Evaluation of a High Protein DDGS Product in Broiler Chickens: Performance, Nitrogen-Corrected Apparent Metabolisable Energy, and Standardised Ileal Amino Acid Digestibility

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ABSTRACT

1. New production processes and additional uses for corn co-products have increased the availability of distiller's dried grains with solubles (DDGS) with varying energy and amino acid digestibility, for use in poultry feed. The objective of this study was to determine the performance, N-corrected metabolisable energy (AME_n), and amino acid (AA) digestibility of a 34% CP (as fed) high-protein DDGS (HP-DDGS) included in poultry diets for Cobb 500 broiler chickens.

2. A total of 832 Cobb 500 broilers were randomly assigned to four dietary treatments containing 5% conventional DDGS (CV-DDGS) as a control or 10, 15, and 20% HP-DDGS and fed for 42 d. After the performance trial, 240 birds from the original 832 were selected for a concurrent AME_n and AA digestibility experiment consisting of two AME_n diets and two AA diets.

3. Birds fed diets containing 15 and 20% HP-DDGS gained less weight than birds fed the CV-DDGS ($P < 0.05$) but did not differ in feed intake (FI), and therefore had a less efficient FCR than the control ($P<0.05$). The AME_n of HP-DDGS was determined to be 11.4 MJ/kg. The standardised ileal amino acid digestibility (SIAAD) of the essential amino acids Lys and Met were determined to be 80.9 and 88.6%, respectively.

4. HP-DDGS can be included in broiler diets up to 10% without any negative impact on performance or requiring supplemental Lys and Arg. The results from the AA digestibility study indicated that HP-DDGS could be a good source of digestible Lys.

Key Words: High protein DDGS, feedstuffs, amino acids, metabolisable energy, digestibility, broilers

INTRODUCTION

Distillers dried grains with solubles (DDGS) are a co-product of the dry-milling process in ethanol production and have been used in poultry diets because they are readily-available and contain significant and concentrated amounts of CP, AA, energy and other important nutrients (Swiatkiewicz and Koreleski 2008). Due to variations in growing conditions, ethanol processing methods and extracting oil, the nutrient composition of DDGS from different sources varies widely (Meloche *et al*. 2013, 2014). As a result, the recommended inclusion rates of DDGS in poultry diets vary. Conventional DDGS can be fed at high inclusion rates of 15-24% of the diet without negatively impacting broiler performance (Min *et al*. 2015; Shim *et al*. 2011).

Intentionally changing the processing method can enhance aspects of the nutrient profile in the final DDGS, such as increased CP and metabolisable energy (ME). Alterations to the processing method and the subsequent enhancement of nutrient profiles have the potential to alter recommended inclusion rates of DDGS in broiler feed and can impact performance (Jung and Batal 2010). To produce high-protein (HP)-DDGS used in this study, fibre was removed using a proprietary technique and the resulting feed ingredient contained ≥34% CP as-fed, compared to an average of 27% CP seen in conventional (CV)-DDGS from the same plant. Additional processing of the DDGS to remove fibre along with initial component analyses suggested improved AA digestibility in the final product. The intent of additional processing was to produce a feed ingredient that could potentially replace high-protein ingredients such as soybean meal while simultaneously reducing the need for supplemental AA. Isocaloric and isonitrogenous diets were formulated to test the effects of increasing inclusion level of HP-DDGS at the expense of feed protein components on broiler performance.

To evaluate the availability of the increased CP and energy content of HP-DDGS, equations for N-corrected apparent metabolisable energy (AME_n) and standardised ileal AA digestibility (SIAAD) were used, based on previous research (Rochell *et al*. 2011; 2013). The objective of this study was to determine the effects of increasing inclusion of HP-DDGS on performance and to examine how the altered processing technique impacted AA digestibility and AME_n of HP-DDGS in feed for broiler chickens.

MATERIALS AND METHODS

Dietary Treatments

Performance Experimental Treatments

Conventional and HP-DDGS from the same producer were obtained and used for performance, AME_n, and AA digestibility experiments (Table 1). For the feeding trial, starter, grower, and finisher diets were formulated to be isonitrogenous and isocaloric within each diet for phase of growth (Tables 2-4). The treatments consisted of four diets containing 5% CV-DDGS as a control and 10, 15, and 20% HP-DDGS test diets. A 5% HP-DDGS diet was included in the original experimental design, but performance was negatively altered independently of any action of the HP-DDGS, therefore, it was excluded from the final analysis.

Digestibility Experimental Treatments

Diets used in the energy digestibility experiment consisted of an 85% complete basal diet ± sucrose control or HP-DDGS (Table 5). Diets used in the AA digestibility experiment consisted of a N-free purified control diet to measure basal endogenous AA losses and a second semi-purified test diet that contained HP-DDGS as the only source of protein (Table 6). All diets in the AA and digestibility experiments contained 0.50% titanium dioxide as an indigestible marker and were formulated to be adequate in energy, AA, and minerals according to the NRC (1994).

Birds and Housing

All animal procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. A total of 832 Cobb 500 broiler chicks (Welp Hatchery, Bancroft, IA) at 1 d of age were housed in 64 floor pens measuring 1.2m x 1.2m containing 13 birds per pen for a total of 42 d. The room temperature was kept at a maximum of 32°C for the first 7 d and was decreased by about 1°C per day until a final temperature of 21°C was reached by experimental d 30. The lighting schedule for the first 7 d was 23 h of light and 1 h of dark before being transitioned to 20 h of light and 4 h of dark for the remainder of the experiment. Each pen was randomly assigned to 1 of 4 dietary treatments with a total of 16 replicates per treatment. Birds were given *ad libitum* access to mash feed and water in a round feeder and nipple drinker.

Performance

The 42-d trial was divided into three feeding phases; a 14-d starter, 21-d grower, and 7-d finisher period. Birds were weighed as a pen at the start of the study and at the end of each of the three feeding phases. Feeders (including feed) were weighed at the start of the study, end of each period, and every time feed was added. The body weight (BW), BW gain (BWG), feed intake (FI), and feed conversion ratio (FCR) per bird was calculated, with FCR calculated as FI per kg BWG.

Nitrogen-Corrected Apparent Metabolizable Energy

At the end of the 42 d performance study, 240 birds were selected from the original 832 and placed in 24 pens measuring 1.2m x 1.2m containing 10 birds each. Birds were selected based on size and gender so that each pen contained five healthy males and five females of approximately the same BW (pen weight standard deviation = 1.1kg). Half of the pens were randomly assigned to one of two diets that differed in energy digestibility for a total of six replicates per treatment. Birds were given *ad libitum* access to food and water through a round feeder and nipple drinker for 7 d. Birds were weighed at the start and end of the 7-d period. Fresh excreta samples were collected at on d 7. The AME_n of the HP-DDGS was calculated using a previously determined equation (Rochell *et al*. 2011). The total AME_n intake of each dietary treatment was calculated using 8.73 as the nitrogen correction factor using the following equation (Titus 1955):

$$\mathrm{AME_n\ intake} = [\mathrm{GE\ intake} - \mathrm{GE\ excretion}] - [8.73\ \times\ (\mathrm{N\ intake} - \mathrm{N\ excretion})]$$

The AME_n for sucrose was obtained from the poultry NRC and supporting publications (NRC 1994; Janssen 1989). The contribution of AME_n from sucrose in the control diet (15.6 MJ) was subtracted from the total consumed AME_n of the control diet to determine the basal AME_n intake by the following equation (Hill and Anderson 1958):

$$\text{Basal AME}_\text{n}\ \text{Intake} = \text{AME}_\text{n}\ \text{of control diet} - 15.6\ \text{MJ of ME/kg of glucose}$$

The equation below was then used to calculate the AME_n of HP-DDGS (Rochell *et al*. 2011):

$$\text{HP} - \text{DDGS AME}_\text{n} = \frac{(\text{Total AME}_\text{n}\ \text{intake} - \text{basal AME}_\text{n}\ \text{intake})}{\text{co} - \text{product intake}}$$

Amino Acid Digestibility

The remaining 240 birds selected for the digestibility study were assigned to control or HP-DDGS test diets, as mentioned previously, for 7 d. Housing, feeding, and water conditions were the same as those for birds in the AME_n assay above. Birds were weighed at the start and end of the 7-d period before being euthanised by carbon dioxide to collect digesta from the lower half of the ileum identified as the section of intestine between Meckel's diverticulum and the caecal junction. Digesta was removed from this section by compressing one end of removed segment and moving along its length until its contents were emptied. All samples were pooled by pen and stored at -20°C until analysis.

The following equation was used to determine the apparent ileal amino acid digestibility (AIAAD) (Lemme *et al*. 2004):

$$\text{AIAAD} = \frac{\left[\left(\frac{\text{AA}}{\text{TiO}_2}\right)_{\text{diet}} - \left(\frac{\text{AA}}{\text{TiO}_2}\right)_{\text{digesta}}\right]}{\left(\frac{\text{AA}}{\text{TiO}_2}\right)_{\text{diet}}}$$

Ileal endogenous AA (IEAA) flow in birds fed the nitrogen-free control diet was calculated by using the equation below (Moughan *et al*. 1992):

$$\text{IEAA, mg per kg of dry matter intake (DMI)} = \text{ileal AA} \times \left(\frac{[\text{TiO}_2]_{\text{diet}}}{[\text{TiO}_2]_{\text{digesta}}}\right)$$

AIAAD coefficients were standardised by the IEAA flows to determine the standardised ileal AA digestibility (SIAAD) using the following equation (Adedokun *et al*. 2007):

$$\text{SIAAD} = \text{AIAAD} + \left[\frac{\text{IEAA Flow (g per kg DMI)}}{\text{Dietary AA content (g per kg DMI)}}\right] \times 100$$

Sample Analysis

After collection, all samples were stored at -20°C until analysis. Diet, digesta, and excreta samples were dried in a 60°C oven (Fisher Scientific, Pittsburgh, PA) and ground through a 1 mm screen (Arthur H. Thomas Company, Philadelphia, PA) prior to analysis. Analyses for DM (AOAC method 930.15), CP (AOAC method 990.03), crude fat (AOAC method 2003.06), and gross energy (GE) were conducted on all the diets from the performance and digestibility experiments. Diets from the energy and AA digestibility experiments were analysed for titanium dioxide concentration using the method describe by Leone (1973). Additionally, the amino acid profile was analysed for the diet with HP-DDGS as the sole source of crude protein.

Excreta samples from the birds fed the energy digestibility diets were analysed for DM, GE (Parr 6200 bomb calorimeter, Parr Instrument Company, Moline, IL), CP, and titanium dioxide concentration. Digesta and excreta samples from the birds fed the N-free and AA digestibility diets were analysed for DM, CP, AA profile, and titanium dioxide concentration. All AA profiles were analysed at a commercial laboratory (University of Missouri Agriculture Experiment Station Chemical Laboratories; Columbia, Missouri).

Statistical Analysis

Performance measurements were analysed using the GLM procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The LSMeans statement with the PDIFF option was used to calculate mean values with an α-value of $P < 0.05$ used to determine significance among means. Means were separated using the MEANS statement with the LINES TUKEY option. An additional non-orthogonal contrast was performed to determine differences between the 5% CV-DDGS diet and all three HP-DDGS treatments. The effects of HP-DDGS inclusion on performance were analyzed by orthogonal contrasts for linear and quadratic effects.

RESULTS

Performance

As expected, HP-DDGS contained a greater concentrations of CP and most AA compared to the conventional-DDGS. In addition, the HP-DDGS contained slightly less crude fat and slightly greater crude fibre than the conventional-DDGS, but the estimated ME was calculated to be similar (Table 1).

At the start of the performance study, there were no significant differences in the BW of the birds (Table 7). During the starter period (d 0 to 14), birds fed the 15% HP-DDGS diet had a 10.3% lower BW ($P<0.0001$), 8.8% reduction in BWG ($P<0.0001$), and 9.4% less efficient FCR than birds fed the control diets ($P<0.0001$). There was a 3.8% decrease in FI between birds fed the 20% HP-DDGS and 15% HP-DDGS diets ($P=0.005$; Table 7).

During the grower period (d 14 to 35), birds fed the 15% HP-DDGS diet had 4.0% lower BW compared to the control diet ($P<0.0001$) but did not differ in BWG or FI. The FCR was 1.8% less efficient than the control, but not different than the 10% HP-DDGS diet, indicating that birds fed 15% HP-DDGS may have recovered from the reduced performance observed in the starter period. Compared to the control group, birds fed the 20% HP-DDGS diet had a 6.0% less efficient FCR ($P<0.0001$).

During the finisher period (d 35 to 42), birds fed the 15 and 20% HP-DDGS diets had a 5.2 and 4.4% lower BW, respectively, compared to birds fed the control diet ($P<0.0001$). Birds fed the 15% HP-DDGS diet had a 4.6% reduced FI compared to the control group ($P=0.02$), while the FI of birds fed 20% HP-DDGS was not different than any other group ($P>0.05$). There were no differences noted in FCR between birds fed the different dietary treatments during the finisher period.

Overall (d 0 to 42), birds fed the 15 and 20% HP-DDGS diets had a 4.9 and 4.2% reduced BWG, respectively, compared to the control group (P<0.0001). Throughout the three feeding periods there were differences in BW, BWG, and FI, but negative effects on FCR for the entire 42-d period were only observed in birds fed diets containing 15 and 20% HP-DDGS. These two groups had a 3.0 and 4.7% less efficient FCR, respectively, compared to the control group (P<0.0001; Table 7). The linear and quadratic effects of HP-DDGS inclusion on performance parameters are shown in Table 7.

AME_n and Amino Acid Digestibility

The HP-DDGS used in this study had a calculated AME_n of HP-DDGS of 11.4 ± 0.7 MJ/kg (Table 8). The determined AME_n, SIAAD, and endogenous losses of each AA are presented in Table 8.

DISCUSSION

Performance

The performance results indicated that HP-DDGS can be fed at inclusion rates of up to 10% of the diet without any negative impact on growth or requiring additional supplemental single amino acids. This is in contrast with another study that reported negative impacts of HP-DDGS on overall performance after 20 days at inclusion rates greater than 6% of the diet (Jung and Batal 2010). This discrepancy between results may be due to differences in the age of the birds and nutrient composition of HP-DDGS utilised.

While overall performance was negatively impacted by HP-DDGS in the study by Jung and Batal (2010), they did not observe changes to BWG in the 7-d starter period at inclusion rates of 12%. This is in contrast to the reduced performances observed at high inclusion rates in the starter period of the current study. Caking of the HP-DDGS in the diet may partially explain changes in bird performance during the starter period, which was ameliorated by further grinding HP-DDGS prior to mixing in the grower and finisher period diets. The textural differences in the higher inclusion diets as a result of caking resulted in sorting of feed by the chicks, which may have unintentionally altered performance in the starter period. Overall reductions in efficiency may partially be explained as carryover from reduced performance during the starter period, when chicks build their skeletal structure to accommodate muscle growth during the grower period. This was supported by the observation that performance differences due to higher HP-DDGS inclusion rates reduced over time (Table 7).

Differences in the AA profile of the test diets could further elucidate the discrepancies in performance that were observed with increasing the inclusion rate of HP-DDGS. The HP-DDGS product was produced by a proprietary method that removed fibre, and was therefore assumed to have higher protein availability and digestibility. The aim for the potential feedstuff was to replace several feed protein components with a more concentrated, digestible ingredient, therefore, all diets in the current study were formulated as simply as possible, and balanced based on total CP and energy for each growth stage. The AA profiles of the diets changed as higher levels of HP-DDGS displaced soybean meal and corn with the assumption of improved digestibility of HP-DDGS. The percentage of two essential AA in poultry diets, Lys and Arg, were altered as HP-DDGS inclusion increased. Unlike Lys, Arg is not a limiting AA in corn and soybean meal-based diets. The soybean meal used in this study contained 3.0% Lys compared to 1.2, 0.7 and 0.2% in HP-DDGS, CV-DDGS, and corn, respectively. In contrast, soybean meal contains 3.6% Arg, HP-DDGS and CV-DDGS 1.1%, and corn 0.86%. As HP-DDGS concentration in the diets increased, the AA profile of corn was over-represented as HP-DDGS displaced soybean meal, resulting in an overall decrease in Lys and Arg. The overall effect was that diets with a higher inclusion of HP-DDGS had a diminished AA profile compared to diets with CV-DDGS or a lower inclusion of HP-DDGS.

The NRC (1994) suggests a range of 0.85 to 1.10% for total Lys as being acceptable for 0 to 6 week- old broilers. The Cobb breeder recommendation suggests that starter, grower and finisher diets contain 1.18%, 1.05, and 0.95% digestible Lys, respectively. The 20% HP-DDGS diet was calculated to contain 1.12, 0.96 and 0.86% digestible Lys, respectively, so diets were within NRC recommendations and within 0.06-0.09% of Cobb 500 recommendations. Arg was affected to a greater degree than Lys at higher inclusion rates of HP-DDGS (15 and 20%, respectively) because no supplemental Arg was added to the diet. It is important to note that Arg is not economical to supplement. Recommended digestible Arg in starter, grower, and finisher diets has been suggested to be 1.24, 1.10, and 1.03%, respectively (Cobb 500 2015) and the NRC (1994) states 1.0 to 1.25% total Arg as being acceptable for 0 to 6 week-old broilers. In the current study, the 15 and 20% HP-DDGS diets in the starter period contained 1.08 and 1.01% digestible Arg, grower diets contained 0.97 and 0.91%, and finisher diets contained 0.90 and 0.84%, respectively. The experimental diets could have been formulated to contain increased amounts of supplemental Lys and Arg as HP-DDGS concentration increased in the diet; however, preliminary analyses and digestibility values suggested an increased digestibility and content of both Lys and Arg in HP-DDGS. Therefore, this experiment was designed to determine if this different DDGS production process could result in a product to replace both SBM and supplemental AA, while maintaining performance and FI in response to textural changes brought about by fibre removal. The results suggested the need to supplement AA in diets where concentrations of HP-DDGS exceed 10%. Consequently, the experimental diet formulation used herein highlighted the need for accurate analysis with new feed ingredients. For practical diet formulations with higher inclusion rates of HP-DDGS, AA deficiencies can be easily corrected by adding supplemental AA.

Determined AME_n

Previous work reported an average AME_n from six sources of DDGS as 11.2 MJ/kg ranging from 9.0 to 13.0 MJ/kg (Rochell *et al*. 2011). Analysis of reduced-oil DDGS from 15 sources reported an average AME_n of 9.7 MJ/kg ranging from 7.8 to 11.8 MJ/kg (Meloche *et al*. 2013). Reported values from 15 sources of CV-DDGS had an average of 11.6 MJ/kg with a range of 8.3 to 15.2 MJ/kg (Meloche *et al*. 2014). Rochell *et al*. (2011) reported an AME_n from two sources of HP-DDGS as 11.3 and 12.3 MJ/kg. The HP-DDGS used in this study had an average AME_n that was greater than the CV-DDGS published by Meloche *et al*. (2013), but similar to values reported by Rochell *et al*. (2011) and Meloche *et al*. (2014). The determined AME_n of the HP-DDGS used in this study was lower than other HP-DDGS, but fell within published ranges for CV-DDGS (Rochell *et al*. 2011; Meloche *et al*. 2013, 2014). Nutrient composition of DDGS varies across different sources and this discrepancy would likely explain the differences in AME_n as a result of different processing methods and total amount of oil removed from the original DDGS product (Swiatkiewicz and Koreleski 2008; Meloche *et al*. 2013, 2014).

Amino Acid Digestibility

Comparisons between the SIAAD of HP-DDGS used in this study and the AA digestibilities of corn and other by-products in published literature are represented in Table 9. Published reports using the same method to measure SIAAD typically freeze-dry digesta to avoid damage or irreversible binding of AA due to high temperatures used during oven-drying. Results published by Dale *et al*. (1985) reported that both drying methods lead to similar results in AME_n and AA availability assays, suggesting that oven-drying the digesta did not influence the SIAAD results in this study.

Notably, the determined SIAAD of Lys at 80.9% was much higher than reported values of 58.3% SIAAD and 69.6% digestibility in CV-DDGS reported by Adedokun *et al*. (2015) and Batal and Dale (2006), respectively. It was also greater than the 73.0% SIAAD and 73.9% digestibility in HP-DDG reported by Applegate *et al*. (2009) and Kim *et al*. (2008), respectively. Additionally, the SIAAD of indispensable AA such as Met at 88.6%, Arg at 90.3%, Thr at 81.2%, and Val at 85.5% were greater than values reported for CV-DDGS (Batal and Dale 2006; Adedokun *et al*. 2015) and HP-DDG (Kim *et al*. 2008; Applegate *et al*. 2009). While Arg had increased digestibility compared to traditional DDGS, the performance results showed a need to add supplemental Arg beyond 10% dietary HP-DDGS. In most cases, the HP-DDGS analysed in this study had better or similar SIAAD or digestibilities compared to varying sources of CV-DDGS and HP-DDG reported elsewhere. With the exception of the average digestibility of Leu in HP-DDGS reported by Kim *et al*. (2008), instances where the reported value was higher than that determined for HP-DDGS in this study were observed in dispensable AA only (Table 9).

The addition of solubles in later stages of DDGS production is unregulated and inconsistent, resulting in varying AA digestibilities between DDGS sources and lower AA digestibilities compared to DDG, which has been observed in both swine and poultry models (Adeola and Ragland 2016; Kim *et al*. 2011). Differences observed between the HP-DDGS used in this study and reported values for HP-DDG may be due to the addition of solubles in the HP-DDGS or the production process and starting materials. Interestingly, the HP-DDGS in this study had greater SIAAD- in most cases- compared to HP-DDG with a greater CP content. Kim *et al*. (2008) utilised HP-DDG with 44.1% CP, while the HP-DDG used by Applegate *et al*. (2009) contained 54% CP. These results indicated that the HP-DDGS used in this study contained more digestible AA and may be used to reduce supplemental AA in broiler diets, but also it is important to consider the high degree of variation in nutrient composition of DDGS from different sources and manufacturing processes.

Compared to whole corn grain, the AA in CV-DDGS are typically concentrated approximately three-fold. The AA profile of corn contains, on average, 0.2% Lys and 0.2% Met, while CV-DDGS contain 0.7% Lys and 0.5% Met. The HP-DDGS used in this study had an AA profile containing 1.2% Lys and 0.7% Met, as fed (Table 1). The concentrations of Lys and Met in CV-DDGS were 0.5% and 0.2% lower, respectively, than those in the HP-DDGS. Published values by Adedokun *et al*. (2015) listed the SIAAD of Lys and Met in three sources of corn with an average of 92.4 and 95.0%, respectively (Table 9). It is important to note that corn contains less Lys and Met, as illustrated above. According to the same study, the digestibility of the same AA from five sources of CV-DDGS was, on average, 58.3% for Lys and 81.7% for Met (Adedokun *et al*. 2015). In comparison to reported values, Lys in corn was 11.5% more digestible than in HP-DDGS, but the Lys in CV-DDGS was 22.6% less digestible than in HP-DDGS. High protein DDGS contained 6.4% less digestible Met than corn but contained 6.9% more digestible Met than DDGS. The results of the current study provided insight into the viability of HP-DDGS as an ingredient in broiler diets. Producers wishing to use HP-DDGS or other new DDGS products in their practical diets must consider diet cost and AA trade-offs. Inclusion rates of the HP-DDGS used in this study above 10% may benefit from supplementation of Lys and Arg through synthetic sources or soybean meal inclusion rates.

From an engineering and function standpoint, the quality of a product formed by new processing and drying methods must not alter the textural properties of the diet in which it is incorporated in such a way that birds can sort feed. This behaviour can lead to unintentionally reduced performance. Feeding HP-DDGS at levels at or below 10% could reduce the need for supplemental AA in broiler diets due to the increased concentration of Lys and Met in HP-DDGS versus CV-DDGS and corn in addition to the high digestibility of indispensable and limiting AA.

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Table 1. Composition of HP- and CV-DDGS used in performance, AA digestibility, and ME experiments, as-fed basis

Item, g/kg[1]	HP-DDGS[2]	CV-DDGS[2,3]
DM	831.0	898.0
Crude fat	79.1	96.3
Crude fiber	83.5	78.5
CP	341.0	271.0
Arg	14.9	11.0
Cys	5.8	4.5
Gly	12.5	6.0
His	8.8	06.2
Iso	12.6	11.5
Leu	43.2	24.0
Lys	11.6	7.0
Met	7.4	5.0
Phe	15.7	13.5
Ser	16.0	13.0
Thr	13.1	9.3
Trp	3.0	2.0
Tyr	13.4	8.0
Val	16.0	14.0
Poultry ME, MJ/kg	11.0	11.0

[1] Analysed by Midwest Laboratories, Omaha, NE
[2] HP-DDGS = high protein DDGS; CV-DDGS=conventional DDGS
[3] CV-DDGS included at 5% of the diet in performance experiment; HP-DDGS included at 10, 15, and 20% of diets used in the performance experiment and 15% for AA digestibility and ME experiments. HP-DDGS were the sole AA source in the AA digestibility experiment.

Table 2. Composition of experimental starter diets fed to Cobb 500 broiler chickens from d 1 to 14.

	Experimental diet, (%)			
Ingredient, g/kg	5% CV-DDGS[1]	10% HP-DDGS[1]	15% HP-DDGS	20% HP-DDGS
Corn	589.3	577.4	557.9	538.3
Soybean meal, 480.0 g/kg CP	300.0	260.0	227.5	195.0
Soybean oil	11.5	12.7	14.6	16.6
Salt	3.3	3.4	3.4	3.3
DL-Met	4.0	4.0	4.0	4.0
L-Lys•HCl	5.0	5.0	5.0	5.0
L-Thr	1.5	1.5	1.5	1.5
Limestone	10.2	9.8	10.1	10.3
Dicalcium phosphate	17.8	18.8	18.7	18.7
Choline chloride-60	1.0	1.0	1.0	1.0
Vitamin-mineral premix[2]	6.3	6.3	6.3	6.3
Calculated Values, g/kg				
Crude fat	43.2	46.2	50.8	55.5
CP	215.2	215.5	215.3	215.0
Digestible Lys	13.2	12.4	11.8	11.2
Digestible Met	6.6	6.5	6.5	6.5
Digestible Arg	12.2	11.4	10.8	10.1
Digestible Thr	8.5	08.2	8.0	7.8
ME (MJ/kg)	12.6	12.6	12.6	12.6
Analysed Values, g/kg				
Moisture	123.0	126.4	127.3	127.4
DM	877.0	873.6	872.7	872.6
Crude fat	35.2	38.0	47.1	31.8
CP	220.1	206.2	197.6	229.8
GE, MJ/kg	15.9	16.1	16.1	16.5

[1]HP-DDGS= high protein DDGS (341.0% CP as fed); CV-DDGS= conventional DDGS (271.0% CP as fed).

[2]Vitamin and mineral premix provided per kg of diet: selenium 250 µg; retinyl acetate (vitamin A) 2.8 mg; cholecalciferol (vitamin D_3) 68.8 µg; α-tocopherol acetate (vitamin E) 13.2 mg; menadione 1.1 mg; vitamin B_{12} 12 µg; biotin 41 µg; choline 447 mg; folic acid 1.4 mg; niacin 41.3 mg; pantothenic acid 11 mg; pyridoxine 1.1 mg; riboflavin 5.5 mg; thiamine 1.4 mg; iron 282 mg; magnesium 125 mg; manganese 275 mg; zinc 275 mg; copper 27.5 mg; iodine 844 µg.

Table 3. Composition of experimental grower diets fed to Cobb 500 broiler chicks from d15 to 35.

	Experimental Diet (%)			
Ingredient, g/kg	5% CV-DDGS[1]	10% HP-DDGS[1]	15% HP-DDGS	20% HP-DDGS
Corn	629.5	580.0	560.0	540.0
Soybean meal, 480.0 g/kg CP	260.0	225.0	195.0	162.5
Soybean oil	20.0	35.0	35.0	38.9
Salt	3.3	4.7	4.7	3.3
DL-Met	3.0	3.0	3.0	3.0
L-Lys•HCl	4.0	4.0	4.0	4.0
L-Thr	1.0	1.0	1.0	1.0
Limestone	3.9	20.0	20.0	20.0
Dicalcium phosphate	18.0	20.0	20.0	20.0
Choline chloride-60	1.0	1.0	1.0	1.0
Vitamin-mineral premix[2]	6.3	6.3	6.3	6.3
Calculated Values, g/kg				
Crude fat	52.2	67.2	70.0	76.4
CP	197.5	197.3	198.2	198.0
Digestible Lys	11.5	10.8	10.2	9.6
Digestible Met	5.5	5.4	5.4	5.4
Digestible Arg	11.1	10.3	9.7	9.1
Digestible Thr	7.5	7.2	7.0	6.8
ME (MJ/kg)	13.4	13.4	13.3	13.3
Analysed Values, g/kg				
Moisture	123.0	123.0	123.4	124.1
DM	877.0	877.0	876.6	875.9
Crude fat	42.3	45.3	51.5	55.5
CP	203.0	205.2	200.3	203.8
GE, MJ/kg	16.5	16.1	16.5	16.5

[1]HP-DDGS= high protein DDGS (341.0 g/kg CP as fed), CV-DDGS= conventional DDGS (271.0 g/kg CP as fed).

[2]Vitamin and mineral premix provided per kg of diet: selenium 250 µg; retinyl acetate (vitamin A) 2.8 mg; cholecalciferol (vitamin D_3) 68.8 µg; α-tocopherol acetate (vitamin E) 13.2 mg; menadione 1.1 mg; vitamin B_{12} 12 µg; biotin 41 µg; choline 447 mg; folic acid 1.4 mg; niacin 41.3 mg; pantothenic acid 11 mg; pyridoxine 1.1 mg; riboflavin 5.5 mg; thiamine 1.4 mg; iron 282 mg; magnesium 125 mg; manganese 275 mg; zinc 275 mg; copper 27.5 mg; iodine 844 µg.

Table 4. Composition of experimental finisher diets fed to Cobb 500 broiler chickens from d 36 to 42.

	Experimental Diet (%)			
Ingredient, g/kg	5% CV-DDGS[1]	10% HP-DDGS[1]	15% HP-DDGS	20% HP-DDGS
Corn	636.5	629.4	610.8	591.9
Soybean meal, 480.0 g/kg CP	240.0	197.7	166.3	134.2
Soybean oil	35.0	35.0	35.0	35.0
Salt	3.1	3.1	3.1	3.1
DL-Met	2.5	2.5	2.5	2.5
L-Lys•HCl	3.5	3.5	3.5	3.5
L-Thr	0.5	0.5	0.5	0.5
Limestone	7.0	8.0	8.0	9.0
Dicalcium phosphate	14.6	13.0	13.0	13.0
Choline chloride-60	1.0	1.0	1.0	1.0
Vitamin-mineral premix[2]	6.3	6.3	6.3	6.3
Calculated Values, g/kg				
Crude fat	66.5	68.8	71.7	74.5
CP	187.5	187.1	187.5	187.5
Digestible Lys	10.6	9.8	9.2	8.6
Digestible Met	4.9	4.9	4.9	4.9
Digestible Arg	10.4	9.6	9.0	8.4
Digestible Thr	6.7	6.4	6.2	6.0
ME (MJ/kg)	13.0	13.0	13.0	13.0
Analysed Values, g/kg				
Moisture	122.9	122.4	124.3	124.8
DM	877.1	877.6	875.7	875.2
Crude fat	32.6	51.9	53.4	54.1
CP	197.0	194.7	188.3	189.0
GE, MJ/kg	16.4	16.6	16.6	17.0

[1]HP-DDGS= high protein DDGS (341.0 g/kg CP as fed), CV-DDGS= conventional DDGS (271.0 g/kg CP as fed).

[2]Vitamin and mineral premix provided per kg of diet: selenium 250 µg; retinyl acetate (vitamin A) 2.8 mg; cholecalciferol (vitamin D_3) 68.8 µg; α-tocopherol acetate (vitamin E) 13.2 mg; menadione 1.1 mg; vitamin B_{12} 12 µg; biotin 41 µg; choline 447 mg; folic acid 1.4 mg; niacin 41.3 mg; pantothenic acid 11 mg; pyridoxine 1.1 mg; riboflavin 5.5 mg; thiamine 1.4 mg; iron 282 mg; magnesium 125 mg; manganese 275 mg; zinc 275 mg; copper 27.5 mg; iodine 844 µg.

Table 5. Composition of experimental diets[1] fed to Cobb 500 broilers for seven consecutive days to determine the energy digestibility of HP-DDGS[2] (d 43 to 49).

Ingredients	Basal diet, g/kg	
Corn	626.3	
Soybean meal, 480.0 g/kg CP	325.9	
Salt	5.2	
DL-Met	2.8	
Limestone	11.3	
Dicalcium phosphate	17.2	
Vitamin-mineral premix[3]	6.3	
Titanium dioxide	5.0	
	Experimental diets, g/kg	
Basal	850.0	850.0
Sucrose	150.0	0.0
HP-DDGS	0.0	150.0
Analysed values, g/kg		
Moisture	108.4	127.2
DM	891.6	872.8
Crude fat	17.7	33.6
CP	157.6	217.2
GE, MJ/kg	15.6	16.1
Titanium dioxide	5.2	5.1

[1]Experimental diets used an 85% complete basal diet ± sucrose or HP-DDGS
[2]HP-DDGS= high protein DDGS (34.1% CP as fed)
[3]Vitamin and mineral premix provided per kg of diet: selenium 250 µg; retinyl acetate (vitamin A) 2.8 mg; cholecalciferol (vitamin D_3) 68.8 µg; α-tocopherol acetate (vitamin E) 13.2 mg; menadione 1.1 mg; vitamin B_{12} 12 µg; biotin 41 µg; choline 447 mg; folic acid 1.4 mg; niacin 41.3 mg; pantothenic acid 11 mg; pyridoxine 1.1 mg; riboflavin 5.5 mg; thiamine 1.4 mg; iron 282 mg; magnesium 125 mg; manganese 275 mg; zinc 275 mg; copper 27.5 mg; iodine 844 µg.

Table 6. Composition of experimental diets fed to Cobb 500 broilers for seven consecutive days to determine the amino acid digestibility of HP-DDGS (d 43 to 49).

	Experimental Diets, g/kg	
Ingredient	**N-Free Control**	**HP-DDGS Test**
HP-DDGS	0.0	542.0
Sucrose	385.3	291.8
Soybean oil	50.0	50.0
Magnesium oxide	1.5	1.5
Potassium chloride	3.4	4.0
Potassium sulfate	8.0	6.0
Sodium bicarbonate	8.4	1.2
Limestone	9.0	5.0
Defluorinated phosphate	18.5	18.5
Choline chloride-60	2.5	2.5
Vitamin-mineral premix[2]	7.5	7.5
Corn Starch	450.9	15.0
Solka Floc	50.0	50.0
Titanium dioxide	5.0	5.0
Analysed Values, g/kg		
Moisture	52.8	86.1
DM	947.2	913.9
Crude fat	15.2	55.0
CP	0.0	208.0
GE, MJ/kg	15.4	18.2
Titanium dioxide	5.0	5.9

[1] HP-DDGS= high protein DDGS (34.1% CP as fed)

[2]Vitamin and mineral premix provided per kg of diet: selenium 250 µg; retinyl acetate (vitamin A) 2.8 mg; cholecalciferol (vitamin D_3) 18.8 µg; α-tocopherol acetate (vitamin E) 13.2 mg; menadione 1.1 mg; vitamin B_{12} 12 µg; biotin 41 µg; choline 447 mg; folic acid 1.4 mg; niacin 41.3 mg; pantothenic acid 11 mg; pyridoxine 1.1 mg; riboflavin 5.5 mg; thiamine 1.4 mg; iron 282 mg; magnesium 125 mg; manganese 275 mg; zinc 275 mg; copper 27.5 mg; iodine 844 µg.

Table 7. Performance of Cobb 500 broiler chickens[1] on a per bird basis fed increasing concentrations of HP-DDGS[2] for 42 consecutive days (d 0 to 42)

	Experimental Diets[1]				Statistics				
					Mean Comparison		Contrasts P-value		
Outcome	5% CV-DDGS	10% HP-DDGS	15% HP-DDGS	20% HP-DDGS	SEM	P-Value[3]	C v. HP[4]	L[5]	Q[5]
D 0 BW, g	37.93	37.74	37.40	37.48	0.122	0.25	0.10	0.33	0.36
Starter, d 0 to14[6]									
D 14 BW, kg	0.39[a]	0.39[a]	0.35[b]	0.37[a]	0.001	< 0.0001	<0.0001	0.03	<0.0001
BWG, kg/bird	0.34[a]	0.35[a]	0.31[b]	0.34[a]	0.009	< 0.0001	0.003	0.03	<0.0001
FI, kg/bird	0.51[ab]	0.53[a]	0.51[b]	0.53[a]	0.005	0.005	0.16	0.51	0.002
FCR	1.49[c]	1.51[c]	1.63[a]	1.58[b]	0.032	< 0.0001	<0.0001	<0.0001	<0.0001
Grower, d 14 to 35									
D 35 BW, kg	1.99[ab]	2.02[a]	1.91[c]	1.93[bc]	0.026	< 0.0001	0.08	0.0008	0.008
BWG, kg/bird	1.61[ab]	1.64[a]	1.56[b]	1.55[b]	0.021	0.0007	0.19	0.0005	0.12
FI, kg/bird	2.66[ab]	2.74[a]	2.63[b]	2.70[ab]	0.024	0.01	0.25	0.29	0.008
FCR	1.66[c]	1.67[bc]	1.69[b]	1.76[a]	0.023	< 0.0001	<0.0001	<0.0001	0.08
Finisher, d 35 to 42									
D 42 BW, kg	2.70[a]	2.72[a]	2.56[b]	2.58[b]	0.041	< 0.0001	0.009	0.002	0.02
BWG, kg/bird	0.70[a]	0.70[ab]	0.65[b]	0.65[b]	0.014	0.004	0.01	0.02	0.18
FI, kg/bird	1.30[a]	1.29[ab]	1.24[b]	1.26[ab]	0.014	0.02	0.05	0.18	0.05
FCR	1.86	1.86	1.91	1.94	0.02	0.08	0.11	0.03	0.78
Overall, d 0 to 42									
BWG, kg/bird	2.65[a]	2.68[a]	2.52[b]	2.54[b]	0.04	< 0.0001	0.02	0.0009	0.02
FI, kg/bird	4.47[ab]	4.55[a]	4.38[b]	4.49[ab]	0.035	0.02	0.91	0.28	0.006
FCR	1.69[c]	1.70[c]	1.74[b]	1.77[a]	0.018	< 0.0001	<0.0001	<0.0001	0.74

[1]Experimental diets were divided into a starter phase (14 d), grower phase (21 d) and finisher phase (7 d) for a total of 42 d. Broilers were fed a CV-DDGS diet (conventional DDGS) and three different test diets with increasing concentration of HP-DDGS (10%, 15%, and 20%). Control and dietary treatments contained 208 broilers each with 16 replicates.
[2] HP-DDGS= High Protein DDGS; crude protein=34.1% as fed.
[3] Model significant (P<0.0001)

[4] Contrast performed to determine difference between the 5% CV-DDGS diet and all 3 HP-DDGS diets; C v. HP[4] = Conventional vs. HP-DDGS '-3 1 1 1'
[5] Orthogonal contrasts were performed to assess the effect of HP-DDGS inclusion on bird performance. Comparisons were made between 10, 15, and 20% HP-DDGS treatments; L = Linear '-1 0 1'; Q = Quadratic '-1 2 -1'
[6] Least square means with differing superscripts are significantly different within a row ($P<0.05$)
[7] Abbreviations: BW= body weight; FI= feed intake; BWG= body weight gain; FCR= feed conversion ratio (kg intake/kg gain)

Table 8. Standard ileal amino acid digestibility (SIAAD)[1], ileal endogenous AA losses (IEAA), and AME_n[4] of HP-DDGS.

AA	SIAAD, %[2]	IEAA, mg/kg DMI[3]
Indispensable amino acids		
Arg	90.32 ± 2.830	213.47
His	85.79 ± 4.996	153.61
Ile	84.27 ± 3.719	411.70
Leu	90.41 ± 1.787	596.20
Lys	80.89 ± 2.002	415.32
Met	88.59 ± 3.616	113.15
Phe	88.26 ± 2.781	347.12
Thr	81.20 ± 4.238	623.67
Val	85.50 ± 4.212	744.76
Dispensable amino acids		
Asp	82.13 ± 4.501	789.14
Ser	87.49 ± 2.431	482.07
Glu	89.78 ± 1.570	1,048.99
Pro	81.99 ± 1.761	598.80
Ala	86.48 ± 3.053	577.44
Cys	80.56 ± 3.575	264.39
Tyr	84.27 ± 3.355	202.73
Trp	82.44 ± 3.457	68.17
AME_n[4](MJ/kg)	11.4±0.7	

[1]Standard ileal amino acid digestibility determined by digesta collected after a 7-day adaptation period (d 43 to 49) and calculated by determining apparent ileal AA digestibility and standardising by basal endogenous AA losses from birds fed a N-free diet
[2]SIAAD presented as average SIAAD of 4 replicate cages ± standard error
[3]Ileal endogenous AA losses (IEAA) determined by digesta collected from birds fed a N-free diet after a 7-day adaptation periods (d 43 to 49); IEAA presented as average of pooled digesta from 6 replicate cages
[4]AME_n determined by excreta collected following a 7-day adaptation period (d 43 to 49) and calculated by determining total AME_n intake for all treatments, AME_n contribution from glucose, and basal diet AME_n before subtracting basal diet AME_n from the total AME_n intake of birds fed an experimental diet with HP-DDGS. AME_n presented as average AME_n of 6 replicate cages ± the standard deviation.

Table 9. Comparison of standard ileal amino acid digestibility (SIAAD) of corn, DDGS, and HP-DDGS

Amino Acid	Digestibility (%)					
	Corn[1]	CV-DDGS (Adedokun *et al.* 2015)[2]	CV-DDGS (Batal and Dale 2006)[3]	HP-DDG (Applegate *et al.* 2009)[4]	HP-DDG (Kim *et al.* 2008)[5]	HP DDGS[6] (Present Study)
Indispensable amino acids						
Arg	95.67	79.18	84.10	81.31	88.10	90.32
His	91.43	73.82	84.10	77.20	79.80	85.79
Ile	95.27	73.00	83.30	78.11	85.00	84.27
Leu	96.10	84.38	88.60	81.02	91.20	90.41
Lys	92.40	58.32	69.60	73.00	73.90	80.89
Met	94.97	81.66	86.80	84.93	84.40	88.59
Phe	103.30	80.34	87.50	80.94	88.10	88.26
Thr	89.83	65.68	74.50	73.02	78.60	81.20
Val	93.33	72.62	79.50	75.76	83.50	85.50
Dispensable amino acids						
Asp	93.60	66.50	74.80	71.45	77.10	82.13
Ser	94.67	75.15	81.90	79.79	84.50	87.49
Glu	96.60	83.82	83.30	80.93	88.10	89.78
Pro	92.27	77.16	83.50	79.34	87.90	81.99
Ala	94.70	81.34	82.80	80.64	87.10	86.48
Cys	93.50	74.78	73.90	76.77	81.30	80.56
Tyr	ND	ND	87.90	82.56	89.90	84.27
Trp	ND	ND	82.80	79.58	90.90	82.44

[1] Average SIAAD from 3 different sources of corn fed to 21-day-old Ross 708 broilers from Table 5 of Adedokun *et al.* (2015)
[2]Average SIAAD from 5 different sources of CV-DDGS fed to 21-day-old Ross 708 broilers from Table 4 of Adedokun *et al.* (2015)
[3]Average percent digestibility (as fed) of the AA from 8 sources of CV-DDGS fed to cecectomised Single Comb White Leghorn roosters from Table 3 of Batal and Dale (2006)
[4] Average SIAAD of HP-DDG (54.0% CP) fed to 22-day-old Ross × Ross 308 broilers from Table 4 of Applegate *et al.* (2009)
[5] Average percent digestibility of the AA in HP-DDG (44.1%CP) fed to 5 cecectomised Single Comb White Leghorn roosters from Table 6 of Kim *et al.* (2008)
[6]Standard ileal amino acid digestibility determined by digesta collected after a 7-day adaptation period (d 43 to 49) and calculated by determining apparent ileal AA digestibility and standardising by basal endogenous amino acid losses from birds fed a N-free diet; SIAAD presented as average SIAAD of 4 replicate cages
Abbreviations: ND = No data