Apparent ileal amino acids digestibility of diets with graded levels of corn DDGS and determination of DDGS amino acids digestibility by difference and regression methods in broilers

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ABSTRACT: This study was conducted to determine apparent ileal amino acids digestibility (AIAAD) of diets with different levels of corn distiller's dried grains with solubles (DDGS) and to determine AIAAD of corn DDGS by difference and regression methods in broiler chickens. One hundred and fifty 30-day-old male broiler chickens were used in the experiment. The corn DDGS were incorporated into basal diets at graded levels (0, 4, 8, 12, and 16%). All birds were killed at the age of 35 days and the contents of the lower half of the ileum were collected. AIAAD was calculated using chromic oxide as the indigestible marker. For AIAAD of DDGS determination, difference and regression methods were used. AIAAD of Lys (78.6%) and Met (91.3%) were the significantly highest in the diet without DDGS in comparison with other diets ($P < 0.01$). AIAAD of the rest of essential and nonessential amino acids were the lowest in the diet with 8% of DDGS in comparison with the diets with 0 or 16% of DDGS ($P < 0.01$). The apparent ileal crude protein digestibility was also the lowest in the diet with 8% (61.8%) of DDGS ($P < 0.01$). The highest differences in AIAAD of DDGS determined by the difference method and in the diet without DDGS (basal diet) determined by the regression methods were found in Lys and Met. There were very low differences between DDGS and basal diet in AIAAD of nonessential amino acids, except Ala and Asp. These results show that higher levels of DDGS decrease AIAAD.

Keywords: chickens; digesta; crude protein; lysine; methionine

Abbreviations: Arg = Arginine, His = Histidine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Thr = Threonine, Val = Valine, Ala = Alanine, Asp = Aspartate, Glu = Glutamate, Gly = Glycine, Pro = Proline, Ser = Serine, Tyr = Tyrosine

Corn distiller's dried grains with solubles (DDGS) is a by-product of the fuel ethanol industry. During the production of alcohol, starch is removed from the grain and converted into alcohol and carbon dioxide. As a result of starch fermentation, the concentration of the remaining nutrients in the grain increases approximately threefold (Spiehs et al., 2002). Increased emphasis on ethanol production in global scale leads to significant increases in the production of DDGS. From 1 kg of corn grains, almost 0.32 kg of ethanol and 0.33 kg of distillers' grains can be gained (McAloon et al., 2000). Fermentation residues from the ethanol industry may be based on mixtures of several grains. Anyway, the DDGS available today usually derive from ethanol plants with corn as the only grain input. However, a high variability in nutritional composition among DDGS samples is presumably caused by differences in the original corn composition, fermentation, and disposition of soluble. Considerable differences are observed among the true amino acids (AA) digestibility of

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the DDGS samples. In general, DDGS samples that were more yellow and lighter in colour had higher total and digestible AA levels (Batal and Dale, 2006; Fastinger et al., 2006). Lys content and digestibility seemed to be reduced to a greater extent in the darker coloured DDGS than the other essential AA, suggesting that the Maillard reaction reduced total Lys content and lowered its digestibility. Fastinger and Mahan (2006) also stated that darker coloured DDGS sources may have lower analyzed Lys contents, as well as lower Lys and essential AA digestibility than lighter coloured DDGS sources.

A lot of studies were conducted to determine amino acids digestibility using different methods; precision-fed cecectomized rooster assay was used by Batal and Dale (2006), Fastinger et al. (2006), Adedokun et al. (2009), and Pahm et al. (2009). Batal and Dale (2006) also used conventional precision-fed rooster assay. Adedokun et al. (2009) applied the direct method in 3-week-old broilers and in 37-week-old laying hens. One year earlier the team of Adedokun et al. (2008) used the direct method in 5-day-old and 3-week-old broilers and turkey poults. In 2012 Kozlowski et al. (2012) used direct method in male growing turkeys, too. Ileal digestibility of feed with different level of DDGS in 6-week-old broilers was tested by Liu et al. (2011). But application of the difference method in calculation of ileal digestibility of DDGS is not common with only few studies dealing with it (Oryschak et al., 2010 – in 4-week-old broiler).

The objective of this study was to evaluate the AIAAD of diets with graded level of DDGS and to determine amino acids digestibility of a DDGS sample by the difference method and the regression method.

MATERIAL AND METHODS

The experiment was conducted on 150 male broilers obtained from conventional farm at 25 days of age and housed in the test cages batteries at Mendel University in Brno. The birds were distributed among 15 cages in two batteries (10 chicks per cage). First five days after transfer to cage technology, during acclimatization period, all chickens were fed the same basal diet (Table 1). The basal diet provided 157.2 g/kg crude protein and 11.7 MJ/kg metabolizable energy. On day 30 the cages were divided into 5 treatments (each treatment in 3 cages). Test period lasted for five days (from day 30 to day 35 of broilers' age) and the birds were fed experimental diets

Table 1. Composition of the basal diet (g/kg)

Wheat	577
Corn	170
SBM	150
Soybean oil	17
DDGS	10
MCP	10
Limestone	10
Salt	3
Cr_2O_3	3
Complex of minerals and vitamins	50

DDGS = distiller's dried grains with solubles, SBM = soybean meal, MCP = monocalcium phosphate

(Table 2). Broilers in the first group were fed only the basal diet, broilers in the next four groups were fed test diets where the basal diet was replaced by DDGS at the levels of 4, 8, 12, and 16%. DDGS used in the study had light yellow colour with the following parameters: lightness (L^*) 70.4, redness (a^*) 9.3, and yellowness (b^*) 41.0. Access to drinking water and feed was provided *ad libitum*. The last day of the experiment birds were fasted for 12 h and then they were allowed to consume the diets *ad libitum* again for 4 h. Then the birds were killed by cervical dislocation. Abdominal cavity was opened immediately and gut content from the lower half of the ileum to 3 cm proximal to the ileocecal junction was gently pushed by fingers to Petri dishes and frozen for next processing. Digesta of the first five randomly chosen broilers from one cage was collected to one Petri dish and digesta of the second five broilers was collected to another one. Finally six replicates of digesta were collected per one treatment. Before the analysis, the digesta samples were freeze-dried and finely ground.

The samples of the diets and ileal digesta were treated by oxidative acid hydrolysis of HCl (c = 6 mol/l). The hydrolysate samples were chromato-

Table 2. Composition of the experimental diets (g/kg)

	Group				
	0	4	8	12	16
Basal diet	1000	960	920	880	840
DDGS	0	40	80	120	160
CP	157.2	166.4	172.4	178.2	182.7
ME (MJ/kg)	11.7	11.5	11.3	11.0	10.8

DDGS = distiller's dried grains with solubles, CP = crude protein, ME = metabolizable energy

graphically analyzed (AAA 400 analyser; INGOS, s.r.o., Prague, Czech Republic) using Na-citrate buffers and ninhydrin to detect the amounts of particular amino acids.

Apparent ileal amino acids digestibility (AIAAD) of feed was calculated with the following formula:

$$AIAAD = 100 - (100 \times Id \times AAdc/Idc \times AAd) (\%)$$

where:

Id = content of indicator in the dry matter of the diet

AAdc = content of amino acid in the dry matter of digesta

Idc = content of indicator in the dry matter of digesta

AAd = content of amino acid in the dry matter of the diet

Apparent ileal digestibility values of amino acids in DDGS (D_{DDGS}) calculated by difference method were determined as follows (Fan and Sauer, 1995):

$$D_{DDGS} = (D_D - D_B \times S_B)/S_A; S_B = 1 - S_A$$

where:

D_D = apparent ileal amino acids digestibility in the test diet (%)

D_B = apparent ileal amino acids digestibility in the basal diet (%)

S_B = contribution level of amino acid from the basal feedstuf to the tested diet (decimal %)

S_A = contribution level of amino acid from the tested feedstuff to the tested diet (decimal %)

For the determination of digestibility coefficients of amino acids in DDGS by the difference method the coefficients of AIAAD of diets with 0 g/kg DDGS (basal diet; D_B) and diets with 160 g/kg DDGS (tested diet; D_A) were used.

For determination of AIAAD coefficients of diets with a different level of DDGS a simple linear regression was used (Fan and Sauer, 1995):

$$D_{D_i} = A + B \times S_{B_i}$$

where:

D_{D_i} = apparent ileal digestibility of an amino acid in the i^{th} tested diet (%)

A = D_A

B = $D_B - D_A$

D_B, D_A = apparent ileal digestibility values of amino acids to be determined for the basal and the tested feedstuffs

S_{B_i} = contribution level of amino acid from the basal feedstuff to the i^{th} tested diet (decimal %)

Kruskal-Wallis analysis followed by LSD test was used for statistical evaluation using software package Unistat (Version 5.1, 2002).

RESULTS AND DISCUSSION

The amino acids composition of DDGS and the diets is presented in Table 3. The apparent ileal digestibility values of amino acids and crude protein of diets with different levels of DDGS are presented

Table 3. Amino acids composition of DDGS and the diets (g/kg)

	DDGS	Diets				
		0	4	8	12	16
Arginine	15.2	9.0	8.8	9.1	9.6	9.5
Histidine	8.0	3.9	4.1	4.4	4.6	4.7
Isoleucine	11.4	5.5	5.5	5.5	6.2	6.3
Leucine	31.3	11.4	11.9	12.8	14.1	15.0
Lysine	9.1	11.0	9.2	10.4	10.1	9.4
Methionine	4.6	5.2	3.8	4.6	3.9	3.6
Phenylalanine	12.6	7.5	7.3	7.1	8.9	8.6
Threonine	10.0	6.7	6.3	7.1	7.1	7.4
Valine	14.1	6.7	6.7	7.3	7.8	7.9
Alanine	19.6	6.6	6.8	7.7	8.3	8.9
Aspartate	20.1	13.2	12.4	13.3	13.3	14.4
Glutamate	42.6	30.3	30.3	29.7	32.9	34.2
Glycine	11.5	6.2	6.3	6.4	6.8	7.1
Proline	47.2	10.4	10.9	11.5	12.3	12.9
Serine	12.1	7.1	6.9	7.4	7.9	8.2
Tyrosine	10.4	5.0	4.9	5.1	6.0	6.1

DDGS = distiller's dried grains with solubles

Table 4. Values of apparent ileal amino acid digestibility in the diets with different levels of DDGS (%)

	Group				
	0	4	8	12	16
Essential amino acids					
Arginine	75.6^{b}	72.2^{b}	63.4^{a}	70.2^{b}	73.4^{b}
Histidine	72.2^{b}	68.8^{ab}	62.3^{a}	68.1^{ab}	73.2^{b}
Isoleucine	58.8^{b}	53.3^{b}	42.4^{a}	54.4^{b}	59.7^{b}
Leucine	74.5^{b}	70.9^{b}	64.4^{a}	71.8^{b}	75.7^{b}
Lysine	78.7^{a}	71.1^{b}	66.2^{b}	70.3^{b}	71.0^{b}
Methionine	91.3^{a}	86.0^{b}	85.1^{b}	83.9^{b}	84.5^{b}
Phenylalanine	77.8^{b}	73.4^{b}	65.1^{a}	75.0^{b}	76.1^{b}
Threonine	69.5^{b}	63.3^{ab}	57.6^{a}	62.9^{ab}	68.8^{b}
Valine	68.6^{b}	63.9^{ab}	57.3^{a}	64.2^{ab}	69.9^{b}
Nonessential amino acids					
Alanine	70.1^{b}	66.6^{ab}	61.9^{a}	69.0^{ab}	73.9^{b}
Aspartate	69.1^{b}	62.2^{ac}	58.0^{a}	61.1^{ac}	67.7^{c}
Glutamate	82.8^{b}	80.3^{b}	74.3^{a}	79.9^{b}	82.4^{b}
Glycine	67.3^{b}	62.6^{b}	54.1^{a}	60.8^{ab}	67.0^{b}
Proline	80.6^{b}	77.8^{b}	65.8^{a}	74.6^{b}	80.8^{b}
Serine	71.0^{b}	66.6^{ab}	59.9^{a}	67.4^{ab}	72.1^{b}
Tyrosine	76.5^{b}	72.3^{b}	64.7^{a}	73.9^{b}	76.2^{b}
Crude protein					
	73.2^{b}	71.6^{b}	61.8^{a}	69.1^{b}	72.0^{b}

DDGS = distiller's dried grains with solubles

$^{a–c}$statistically significant difference between groups ($P < 0.01$)

in Table 4. AIAAD of Lys and Met were significantly the highest in the diet without DDGS in comparison with other diets ($P < 0.01$). Coefficients of AIAAD of His and Thr were significantly lower in the diet with 8% of DDGS than in the diets with 0 and 16% of DDGS ($P < 0.01$). In the rest of essential amino acids (Arg, Ile, Leu, Phe, Val) the AIAAD were the lowest in the diet with 8% of DDGS in comparison with all other diets ($P < 0.01$). AIAAD of nonessential amino acids were also the lowest in the diet with 8% of DDGS ($P < 0.01$). The apparent ileal crude protein digestibility was the lowest in the diet with 8% (61.8%) of DDGS ($P < 0.01$), too. Ile was poorly digested in all groups and its digestibility ranged 42.4–59.7%. The highest digestibility was found in Met ranging 83.9–91.3%.

Ileal amino acids digestibility of DDGS was calculated by the difference method and the regression method according to Fan and Sauer (1995). Regression equations for calculation of AIAAD of each amino acid in the diets with different levels of DDGS were determined according to Fan and Sauer (1995), too. Results of the methods are shown in Table 5.

The highest differences between AIAAD of DDGS determined by the difference method and the basal diet without DDGS were found in Lys and Met. According to the difference method the AIAAD was relatively low (Met 48.8%, Lys 30.1%). This reflects the fact that apparent ileal digestibilities of Lys and Met were the highest in the diet with 0% of DDGS. The differences in AIAAD between DDGS and the basal diet higher than 10 percent points were observed in Arg (13.6%) and Phe (10.4%). In the rest of essential amino acids the differences between the methods were lesser than 10 percent points. The lowest difference between the DDGS and the basal diet was determined in Thr (4.6%). In nonessential amino acids the highest difference between the DDGS and the basal diet in AIAAD was for Ala. In Glu, Gly, Pro, and Tyr the differences were lower than 2.5%.

These results show that at the higher level of inclusion, the digestibility values in DDGS reduce the AIAAD of the diets. The reduction is higher for amino acids that are present in lower amounts in DDGS (Lys, Met). As Fan and Sauer (1995) con-

Table 5. Apparent ileal amino acid digestibility values of DDGS determined by difference and regression methods (%)

	Difference method	Regression method*	Regression equation
Arginine	62.0	75.6	$y = 62.0 + 13.62x$
Histidine	77.9	72.2	$y = 77.9 - 5.69x$
Isoleucine	64.7	58.8	$y = 64.7 - 5.94x$
Leucine	81.8	74.5	$y = 81.8 - 7.33x$
Lysine	30.1	78.7	$y = 30.9 + 47.78x$
Methionine	48.8	91.3	$y = 48.8 + 42.48x$
Phenylalanine	67.4	77.8	$y = 67.4 + 10.37x$
Threonine	64.9	69.5	$y = 64.9 + 4.56x$
Valine	77.1	68.6	$y = 77.1 - 8.51x$
Alanine	93.7	70.1	$y = 93.7 - 23.59x$
Aspartate	60.4	69.1	$y = 60.4 + 8.71x$
Glutamate	80.7	82.8	$y = 80.7 + 2.12x$
Glycine	65.1	67.3	$y = 65.1 + 2.23x$
Proline	81.2	80.6	$y = 81.2 - 0.57x$
Serine	77.2	71.0	$y = 77.9 - 6.86x$
Tyrosine	74.7	76.5	$y = 74.7 + 1.82x$

DDGS = distiller's dried grains with solubles

*S_{B_i} (contribution level of amino acid from the basal feedstuff to the i^{th} tested diet) = 1 decimal %

cluded, the reliability of the determination of the apparent ileal digestibility values of amino acids with the difference method is dependent on the contributions of amino acids in the assay feedstuff to their total dietary contents. Therefore for the determination of DDGS AIAAD the results of AIAAD of the diet with the highest level of DDGS (16%) were used in this study.

Concerning the essential amino acids, the values of AIAAD determined by the difference method were lower in this study than reported by Oryschak et al. (2010), except His and Thr. In comparison with the present study, Kozlowski et al. (2012) published higher standardized AIAAD of wheat DDGS in growing turkeys except Leu, Val, Ala, and Pro. The values of AIAAD can be influenced by the content of amino acids in the feedstuff, which was also found by Fan et al. (1994).

The AIAAD is affected also by the colour of the DDGS. Batal and Dale (2006) found correlations ($P < 0.01$) between AIAAD and L^* values ($r = 0.87$) and b^* values ($r = 0.96$) but not with a^* values. More lightness ($L^* = 60.3$) and more yellowness ($b^* = 25.9$) were associated with a 76.8% Lys digestibility, whereas darker ($L^* = 50.4$) and less yellow ($b^* = 7.41$) samples were associated with 45.8% Lys digestibility. In this study, also a high AIAAD of Lys was determined by the regression method (78.7%) and L^* and b^* values were also higher (70.4 and 41.0), which confirms the conclusion of the mentioned authors. Correlation between colour and AIAAD was also found by Fastinger et al. (2006).

AIAAD is affected by individual feed ingredients and type of bird (meat vs. egg) or methods by which they are determined (Adedokun et al., 2009) and digestibility measurements may be influenced by a number of factors such as rate of passage, physiological status as related to growth or maintenance, feed consumption, and nutritional adequacy of test diets (Huang et al., 2007).

Based on the results of the present study it can be concluded that higher levels of DDGS decreased AIAAD of the diets, but if included up to 16%, DDGS did not negatively affect AIAAD except Lys and Met.

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