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Apparent and standardised ileal amino acids digestibility of wheat distillers dried grains with soluble with- or without exogenous protease in broilers and turkeys

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ABSTRACT

1. The apparent- (**AIAAD**) and standardised ileal amino acids digestibility (**SIAAD**) of wheat distillers dried grains with soluble (**wheat-DDGS**) was determined for broilers and turkeys at 28 days using two experiments.

2. A total of 84 male Ross 308 broiler chicks (Experiment 1) or 84 male BUT 10 turkeys poults (Experiment 2) were offered a nutrient-adequate diet from d 1 to 23. On d 23, birds in each experiment were allocated to four treatments consisting of a nitrogen-free diet (with- or without protease) and an assay diet containing wheat-DDGS as the only source of amino acids (with- or without protease) in a randomized complete block design.

3. In experiments 1 and 2, the coefficient of AIAAD or SIAAD of Lys and Asp were low irrespective of protease. In experiment 1, the coefficient of AIAAD ranged from 0.35 (Ala) to 0.75 (Pro) without protease whereas the range was 0.42 (Thr) to 0.82 (Pro) with protease. Protease improved ($P < 0.05$) the coefficient of AIAAD for Arg and Pro and tended to improve the ($P < 0.10$) coefficient of AIAAD for Met. Without protease, coefficient of SIAAD ranged from 0.51 (Ala) to 0.84 (Pro) whereas the range was from 0.65 (Ala) to 0.93 (Pro) with protease. Protease improved ($P < 0.05$) the coefficient of SIAAD of Arg, Leu, Phe, Met, Val and Pro.

4. In experiment 2, the coefficient of AIAAD was lower than 0.50 for all amino acids except for Glu (0.70) and Pro (0.81) without protease. On the other hand, 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 supplementation. With the exception of Cys

and Pro, protease improved ($P < 0.05$) the coefficient of AIAAD and SIAAD of all other amino acids from between 0.05 to 0.19.

5. It was concluded that the AIAAD and SIAAD of wheat-DDGS are variable and are generally greater in broilers compared with turkeys at 28 days of age. Protease improved the ileal digestibility of a large number of amino acids in wheat-DDGS for broilers and turkeys.

INTRODUCTION

Wheat distillers dried grains with soluble (**wheat-DDGS**) is the main co-product of bioethanol produced from wheat grain by the dry-grind process. The removal of the starch in wheat during bioethanol production concentrates other nutrients in wheat-DDGS (Liu, 2011). Therefore, wheat-DDGS contains greater quantities of crude protein (**CP**) and amino acids (**AA**) compared with wheat grain. Maize-DDGS is a good source of AA for broilers and turkeys (Lumpkins *et al*., 2004; Batal and Dale, 2006; Fastinger *et al*., 2006) and it is expected that wheat-DDGS will likewise be a good source of AA. Due to differences between broiler and turkey both in the post hatch development of their gastrointestinal tract as well as their capacity to utilise dietary nutrients (Uni *et al*., 1999) it will be expected that their ability to utilise amino acids in wheat-DDGS will differ. Adedokun *et al*. (2008) reported that the AIAAD of maize-DDGS for broilers and turkeys are different at 21 days of age. However there is a dearth of information about the apparent- (**AIAAD**) or standardised ileal amino acids digestibility (**SIAAD**) of wheat-DDGS for broiler and turkeys. This information is needed when diets need to be formulated on digestible amino acids basis.

There is opportunity, using exogenous enzymes, to increase the nutritive value of feed ingredients that may otherwise be of limited use in poultry. The objectives of supplementing enzymes in diets are to reduce negative effects of the anti-nutritive factors in feedstuffs (Bedford and Schulze, 1998), enhance the overall digestibility of the feed (Selle *et al*., 2009), increase nutrients bioavailability (Martinez-Amezcua *et al*., 2006), and reduce nutrient excretion (Adeola and Cowieson, 2011). There is currently no information on the benefits of protease supplementation on protein and AA digestibility of wheat-DDGS for broilers and turkeys.

Therefore the objective of the current experiments was to determine the AIAAD and SIAAD of wheat-DDGS without- or with protease supplementation in broilers and turkeys.

MATERIALS AND METHODS

Animals and management

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

A total of 84 Ross 308 male broiler chicks (Experiment 1) or 84 BUT 10 male turkey poults (Experiment 2) were used to determine the AIAAD and SIAAD of wheat-DDGS. The birds were reared in a house with facilities to control temperature, light, and humidity. In each of the experiments, birds were offered a pre-experimental diet formulated to meet energy and nutrient requirements of the specific breed. In each experiment, birds were allocated to one of 4 experimental diets in a randomised

complete block design using initial bodyweight as blocking criterion. Each treatment had seven replicate cages and three birds per replicate cage. Birds had *ad libitum* access to diets and water during the entire experimental period.

Diets and sample collection

The pre-experimental diet offered in both experiments (as-is) contained, 12.7 MJ/kg of metabolisable energy, 230 g/kg of CP and 6.8 g/kg of P. 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. The chemical composition of the wheat-DDGS used in the current study is presented in Table 1. The subtilisin protease used in the current study was produced and expressed in *Bacillus subtilis*. The protease was added in the diet at a rate to supply 4,000 units per kg and one protease unit was defined as the quantity of the enzyme that solubilises one mg of azo-casein per minute. Basal ileal endogenous amino acids loss was analysed from ileal digesta of birds offered nitrogen free diets.

Titanium dioxide was added to the experimental diets (3 g/kg of diet) as an indigestible marker to enable determination of AIAAD by the index method. 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 2. On d 28, ileal digesta was collected by gently flushing the mid ileum with water. Mid ileum is defined as 5cm distal to Meckel's diverticulum to approximately 5 cm proximal

to the ileo-caecal junction. Ileal digesta was pooled per cage and stored frozen (-20 ^{o}C) prior to chemical analysis.

Chemical analysis

Samples of diets, wheat-DDGS and ileal digesta were analysed for DM, Ti, N, and AA. Ileal digesta samples for AA analysis were lyophilized and ground to pass through 0.5 mm screen using a mill grinder (Retsch ZM 100, F. Kurt Retsch GmbH & Co.KG, Haan, Germany) before chemical analysis. To determine DM content, samples were dried at 105^{o}C for 24 hours in a drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England. UK). Nitrogen was determined by the combustion method (Leco Model 2000 CHN analyser, Leco Corp., St. Joseph, MI, USA). Samples for AA analysis were hydrolysed for 24 hours in 6 N hydrochloric acid at 110 ^{o}C under an atmosphere of N. For Met and Cys, performic acid oxidation was carried out before acid hydrolysis. The AA in the hydrolysate were determined by HPLC after post-column derivatization [(AOAC International 2000, method 982.30E (a, b, c)]. Analysis for Ti was performed as described by Short *et al*. (1996). Protease activity was measured using the modified method of Lynn and Clevette-Radford (1984) with azocasein used as substrate.

Calculations and statistical analysis

Apparent ileal amino acid digestibility in wheat-DDGS in the assay diets was calculated using the index method. Basal endogenous ileal amino acid flow was also calculated using the index method whereas standardised ileal amino acid digestibility was

determined from the correction of AIAAD values for basal endogenous ileal amino acid losses. Data were analysed using Generalized Linear Models of Genstat Statistical Package (11th edition, VSN International) to test for treatment effects. Statistical significance was set at $P < 0.05$ and tendency at $0.05 < P < 0.1$.

RESULTS

Experiment 1

Ileal endogenous AA flow was not different ($P > 0.05$) between broilers or turkeys fed NFD without or with protease, therefore, the average of the two treatments are presented for each of the species in Table 3. Ileal endogenous flow of Glu was greatest whereas Cys was lowest. Amongst the indispensable AA, the ileal endogenous flow of Met was the lowest.

The AIAAD and SIAAD of wheat-DDGS without or with protease in broilers are presented in Table 4. On the 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.

Regardless of protease, the coefficient of AIAAD or SIAAD for Lys was low and ranged from -0.28 to 0.03. 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 (excluding Lys), the greatest AIAAD coefficient for wheat-DDGS without protease was 0.56 for Phe (0.56) whereas the lowest value of 0.37 was observed for Met and Thr. 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 but had no effect on the other AA.

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 for 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.

Experiment 2

The ileal endogenous AA flow for the turkeys are presented in Table 3 and expounded above.

The coefficients of AIAAD for turkeys receiving wheat-DDGS without- or with protease supplementation are presented in Table 5. Regardless of protease supplementation, the coefficient of AIAAD or SIAAD for Lys was low and ranged from -0.11 to 0.03. Coefficients of AIAAD and SIAAD were also low and highly variable for Asp (ranging from 0.04 to 0.45). The coefficients of AIAAD in the wheat-DDGS, without protease, for turkeys were lower than 0.50 in all AA except for Glu (0.70) and Pro (0.81). The coefficient of AIAAD, in wheat-DDGS with protease, ranged from 0.35 (Thr) to 0.80 (Pro). Of the indispensable AA, the greatest and lowest AIAAD coefficients were for Phe (0.47) and Thr (0.19), respectively.

The coefficients of SIAAD of the wheat-DDGS without protease ranged from 0.41 (Thr) to 0.89 (Pro) whereas the range was from 0.56 (Arg) to 0.88 (Pro) when protease was added. Except for Cys and Pro, protease improved ($P < 0.05$) the coefficient of AIAAD and SIAAD of all other AA. Exceptions are the tendency for improvement ($P < 0.10$) observed for Arg, and Leu. On the average, protease increased the coefficient of AIAAD or SIAAD in the wheat-DDGS by 0.11.

DISCUSSION

The objective of the current study was to determine the AIAAD and SIAAD of wheat-DDGS without or with exogenous protease in broilers and turkeys. It is attractive to use wheat-DDGS as a feedstuff for poultry because it has greater nutrient content, is becoming more readily available and costs less compared with wheat grain.

The processes involved in the production of DDGS causes variation in its chemical composition among sources (Liu, 2011). Olukosi and Adebiyi (2013) summarised the chemical composition of 463 samples of maize- or wheat-DDGS and observed that the chemical composition of DDGS may vary substantially among sources. However, the CP and AA content in the wheat-DDGS used in the current study is comparable with those reported by Fastinger *et al*. (2006) and Bandegan *et al*. (2009).

Standardised amino acids coefficients are determined from the correction of AIAAD values for AA that are of endogenous origin. Endogenous ileal AA flow was determined using NFD in broilers and turkeys in the current study. Endogenous ileal AA flow in the current study were similar to those reported by Ravindran *et al*. (2004; 2009). Met was

observed to have the lowest basal endogenous flow in the current study and those of Ravindran *et al*. (2004) and (2009). However, endogenous ileal AA flow may be expected to vary slightly among studies due to differences species, age of the birds or analytical methods (Adedokun *et al*., 2008).

In the current study, the AID of N in wheat-DDGS was 49% for broilers and is lower than the average AID of N of five wheat-DDGS (67%) reported by Bandegan *et al*. (2009). Similarly, the SID of N in broilers (51%) in the current study was 18 and 13 percentage points lower than the values reported by Bandegan *et al*. (2009) and Kluth and Rudehutscord (2010), respectively. Of the indispensable AA, the SID of Phe was greatest in the current study and is similar to observations by Bandegan *et al*. (2010) in broilers and Lan *et al*. (2008) in finishing pigs. The AID and SID of Lys in the wheat-DDGS fed to broilers and turkeys in the current study ranged from -0.28% to 3%. Bandegan *et al*. (2009) reported the average SID of Lys in five samples of wheat-DDGS to be 40% in broilers whereas the SID of Lys ranged from -0.04% to 71% in five samples of wheat-DDGS for broilers in Cozannet *et al*. (2011) study.

The AIAAD and SIAAD in wheat-DDGS reported in the current study are generally lower compared with those reported in the study by Bandegan *et al*. (2009) and Cozannet *et al*. (2011). The differences in AA digestibility of wheat-DDGS in the current study and those of Bandegan *et al*. (2009) and Cozannet *et al*. (2011) may be due to differences in heat treatment or the quantity of condensed solubles added to distiller's grains (Fastinger *et al*., 2006). As earlier mentioned, the chemical characteristics of DDGS often vary among plants and within the same plant. For example, the coefficient

of variation of SIAAD in five samples of wheat-DDGS from the same plant ranged from 5.6 to 24.1% in the study of Bandegan *et al*. (2009).

Protease supplementation improved AA digestibility in the wheat-DDGS used in the current study and the digestibility values of AA in the protease-supplemented diet in the current study are similar to the values reported by Bandegan *et al*. (2009) for non-enzyme supplemented wheat-DDGS. It appears therefore that exogenous enzymes may be used to reduce the variation in AIAAD and SIAAD of wheat-DDGS among sources. It is known that exogenous enzymes can both improve nutrient utilisation of poor quality feedstuffs (Classen *et al*., 1995; Bedford and Schulze, 1998) and reduce variability in digestibility of feed ingredients for poultry (Bedford, 2000).

As mentioned earlier, 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 implicated for the reduction and variability in the digestibility of protein and AA in DDGS (Fastinger *et al*., 2006; Cozannet *et al*., 2010). Excessive heat treatment of DDGS used for poultry reduces the digestibility of AA in 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 during 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 measure the colour of wheat-DDGS in the current study, but visual comparisons using a maize-DDGS colour score chart as a guide (Shurson, 2011) indicated that the wheat-DDGS used is relatively darker 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 in broilers than darker coloured maize-DDGS (Ergul *et al*., 2003; Batal and Dale, 2006) and caecectomized roosters (Fastinger *et al*., 2006; Cozannet *et al*., 2011). On the other hand, it is noteworthy that although the colour of 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).

The coefficients of AIAAD and SIAAD of wheat-DDGS were greater in broilers compared with turkeys. On the average, coefficient of AIAAD or SIAAD of wheat-DDGS was 0.13 or 0.10, respectively, greater in broilers compared with turkeys and the largest differences in AA digestibility were observed for His, Thr, Cys, Ser, Gly and Asp. On the other hand, there were narrow differences in the AIAAD and SIAAD of Pro and Glu between broilers and turkeys.

Adedokun *et al*. (2008) reported that the AIAAD of five plant feedstuffs were greater at d 5 and 21 post-hatch in broilers compared with turkeys. The differences in the utilisation of AA in the wheat-DDGS between broilers and turkeys in the current study may be due to differences in physiological development at 28 days of age. Uni *et al*. (1995; 1999) reported that post hatch development of the small intestine in turkeys is slower compared with that of the broiler chick. It is speculated that broilers being physiological more mature on day 28 were able to utilise AA in the wheat-DDGS more efficiently compared with turkeys 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 turkeys. Indeed, protease supplementation increased the SIAAD of a large number of AA in the wheat-DDGS for broilers and turkeys by up to 21 percentage points. The greatest improvements in the coefficients of AIAAD to protease were observed for Met (+ 0.12) and Arg (+ 0.15). Similar observation was noted for the coefficient of SIAAD where Met (+ 0.16) and Arg (+ 0.21) were also the most responsive to protease in broilers.

On the other hand, Asp, Gly, Ser and Glu were common to both AIAAD and SIAAD as the least responsive to protease in the current study. In turkeys, the most pronounced responses to protease were observed in the coefficients of AIAAD and SIAAD of Gly (+ 0.16), Ser (+ 0.16), Met (+ 0.17), Thr (+ 0.17), Ala (+ 0.17) and Asp (+ 0.19). Proline

and Glu were common to both AIAAD and SIAAD as the least responsive to protease in turkeys. The effect of exogenous enzymes reduces as the digestibility of the substrate increases (Cowieson, 2010), as such, the lesser response of Pro and Glu to protease, relative to the other AA, in the current study is likely due to the greater (> 70%) digestibility of Pro and Glu in the treatments without protease. Comparing broilers and turkeys, it was noted that Met was common in both species as the AA with the greatest response to protease whereas Glu was common to both as the AA that is least responsive to protease. Thus it is likely that although broilers will have greater AA digestibility values when compared to turkeys at the same age, the pattern of the digestibility values of the AA and their responses to protease supplementation are quite similar.

Cowieson (2010) observed that the digestibility of AA in corn and wheat-based diets for poultry follows a pattern where Arg and Met are the most digestible AA and Cys is the least digestible. The corresponding effect is that Met and Arg respond the least to phytase and xylanase supplementation in corn and wheat-based diets (Cowieson, 2010). In the current study, Met is among the AA with greatest response to protease supplementation. In addition to the fact that the mode of action of protease differs from that of phytase and xylanase (Adeola and Cowieson, 2011), the marked response of Met to protease supplementation in the current study is most likely due to its low digestibility in the wheat-DDGS because it can be expected that the efficacy of exogenous enzymes will be greater in feedstuffs or diets with low AA digestibility (Cowieson and Bedford, 2009). In the case of Lys, where a negative AID and SID value was observed for broilers and turkeys, a substantial improvement would have been expected with protease supplementation in the current study. Protease increased the

coefficient of AID and SID of Lys from a negative value up to 0.03, which suggests that protease reduced endogenous loss of Lys. However, it appears that majority of the Lys in the wheat-DDGS used in the current study were bound in insoluble Lys-carbohydrate complexes, and this may partly explain the low digestibility and lack of marked response of Lys to protease.

Proteases are often supplemented in the diet as part of an admixture of xylanase, amylase and protease; as such, data on improvement in AA digestibility of feedstuffs when protease alone is supplemented are scarce. The observed improvement in N and AA digestibility in the wheat-DDGS 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 utilizes 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 utilized 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 is concluded from the observations in the current study that AA digestibility in the wheat-DDGS for broilers and turkeys is generally low and variable. Because of the low digestibility of a large number of indispensable AA in wheat-DDGS, the use of crystalline AA may be necessary when wheat-DDGS is used as a source of AA for

broilers and turkeys. On the average, protease improved the digestibility of N and AA in the wheat-DDGS from 5 to 21 percentage units in broilers and turkeys. The magnitude of improvement in individual AA digestibility to protease varied, most likely due to the inherent low digestibility of AA in the wheat-DDGS. To achieve the greatest benefits, wheat-DDGS may not be used as the main source of AA but as a partial substitute for soyabean meal in a protease supplemented diet for broilers and turkeys.

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Table 1. Analysed nutrient composition of wheat distillers dried grains with soluble (as-is basis)

Item	g/kg
Dry matter	858
Crude protein	326
Gross energy (MJ/kg)	18.5
Crude fibre	80.0
Ether extract	72.5
Neutral detergent fibre	389
Acid detergent fibre	223
Ca	1.10
P	6.50
K	11.3
Na	5.20
Indispensable amino acids	
Arg	11.8
His	8.30
Ile	13.7
Leu	22.6
Lys	7.70
Met	4.50
Phe	15.8
Thr	11.5
Trp	3.80
Val	16.2
Dispensable amino acids	
Ala	14.0
Asp	18.3
Cys	5.90
Glu	84.9
Gly	14.9
Pro	30.2
Ser	17.0
Tyr	10.2

Table 2. Composition of nitrogen-free and assay diets used in the experiments.

	Exp. 1, Broilers		Exp. 2, Turkeys	
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 maizestarch 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 soluble as the only source of amino acid

Table 3. Ileal endogenous amino acids flow (mg/kg of DM intake) in broilers and turkeys fed nitrogen free diets[1]

Item	Broilers	Turkeys
Indispensable amino acids		
Arg	269.0	175.3
His	204.4	172.5
Ile	253.3	199.9
Leu	445.4	315.4
Lys	308.0	216.2
Phe	296.3	218.4
Thr	297.5	248.5
Met	120.7	91.20
Val	324.0	267.9
Dispensable amino acids		
Ala	294.3	272.1
Cys	113.5	79.40
Glu	769.8	591.1
Gly	313.2	289.0
Pro	343.9	270.9
Ser	352.9	395.8
Tyr	262.8	201.8
Asp	444.9	396.0

[1]For both broilers and turkeys, there was no difference ($P > 0.05$) between birds fed a nitrogen free diet without- and those fed the diet with protease added, therefore, the average of the two treatments are presented.

Table 4. Coefficients of apparent- and standardised ileal amino acids digestibility (%) of wheat distillers dried grains with soluble 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	0.53	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
Lys	-0.28	0.02	0.12	0.049	-0.01	0.03	0.08	0.245
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 5. Coefficients of apparent- and standardised ileal amino acids digestibility of wheat distillers dried grains with soluble without- or with protease supplementation for turkeys

	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
Lys	-0.11	0.00	0.04	0.021	-0.05	0.03	0.04	0.048
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