DIGESTIBILITY METHODS

Metabolism and Nutrition

Influence of the in vivo method and basal dietary ingredients employed in the determination of the amino acid digestibility of wheat-DDGS with broilers

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ABSTRACT

As distillers dried grains with solubles (DDGS) become increasingly available, it is important to determine their nutritional value for precise feed formulation. The accurate determination of digestibility is crucial and it is known that the methods used will affect the values obtained. An experiment was designed to determine and compare the standardized ileal digestibility (SID) of amino acids from wheat-DDGS using a semi-synthetic diet and a difference method using four further diets based on either corn, wheat, corn-DDGS and wheat-DDGS. Eighty day-old male broilers were fed a commercial starter diet until day (d) 21 and then an adaptation on diet to day 23. The trial period took place between d 24 and 27. Feed intake was measured, excreta collected and at d 27 all birds were culled and ileal digesta was collected for the determination of apparent ileal digestibility (AID) and SID of amino acids. Values determined were similar to those reported elsewhere in the literature, although SID values for lysine were particularly low, being 0.26, 0.27 or 0.32, measured in semi-synthetic, corn or wheat diet backgrounds, respectively. It appeared that diet type employed was influential in the values obtained. The SID values for methionine, cysteine, methionine plus cysteine and arginine were significantly lower ($P < 0.05$) when measured in semi-synthetic diet backgrounds than wheat or corn-based diets. It does appear that dextrose and possibly purified starch have a detrimental impact on the broiler digestive tract. This may impact upon all digestibility methodologies where such a diet base is used.

Key words

Amino acid, Broiler, Diet, Digestibility, Lysine, Methionine, Wheat-DDGS

Abbreviations

AA, Amino Acids; AID, Apparent Ileal Digestibility; DDGS, Distiller Dried Grains with Solubles; SID, Standardised Ileal Digestibility

INTRODUCTION

As worldwide pressure on energy supply to broiler diets continues, and cereals are increasingly directed towards the ethanol industry, there is great interest in the co-products that are generated from that industry. Distillers dried grains with solubles (DDGS) are a high-protein high-fibre product, usually from corn or wheat origin, that remain after fermentation to ethanol, either in the bio-ethanol or potable ethanol sectors, and have potential value to the feed industry. An understanding of the nutritional content of all ingredients is necessary for the accurate formulation of diets for animals. As novel ingredients such as DDGS become available from various sources, it is important to determine their nutritional value so they can be most efficiently used. The accurate determination of digestibility of nutrients is crucial for best cost diet formulation and optimum animal performance (Mosenthin et al., 2000). It is known that the methods used to determine digestibility of energy and amino acids (AA) will affect the values obtained (Adeola and Ileleji, 2009; Kim et al., 2011) and that this may depend on the ingredient of interest (Kim et al., 2012). The regression method is commonly used to determine the digestibility of AA and uses diets with increasing levels of a test ingredient based on the linear relationship between the dietary content of apparent ileal digestible (AID) and total AA derived from the graded level. The content of digestible AA or energy in each diet is calculated and regressed against inclusion ratio. The relationship is then extrapolated to 1000 g/kg of that ingredient to determine the apparent digestibility value. Extrapolation to zero inclusion will allow estimation of endogenous loss, which will allow correction of the apparent value to a true value (Batterham et al., 1979; Short et al., 1999; Wiseman et al., 2003). Similarly, a nitrogen-free diet could be fed to estimate endogenous loss (Adedokun et al., 2008); any AA or protein in digesta and excreta are assumed to be of endogenous origin. Another commonly used method is the direct method in which the assay diet is formulated in such a way that the assay feedstuff provides the sole source of dietary

AA. Standardized Ileal Digestibility (SID), which accounts for basal endogenous loss, can be determined by the method described by Lemme et al. (2004).

As such, it is clear that the method used to determine AA digestibility should be carefully considered and the specifics of that method, such as the diet chosen. Anecdotally, the authors have observed blood and tissue in the excreta of broilers when fed semi-synthetic diets. This was assumed to be indicative of irritation to the gut, perhaps induced by the purified fraction of the diet. In studies designed to investigate semi-synthetic diets for their use in such experiments, Becker et al. (1955) suggested that the use of starch and glucose in piglet diets was not appropriate and noted increased mortality attributed to gastric upset specifically attributable to the high glucose component of the diet. Despite this important finding, the paper of Becker et al. (1955) has not been widely cited and it is common to use high levels of starch and/or di monosaccharides (sucrose, glucose) in semi-synthetic diets. These ingredients are included in diets with the assumption that they provide digestible energy and are neutral in their effects on the digestive tract.

An experiment was designed to determine and compare the SID values of AA from wheat-DDGS using a semi-synthetic diet and by a difference method using four further diets based on either corn, wheat, corn-DDGS or wheat-DDGS to allow comparison of the values by different methodologies but also add values to the growing body of literature on this topic.

MATERIALS AND METHODS

Birds

Eighty day-old male Ross 308 broilers were obtained (PD Hook Hatcheries Ltd, Thirsk, UK) and were group-housed in groups of twenty until d 21. On d 21, birds were re-housed in pairs based on similar weight. Each treatment was fed to 8 replicate cages of two birds per cage. Cages were 37 cm wide by 42 cm tall by 30 cm deep, contained a roost and were wire bottomed. From dy 1 to 21, prior to the trial period, chicks were fed a corn:soybean meal mash diet (Table 1), formulated to be sufficient in energy, AA, vitamins and minerals. At d 21 the birds were assigned to trial diets. From d 24 to 27, (a total of 72 hours) feed intake was measured and excreta collected. At all times, feed and water were provided on an *ad libitum* basis. During the trial period, temperature was maintained at 21ºC and the birds were kept under artificial light for 23 hours per day, with one hour of dark. The air in the metabolism room was continuously circulated and humidity monitored. All birds were culled on d 28 of the experiment by asphyxiation with carbon dioxide and cervical dislocation to confirm death. The weight of each carcass was recorded and the ileal region of the gut was dissected out from the Meckel's diverticulum to the ileal-caecal junction.Ileal digesta was collected to determine the AID and the SID of crude protein (CP) and AA. Digesta were pooled per cage (two birds). All bird protocols were approved by the relevant Ethical Review Committee and all experimental conditions followed official guidelines for the care and management of birds.

Treatment diets

There were 5 dietary treatments (Table 2), which were designed to allow determination of AA digestibility of wheat DDGS in different diet backgrounds. Diet S-DDGS was a semi-synthetic diet including 500 g wheat DDGS/kg and 205 g starch and 200 g glucose/kg. Diets C and W were based on 660 g corn or wheat/kg and 245 g soybean meal (SBM)/kg, respectively. Diets C-DDGS and W-DDGS contained corn or wheat (respectively) at 295g/kg, together with 110 g SBM/kg and500g wheat DDGS/kg. With the exception of the semi-synthetic diet, the proportions of cereal to SBM in the diets were maintained at a constant ratio. All treatments contained a vitamin and mineral premix designed for semi-synthetic diets (Target Feeds, Whitchurch, Shropshire, UK), soya oil to bind the diet and reduce dustiness and titanium dioxide (5 g/kg) as an indigestible marker. All experimental diets were manufactured on site at the University of Nottingham, Sutton Bonington Campus. Cereals were ground using a Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany) fitted with a 4 mm screen and then diets were mixed using a commercial planetary dough mixer. All diets were stored at ambient temperature.

Chemical analyses and calculations

For samples of diets, dry matter (DM) was determined in triplicate. Samples weighing approximately 500 mg were dried to a constant weight at 100°C in a forced air convection oven. Due to their small sample size and collection directly into plastic containers, digesta samples were frozen and then freeze-dried to a constant weight when determining dry matter. The concentration of titanium dioxide (employed as an inert marker) was determined in diet and digesta samples using the spectrophotometric method described by Short et al. (1996). Amino acids analysis was conducted as follows: briefly, diet and digesta samples (~500 mg) were oxidized overnight with a hydrogen peroxide/formic acid/phenol solution, before neutralisation with sodium metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were released from the samples by hydrolysis with 6 N HCl for 24 h at 110°C. Following acid hydrolysis, 7.5N NaOH was added to each sample and the hydrolysate adjusted to pH 2.20, centrifuged (3000 rpm/2 min) and filtered (0.22µm syringe filter). The AA contents in the diets and ileal digesta were determined by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm.

The AID of AA in the assay diets were calculated according to equation:

$AID_D = 1- [(I_D \times A_I)/(A_D \times I_I)]$

where AID_D = AID of AA in the diet, I_D = marker concentration in the assay diet (g/kg DM), A_I = AA concentration in ileal digesta (g/kg DM), A_D = AA concentration in the assay diet (g/kg DM) and I_I = marker concentration in ileal digesta (g/kg DM).

The final SID values attributed to each diet background (Table 5) were calculated in two stages as follows:

Part 1. $SID_D = AID_D + [EELaa\ (g/kg\ DMI)/A_D]$

where SID_D = the SID of AA in the individual 5 diets, AID_D=AID of AA in those diets, EELaa = the mean assumed endogenous loss of AA/kg DM intake (Lemme et al., 2004) and A_D = AA concentration g/kg in the assay diet.

Part 2. The content of SID of each AA in each diet (Table 4) was then calculated by multiplying the content of that AA in the diet by its SID_D value. The coefficient of SID of AA attributed to the wheat DDGS was assumed directly in the S-DDGS diet (SID multiplied by 2 as it was included at 500g/kg of diet and was the only AA source present). It was calculated by difference between the 2 corn and 2 wheat diets according to the difference method (Fan and Sauer, 2005).

Statistical Analysis

All data were exported to JMP v10.0 Pro (SAS Institute, Cary, NC, USA) and subjected to analysis of variance. Means were separated by students t-test and were considered significant at $P<0.05$.

RESULTS

Apparent Ileal Digestibility

Generally, the S-DDGS diet showed very low AID for all AA (Table 3). The AID values for birds fed the other diets were generally higher ($P < 0.05$), particularly for those diets that had no DDGS (Diets C and W). For lysine, methionine, threonine, isoleucine, leucine, valine, histidine, arginine and phenylalanine, the S-DDGS value was lower ($P < 0.05$) than all other diets. The AID values determined in C and W were the highest. The value determined in C-DDGS and W-DDGS were intermediate and significantly different from the highest and lowest. For cysteine, the S-DDGS had the lowest value for AID and the W and W-DDGS the highest. Diets C and C-DDGS were intermediate but not significantly different from the highest or lowest value ($P > 0.05$). For methionine plus cystine, the S-DDGS diet also had significantly lower values than all the other diets ($P < 0.05$), which were not different from each other.

Standardised Ileal Digestibility

The SID content for each experimental diet is shown in Table 4 for reference. The SID values of DDGS for lysine, threonine, isoleucine, leucine, valine, histidine and phenylalanine were not significantly different ($P > 0.05$), when measured in different diet backgrounds (Table 5). However the SID values of methionine, cysteine, methionine plus cystine and arginine of DDGS were significantly affected by the diet ($P < 0.05$), with birds fed the semi-synthetic diet (direct method) exhibiting significantly lower SID values than those fed a corn or wheat-based diet (difference method), which did not differ ($P > 0.05$) between each other.

DISCUSSION

Corn, wheat and sorghum are examples of ingredients used as bioethanol feedstock. Whatever the source, DDGS is relatively high in CP and fibre because starch is removed during ethanol production, concentrating other components. It appears that DDGS from corn is lower in CP, compared to that from wheat, probably reflecting the CP content of the starting material. Olukosi and Adebiyi, (2013) compared corn and wheat DDGS and found that although corn DDGS was lower in CP the content was much less variable between samples. The AA digestibility of corn DDGS may be greater however, and extrusion will improve the digestibility in both types (Oryschak et al., 2010). The values of AID and SID of AA determined in this study were similar to, although slightly lower, than those expected. Bandegan et al. (2009) measured the AID and SID value of 5 samples of wheat DDGS, in a semi-synthetic background using the direct method similar to that used in the current study. For the AID coefficients of lysine they reported variability between 0.24 and 0.46; methionine 0.69 and 0.76 and methionine plus cystine 0.63 and 0.71. The low end values of Bandegan et al. (2009) are higher but not dissimilar to the values currently reported (0.16, 0.61 and 0.55 respectively). Bandegan et al., (2009) reported variation in SID of AA for lysine of 0.29 and 0.50; methionine 0.71 and 0.78 and methionine plus cystine, 0.66 and 0.74. This shows somewhat greater deviation in the current data which generated values of 0.26, 0.64 and 0.58 respectively. Interestingly, in both the current study and that of Bandegan et al. (2009), these values for lysine are considerably lower than expectation, and certainly when compared to the AID of lysine for whole wheat, for example. These low values may be due to heat damage of the DDGS. Cozannet et al., (2010) showed a significant correlation between wheat DDGS colour and lysine digestibility in pigs; those that were lighter had higher lysine digestibility. This may be due to Maillard reaction reducing lysine availability (Friedman, 1996). For corn DDGS, Adedokun et al. (2008) reported low SID lysine, with particularly low levels for dark DDGS compared to light (light, 0.60; dark, 0.31) in broiler chickens. Similarly, with severe heat treatment Amezcua and Parsons (2007) reported lysine digestibilities for corn DDGS as low as 0.08 in broilers. It has been suggested that the AID of lysine from corn origin may be significantly higher than that from wheat (Oryschak et al., 2010). The method employed may also affect the values obtained, for example, the type of animal used for determination. In a study investigating corn DDGS, Adedokun et al., (2009) measured variation between AID of lysine obtained in broilers (0.49), layers (0.43) and force fed roosters (0.15). Li et al., (2013) reported true digestible AA values for lysine in corn DDGS to be approximately 0.46.

It appears that the values obtained for SID of some essential AA depends on the method used and, particularly, the diet background. The SID values for some AA in the current study were significantly reduced when derived in a semi-synthetic background that contained glucose and starch at 200 and 205 g/kg, respectively compared to a corn or wheat-based diet.. Presumably this is attributable to the semi-synthetic portion of the diet and is supportive of previous anecdotal findings. Recently, Kong and Adeola (2013) investigated the effect of varying the ratio of dextrose and starch in a semi-purified, nitrogen-free diet on endogenous losses in broilers. When the ratio of starch to dextrose was 849:0, 566:283 or 283:566 in the diet (g/kg), endogenous losses of AA were not affected. However, when the ratio was 0:849 (high dextrose) a significant increase in endogenous loss was observed. This is interesting as it suggests that purified ingredients, in this case dextrose, can influence endogenous losses. This problem could be via a direct osmotic effect or, as suggested by Becker et al. (1955), through encouraging yeast proliferation and fermentation of the sugar to ethanol causing bloat and damage to the epithelium. Unfortunately there was not a non-purified control in the trial of Kong and Adeola (2013). In fact, such a control diet does not exist as any non-synthetic diet would contain protein and, as a result, endogenous losses of amino acids could not be precisely separated from that derived from feed sources. However, it does raise the question as to whether purified starch may also promote greater endogenous losses compared with conventional ingredients. In contrast, Fan and Sauer (1995) did not find any differences in the SID of AA in canola meal fed to growing pigs based on either the direct method (using 517g corn starch/kg) or derived by the difference method.

Purified starch presents a particle size of approximately 20 microns, ie the size of a single granule (M. Bedford and H. Masey O'Neill, personal communication). Such particles are so small that they will not be retained in the gizzard and thus flow rapidly into the small intestine without significant time for hydration. As a result it is conceivable that purified starch presents a separate problem, distinct from that of glucose. Starch may enter the small intestine in a poorly digested state and thus a large proportion may evade digestion and possibly, over time as the gut adapts, become a significant fermentation source, the consequences of which are manifold. Indeed, Ren et al. (2012) measured the true metabolisable energy (TME) of purified corn starch in force-fed roosters using either a 25 g or a 40 g bolus. The value derived on a 40 g bolus was lower than that of a 25 g bolus. This suggests that when a large amount of starch is fed, the ability of nutrients to be digested and absorbed from the lumen is reduced and that purified starch, as well as dextrose, could be an irritant to gut mucosae.. This hypothesis is highly relevant when considering a difference or regression method for calculating the AME (or phosphorous or nitrogen digestibility) of an ingredient. The varying levels of test ingredient used in the suite of diets for the regression method will also result in graded levels of the "inert" carbohydrate filler, ie purified starch and/or glucose. The latter may have a disproportionate effect on endogenous losses from the tract, leading to an incorrect slope and therefore an incorrect SID values. Rochell et al., (2012) observed generally decreased AA digestibilities in meat and bone meal (MBM)-containing diets that included 500 g dextrose/kg, compared to those that were based on commercial formulations. This could have been due to the sample of MBM being of poor nutritional quality but the fact that digestibilities of some AA were not different, and others significantly so, suggests the effects of diet base were not consistent. For example there were no differences in glycine digestibilities between diet bases. However, the digestibility of cysteine, a key component of mucin (Selle et al., 2000), was less than half that of the commercial diet in the dextrose diet. Disproportionate endogenous losses, brought on by the dextrose diet, could explain such findings. Certainly, with the feeding of a semi-synthetic diet, feeding behaviours and conditions within the tract are affected. Becker et al., (1955) have proposed one such change in behaviour relates to an increase in yeast organisms when dextrose is fed. Vissia and Beynen (2000) suggested that, with a glucose-based diet as opposed to a starch-based diet, intake and faecal output of rats were significantly increased. Digestibility was reduced and presumably these effects indicate a more liquid digesta and as a result increased rate of passage. However, it is possible in the context of the current discussion that increased faecal output could also equate to increased endogenous loss. The current programme suggests that digestibility assays that are based on purified starch and dextrose may be affected by intake and this should always be considered when comparing digestibility values. Further, other monosaccharides have also been shown to be detrimental when included in animal feed (Malone et al., 1971; Douglas et al., 2003; Peng et al., 2004). As such, dextrose and to a lesser extent starch, may be acting as anti-nutritional factors in purified or semi-synthetic diets once a certain inclusion rate threshold is breached. Myrie et al. (2008) suggested that a source of hemicellulose may have an important impact on endogenous losses and should be considered when designing a digestibility diet. Similarly, Cowieson et al. (2004) and Woyengo and Nyachoti (2013) showed an increase in endogenous losses caused by phytate. As such all these potential anti-nutritional factors (ANFs) should be considered and their level in the purified portion of the diet (considered to be neutral) should be minimised, where possible, when using a by-difference or regression method. In the current study, the SID of AA were estimated by correcting the same mean values of basal endogenous AA losses (Lemme et al., 2004) from the AID of AA in the diets. Thus, a lower SID of some AA in wheat DDGS measured by the direct method seems to suggest that the semi-purified diets that contained 200 g glucose/kg may influence endogenous losses of some AA than the diets based on commercial raw materials.

CONCLUSION

The current study was designed to determine the SID of AA from a UK produced wheat-DDGS. The values were derived and compared using a semi-synthetic diet and by a difference method using four further diets based on either corn or wheat to address the question of whether the semi-synthetic or purified portion of an experimental diet has any negative effect on the digestive tract. It does appear, along with anecdotal reports of poor gut health, that dextrose and possibly purified starch could have a detrimental impact on the broiler digestive tract. The SID of some AA (methionine, cystine and arginine) were lower in W-DDGS when determined by a direct method and using semi-synthetic diet (200 g/kg glucose) compared with values derived through a by-difference method based on corn or wheat. This may suggest that the semi-purified diets that contained 200 g glucose/kg may promote higher endogenous losses of some AA compared with using the commercial-type of diets in broilers.

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Table1. Starter feed diet formulation, g/kg except where stated

Ingredients		Calculated composition (of diet, all expressed as total)	
Corn	624.5	ME, kcal/kg	3084
Soybean meal	260.0	CP	204.3
Fullfat soy	50.0	Calcium	9.40
Soybean oil	20.0	Available phosphorous	4.80
L-Lysine.HCl	4.0	Sodium	1.60
DL-Methionine	4.0	Crude fat	55.1
L-Threonine	1.5	Crude fibre	27.2
Limestone	12.5	Lysine	13.9
Monocalcium phosphate	15.0	Methionine + Cystine	10.4
Sodium bicarbonate	1.5	Threonine	9.20
Sodium chloride	2.5	Tryptophan	2.15
Vitamin and mineral premix[1]	4.0		
Elancoban[2]	0.5		

[1]Vitamin and mineral pre-mix provided the following (per kg of diet): Vitamin A, 13500IU; Vitamin D3, 5000 IU; vitamin E 100IU; vitamin B1, 3mg; vitamin B2, 10mg; vitamin B6 3mg; vitamin B12, 30ug; vitamin K, 5mg; niacin, 60mg; pantothenic acid, 15mg; folic acid, 1.5ug; biotin, 251ug; choline, 250mg; iron, 20mg; manganese, 100mg; copper, 10mg; zinc, 80mg; iodine 1mg; selenium, 0.25mg; calcium, 1000mg.

[2]Supplied 100 ppm of monensin per kg of diet.

Table 2. Experiment diet formulations (g/kg diet)

	DDGS	Dietary treatments				
		S-DDGS	C	C-DDGS	W	W-DDGS
Corn			660	295		
Wheat					660	295
Soybean meal			245	110	245	110
Wheat DDGS		500		500		500
Wheat Starch		205				
Glucose		200				
Soya Oil		50	50	50	50	50
Vitamin and Mineral Premix[1]		40	40	40	40	40
Ti0$_2$		5	5	5	5	5
Analyzed amino acid composition[2]						
Lysine	6.7	3.3	10.8	8.2	11.5	8.5
Methionine	5.3	2.7	2.9	4.0	3.1	4.1
Cystine	13.3	6.7	6.0	9.3	7.0	9.8
Methionine + Cystine	18.6	9.3	8.9	13.3	10.1	13.8
Threonine	12.2	6.1	7.9	9.6	8.2	9.8
Isoleucine	12.6	6.3	7.7	9.8	8.9	10.3
Valine	16.4	8.2	8.9	12.2	10.2	12.8
Leucine	26.2	13.1	18.3	21.3	16.9	20.7
Histidine	7.3	3.6	5.5	6.1	5.9	6.3
Phenylalanine	18.1	9.0	10.0	13.5	11.2	14.1
Arginine	14.7	7.3	13.1	13.2	14.5	13.8

[1]Vitamin and mineral pre-mix provided the following (per kg of diet): phosphorus, 5g; magnesium, 90mg; calcium, 7.5g; sodium, 1.5g; copper, 0.6mg (as copper sulphate); selenium, 160µg (as selenium BCP); vitamin A, 7500 IU; vitamin D3, 1500 IU; vitamin E, 10 IU (as α-tocopherol acetate); vitamin B1, 5mg; vitamin B2, 4mg; vitamin B6, 4mg; vitamin B12, 10 µg; pantothenic acid, 9mg; folic acid, 1.5mg; biotin, 150 µg; choline, 1500mg.

[2] Values expressed as g/kg (100% DM basis).

Table 3. The coefficient of apparent ileal digestibility (AID) of amino acids of the experimental diets measured in broilers

	Dietary treatments[1]						
Amino acids	S-DDGS	C	C-DDGS	W	W-DDGS	P	RMSE
Lysine	0.16c	0.82a	0.58b	0.79a	0.59b	<0.001	0.059
Methionine	0.61c	0.85a	0.74b	0.83a	0.74b	<0.001	0.067
Cystine	0.49b	0.59ab	0.62ab	0.70a	0.68a	0.040	0.130
Methionine + Cystine	0.55b	0.72a	0.68a	0.76a	0.71a	0.003	0.095
Threonine	0.47c	0.68a	0.57b	0.68a	0.58b	<0.001	0.048
Isoleucine	0.56c	0.76a	0.65b	0.77a	0.66b	<0.001	0.058
Leucine	0.63c	0.79a	0.71b	0.78a	0.71b	<0.001	0.043
Valine	0.46c	0.69a	0.58b	0.69a	0.57b	<0.001	0.065
Histidine	0.54c	0.76a	0.64b	0.77a	0.66b	<0.001	0.048
Phenylalanine	0.70d	0.79ab	0.74c	0.80a	0.75bc	<0.001	0.036
Arginine	0.58d	0.85a	0.72c	0.81b	0.71c	<0.001	0.035

[1]S-DDGS, semisynthetic diet containing DDGS; C, corn diet; C-WDDGS, corn diet containing DDGS; W, wheat diet; W-DDGS, wheat diet containing DDGS.
[a-d] Within a row, means without common superscripts are significantly different as indicated by the P value.

Table 4. The content of standardised ileal digestible amino acidsin each diet (g/kg)

	Dietary treatments[1]				
Amino acids	S-DDGS	C	C-DDGS	W	W-DDGS
Lysine	0.79	9.11	4.97	9.29	5.22
Methionine	1.71	2.56	3.02	2.67	3.07
Threonine	3.43	5.94	6.05	6.13	6.27
Isoleucine	3.92	6.23	6.72	7.27	7.23
Valine	4.21	6.61	7.51	7.45	7.79
Leucine	8.65	14.82	15.53	13.54	14.96
Histidine	2.19	4.39	4.11	4.71	4.33
Phenylalanine	6.56	8.14	10.32	9.16	10.82
Arginine	4.45	11.39	9.71	11.98	10.08
Cystine	3.45	3.70	5.95	5.03	6.76
Methionine + Cystine	5.41	6.66	9.30	7.96	10.01

[1]S-DDGS, semisynthetic diet containing DDGS; C, corn diet; C-WDDGS, corn diet containing DDGS; W, wheat diet; W-DDGS, wheat diet containing DDGS.

Table 5. The coefficient of standardised ileal digestibility (SID) of amino acid in wheat DDGS measured broilers affected by diet type

	Diet types							
	Semi-synthetic[1]		Corn[2]		Wheat[2]			
	Mean	SD	Mean	SD	Mean	SD	P	RMSE
Lysine	0.26	0.102	0.27	0.036	0.32	0.039	0.056	0.064
Methionine	0.64b	0.046	0.70a	0.012	0.71a	0.039	0.004	0.035
Cystine	0.52b	0.058	0.65a	0.049	0.68a	0.049	<0.001	0.057
Methionine + Cystine	0.58b	0.048	0.68a	0.029	0.69a	0.049	<0.001	0.043
Threonine	0.56	0.058	0.56	0.022	0.58	0.024	0.463	0.037
Isoleucine	0.62	0.061	0.62	0.023	0.63	0.031	0.871	0.040
Leucine	0.66	0.038	0.68	0.021	0.68	0.023	0.357	0.028
Valine	0.52	0.073	0.56	0.027	0.54	0.037	0.250	0.048
Histidine	0.6	0.057	0.59	0.022	0.61	0.029	0.548	0.038
Phenylalanine	0.73	0.049	0.74	0.014	0.74	0.014	0.491	0.029
Arginine	0.58b	0.042	0.68a	0.018	0.69a	0.022	<0.001	0.043

[a,b] Within a row, means without common superscripts are significantly different as indicated by the P value.

[1]Using direct method; [2]Using the difference method.