offered in the morning and evening (08:00 and 20:00, respectively). Pigs were given *ad libitum* access to water throughout the study. Each experimental period lasted for seven days consisting of five days of adaptation to the diets and two days of ileal digesta collection. Ileal digesta was collected for 12 hours on both days (d 6 and 7). Ileal digesta were collected in whirlpak® bags containing 10 ml of 10% formic acid and stored frozen (-20^{o}C) prior to further analyses. The pigs were housed individually in smooth-walled pens within a facility equipped with temperature, light, and humidity control during the study.

6.2.3 Chemical Analysis

Samples of the diets and ileal digesta were analysed for dry matter (DM), N, amino acids (AA) and chromium. Ileal digesta samples for AA analysis were freeze dried. Diet and ileal digesta samples were ground to pass through a 0.5 mm screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis. Dry matter content in the diets and ileal digesta was determined by drying samples at 100^{o}C for 24 hours. Nitrogen was determined by combustion method (AOAC International 2006, method 968.06). For AA analyses, samples were hydrolysed for 24 hours in 6N hydrochloric acid at 110^{o}C under an atmosphere of N. For Met and Cys, performic acid oxidation was carried out before acid hydrolysis. The AA in the hydrolysate were determined by HPLC after post-column derivatisation [(AOAC International 2000, method 982.30E (a, b, c)]. Chromium was determined using the inductively coupled plasma atomic emission spectroscopy method

following nitric/perchloric acids wet ash digestion (AOAC International 2000, method 990.08).

6.2.4 Calculations

Basal ileal AA flow was calculated using the following equation:

1. $\text{EAAF} = \text{AAo} \times \left(\frac{\text{Cri}}{\text{Cro}}\right)$

where EAAF is endogenous ileal AA flow (mg/kg of DM intake); AAo is the concentration of AA in ileal digesta; Cr_i is the concentration of chromium in diet (mg/kg); Cr_o is the concentration of the chromium in ileal digesta (mg/kg).

Apparent ileal AA digestibility (AIAAD) was calculated using the following equation:

2. $\text{AIAAD} = \left[1 - \left(\frac{\text{Cri}}{\text{Cro}}\right) \times \left(\frac{\text{AAo}}{\text{AAi}}\right)\right] \text{x } 100$

where AIAAD is apparent ileal amino acid digestibility (%); Cr_i is the concentration of chromium in diet (mg/kg); Cr_o is the concentration of the chromium in digesta (mg/kg); AA_o is the concentration of nutrient in the digesta (g/kg of DM) and AA_i is the concentration of nutrient in the diet (g/kg of DM).

Standardised ileal AA digestibility (SIAAD) was calculated using the following equation:

3. $\text{SIAAD} = \text{AIAAD} + \left[\left(\frac{\text{EAAF}}{\text{AAi}}\right) \text{x } 100\right]$

where SIAAD is standardised ileal AA digestibility (%); AIAAD is apparent ileal AA digestibility (%); EAAF is the endogenous basal ileal AA flow (g/kg of DM intake) and AA_i is the AA concentration in the diet (g/kg of DM).

6.2.5 Statistical Analysis

Data was analysed using the Generalised Linear Models of Genstat 11 program as a 6 × 2 Youden square design and least squares means were separated using the Tukey test with $P < 0.05$ indicating statistical significance. Interactions between dietary fibre type and crude protein level on AIAAD and SIAAD were also tested.

6.3 RESULTS

The ingredient and chemical compositions of the experimental diets used in the current study are presented in Table 6-1. The diets were isocaloric and isonitrogenous for the treatments containing adequate or reduced CP levels. Regardless of CP level, crude fibre, NDF, ADF and ADL were lowest in the SBM diet. On the other hand, the CM diet contained the greatest levels of ADF and ADL whereas NDF was greatest in the maize-DDGS diet.

Dry matter utilisation and the apparent ileal digestibility (AID) of N and indispensable AA for growing pigs receiving diets that differed in fibre composition and crude protein level are presented in Table 6-2. Corresponding AID for dispensable AA and total amino acids (TAA) are presented in Table 6-3. With the exception of Met, Trp, Cys and Pro, AIAAD generally decreased ($P < 0.05$) in the order SBM>maize-DDGS>CM diet in the current study. Ileal DM utilisation was greater ($P < 0.05$) in the SBM diet compared with either the CM diet or the maize-DDGS diet. With the exception of Met, Trp, Cys and Pro, AIAAD were greater ($P < 0.05$) for the SBM diet compared with the CM diet. Ileal DM utilisation and AID of Gly and Asp were greater ($P < 0.05$) for the SBM diet compared with the maize-DDGS diet. The AID of the following AA was greater in the maize-DDGS diet compared with the CM diet: Ile, Leu, Phe, Val, Ala, Tyr and Asp. The AID of TAA was greater ($P < 0.01$) for the SBM diet compared with the CM diet, but did not differ significantly from that of the maize-DDGS diet. There was fibre type × protein level interaction ($P < 0.05$) for the AID of Lys. This was because the AID of Lys was different ($P < 0.05$) amongst the CP-adequate dietary treatments, whereas the AID of Lys was not different amongst the dietary treatments marginal in CP.

The standardised ileal digestibility (SID) of N and indispensable AA for growing pigs receiving diets that differed in fibre composition and CP level are presented in Table 6-4. Corresponding SID for dispensable AA and TAA are presented in Table 6-5. With the exception of Trp and Pro, SIAAD was different amongst the dietary fibre sources with SIAAD generally greater in the SBM diet, intermediate in the maize-DDGS diet and lowest in the CM diet. Standardised ileal amino acid digestibility for the SBM diet were greater ($P < 0.05$) than those of the CM diet except for Trp and Pro, whereas Gly and Asp were more digestible ($P < 0.05$) in the SBM diet compared with the maize-DDGS diet. The SID of the following AA was greater for the maize-DDGS diet compared with the CM diet: Ile, Leu, Val, Ala, Tyr and Asp. The SID of TAA was greater ($P < 0.05$) for the SBM diet compared with the CM diet. On the other hand, SID of TAA was not different between the SBM and

maize-DDGS diet. Reducing the dietary protein level from 18 to 14 % did not affect ileal DM utilisation, AIAAD or SIAAD in the current study.

Table 6-1. Ingredient and chemical composition of experimental diets to determine the effect of dietary fibre type and crude protein level on apparent- or standardised ileal amino acids digestibility of growing pigs.

	Adequate CP			Reduced CP		
Ingredients, g/kg	Soyabean meal	Canola meal	DDGS	Soyabean meal	Canola meal	DDGS
Maize	670	584	429	771	728	632
Soybean meal	265	0	0	160	0	0
Canola meal	0	370	0	0	220	0
Maize-DDGS	0	0	510	0	0	300
Soybean oil	12	0	4	14	0	8
Limestone (38% Ca)	11	11	14	10	11	12
Monocalcium phosphate[1]	10	0	5	13	6	10
Others[2]	31	31	31	31	31	31
L-Lysine HCl	1	4	7	1	4	7
Calculated nutrients and energy						
Protein, g/kg	184	184	183	142	143	142
Metabolisable energy, MJ/kg	13.9	14.1	14.0	13.9	13.9	14.0
Digestible energy, MJ/kg	14.4	14.6	14.4	14.4	14.3	14.3
Calcium, g/kg	6.79	6.85	6.82	6.57	6.81	6.65
Total phosphorus g/kg	5.78	5.83	5.94	5.92	5.73	5.99
non-phytate P, g/kg	3.32	1.34	3.67	3.65	2.25	3.84
Crude fibre, g/kg	24.0	48.1	44.9	21.9	36.3	34.1
NDF, g/kg	84.6	143	207	85.2	120	157
ADF, g/kg	33.9	82.4	72.9	31.2	60.2	54.0
ADL, g/kg	5.12	29.0	14.7	4.29	18.5	9.92

[1]Contain 21% Ca and 18% P.

[2]Others: 3 g/kg of common salt, 1.5 g/kg of vitamin premix (contains per gram of premix: vitamin A, 2640 IU; vitamin D3, 264 IU; vitamin E, 17.6 IU; vitamin K activity, 2.4 mg; menadione, 880 μg; vitamin B12, 15.4 μg; riboflavin, 3.52 mg; D-pantothenic acid, 8.8 mg; niacin,13.2 mg), 1 g/kg of mineral premix (contains per gram of premix: Cu (as copper chloride), 9 mg; I (as Ethylenediamine Dihydroiodide (EDDI)), 0.36 mg; Fe (as ferrous carbonate), 194 mg; Mn (as manganese oxide), 17 mg; and Zn (as zinc oxide), 149 mg), 0.5 g/kg of selenium premix (supplied 300 μg of Se per kilogram of diet), 25 g/kg of chromic oxide premix (prepared as 1 g chromic oxide added to 4 g of cornstarch). DDGS; maize-distillers dried grains with solubles, NDF; neutral detergent fibre, ADF; acid detergent fibre, ADL; acid detergent lignin.

Table 6-2. Dry matter utilisation and apparent ileal digestibility (%) of nitrogen and indispensable amino acids for growing pigs in response to dietary fibre type and crude protein level.

	DM	N	Arg	His	Ile	Leu	Lys	Phe	Thr	Met	Trp	Val
Effect of fibre type												
Soyabean meal	67.9^{b}	74.2^{b}	86.3^{b}	83.3^{b}	79.7^{b}	82.3^{b}	82.2^{b}	81.6^{b}	69.7^{b}	83.4	79.4	74.1^{b}
Canola meal	56.5^{a}	62.0^{a}	79.4^{a}	75.0^{a}	66.8^{a}	73.1^{a}	72.0^{a}	71.3^{a}	56.4^{a}	77.4	74.6	62.6^{a}
Maize-DDGS	57.0^{a}	69.0^{ab}	81.4^{ab}	78.8^{ab}	74.6^{b}	82.3^{b}	77.4^{ab}	78.5^{b}	64.1^{ab}	81.5	72.4	71.4^{b}
s.e.d	4.63	3.66	2.35	2.62	3.00	2.63	2.54	2.89	4.00	2.15	3.29	3.33
Effect of crude protein level												
Adequate	60.2	70.2	83.6	79.7	74.3	79.8	76.7	77.7	65.0	82.2	77.3	70.3
Reduced (-4%)	61.0	66.8	81.3	78.5	73.4	78.9	78.0	76.8	62.1	79.4	73.5	68.8
s.e.d	3.75	2.96	1.90	2.12	2.43	2.13	2.06	2.29	3.24	2.04	2.67	2.70
P values for main effects and interaction												
Fibre type	0.030	0.009	0.017	0.013	<0.001	0.002	0.002	0.003	0.009	0.068	0.102	0.005
Protein level	0.823	0.261	0.227	0.578	0.699	0.678	0.532	0.697	0.389	0.190	0.170	0.573
Fibre type × protein level	0.461	0.233	0.208	0.267	0.388	0.379	0.045	0.391	0.321	0.813	0.333	0.359

DM; dry matter, Maize-DDGS; maize distillers dried grains with solubles, s.e.d; standard error of difference of means.
[a,b]Means within a column without a common superscript differ significantly ($P < 0.05$).

Table 6-3. Apparent ileal digestibility (%) of total- and dispensable amino acids for growing pigs in response to dietary fibre type and crude protein level.

	Ala	Cys	Glu	Gly	Pro	Ser	Tyr	Asp	TAA
Effect of fibre type									
Soyabean meal	75.9^{b}	73.2	84.8^{b}	69.1^{b}	67.1	76.9^{b}	79.9^{b}	77.4^{c}	77.9^{b}
Canola meal	67.4^{a}	64.0	78.3^{a}	58.1^{a}	64.6	63.8^{a}	69.3^{a}	60.4^{a}	68.2^{a}
Maize-DDGS	77.3^{b}	71.5	81.4^{ab}	59.1^{a}	74.1	71.3^{ab}	77.7^{b}	68.4^{b}	74.9^{ab}
s.e.d	3.08	3.87	2.44	3.56	4.55	3.28	2.87	3.56	3.21
Effect of crude protein level									
Adequate	74.3	70.1	82.1	64.1	71.7	71.5	76.5	69.2	74.7
Reduced (-4%)	73.0	69.4	81.1	60.2	65.5	70.2	75.1	68.8	73.0
s.e.d	2.50	3.13	1.98	2.82	3.69	2.66	2.33	2.89	2.60
P values for main effects and interaction									
Fibre type	0.007	0.057	0.041	0.006	0.111	0.002	0.002	<0.001	0.017
Protein level	0.582	0.838	0.644	0.182	0.106	0.625	0.548	0.911	0.529
Fibre type × protein level	0.331	0.492	0.442	0.586	0.652	0.220	0.264	0.233	0.223

Maize-DDGS; maize distillers dried grains with solubles, s.e.d; standard error of difference of means.
[a,b,c]Means within a column without a common superscript differ significantly ($P < 0.05$)

Table 6-4. Standardised ileal digestibility (%) of nitrogen and indispensable amino acids for growing pigs in response to dietary fibre type and crude protein level.

	N	Arg	His	Ile	Leu	Lys	Phe	Thr	Met	Trp	Val
Effect of fibre type											
Soyabean meal	81.2^{b}	90.4^{b}	86.3^{b}	83.2^{b}	84.9^{b}	86.3^{b}	84.7^{b}	76.4^{b}	87.8^{b}	83.9	79.5^{b}
Canola meal	68.8^{a}	83.7^{a}	77.8^{a}	70.5^{a}	75.8^{a}	75.4^{a}	74.6^{a}	62.4^{a}	79.5^{a}	79.3	67.5^{a}
Maize-DDGS	76.0^{ab}	86.4^{ab}	81.6^{ab}	78.3^{b}	84.3^{b}	80.9^{ab}	81.5^{ab}	70.6^{ab}	84.5^{ab}	78.4	76.3^{b}
s.e.d	3.66	2.35	2.62	3.00	2.63	2.54	2.89	4.00	2.51	3.29	3.33
Effect of crude protein level											
Adequate	76.4	87.6	82.3	77.6	82.1	80.2	80.6	70.7	83.9	81.8	74.9
Reduced (-4%)	74.6	86.20	81.7	77.4	81.6	81.9	80.3	69.3	81.6	79.3	74.4
s.e.d	2.96	1.90	2.12	2.43	2.13	2.06	2.29	3.24	2.04	2.67	2.70
P values for main effects and interaction											
Fibre type	0.008	0.026	0.010	<0.001	0.002	<0.001	0.004	0.006	0.047	0.205	0.004
Protein level	0.561	0.482	0.764	0.940	0.807	0.397	0.886	0.673	0.253	0.363	0.866
Fibre type × protein level	0.225	0.191	0.258	0.359	0.371	0.055	0.375	0.314	0.760	0.315	0.333

Maize-DDGS; maize distillers dried grains with solubles, s.e.d; standard error of difference of means.
[a,b]Means within a column without a common superscript differ significantly ($P < 0.05$).

Table 6-5. Standardised ileal digestibility (%) of total- and dispensable amino acids for growing pigs in response to dietary fibre type and crude protein level.

	Ala	Cys	Glu	Gly	Pro	Ser	Tyr	Asp	TAA
Effect of fibre type									
Soyabean meal	80.9[b]	77.6[b]	87.2[b]	85.0[b]	87.4	82.3[b]	83.6[b]	81.4[c]	84.3[b]
Canola meal	72.2[a]	67.2[a]	80.6[a]	71.5[a]	82.3	69.5[a]	73.1[a]	65.6[a]	74.4[a]
Maize-DDGS	80.9[b]	75.1[ab]	83.8[ab]	74.1[a]	89.6	77.0[ab]	81.0[b]	73.7[b]	80.9[ab]
s.e.d	3.08	3.87	2.44	3.49	4.55	3.28	2.87	3.56	3.21
Effect of crude protein level									
Adequate	78.5	73.5	84.2	77.3	88.2	76.6	79.8	73.4	80.3
Reduced (-4%)	77.8	73.5	83.7	76.8	84.8	76.3	79.0	74.2	79.8
s.e.d	2.50	3.13	1.98	2.82	3.69	2.66	2.33	2.89	2.60
P values for main effects and interaction									
Fibre type	0.011	0.031	0.039	0.001	0.274	0.002	0.003	<0.001	0.015
Protein level	0.772	0.993	0.793	0.869	0.370	0.923	0.738	0.807	0.845
Fibre type × protein level	0.315	0.437	0.433	0.595	0.629	0.226	0.271	0.218	0.210

Maize-DDGS; Maize distillers dried grains with solubles, s.e.d; standard error of difference of means.
[a,b]Means within a column without a common superscript differ significantly ($P < 0.05$)

6.4 DISCUSSION

The objective of the current study was to determine the effect of dietary fibre type and CP level on ileal AA digestibility in growing pigs.

Dietary fibre consists of the structural (cellulose, hemicellulose, and pectin) and non-structural (gums and mucilages) polysaccharides and lignin. These fibre types may also be categorised based on their solubility in water into either soluble (hemicellulose) or insoluble (cellulose and lignin). Cellulose, hemicellulose, pectin and lignin are the main dietary fibre types in feed ingredients used in swine diet and the physical and chemical characteristics of these fibres are different. For this reason, the digestibility of dietary fibre as well as the effects of the different fibre types on the digestibility of other dietary components often varies. Soyabean meal, CM and maize-DDGS were selected to determine the effect of dietary fibre type on ileal AA digestibility in the current study because these feed ingredients are the most commonly used as protein sources in pigs diet and more importantly, their fibre characteristics are different.

Neutral detergent fibre consists of cellulose, hemicellulose and lignin. The hemicellulose fraction in NDF are highly water soluble and when ingested they may cause an increase in digesta viscosity in the gastrointestinal tract, reduce the rate of digesta transit or cause a reduction in nutrient absorption by encapsulation of nutrients and digestive enzymes in a gel matrix. Excessive levels of dietary soluble fibre may compromise protein and AA digestibility in the gastrointestinal tract by increasing digesta viscosity which may lead to a reduction in the mixing of digesta, a reduction in contact between proteases and dietary protein or a reduction in contact between the absorptive surface and digesta (Choct *et al*., 2004). Acid detergent fibre consists mainly of cellulose and lignin whereas ADL consist almost entirely of lignin. Both ADF and ADL are insoluble in water and poorly digested by non-ruminant animals. Dietary ADF and ADL may increase digesta transit in the gastrointestinal tract or form insoluble bonds with dietary nutrients and in the process making them unavailable for absorption or decrease DM utilisation (Wilfart *et al*., 2007).

It is common to use purified cellulose, pectin, straws, hulls or sugar beet pulp to modify the fibre composition of the diet when determining the effect of fibre on nutrient digestibility (Zervas and Zijlstra, 2002; Zhang *et al*., 2013). However it is often the case that there is a disproportionate increase in the concentration of a type of fibre (soluble or insoluble) which is not characteristic of changes that occur when conventional feed ingredients are being used.

Also, because supplemental fibre sources such as purified cellulose are not chemically integrated with the feed ingredients in the diet, it is less likely that purified cellulose will impair access of digestive enzymes to dietary nutrients compared with what may be expected from dietary fibre that are chemically bound to other nutrients in feed ingredients.

Calculated hemicellulose content (NDF minus ADF) was 5.1, 6.1 and 13.4% in the CP-adequate SBM, CM and maize-DDGS diets, respectively in the current study. Corresponding values in the reduced-CP diets were 5.4, 6.0 and 10.3, respectively. On the other hand, ADF contents in the SBM, CM and maize-DDGS diets were 3.4, 8.2, and 7.3% in the CP-adequate diets or 3.1, 6.0 and 5.4% in the reduced-CP diets, respectively. These observations indicate that the soluble fibre content in a diet formulated using maize-DDGS as protein source is approximately two-three times greater compared with using SBM and there was approximately a 100% increase in the level of insoluble fibre when CM or maize-DDGS are used as protein sources compared with using SBM. The differences in the soluble and insoluble fibre contents in the experimental diets used in the current study indicate that the choice of protein source/s used in the pig diet influences its fibre characteristics which in turn, may affect AA digestibility.

Because the SBM diet contained the lowest levels of both the soluble and insoluble fibre types in the current study, it may be expected that the AIAAD and SIAAD will be greater compared with either the maize-DDGS or CM diets. It was noted that about half of the AA in the maize-DDGS were more digestible compared with the CM diet whereas, AIAAD and SIAAD were generally similar between the maize-DDGS diet and the SBM diet. The insoluble fibre content (ADL) was six times greater in the CM diet compared with the SBM diet or two times greater compared with the maize-DDGS diet. Ileal DM utilisation was greater in the SBM diet compared with either the maize-DDGS or CM diet. The lower ileal DM utilisation observed for the diets containing greater levels of dietary fibre (CM and maize-DDGS) in the current study is consistent with reports that increased fibre levels reduces ileal DM utilisation in pigs (Lenis *et al*., 1996; Zhang *et al*., 2013).

As earlier mentioned, NSP may reduce AA digestibility by formation of a gel causing an increase in digesta viscosity or a reduction in nutrient absorption by encapsulation of AA and digestive enzymes within the gel medium (Choct *et al*., 2004). Increased levels of dietary soluble fibre levels have been reported to reduce palatability and voluntary feed intake (Zhang *et al*., 2013), increase endogenous N and AA flow (Lenis *et al*., 1996), decrease energy utilisation (Sauer *et al*., 1991) and protein and AA digestibility (Dierick *et al*., 1983;

Mosenthin *et al*., 1994; Zervas and Zijlstra, 2002) for growing pigs. Because the calculated hemicellulose levels in the SBM and CM diet were similar in the current study, then it is more likely that the inferior AIAAD or SIAAD observed in the CM diet compared with the SBM diet was due to the anti-nutritive properties associated with the greater levels of insoluble fibre in the CM diet. On the other hand, it is surprising that there were minimal differences between the AIAAD and SIAAD of the SBM and maize-DDGS diets considering that the latter contained greater levels of soluble fibre.

In the review by Stein and Shurson (2009), it was noted that the inclusion of maize-DDGS in pig's diet may not necessarily compromise energy utilisation and nutrient digestibility in spite of its high fibre content. This may be due to the fact that during bioethanol production, it is common to treat the maize grain with fibre degrading enzymes to break down structural carbohydrates into simple sugars in order to increase ethanol yield. Polizeli *et al*. (2005) noted that because the structure of hemicelluloses is heterogeneous, a complete hydrolysis of hemicellulose is hardly achievable and may require a multi-enzyme complex containing a broad variety of enzyme activities. The molecular structure of a xylan for example, in terms of the chain length and the degree of branching, may affect the efficacy of a hemicellulase complex that may lead to the production of intermediate products such as β-D-xylopyranosyl oligomers (Polizeli *et al*., 2005). It is therefore speculated that although the soluble fibre levels in the maize-DDGS diet was greater compared with that of the SBM diet in the current study, a significant proportion of the fibre in the maize-DDGS diet may have been present as oligosaccharides that are less able to cause the anti-nutritive effects characteristic of arabinoxylans or β-glucans.

Feed ingredients used in pig's diet are heat treated for a number of reasons, two of which are 1) to reduce the moisture content which improves the palatability and shelf life of the feed ingredient, 2) to reduce the concentration of anti-nutrients such as glucosinolates and protease inhibitors in the feed ingredient. Excessive heat treatment causes a Malliard reaction that reduces the digestibility of AA in feed ingredients. There was no information about the level of heat treatment for the three protein sources used in the current study, however, because the AID and SID of Lys and Cys (the most susceptible AA to heat damage) were not comparably low in the dietary treatments in the current study, it is may be plausible to rule out the differences in AIAAD or SIAAD to the effects of heat treatment.

The majority of cereal grains and oil seeds used in non-ruminant diets contain phytate at levels that range between 1 to 5% (Cheryan, 1980). Phytate is poorly utilised by non-

ruminants and as a result, nutrients bound to phytate are not available to the animal (Selle *et al*. 2000). Phytate may negatively affect the digestibility of AA by forming complexes with AA, therefore making them unavailable for utilisation or it may increase mucin production potentially increasing endogenous AA losses or compromise the intestinal absorption of AA by binding de novo to AA (Selle and Ravindran, 2008). Selle *et al*. (2000) observed that phytate may bind to the α-NH2 groups and side groups of basic AA (Arg, His, and Lys) and as a result, may reduce the digestibility of these AA. The AID and SID of Arg, His and Lys were amongst those that were significantly greater in the SBM and maize-DDGS diets compared with the CM diet in the current study. This may suggest that the CM diet contained greater levels of phytate compared with the SBM or maize-DDGS diets, which may be responsible in part, for the lower AA digestibility noted for the CM diet. It could be expected that the maize-DDGS diet would contain low levels of phytate because the fermentation process involved in the production of maize-DDGS hydrolyses a large proportion of the phytate in the maize grain by the actions of yeast phytase (Liu, 2011). However, there is an understanding that processes such as heat and enzyme treatment may also affect the phytate content in the SBM, CM and maize-DDGS and that these feed ingredients were incorporated in a mixed diet containing maize in the current study.

The nutritional gain from AA degraded in the large intestine of pigs is not significant (Sauer *et al*., 1991), for this reason; ileal measurements are more accurate to define the AA digestibility of a diet. Because excessive loss of N in pig manure is detrimental to the environment, interventions that do not affect ileal AA digestibility but may reduce undigested protein flow to the large intestine with a view to reduce protein excretion are of particular interest. In the current study, reducing the CP level of the diet by 4 percent did not affect ileal AA digestibility. Therefore, it can be inferred that pigs are able to utilize AA to the same efficiency even though the diet was marginal in CP. Otto *et al*. (2003) on the other hand, reported an increase in ileal AA digestibility in growing pigs receiving a maize-SBM diet containing 6% CP. The difference between the observation in the current study and that of Otto *et al*. (2003) may be due to the fact that in the latter there was a much greater reduction in dietary CP level which may have triggered a greater response from the pigs in the order to meet nutrient requirement.

Except for the AID of Lys, there was no interaction between dietary fibre type and CP level to affect ileal AA digestibility in the current study. The interaction observed for the AID of Lys was due to a reduction in the AID of Lys from 85% in the CP-adequate SBM diet to 79% in the reduced-CP SBM diet. Notably, reducing the CP level in the SBM diet by 4% reduced the

Lys content from 1.03 to 0.75% but caused minimal changes to dietary fibre levels in the current study. It is possible therefore that the increase in dietary fibre content relative to Lys composition in the reduced-CP SBM diet was responsible for the reduction in the AID of Lys. However, in the study of Htoo *et al*. (2007), a reduction in dietary CP from 24 to 20% decreased the AID of most AA except Lys, Met, Thr, Val and Pro. The difference between the results noted in the current study and that of Htoo *et al*. (2007) may be due to differences in diet composition and dietary CP levels used. Changes in the small intestinal absorptive structure of pigs to receiving diets marginal in dietary protein may help to understand how pigs respond to a reduction in dietary CP levels.

It was concluded that the level and type of fibre in protein feed ingredients affects AA digestibility for growing pigs. The use of either SBM or maize-DDGS as protein source in the growing pig diet produced similar ileal AA digestibility but CM was inferior to both SBM and maize-DDGS. In addition, reducing dietary protein level from 18 to 14% does not affect ileal AA digestibility for growing pigs.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 GENERAL DISCUSSION

A review of the literature indicates that the chemical compositions of bioethanol co-products such as maize- and wheat-DDGS vary among sources. Chapter 2 of this thesis evaluated the possibility to use mathematical models to predict the amino acid (AA) composition of maize- and wheat-DDGS from their chemical compositions. Chapter 3, 4 and 5 in this thesis described in detail the nutritional value of wheat-DDGS as a feed ingredient for poultry. In the UK in particular, greater quantities of wheat-DDGS are available as feed ingredients for poultry but unlike maize-DDGS, there are comparatively no data available about the metabolisable energy (ME) value and nutrient digestibility of wheat-DDGS for poultry. It is common to use exogenous enzymes to improve the nutritional value of feed ingredients or the entire diet for poultry. The studies reported in this thesis reported the importance of exogenous enzymes on the nutrient digestibility, growth performance and gastrointestinal tract characteristics of bird receiving diets containing wheat-DDGS. The use of DDGS in the pig diet is becoming more common and majority of the studies in this area have described its nutritional value and optimum inclusion rates for pigs. Because the physical and chemical characteristics of dietary fibre may affect the digestibility of nutrients in the diet, Chapter 6 of this thesis determined if the choice of protein source, including maize-DDGS has an effect on AA digestibility in pigs.

One of the limitations of DDGS as a feed ingredient for poultry is the wide variability in its chemical composition among sources. The variability in DDGS composition is due to a wide variety of factors with the most important factor being the differences in processing techniques among sources. It is however impracticable and expensive to determine the chemical composition of every single DDGS before it is used in the diet. The result from Chapter 2 in this dissertation showed that prediction models are a useful tool in predicting the AA contents of maize- and wheat-DDGS. A compilation of data from a wide range of sources in the aforementioned study also helped to describe in full the wide variability and the relationship between the chemical components in maize- and wheat-DDGS. Even though the variability in the chemical composition of DDGS among sources has been widely reported in the literature, this study was the first to develop prediction models that are useful to determine their AA contents. It is expected that this mathematical models will be useful to feed nutritionists when formulating diets containing maize- or wheat-DDGS for both non-ruminants and ruminants.

When formulating diets for poultry, it is essential to ensure that dietary nutrients are provided at optimum levels because either a deficiency or excessive supply of nutrients may compromise bird performance. For this reason, the energy value, AA digestibility and P utilisation of feed ingredients are needed when formulating diets for broilers and turkey. The energy value and nutrient digestibility of maize-DDGS for poultry has been studied and is well defined mainly because maize grains are more readily available in the USA and because bioethanol production in the USA is older than in the UK. Wheat is used for bioethanol production in the UK and the nutritive value of wheat-DDGS is not known in spite of the possibility to use this co-product as a feed ingredient for poultry.

The result in Chapter 3 and 4 in this dissertation indicated that the ME in wheat-DDGS is comparable to that of wheat grain and that wheat-DDGS is a good source of digestible P for broilers and turkey. These observations are important because wheat-DDGS may be used as an alternative to wheat grain as source of energy for poultry especially in cases where the demand for wheat as a feedstock for bioethanol production reduces the quantity available for poultry. In addition, the inclusion of wheat-DDGS in poultry diets may reduce the level of inorganic P sources needed to be used in the diet which in turn will reduce feed cost.

One of the factors that make DDGS an attractive feed ingredient for poultry is its greater protein and AA content compared with maize/wheat grain. On the downside, the crude protein (CP) and AA content and digestibility of DDGS for poultry may vary among sources due to the negative effects of some production processes including heat treatment. Nonetheless, little is known about the apparent- or standardised ileal AA digestibility (AIAAD or SIAAD, respectively) of wheat-DDGS for broilers and turkey. The results in Chapter 3 and 4 in this dissertation showed that the AIAAD and SIAAD of wheat-DDGS for broilers and turkey are variable and nil for Lys. These are important data that show the need for supplemental AA when formulating diets containing wheat-DDGS for broilers and turkey.

The growth performance of poultry in response to a particular diet attracts the greatest commercial and industry interest. Efforts to determine the optimal inclusion rates of wheat-DDGS for broilers and turkey can be found in the literature. However, the majority of studies in the literature have used total nutrient values, although it is accepted that diet formulation that is based on digestible nutrient values best supports growth performance and minimises nutrient losses in manure. The results reported in Chapter 5 of this dissertation showed that the inclusion of up to 25% wheat-DDGS in broiler diet produced similar growth performance compared with birds receiving no wheat-DDGS in their diet when the diets are formulated

using metabolisable energy and digestible nutrient values of all feed ingredients. The studies in this dissertation helped to show that wheat-DDGS can be used in the diet of broilers at up to 25% inclusion rate.

The use of exogenous enzymes to improve the nutritional value of a feed ingredient or the entire diet for poultry is commonplace and has been well studied. Carbohydrases in particular, may be effective at improving the nutritional value of DDGS for poultry due to its high soluble fibre content relative to the cereal grain. The studies in Chapter 3 and 4 of this dissertation were the first in the literature to evaluate the improvements in metabolisable energy value, AA digestibility and P utilisation of wheat-DDGS using exogenous enzymes for broilers and turkey. The results from the aforementioned studies indicated that a mixture of carbohydrases and protease will improve the metabolisable energy in wheat-DDGS whereas protease will improve the AIAAD and SIAAD of wheat-DDGS for broilers and turkey. This connotes that broilers receiving wheat-DDGS in their diets will derive greater benefits from supplementation of carbohydrases and proteases.

The study in Chapter 5 in this dissertation evaluated the improvement in growth performance and gastrointestinal tract characteristics of broilers in response to supplemental carbohydrases, protease and phytase. A mixture of carbohydrases and protease improved the growth performance of broilers in this study and this result corroborated the improvement reported for the energy value and AA digestibility of wheat-DDGS using the same enzymes in this dissertation. These have important production and environmental significance because improvements in nutrient utilisation or growth performance using exogenous enzymes are often associated with a reduction in feed cost or a reduction in nutrient losses in manure.

The effect of exogenous enzymes on gastrointestinal tract health of poultry is not well understood. It is realistic to expect that ameliorating the anti-nutritive properties of a feed ingredient/diet may lead to improvements in the absorptive structure of the gastrointestinal tract, but the mechanisms involved may not be as straight forward. More work is needed to ascertain the relationship between anti-nutrients and intestinal morphology in broilers and equate the improvements in nutrient digestibility due to exogenous enzymes to changes in small intestinal morphology.

The effect of dietary fibre on nutrient digestibility for pigs has been well studied, reported in the literature and reasonably understood. In spite of the progress in this area, the effect of dietary fibre associated with protein source has been completely overlooked whereas the main

focus has been on the fibre associated with cereal grains. The possible effect of protein-source dietary fibre type cannot be over-emphasized because the fibre characteristics of protein feed ingredients used in pig diets are different and because these feed ingredients often make up approximately 50% of the diet. Evaluation of the effect of dietary fibre associated with protein feedstuffs on AA digestibility may help to clarify some of the differences in growth performance when different protein sources are used in the pig's diet.

Due to the increase in the availability of maize-DDGS, this co-product is increasingly being used to partially replace soyabean meal (SBM) or used in the place of canola meal (CM) in the pig diet. The results from Chapter 6 in this dissertation indicated that ileal AA digestibility was similar when using either SBM or maize-DDGS in growing pig's diet. This observation adds value to the fact that maize-DDGS is a viable feed ingredient for pigs. However, it is necessary to determine the effect on ileal AA digestibility when wheat-DDGS is used to substitute SBM in the pig's diet, considering that wheat-DDGS may contain greater levels of fibre compared with maize-DDGS. In addition, a similar study that evaluates the effect on ileal AA digestibility when maize- or wheat-DDGS are included in poultry diet is required.

It is desirable to reduce the quantity of nutrients lost in pig manure due to its negative implications on the environment. A good strategy will be one that does not compromise pig performance but at the same time reduces nutrient loss in manure. The results in Chapter 6 in this dissertation showed that reducing the level of dietary CP by 4% does not affect ileal AA digestibility. Whilst, it might be necessary to conduct a more robust study that determines the effect of reducing dietary CP level on energy utilisation, nutrient retention and excretion to reach a firm conclusion, the results in Chapter 6 takes a step forward in understanding the strategies that may be used to mitigate nutrient loss by pigs.

7.2 CONCLUSIONS AND RECOMMENDATIONS

From the results of studies reported in this dissertation it is collectively concluded or recommended that:

1. Prediction models can be used to determine the AA content of maize- and wheat-DDGS from their chemical composition with reasonable accuracy.
2. Maize- or wheat-DDGS can be used as feed ingredients for poultry and pigs because of their comparatively similar energy value or nutrient digestibility compared with conventional feed ingredients such as wheat and SBM.
3. Wheat-DDGS is an exceptionally good source of digestible P for broilers and turkey.

4. The effect of exogenous enzymes on nutrient digestibility, the morphology of the small intestinal absorptive structure and gastrointestinal tract characteristics of poultry is not consistent and requires further investigation.
5. The effects of reducing dietary CP level on the morphology of the small intestinal absorptive structure of pigs may improve understanding about how pigs respond to a CP-marginal diet.
6. The use of wheat-DDGS as a low cost alternative for wheat, maize and inorganic P sources for poultry may reduce feed cost and may also reduce competition between poutltry and bioethanol for wheat.
7. The use of exogenous enzymes in diets containing DDGS will increase the nutritive value and limit nutrient loss in manure.
8. The use of wheat-DDGS as a source of protein for poultry will reduce dependency on SBM import and consequentially improve sustainability.

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