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The nutritional value of biofuel co-products for poultry

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CONTENTS

1. Abstract

A total of eight experiments were used to provide data about the nutritional value of wheat-distillers' dried grains with solubles (DDGS) produced in the UK as a feedstuff for broilers and turkey. In the first study, 499 data sets were compiled and used to develop regression models to predict the amino acid (AA) contents of maize- and wheat-DDGS from their crude protein (CP) contents because of the variability in the chemical composition of DDGS among sources. Using a validation exercise, it was noted that the AA contents of maize- and wheat-DDGS can be predicted with reasonable accuracy using the mathematical equations generated in this study. Using a total of six experiments, the apparent metabolisable energy (AME), ileal amino acid (AA) digestibility and phosphorus (P) digestibility in wheat-DDGS without- or with exogenous enzymes was determined for broilers and turkeys.

The AME content in wheat-DDGS without or with enzymes were 15.0 or 15.5 MJ/kg, respectively, for broilers and 14.0 or 14.4 MJ/kg, respectively, for turkey. Nitrogen corrected-AME (AME_n) without or with enzymes were 14.0 or 14.5 MJ/kg, respectively, for broilers; 13.0 and 13.5 MJ/kg, respectively, for turkey. For broilers, apparent ileal AA digestibility (AIAAD) ranged from 35% (alanine (Ala)) to 75% (proline (Pro)) without protease, whereas the range was 42% (threonine (Thr)) to 82% (Pro) with protease supplementation. Supplemental protease improved ($P < 0.05$) the AIAAD of arginine (Arg) and Pro. Standardised ileal amino acids digestibility (SIAAD) ranged from 43% (aspartic acid (Asp)) to 84% (Pro), whereas the range was from 54% (Asp) to 93% (Pro) with added protease. Protease addition improved ($P < 0.05$) the SIAAD of Arg, leucine (Leu), phenylalanine (Phe), methionine (Met), valine (Val), and Pro. For turkey, AIAAD was lower than 50% for all AA except for glutamic acid (Glu) (70%) and Pro (81%) without protease whereas, SIAAD ranged from 41% (Thr) to 89% (Pro) without protease; and 56% (Arg) to 88% (Pro) with protease. Protease improved ($P < 0.05$) the AIAAD and SIAAD (except cystine (Cys) and Pro) from between 5 to 19 percentage points. Phosphorus digestibility (ileal) in wheat-DDGS was 94 or 96% without- or with phytase, respectively, for broilers or 76% or 82%, respectively, for turkey.

On the other hand, total tract phosphorus retention was 92% or 94%, respectively, for broilers and 71% or 81.6%, respectively, for turkeys. Phytase did not improve phosphorus digestibility or retention of wheat-DDGS for broilers and turkeys. The effect of enzyme supplementation on growth performance and gastrointestinal tract (GIT) characteristics of broilers receiving a wheat-soyabean meal (wheat-SBM) based diet containing up to 25% wheat-DDGS, was determined in a final experiment. Supplementation of a mixture of carbohydrases and protease modestly improved the growth performance of broilers from day (d) 1 to 42 but phytase had no effect. In addition, enzyme supplementation had no marked effect on jejunal villi height and crypt depth, intestinal pH or caecal volatile fatty acids production in broilers.

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 metabolisable energy (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.

2. 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 feedstuff for poultry. Although co-products of ethanol production from beverage-ethanol facilities have been available for decades, they have usually been used for feeding ruminants. The anticipated increase in DDGS availability, coupled with its high crude protein (CP) and non phytate P content (Nyachoti *et al*., 2005; Thacker and Widyaratne, 2007) relative to conventional protein feedstuffs, there is possibility that DDGS will be a viable feedstuff for poultry. So far, the preponderance of published literature (mainly from North America) 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 for poultry in the UK, data about its nutritional value for broilers and turkey is essential.

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.

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. 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) content in feedstuffs based on their chemical compositions.

The specific objectives in the current project were;

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

2. Evaluate the energy value and apparent, true or standardised 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

3. Materials and methods

3.1. Experiment 1

3.1.1. Prediction models

Nutrient composition data of 499 samples of maize- and wheat-DDGS were compiled from 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, total indispensable amino acids (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 C_p 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 C_p 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 C_p 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 C_p value (or ideally with C_p 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.

3.2. Experiments 2, 3, 4, 5, 6, 7

3.2.1. Nutritional value of wheat-DDGS for broiler and turkey

The wheat-DDGS used in the current project was produced by a bioethanol plant in the UK. The chemical composition of the wheat-DDGS is presented in Table 1.

Metabolisable energy content – Experiment 2 & 5

A total of 126 male Ross 308 broiler (Experiment 2) or 126 BUT 10 male turkey poults (Experiment 5) were fed a nutrient-adequate starter diet from day 1 to 14. On day 14, the birds were assigned to 6 dietary treatments consisting of 3 levels of wheat-DDGS (0, 300, or 600 g/kg) and 2 levels of an enzyme mixture containing xylanase, amylase and protease activities (XAP) (0 or 250 mg/kg of diet) in a randomised complete block design with 7 replicate pens and 3 birds per replicate pen. The diets were fed for 7 d. The ingredient and chemical compositions of the experimental diets used in both experiments are shown in Table 2. Energy-yielding ingredients in the wheat-soybean meal reference diet were replaced by wheat-DDGS in a way that their ratios were the same across all the diets. Grab excreta samples were collected from each pen from d 18 to 20 to determine the AME of the diet by the index method. The apparent metabolisable energy (AME) or nitrogen corrected-AME (AME_n) of wheat-DDGS was determined from the slope of regression of wheat-DDGS-associated energy intake (MJ) against wheat-DDGS intake (kg).

Phosphorus digestibility and retention – Experiment 3 & 6

A total of 126 male Ross 308 broiler (Experiment 3) or 126 BUT 10 male turkey poults (Experiment 6) were offered a nutrient-adequate diet from d 1 to 14. On day 14, birds in each experiment were allocated to six treatments consisting of three levels of wheat-DDGS (200, 400, or 600 g/kg) and two levels of phytase (0 or 1000 U/kg) in a randomised complete block design. The ingredient and chemical compositions of the experimental diets used in both experiments are shown in Table 3. True P digestibility or retention of wheat-DDGS was determined from the regression of P output (g/kg DM intake) against P intake (g/kg DM). In both experiments, total excreta was collected daily from each cage for 3 d (d 18 to 20), dried and pooled for each cage prior to analysis. Ileal digesta were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction by flushing with distilled water on d 21 and pooled for each cage.

Amino acids digestibility – Experiment 4 & 7

A total of 84 Ross 308 male broiler chicks (Experiment 4) or 84 male BUT 10 turkey poults (Experiment 7) were used for the determination of the apparent ileal amino acids digestibility (AIAAD) and standardised ileal amino acids digestibility (SIAAD) of wheat-DDGS. Four diets were

used in each of the two experiments. The diets consisted of two nitrogen-free diets (without or with protease) and two assay diets (without or with protease). Wheat-DDGS was the only source of AA in the assay diets. Basal ileal endogenous amino acids flow of birds offered the nitrogen free diets was used to calculate SIAAD from AIAAD. Experimental diets were offered from d 23 to 28 in both experiments. The ingredient and chemical compositions of the experimental diets used in both experiments are shown in Table 4. Ileal digesta were collected from the Meckel's diverticulum to approximately 1 cm proximal to the ileo-caecal junction by flushing with distilled water on d 28. Ileal digesta samples were pooled per cage.

3.3. Experiment 8

3.3.1. Growth performance and gastrointestinal tract characteristics of broilers offered diets containing wheat-DDGS

The AME, SIAAD and P digestibility values determined in experiments 2, 3 and 4 were used to formulate diets for broilers to determine growth performance response and gastrointestinal tract characteristics. A total of 288 male Ross 308 broiler chicks were used in the current 42-d study. On day 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 finisher (d 25 to 42), respectively, in order to account for the changing nutrient requirements of the bird. Birds had *ad libitum* access to the experimental diets and water throughout the study. The diets were provided in mash form.

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 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, grower or finisher phases. The ingredient and chemical composition of the PC and NC diets are presented in Tables 5, 6 and 7 for the starter, grower and finishing periods, respectively.

3.4. Chemical analysis

Where necessary, diet, wheat-DDGS, ileal digesta and excreta samples were analysed for gross energy (GE), dry matter (DM), titanium (Ti), nitrogen (N), AA and P. Except for the ileal digesta samples for AA analysis that were lyophilised, 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 high performance liquid chromatography (HPLC) after post-column derivatisation [(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 1. 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)	4,422
Crude fibre	80.0
Ether extract	72.5
Neutral detergent fibre	389
Acid detergent fibre	223
Ash	46.0
Calcium	1.10
Phosphorus	6.50
Potassium	11.3
Sodium	5.20
Amino acids	
Alanine	14.0
Arginine	11.8
Aspartic	18.3
Cystine	5.90
Glutamic acid	84.9
Glycine	14.9
Histidine	8.30
Isoleucine	13.7
Leucine	22.6
Lysine	7.70
Methionine	4.50
Phenylalanine	15.8
Proline	30.2
Serine	17.0
Threonine	11.5
Tyrosine	10.2
Tryptophan	3.80
Valine	16.2

Table 2. Ingredient and analysed nutrient composition of experimental diets to determine apparent metabolisable energy content of wheat distillers' dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease for broilers and turkey.

	Broilers			Turkey		
Item	0	300	600	0	300	600
Wheat	561	385.2	209.2	484.5	328.9	173.5
Soybean meal (48% CP)	291.2	199.9	108.6	340	230.9	121.7
Soybean oil	54.2	37.2	20.2	30	20.4	10.7
Gluten meal[1]	31.6	15.7	0	58	32.3	6.6
Wheat-DDGS	0	300	600	0	300	600
Limestone (38% Ca)	18.5	18.5	18.5	13	13	13
Dicalcium phosphate[2]	14	14	14	35	35	35
Common salt	1	1	1	3	3	3
Vitamin/mineral premix[3]	3	3	3	4	4	4
DL-methionine	1	1	1	1.5	1.5	1.5
L-lysine HCl	2.5	2.5	2.5	6	6	6
Marker premix[4]	15	15	15	15	15	15
XAP premix	7	7	7	10	10	10
Total	1000	1000	1000	1000	1000	1000
Analysed energy and nutrient composition[5]						
Dry matter, g/kg	880	880	870	883	883	874
Gross energy, MJ/kg	4,143	4,265	4,262	4,001	4,078	4,195
CP (N x 6.25), g/kg	226	256	276	258	277	293

[1]XAP premix replaced gluten meal at 7 g/kg.

[2]Contains 21.3% Ca and 18.7% P.

[3]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]Contained 1 g of titanium dioxide added to 4 g of gluten meal.

[5]Values are means of duplicate analyses.

Table 3. Ingredient and chemical composition of experimental diets to determine the true phosphorus utilisation in wheat distillers' dried grains with solubles for broilers and turkey.

	Wheat distillers' dried grains with solubles inclusion level, g/kg		
Item	200	400	600
Ingredients, g/kg			
Maize starch[1]	516	293.5	77
Wheat-DDGS	200	400	600
Soybean oil	18	36	48
Dextrose	100	100	100
Sucrose	130	130	130
Vitamin/mineral premix[2]	2.5	2.5	2.5
Limestone	4.5	9	13.5
Common salt	4	4	4
Marker premix[3]	15	15	15
Phytase premix	10	10	10
Total	1000	1000	1000
Analysed composition[4]			
Dry matter, g/kg	880	890	885
Phosphorus, g/kg	2.0	2.9	4.2
Calcium, g/kg	3.5	4.7	6.9
Phytase activity, FTU/kg	962	810	933

[1]Phytase premix replaced maize-starch at 10 g/kg.

[2]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.

[3]Contained 1 g of titanium dioxide added to 4 g of maize-starch.

[4]Values are means of duplicate analyses

Table 4. Ingredient and analysed chemical composition of nitrogen-free and assay diets used in the experiments.

	Broilers		Turkey	
Item	NFD	Assay	NFD	Assay
Ingredients, g/kg				
DDGS	0	675	0	743
Maize starch[1]	568	12	453	12
Dextrose	200	200	200	132
Vitacell[2]	85	0	200	0
Soybean oil	50	50	50	50
Vitamin-mineral premix[3]	5	5	5	5
Dicalcium phosphate[4]	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
TiO_2	3	3	3	3
Protease premix	±10	±10	±10	±10
Total	1,000	1,000	1,000	1,000
Analysed protease activity, units/kg	3,177	3,459	3,418	3,291

[1]Protease premix replaced maize-starch at 10 g/kg.

[2]Vitacell: Purified cellulose

[3]Vitamin and mineral premix provided (mg per kg of diet): retinol, 3.3; cholecalciferol, 0.06; dl-α tocopherol, 25; menadione, 3.3; thiamin, 2.2; riboflavin, 5.75; pyridoxine, 4.63; cobalamin, 0.018; biotin 0.18; pantothenic acid, 18; folic acid, 1.25; nicotinic acid, 27.8; choline 300; Mn, 60; Cu, 8; Fe, 50; I, 0.45; Se, 0.2; Zn, 55.

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

NFD; Nitrogen free diet, assay diets contained wheat distillers' dried grains with solubles as the only source of amino acid

Table 5. 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 6. 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 7. 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, MJ/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

3.5. 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.

3.5.1. Experiment 2 & 5

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. $$\mathrm{AME} = \mathrm{MEc} \mathrm{x} \mathrm{GEdiet}$$

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

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

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

By solving for Cti,

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

Substituting 1 – Pti for Pbd;

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

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.

3.5.2. Experiment 3 & 6

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

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;

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

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

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

3.5.3. Experiment 4 & 7

Basal ileal AA flow was calculated using the following equation:

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

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:

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

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

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 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.

4. Results

4.1. Prediction models

The range, mean, standard deviation (SD) and coefficient of variation (%CV) of the chemical components of maize- and wheat-DDGS are presented in Table 8. For both maize- and wheat-DDGS, calcium (Ca) and acid detergent fibre (ADF) were the most variable whereas CP was the least variable. However, Ca was more variable in maize- compared with wheat-DDGS. The variability in ash content was closest for both types of DDGS. Table 9 shows the range, mean, SD and %CV of the AA of maize- and wheat-DDGS. For maize-DDGS, Glu, lysine (Lys) and Met were most variable, whereas, Thr, Leu and Val were the least variable. For wheat-DDGS, the most variable AA were Lys, Cys and Phe, whereas, tryptophan (Trp), Thr and Asp were the least variable. For both types of DDGS, Lys and Thr were common as the most and least variable AA, respectively. In addition, maize-DDGS had greater Leu and Lys values whereas the values for all remaining indispensable AA were higher in wheat-DDGS.

Except for Arg, isoleucine (Ile), Lys and Trp, there were positive correlations ($P < 0.05$) between CP and other indispensable AA (data not shown). In addition, there were positive correlations ($P < 0.01$) between all the indispensable AA (except Trp) and total indispensable as well as total AA in maize-DDGS with r ranging from 0.58 to 0.96. There were positive correlations ($P < 0.05$) between CP and all of the indispensable AA in wheat-DDGS except for Trp (data not shown). In addition, the correlations between Lys and Met, Phe as well as Thr were not significant.

Equations for predicting the indispensable AA contents of maize- and wheat-DDGS from their CP composition are presented in Table 10. For maize-DDGS, R^2 ranged from 0.02 to 0.64, and was only greater than 0.50 in the models predicting Met, Phe and total AA. Crude protein did not explain any of the variation in the Lys content of maize-DDGS; and as such, the prediction model using CP alone was not developed for Lys. For 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 other AA.

The prediction models developed were validated using independent data and results are presented in Table 11. For maize-DDGS, the predicted values were close to analysed values for total indispensable and total AA (except Leu). Similarly, except for Met and Lys in wheat-DDGS, the predicted values were close to analysed values for all other indispensable AA. For both types of DDGS, the predicted- and analysed values were closest for total AA, Arg, Thr, Trp and Val.

4.2. Apparent metabolisable energy content

The AME and AME_n values of wheat-DDGS without- or with supplemental XAP for broilers and turkey are presented in Table 12. From the slope of the linear regression equations, the AME (MJ/kg DM) of wheat-DDGS for broilers without- or with supplemental XAP were 15.0 or 15.5, respectively. Corresponding AME_n (MJ/kg DM) were 14.0 and 14.5, respectively. Addition of XAP did not increase the AME and AME_n value of wheat-DDGS for broilers.

The AME ± SEM values (MJ/kg DM) of wheat-DDGS without- or with supplemental XAP for turkey were 14.0 or 14.9, respectively. Corresponding AME_n values (MJ/kg DM) were 13.0 or 13.8, respectively. Supplemental XAP did not increase the AME and AME_n value of wheat-DDGS for turkey.

4.3. True phosphorus digestibility and retention

True P digestibility and retention in wheat-DDGS without or with supplemental phytase for both broilers and turkey is presented in Table 13. For broilers, P digestibility in wheat-DDGS without or with supplemental phytase was 93.6% or 96.0%, respectively. Corresponding values at the total tract was 92.4 and 93.5%, respectively. Phosphorus digestibility and retention was not different between treatments without and those with supplemental phytase. The digestible P and retainable P (DP and RP, respectively) contents in the wheat-DDGS were calculated as the coefficient of P digestibility or retention multiplied by the analysed P content (%) of the wheat-DDGS. The DP content (%) in the wheat-DDGS for broilers without or with phytase was 0.60 or 0.62, respectively, whereas RP content (%) was 0.60 or 0.61, respectively.

For turkey, ileal true phosphorus utilisation (TPU) of wheat-DDGS without or with phytase was 75.8% and 82.1%, respectively. Respective values at the total tract level were 70.7% and 81.6%. True P utilisation was not different between the treatments without and those with phytase at the ileal and total tract. Digestible P content (%) in the wheat-DDGS without or with phytase for turkey was 0.49 or 0.53, respectively, whereas RP (%) content was 0.46 or 0.53, respectively.

4.4. Amino acids digestibility in wheat-DDGS for broiler and turkey

4.4.1. Broilers

The AIAAD and SIAAD of wheat-DDGS without- or with protease for broilers are presented in Table 14. On average, protease increased ($P < 0.05$) the coefficient of apparent ileal digestibility (AID) and the coefficient of standardised ileal digestibility (SID) of N by 0.12.

The coefficient of AIAAD and SIAAD of Lys was zero or less. Coefficients of AIAAD and SIAAD were also low for Asp. The coefficient of AIAAD ranged from 0.34 (Ala) to 0.75 (Pro) without

protease whereas the range was 0.42 (Thr) to 0.82 (Pro) with protease supplementation. Of the indispensable AA, the greatest and lowest AIAAD coefficients, without protease, were observed for Phe (0.56) and Met (0.37), respectively. Protease improved ($P < 0.05$) the coefficient of AIAAD of Arg and Pro by 0.15 and 0.07, respectively, and tended to improve ($P < 0.10$) the coefficient of AIAAD of Met.

The greatest and lowest SIAAD coefficients, without protease, were observed for Pro (0.84) and Asp (0.43), respectively, whereas the range was from 0.93 (Pro) to 0.54 (Asp) with protease. The greatest SIAAD coefficients for the indispensable AA were recorded in histidine (His) (0.72) and Phe (0.71). Protease addition improved ($P < 0.05$) the coefficient of SIAAD of Met, Arg, Leu, Phe, Val and Pro by 0.26, 0.21, 0.14, 0.13, 0.13 and 0.10, respectively.

The AIAAD of wheat-DDGS without- or with protease supplementation for turkey are presented in Table 15. The coefficient of AIAAD and SIAAD of Lys in the wheat-DDGS was zero or less. Coefficients of AIAAD and SIAAD were also low for Asp. The coefficients of AIAAD in the wheat-DDGS for turkey was lower than 0.50 for all AA, except for Glu (0.70) and Pro (0.81) without protease. The coefficient of AIAAD ranged from 0.35 (Thr) to 0.80 (Pro) with protease. Of the indispensable AA, the greatest and lowest AIAAD coefficients were noted for Phe (0.47) and Thr (0.19), respectively.

The coefficient of SIAAD ranged from 0.41 (Thr) to 0.89 (Pro) without protease whereas the range was from 0.56 (Arg) to 0.88 (Pro) with protease added. Except for Cys and Pro, protease improved ($0.05 < P < 0.1$) the coefficient of AIAAD and SIAAD of all other AA. On average, protease increased the coefficient of AIAAD or SIAAD in the wheat-DDGS by 0.11.

4.5. Growth Performance and gastrointestinal tract characteristics

Growth performance responses of broilers receiving wheat-DDGS, XAP and/or phytase from d 1 to 24 are presented in Table 16. Body weight gain (BWG), final body weight (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 17. Bodyweight gain and FBW were similar for birds receiving the PC1 and PC2 diets. On the other hand, gain:feed (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 18. 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.

Digesta pH at the duodenum and caecum of broilers in response to the dietary treatments are presented in Table 19. 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 volatile fatty acids (VFA) produced in the caeca of broilers in response to the dietary treatments in the current study are presented in Table 20. The VFA produced in lesser quantities in

the caeca of broilers in the current study are presented in Table 21. 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 containing wheat-DDGS and supplemental XAP or phytase are presented in Table 22. The micrographs of the villi and crypt of broilers receiving the dietary treatments in the current study are shown in Figure 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 8. Prediction models for the amino acids contents of maize- and wheat-distillers' dried grains with solubles.

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)

TIAA – total indispensable amino acids; TAA – total amino acids

Table 9. Predicted and analysed amino acids values (g/kg DM) for prediction models developed from the crude protein content of maize- and wheat-distillers' dried grains with solubles.

Amino acid	Analysed	Predicted
Maize-DDGS		
Arg	14.7	14.4
His	8.3	9.2
Ile	12.4	11.4
Leu	39.4	35
Lys	11.5	-
Met	7.3	6.6
Phe	16.5	15.8
Thr	12.2	11.7
Trp	2.4	2.5
Val	16.3	16.2
TIAA	141	133.5
TAA	302.9	294.8
Wheat-DDGS		
Arg	16	16.4
His	7.9	9.1
Ile	12.1	14.3
Leu	26.6	27.5
Lys	10	8.1
Met	7.9	5.8
Phe	17.9	18.8
Thr	12.7	12.2
Trp	4.3	4.2
Val	18.6	17.9
TIAA	134	133.9

Values are expressed on a dry matter basis; analysed data for maize-DDGS are from Soares *et al.* (2011) whereas analysed data for wheat-DDGS are from Slominski *et al*. (2010)

Table 10. Chemical composition (g/kg DM) of the maize- and wheat-distillers' dried grains with solubles.

	Maize-DDGS, g/kg						Wheat-DDGS, g/kg					
	n	Max	Min	Mean	SD	CV,%	n	Max	Min	Mean	SD	CV, %
Crude protein	44	347	233	279	24.0	8.5	18	463	321	380.6	40.9	10.8
Crude fibre	33	113	62	74	11.0	15.1	12	86	61	77	9.0	11.0
Neutral detergent fibre	17	510	277	366	58.0	15.7	11	468	218	326	75.0	23.1
Acid detergent fibre	19	185	86	136	33.0	24.2	10	223	74	140	53.0	37.8
Ether Extract	37	177	32	108	24.0	22.0	15	70	29	54	11.0	20.0
Ash	36	59	31	45	6.0	13.6	15	66	46	53	6.00	11.9
Total Phosphorus	25	9.8	6.9	8.0	0.7	8.8	11	11.1	6.5	9.2	1.40	14.8
Calcium	21	0.8	0.2	0.4	0.2	53.5	9	2.4	1.0	1.6	0.50	31.5

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 (%).

Sources of data: ADM (2011); Arvalis (2006); Avelar *et al*. (2010); Bandegan *et al.* (2009); Batal and Dale (2006); Belyea *et al.* (2004); Chrenkova *et al.* (2011); Cozannet *et al.* (2010); Cromwell *et al.* (2011); Fastinger *et al*. (2006); Han and Liu (2010); Janicek *et al.* (2008); Kim *et al.* (2008); Kleinschmit *et al.* (2006); Kluth and Ruderhutscord (2010); Kong and Adeola (2010); Lan *et al.* (2008); McKinnon and Walker (2008); Mjoun *et al.* (2010); Noll *et al.* (2007); Nyachoti *et al.* (2005); Olukosi *et al*. (2010); Oryschak *et al.* (2010); Pineda (2008); Randall and Drew (2010); Robertson *et al.* (2005); Rochell *et al.* (2011); Szczurek (2009); Shurson and Noll (2006); Spiehs *et al.* (2002); Stein (2007); Szczurek (2010); Thacker and Widyaratne (2007); University of Maryland (unpublished).Vilarino *et al.* (2007); Widyaratne and Zijlistra (2007); Youssef *et al.* (2009)

Table 11. Amino acid composition (g/kg) of the maize- and wheat-distillers dried grains with solubles.

	Maize-DDGS, g/kg						Wheat-DDGS, g/kg					
	n	Max	Min	Mean	SD	CV, %	n	Max	Min	Mean	SD	CV, %
Indispensable amino acids												
Arg	26	14.6	10.6	12.2	1.0	8.0	16	20.1	11.8	15.4	2.0	13.0
His	24	9.1	6.5	7.4	0.7	9.4	13	10.2	6.6	8.2	1.2	14.2
Ile	27	12.5	9.6	10.7	0.7	6.7	16	16.6	10.9	13.3	1.7	12.9
Leu	24	36.2	28.9	32.1	2.1	6.6	16	31.3	20.9	25.5	3.3	12.8
Lys	28	11.1	6.2	9	1.2	13.1	16	11.7	6.0	7.7	1.5	20.6
Met	28	7.2	4.4	5.2	0.6	12.0	14	7.1	4.2	5.5	0.9	16.9
Phe	24	15.1	10.9	12.9	1.2	9.6	16	22.2	11.1	17	3.0	17.3
Thr	28	11.6	9.3	10.3	0.7	6.5	16	14.1	9.9	11.7	1.1	9.6
Trp	27	2.6	1.6	2.2	0.2	10.3	9	4.4	3.6	3.9	0.3	7.0
Val	26	16.1	13	14.2	0.9	6.7	16	20.9	13.7	16.4	2.1	12.6
Dispensable amino acids												
Ala	21	21	15.6	18.3	1.4	7.6	13	17.7	11.9	13.8	1.5	10.8
Asp	21	19.7	14.9	17.3	1.3	7.6	13	22.5	16.0	18.5	1.9	10.2
Cys	26	7.0	4.1	5.1	0.6	11.1	14	10.0	5.7	7.3	1.3	18.3
Glu	21	54.8	29.3	36.1	6.2	17.1	13	120	81.7	97.9	12.9	13.2
Gly	21	12.4	9.5	10.8	0.7	6.8	13	19.2	12.8	15.1	1.7	11.1
Pro	21	22.1	16.6	19.3	1.7	8.7	8	41.1	26.3	33.3	5.0	15.1
Ser	22	14.5	10.1	11.7	1.1	9.1	13	20.8	14.5	16.8	1.7	10.2
Tyr	22	12	9.1	10.1	0.7	7.2	8	13.5	9.0	10.7	1.5	13.6

SD - standard deviation; CV – coefficient of variation (%). Sources of data: ADM (2011); Arvalis (2006); Avelar *et al.* (2010); Bandegan *et al*. (2009); Batal and Dale (2006); Cromwell *et al*. (2011); Fastinger *et al.* (2006); Han and Liu (2010); Kluth and Ruderhutscord (2010); Kim *et al.* (2008); Kong and Adeola (2010); Lan *et al.* (2008); Noll *et al.* (2007); Nyachoti *et al.* (2005); Olukosi *et al.* (2010); Oryschak *et al.* (2010); Pineda *et al.* (2008); Shurson and Noll (2005); Spiehs *et al.* (2002); Stein *et al.* (2007); Szczurek (2010); Thacker and Widyaratne (2007); University of Maryland (2011);Vilarino *et al.* (2007); Widyaratne and Zijlistra (2007)

Table 12. Regression equations for the apparent metabolisable energy content of wheat distillers' dried grains with solubles without or with supplementation of a mixture of carbohydrases and protease for broiler and turkey[1,2]

Measurements	Regression equation	SE of slope	r^2	P-value
Broiler				
AME, MJ/kg DM				
Without XAP	Y = 15.0X + 0.013	0.246	0.995	<0.001
With XAP[3]	Y = 15.5X – 0.01	0.366	0.989	<0.001
AME_n, MJ/kg DM				
Without XAP	Y = 14.0X + 0.021	0.219	0.995	<0.001
With XAP[3]	Y = 14.5X – 0.005	0.323	0.990	<0.001
Turkey				
AME, MJ/kg DM				
Without XAP	Y = 14X + 0.201	0.382	0.985	<0.001
With XAP[3]	Y = 14.9X + 0.034	0.323	0.991	<0.001
AME_n, MJ/kg DM				
Without XAP	Y = 13X + 0.184	0.342	0.986	<0.001
With XAP[3]	Y = 13.8X + 0.04	0.285	0.992	<0.001

[1]AME and AME_n values of wheat-DDGS determined from regression of 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 DM. The slope of the regression equation is the AME or AME_n value of the wheat-DDGS.

[2]Supplemental XAP did not significantly ($P > 0.05$) increase the AME or AME_n values of wheat-DDGS for broiler and turkey

[3]Average analysed enzyme activities were 1421 U/kg of xylanase, 262 U/kg of amylase and 3064 U/kg of protease, respectively

S.E - standard error of difference of mean

Table 13. True phosphorus digestibility and retention of wheat distillers' dried grains with solubles without or with phytase supplementation for broilers and turkey.

	Regression equation[1]	r^2	SE of slope[2]	PD/PR[3], %	DP/RP[4], %
Broilers					
Ileal					
Without phytase	Y= 0.064X - 476	0.661	0.010	93.6	0.60
With phytase	Y = 0.040X + 174	0.725	0.005	95.9	0.62
Total tract					
Without phytase	Y = 0.063X - 625	0.534	0.016	92.4	0.60
With phytase	Y = 0.065X - 201	0.689	0.010	93.5	0.61
Turkey					
Ileal					
Without phytase	Y = 0.242X - 430	0.650	0.039	75.8	0.49
With phytase	Y = 0.179X - 98	0.422	0.047	82.1	0.53
Total tract					
Without phytase	Y = 0.294X - 293	0.612	0.056	70.7	0.46
With phytase	Y = 0.184X + 451	0.375	0.054	81.6	0.53

[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 is endogenous P loss (mg/kg of DMI) whereas the slope is true P indigestibility.

[2]Standard error of regression components

[3]Calculated as 100 x (1 - true P indigestibility); True P digestibility or retention not improved by phytase supplementation.

[4]DP and RP are digestible P and retainable P contents of wheat-DDGS, respectively. Calculated as (true P utilisation (%) /100) multiplied by analysed P content in wheat-DDGS (%).

Table 14. Coefficients of apparent and standardised ileal amino acids digestibility (%) of wheat distillers' dried grains with solubles without- or with protease supplementation for broilers

	Apparent				Standardised			
Item	Without protease	With protease[1]	s.e.d	Protease effect	Without protease	With protease[1]	s.e.d	Protease effect
Nitrogen	0.49	0.60	0.04	0.017	0.51	0.63	0.04	0.013
Indispensable amino acids								
Arg	0.38	0.53	0.06	0.026	0.54	0.75	0.06	0.004
His	0.52	0.56	0.06	0.524	0.72	0.79	0.06	0.286
Ile	0.44	053	0.06	0.182	0.57	0.71	0.06	0.059
Leu	0.50	0.59	0.06	0.115	0.64	0.78	0.06	0.029
Phe	0.56	0.65	0.06	0.110	0.70	0.83	0.06	0.043
Thr	0.37	0.42	0.06	0.478	0.52	0.66	0.07	0.081
Met	0.37	0.49	0.07	0.094	0.58	0.74	0.07	0.032
Val	0.44	0.54	0.06	0.106	0.59	0.73	0.06	0.029
Dispensable amino acids								
Ala	0.35	0.45	0.07	0.194	0.51	0.65	0.07	0.067
Cys	0.47	0.53	0.07	0.371	0.63	0.70	0.07	0.303
Glu	0.75	0.79	0.03	0.175	0.82	0.88	0.03	0.062
Gly	0.49	0.48	0.06	0.869	0.66	0.68	0.06	0.75
Pro	0.75	0.82	0.03	0.041	0.84	0.93	0.03	0.01
Ser	0.54	0.56	0.08	0.843	0.71	0.75	0.08	0.633
Tyr	0.45	0.54	0.07	0.182	0.64	0.79	0.07	0.057
Asp	0.34	0.31	0.06	0.644	0.44	0.54	0.07	0.197

[1]Analysed protease activity was 3,459 U/kg of diet

s.e.d - standard error of difference

Table 15. Coefficients of apparent and standardised ileal amino acids digestibility of wheat distillers' dried grains with solubles without- or with protease supplementation for turkey

	Apparent				Standardised			
Item	Without protease	With protease[1]	s.e.d	Protease effect	Without protease	With protease[1]	s.e.d	Protease effect
Indispensable amino acids								
Arg	0.30	0.40	0.04	0.055	0.46	0.56	0.04	0.055
His	0.33	0.44	0.05	0.039	0.55	0.66	0.05	0.039
Ile	0.35	0.46	0.04	0.028	0.50	0.61	0.04	0.028
Leu	0.41	0.49	0.04	0.062	0.55	0.64	0.04	0.062
Phe	0.47	0.57	0.03	0.018	0.62	0.71	0.03	0.018
Thr	0.19	0.35	0.05	0.006	0.41	0.58	0.05	0.006
Met	0.24	0.41	0.05	0.008	0.47	0.63	0.05	0.008
Val	0.33	0.43	0.04	0.047	0.51	0.60	0.04	0.047
Dispensable amino acids								
Ala	0.24	0.41	0.04	0.003	0.44	0.61	0.04	0.003
Cys	0.31	0.44	0.07	0.112	0.45	0.57	0.07	0.112
Glu	0.70	0.75	0.02	0.029	0.77	0.82	0.02	0.029
Gly	0.32	0.48	0.04	0.006	0.53	0.68	0.04	0.006
Pro	0.81	0.80	0.02	0.713	0.89	0.88	0.02	0.713
Ser	0.34	0.50	0.05	0.012	0.58	0.75	0.05	0.012
Tyr	0.40	0.51	0.05	0.049	0.61	0.72	0.05	0.049
Asp	0.04	0.22	0.06	0.009	0.26	0.45	0.06	0.009

[1]Analysed protease activity was 3,291 U/kg of diet

s.e.d - standard error of difference

Table 16. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers' dried grains with solubles supplemented with an 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 17. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers' dried grains with solubles supplemented with an 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 18. Growth performance of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers' dried grains with solubles supplemented with an 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

Table 19. 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 an 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 20. 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 an 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 21. 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 an 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

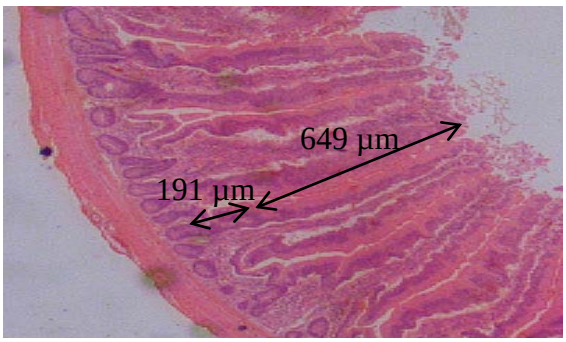
[1]PC1 - wheat-SBM based diet adequate in metabolisable energy (ME) and nutrients; PC2 - wheat-SBM-wheat-DDGS based diet adequate in ME and nutrients; NC1 - wheat-SBM-wheat-DDGS based diet marginal in ME (-0.63 MJ/kg); NC2 - wheat-SBM-wheat-DDGS based diet marginal in P (-0.15% non-phytate P); NC3 - 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 22. Jejunal morphology of broilers receiving a wheat-soyabean meal based diet containing wheat-distillers' dried grains with solubles supplemented with an enzyme mixture containing xylanase, amylase and protease activities or phytase alone or a combination of both.

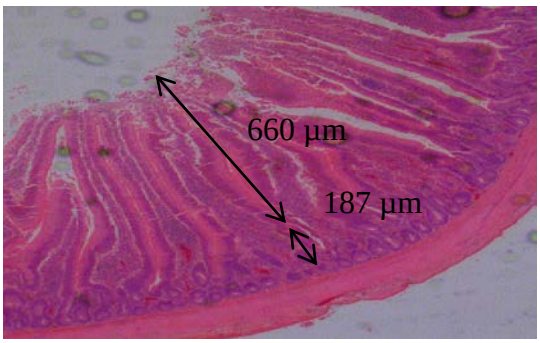
Diets[1]	VH, µm	CD, µm	VH:CD
PC1.	649	191	3.49
PC2	660	187	3.61
NC1	606	144	4.21
NC1 plus XAP	731	212	3.62
NC2	564	168	3.4
NC2 plus phytase	648	207	3.26
NC3	692	194	3.79
NC3 plus XAP and phytase	634	174	3.81
s.e.d	96.9	30.0	0.58
P-values for main effect of diet	0.793	0.387	0.816
P-values for contrast			
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

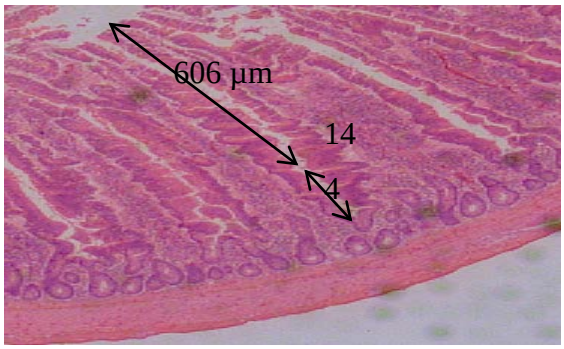
VH: villi length; CD: crypt depth; s.e.d: standard error of difference



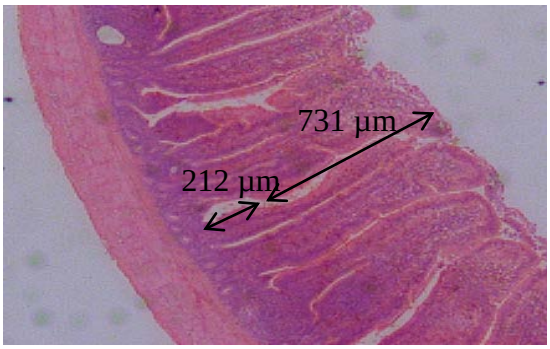
PC1



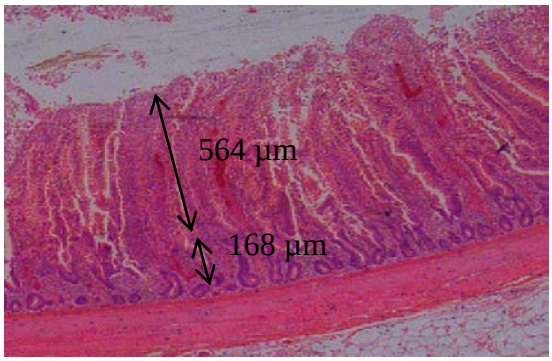
PC2



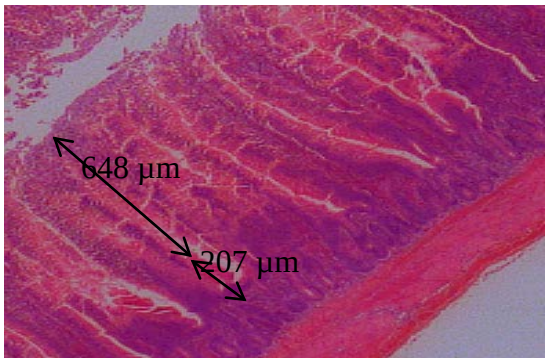
NC1



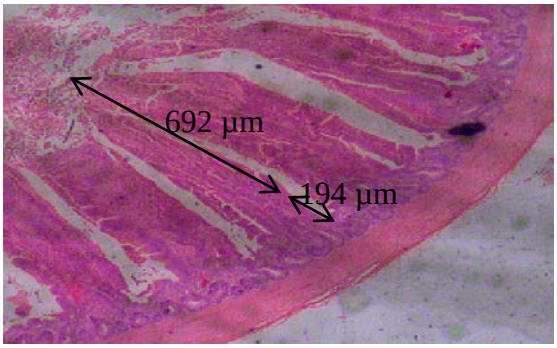
NC1 plus XAP



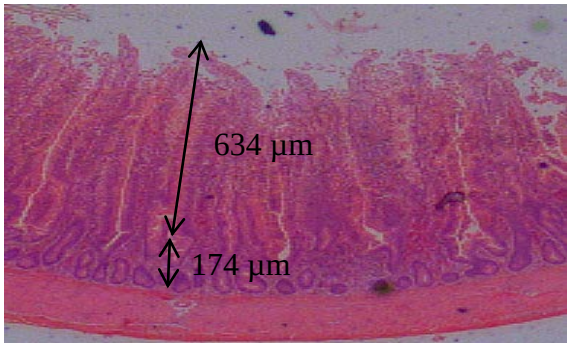
NC2



NC2 plus phytase



NC3



NC3 plus XAP and phytase

Figure 1. Micrographs of the jejunal villi height and crypt depth for broilers receiving diets containing wheat-DDGS.

5. Discussion

5.1. Experiment 1 – Prediction models

The objectives of the current study were to examine the variability in the chemical components of maize- and wheat-DDGS, evaluate the relationships between the chemical components as well as develop prediction equations for the AA contents in maize- and wheat-DDGS from their CP content. It was anticipated that the use of data from a wide range of sources will capture most of the variability in the chemical composition of both maize- and wheat-DDGS, resulting in a robust prediction.

Factors that may affect the chemical composition of DDGS include the 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' solubles (CDS) in the DDGS, as well as 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). Among all these factors however, the ratio of WDG and CDS in DDGS may be the most important. This is because WDG is composed mostly of CP and crude fibre (CF) whereas CDS contains mainly the ether extract (EE), ash and residual sugar fractions (Kim *et al*., 2008).

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 total AA in DDGS (Kim *et al*., 2008; Liu, 2011). In ascending order of variability, Met, Lys and Glu on one hand, and Phe, Cys and Lys on the other were the most variable AA in maize- and wheat-DDGS, respectively. On the other hand, the least variable AA were Thr and Leu for maize-DDGS and Trp and Thr for wheat-DDGS. There does not appear to be an obvious explanation for which AA were the most or least variable but there is a pattern for Lys to be among the most variable and Thr among the least variable. It is possible that the more variable AA are affected more by factors causing variability in the chemical composition of the DDGS (the factors mentioned earlier). In addition, formation of insoluble Lys-carbohydrate moieties and partial destruction of Cys reported in oilseeds and animal protein meals as a result of excessive heat treatment as reported in literature (Cromwell *et al.*, 1993; Cozannet *et al*., 2010) also affect the AA in DDGS as well.

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 analysed CP value of the DDGS. Because the CP and AA profile of DDGS are the result of mixture of the grain and yeast, the proportional contribution of the two may confer considerable variability in amino acid and protein contents of DDGS. Belyea *et al.* (2004) observed that yeast protein contributes up to 55% of the total protein content in maize-DDGS. It is likely that the value reported by Belyea *et al.* (2004) may have overestimated the contribution of yeast protein to total protein in maize-DDGS because the authors did not account for dispensable AA. However, Belyea *et al.* (2004) showed that AA such as Lys, which is typically low in maize and higher in yeast protein (2.4 vs. 33.2 g/kg in maize and yeast, respectively), increased substantially in maize-DDGS after yeast fermentation. Liu (2011) similarly observed that after fermentation of maize by yeast, there were rapid increases in the concentration of some, although not all, AA. On the other hand, Martinez-Amezcua (2005, Unpublished Ph.D. thesis) reported that only about 10% of the total AA in maize-DDGS is contributed by the yeast AA.

In the current study, there were significant positive correlations among all indispensable AA (except Trp) for both maize- and wheat-DDGS. Weak correlations between Trp and the proximate fractions, as well as other AA in maize-DDGS, have been reported previously (Fiene *et al*., 2006). The reasons for the poor correlation between Trp and other indispensable AA 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 reported (Nurit *et al*., 2009). The effect of variable or incomplete extraction of Trp during AA analysis of DDGS may also be an important factor considering the fact that the concentration of Trp is low compared with the concentration of other indispensable AA in DDGS.

It is desirable to be able to predict the level of individual AA from the CP content of DDGS or to predict some essential AA from other AA with high accuracy. Fiene *et al.* (2006) reported that it is possible to predict the content of indispensable AA of maize-DDGS from its CP, EE and CF levels. Consequently, in the current study prediction models for indispensable 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 Met and Phe. On the other hand, R^2 for predicting individual AA from CP content of wheat-DDGS was generally > 0.60 (except for Lys and Trp). In Fiene *et al.* (2006) studies, R^2 of the prediction models ranged from 0.31 to 0.86 and were lowest for Trp and Lys. The reasons for low R^2 for Trp may be due to reasons given previously whereas that of Lys may be because of the wide variability in Lys concentration caused by the formation of insoluble carbohydrate-Lys compounds during the drying process.

The validity of the prediction models developed in the current study was confirmed by comparing predicted with analysed values from independent data (Slominski *et al*., 2010; Soares *et al*., 2011). Most of the AA predicted from the protein content of the DDGS were within 5% of the analysed values of corresponding AA. The amino acids that were outside 10% of the analysed value for both maize and wheat-DDGS were Ile, His and Met. The general agreement between predicted and analysed AA values as shown in the current study, indicates that the prediction models developed in the current study are reliable and can be used for rapid determination of AA content of DDGS, provided the CP content of the DDGS has been determined accurately.

5.2. Experiment 2 and 5 – Metabolisable energy content in wheat-DDGS for broiler and turkey

The AME value of wheat-DDGS for broilers was determined to be 15.0 MJ/kg DM in the current study. This value is greater than 11.1 or 9.27 MJ/kg DM for two wheat-DDGS samples, reported in the Bolarinwa and Adeola (2012) study, as well as the range of 8.97 to 12.0 MJ/kg DM for 10 samples of wheat-DDGS noted in the Cozannet *et al.* (2010) study. 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 for broilers in the current study, which is similar to the 7% reduction reported by Bolarinwa and Adeola (2012). The AME_n value of wheat-DDGS for broilers was determined to be 14.04 MJ/kg 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 DM reported by Bolarinwa and Adeola (2012), Cozannet *et al.* (2010) and Vilarino *et al.* (2007), respectively, for broilers. The AME and AME_n value of wheat-DDGS for turkey was determined to be 14.04 and 14.89 MJ/kg DM, respectively, in the current study. Cozannet *et al*. (2010) used the difference method in their study and determined the AME value of 10 samples of wheat-DDGS to range from 7.70 to 11.5 MJ/kg DM for turkey. Furthermore, Cozannet *et al*. (2010) reported the AME_n values of wheat-DDGS for turkey to range from 7.40 to 10.70 MJ/kg DM.

The gross energy 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) (21.60 vs 18.90 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) for broilers. It appears, therefore, that the gross energy content of wheat-DDGS is influential in defining its AME value for broilers. On the other hand, although the gross energy content in the wheat-DDGS used in the current study were similar to those used in the study of Cozannet *et al.* (2010) (21.6 vs. 20.8 MJ/kg

DM, respectively), energy metabolisability in the wheat-DDGS for turkey was greater in the current study (65 vs. 47%, respectively).

It was noted that the AME or AME_n values of wheat-DDGS were 0.97 or 1.03 MJ/kg DM, respectively, greater for broilers compared with turkey in the current study. Similarly, Cozannet *et al.* (2010) observed that the mean 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 d of age compared with turkey at 13 wks old. It is speculated that the difference in energy utilisation in wheat-DDGS between broilers and turkeys in the current study is due to differences in physiological maturity between the two species at 21 d of age. 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 reported energy values of wheat-DDGS show the need to develop a standardised method for determining energy value of wheat-DDGS for poultry. Although the differences in the nutrient composition of wheat-DDGS among sources might be implicated in causing variability to the utilisable energy value of the co-product, the methodology, age and species of poultry used for determining its energy value are also potential sources of variation.

Exogenous enzymes, such as carbohydrases and proteases or a combination of these, are often incorporated into poultry diets; however, there is a dearth of information on the efficacy of these enzymes to improve the nutritive value of wheat-DDGS. In addition to improving energy value and nutrient digestibility, supplementing diets containing wheat-DDGS with exogenous enzymes may reduce variability in the nutritive value of wheat-DDGS. The efficacy of exogenous enzymes to improve the nutritive value of bioethanol co-products has been determined mostly for corn-DDGS (Adeola and Ileleji, 2009; Adeola *et al*. 2010; Liu *et al*., 2011) but greater benefits may be derived from using exogenous enzymes in diets containing wheat-DDGS because wheat contains greater levels of NSP than corn.

In the 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 fibre 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 high utilisable energy content in the wheat-DDGS used in the current study was partly responsible for the marginal effect of XAP. Also, analysed 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 fibre, it is unlikely that a combination of carbohydrases and proteases will not significantly improve its utilisable 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.

5.3. Experiment 3 and 6 Phosphorus digestibility and retention of wheat-DDGS for broiler and turkey

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 P content of wheat-DDGS highlights the variability in its chemical composition among sources and these variations are likely due to differences in the P composition in the wheat used or to differences in processing techniques.

Increasing the inclusion level of wheat-DDGS reduced dietary DM utilisation for broilers and turkeys in the current study. The increase in dietary fibre as wheat-DDGS replaced corn starch in the diets may explain the reduction in DM utilisation noted in the current study. Increased levels of dietary fibre have been reported to reduce DM and nutrient utilisation in poultry (Choct *et al*., 2004). 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.

Phytase did not improve dietary P digestibility and retention for broilers and turkeys in the current study. The efficacy of supplemental phytase to release P bound to phytate for poultry and pig have been described extensively in the literature and reviewed (Selle and Ravindran, 2007; Woyengo and Nyachoti, 2011). The lack of improvement in dietary P utilisation noted in the current study may be due to the characteristics of the wheat-DDGS. The majority of the phytate bound P in the wheat-DDGS may have being released by the actions of yeast phytase during the fermentation process in bioethanol production; thus the lack of effect from the supplemented phytase.

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 (Liu and Han, 2011) observed that during the fermentation process, percentage phytate-P in total P decreased significantly, whereas percentage non phytate-P in total P increased. These observations suggested that phytate underwent degradation through the actions of yeast phytase. In addition, 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 DDGS. Information about the phytate P content or temperature used to treat 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.

It is possible to extrapolate the TPU and basal endogenous P loss from the linear relationship between undigested P and dietary P intake using the regression method. In the current study, there was a strong relationship between undigested P and dietary P intake, which is important when using the regression method. The regression method has been used to determine TPU of feed ingredients for broilers (Dilger and Adeola, 2006) and swine (Akinmusire and Adeola, 2008). True P utilisation of wheat-DDGS was greater than 90% for broilers or 70% for turkey in the current study. The difference in TPU between broilers and turkey in the current study is probably due to differences in physiological maturity between the two species at 21 d of age. The TPU of wheat-DDGS noted for broilers and turkey in the current study indicate that the majority of P in wheat-DDGS was bioavailable. Supplemental phytase did not affect TPU for broilers and turkey in the current study. The high TPU of wheat-DDGS in the current study is an indication that the level of phytate in the wheat-DDGS would have been low and this may explain the lack of phytase effect.

In conclusion, the results from the current study indicate that wheat-DDGS is a good source of utilisable P for broilers and turkey; thus the inclusion of wheat-DDGS in the diet will reduce the use of inorganic P sources. Supplemental phytase did not improve the TPU of the wheat-DDGS for broilers and turkey most likely because the wheat-DDGS contained low levels of phytate-bound P.

5.4. Experiment 4 and 7 – Amino acids digestibility of wheat-DDGS for broiler and turkey

It is possible to use wheat-DDGS as a feedstuff for poultry because of its greater nutrient content compared with wheat grain. Wheat-DDGS is even more attractive because of the increase in its availability and lower cost compared with wheat grain. The objective of the current study was to determine the AIAAD and SIAAD of wheat-DDGS without or with exogenous protease for broilers and turkey.

In the current study, the AID of N in wheat-DDGS was 49% for broilers and is lower than the average AID of N for five samples of wheat-DDGS (67%) reported by Bandegan *et al.* (2009). Similarly, the SID of N for broilers (51%) in the current study was lower compared with those reported by Bandegan *et al*. (2009) (69%) and Kluth and Rudehutscord (2010) (64%). The ileal digestibility of Lys in the wheat-DDGS for broilers and turkey in the current study was nil. Similar nil digestibility for Lys in wheat-DDGS in broilers have been reported by Cozannet *et al.* (2011). It was noted in the current study that the coefficient of AIAAD of Lys and Asp were lowest in wheat-DDGS for broilers. The observation that Lys is the least digestible AA in wheat-DDGS is consistent with those by Bandegan *et al.* (2009) and Cozannet *et al*. (2011) as well as with studies using maize-DDGS in broilers (Lumpkins *et al*., 2004; Batal and Dale, 2006). Of the indispensable AA, the coefficient of SIAAD of Phe was the greatest in the current study and is similar to observations by Bandegan *et al*. (2009) for broilers and Lan *et al.* (2008) for finishing pigs.

Protein and AA digestibility in different samples of maize- or wheat-DDGS may vary substantially in poultry (Batal and Dale, 2006; Cozannet *et al*., 2010). Heat treatment during the production of wheat-DDGS has been widely implicated for the reduction, or variability, in the digestibility of protein and AA in DDGS (Fastinger *et al*., 2006; Cozannet *et al*., 2010). Excessive heat treatment of feed ingredients used for poultry reduces the digestibility of AA for poultry due to the formation of insoluble carbohydrate-protein complexes in a Maillard reaction. The negative effects of excessive heat treatment may be exacerbated in DDGS because a number of steps in the bioethanol production (cooking, liquefaction, saccharification, drying) require the application of heat. Indeed, Liu and Han (2011) noted that the formation of carbohydrate-protein complexes in maize-DDGS was not solely limited to the drying process, because a proportion of Lys in wet distiller's grains and condensed solubles was already bound to carbohydrates before drying.

The colour of DDGS may be used as a measure of the intensity of heat treatment (Fastinger *et al*., 2006). Colour measuring tools were not used to define the colour of the wheat-DDGS used in the current study, but comparisons using a maize-DDGS colour score chart (Shurson, 2011) indicated that the wheat-DDGS is relatively dark in colour. 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 for broilers than the darker coloured maize-DDGS (Ergul *et al*., 2003; Batal and Dale, 2006) and caecectomised roosters (Fastinger *et al*., 2006; Cozannet *et al*., 2011). On the other hand, it is noteworthy that although the colour of the DDGS is mainly affected by 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 also define the colour of DDGS (Liu, 2011; Shurson, 2011).

It was noted in the current study that the coefficient of AIAAD and SIAAD of wheat-DDGS were greater in broilers compared with turkey. On the average, the coefficient of AIAAD or SIAAD of wheat-DDGS was 0.13 or 0.10, respectively, and greater in broilers compared with turkey; the largest differences in AA digestibility were observed for His, Thr, Cys, serine (Ser), glycine (Gly) and Asp. The differences in the utilisation of AA in the wheat-DDGS between the two species may be due to differences in physiological development at 28 days of age. Uni *et al*. (1995; 1999) reported that the post hatch development of the small intestine of turkey poults is slower compared with that of the broiler chick. It is speculated that broilers, being physiologically more mature on day 28, were able to utilise AA in the wheat-DDGS more efficiently compared with turkey at the same age.

The benefits of using supplemental enzymes in poultry diets are to increase the nutritional value of the diet or feed ingredients or reduce the variation in the nutrient quality of feed ingredients whilst reducing nutrient losses in manure (Bedford, 2000). It was hypothesized in the current study that protease will improve AIAAD and SIAAD in wheat-DDGS for broilers and turkey. Indeed, protease supplementation increased the coefficient of SIAAD of a large number of AA in the wheat-DDGS for broilers and turkey by up to 0.21.

Proteases are often supplemented in the diet as part of an admixture of xylanase, amylase and protease; as such, data about improvement in AA digestibility of feed ingredients when protease is used alone, are scarce. 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, thus reducing the requirement for AA and energy; proteases may 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). Because supplemental enzymes are often more likely to be effective at improving the value of poorly utilised feedstuffs or diet (Classen *et al*., 1995; Bedford and Schulze, 1998), it is possible that the effectiveness of protease supplementation in the current study was due to the inherent poor AA digestibility in the wheat-DDGS. It will be worthy to establish whether dark coloured wheat-DDGS respond better to protease supplementation than light coloured wheat-DDGS.

5.5. Experiment 8 – Growth performance and gastrointestinal tract characteristic of broilers fed diets containing wheat-DDGS

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 similarly 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 formed a complex 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 non-starch polysaccharides (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 post-hatch 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). It was reported earlier in this report that supplemental phytase did not improve the digestible P content in wheat-DDGS and that 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 was 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).

5.6. Conclusions

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.

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