J. Dairy Sci. 92:6056–6067
doi:10.3168/jds.2008-1885

Intestinal digestibility of amino acids in rumen-undegraded protein estimated using a precision-fed cecectomized rooster bioassay: II. Distillers dried grains with solubles and fish meal[1]

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ABSTRACT

The objectives of this experiment were to measure intestinal digestibility of AA in the rumen-undegraded protein fraction (RUP-AA) of distillers dried grains with solubles (DDGS) and fish meal (FM) samples and to determine whether these feeds contain a constant protein fraction that is undegradable in the rumen and indigestible in the small intestine, as assumed in the French Institut National de la Recherche Agronomique (Paris, France) and Scandinavian AAT-PBV (AAT = AA absorbed from small intestine; PBV = protein balance in the rumen) models. Five sources of DDGS and 5 sources of FM were obtained from Feed Analysis Consortium, Inc. (Champaign, IL). To obtain the rumen-undegradable protein fraction, samples were ruminally incubated in situ for 16 h in 4 lactating cows, and the collected rumen-undegraded residues (RUR) were pooled by sample. Subsamples of the intact feeds and RUR were crop-intubated to 4 cecectomized roosters, and total excreta were collected for 48 h. Intact feeds, RUR, and excreta were analyzed for AA. Basal endogenous AA loss estimates were obtained from fasted birds and were used to calculate standardized digestibility of RUP-AA and AA in the intact feeds. Indigestibility coefficients of the intact feeds were calculated as (100 − % standardized AA digestibility), and indigestibility of the RUR was calculated as [(100 − % ruminal degradation of AA) × (100 − % standardized RUP-AA digestibility)/100]. Results indicate that standardized digestibility of feed-AA differs from RUP-AA for DDGS samples but not for FM samples, and that standardized digestibility of individual AA differs within samples. For the DDGS samples, standardized feed-AA and RUP-AA digestibility values were most often lowest for His and Lys and highest for Met and Trp. For FM samples, standardized feed-AA and RUP-AA digestibility values were most often lowest for His and highest for Trp. Results also indicate that DDGS and most FM samples do not contain a constant protein fraction that is both undegradable in the rumen and indigestible in the small intestine. Indigestibility values of RUR were lower than in intact feeds, suggesting that the feed ingredients used in this experiment contain a protein fraction that is indigestible in the intestine but partly degradable in the rumen or digestible in the intestine after rumen incubation, or both.

Key words: amino acid digestibility, distillers dried grains with solubles, fish meal

INTRODUCTION

Currently, there is limited data reported in the literature on intestinal digestibility of individual AA in the RUP fraction (**RUP-AA**) of feedstuffs. Therefore, more research is needed to measure the variation in intestinal RUP-AA digestibility within and among feeds. Determining digestibility of RUP-AA in distillers dried grains with solubles (**DDGS**) is important because of the variability of AA digestibility in DDGS reported in the swine and poultry literature (Stein et al., 2006; Martinez Amezcua and Parsons, 2007) and the increased feeding of DDGS to dairy cows. Fish meal (**FM**) is a high-quality protein supplement that is fed to dairy cows for its high RUP content. Therefore, determining digestibility of RUP-AA in FM will also be beneficial to the dairy industry.

In the Institut National de la Recherche Agronomique (**INRA**) model (Vérité and Peyraud, 1989) and in the Scandinavia AAT-PBV model (Madsen et al., 1995), it is assumed that feedstuffs contain a constant protein fraction that is totally indigestible in the small intes-

Received November 10, 2008.
Accepted July 27, 2009.
[1]This is scientific contribution number 2390 from the New Hampshire Agricultural Experiment Station (Durham), contributing to regional research projects NC-1009 and NC-1119.
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tine and also completely undegradable in the rumen. However, Prestløkken and Rise (2003) reported that the assumption that feedstuffs contain a constant undegradable and indigestible protein fraction is not true for all feeds. This hypothesis has not been evaluated for DDGS; therefore, further evaluation of this hypothesis using a variety of feedstuffs is warranted.

The objectives of this experiment were 1) to determine digestibility of RUP-AA in DDGS and FM using a precision-fed cecectomized rooster assay and 2) to determine whether these feedstuffs contain a constant protein fraction that is both undegradable in the rumen and indigestible in the small intestine. A similar experiment using soybean meal and SoyPlus (West Central, Ralston, IA) samples was conducted, and these results are reported in a companion paper (Boucher et al., 2009a).

MATERIALS AND METHODS

Feed Samples

Two kilograms each of 5 sources of DDGS and 5 sources of FM were obtained from Feed Analysis Consortium, Inc. (Champaign, IL). The 5 sources of FM included 1 source of anchovy (**ANVY**), 1 source of catfish (**CFSH**), 2 sources of menhaden (**MNHN**), and 1 source of pollock (**PLCK**). Each sample was ground to pass a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). To assess the effects of excessive heat on intestinal digestibility of RUP and RUP-AA, 1 of the DDGS samples was heated at 140°C for 60 min to depress RUP and RUP-AA digestibility. The FM samples were not heated so that the variation among FM types could be determined.

Ruminal Incubation

Procedures for the ruminal cannulation surgery and experimental protocol were approved by the Institutional Animal Care and Use Committee at the University of New Hampshire. The procedures used for the ruminal incubation of the DDGS and FM samples were identical to the ruminal incubation procedure described in the companion paper (Boucher et al., 2009a). Briefly, to provide rumen-undegraded residues (**RUR**) for use in the precision-fed cecectomized rooster assay and for use in experiments reported elsewhere to evaluate in vitro methods for estimating RUP-AA digestibility (Boucher et al., 2009b,c), 1.2 kg of sample (ground to 2 mm) was weighed equally into 150 polyester bags so that each bag contained 8 g of sample. The bags (Ankom Technologies, Macedon, NY) had a mean pore size of 50 μm and a dimension of 10 × 20 cm. The bags were inserted into the rumen of 4 ruminally cannulated lactating cows averaging (mean ± SD) 48 ± 4 DIM and fed a 55% forage, 45% concentrate diet. Five separate ruminal incubations were required to incubate the 10 samples using 4 ruminally cannulated cows. Intervals of 1 wk were allotted between each incubation.

Samples were ruminally incubated for 16 h. One time point was selected to be representative of total RUP. The 16-h ruminal incubation time was selected for several reasons. In a literature search, 9 studies were identified that determined RUP digestibility of FM using a single time point for ruminal incubation. Of the 9 studies, a 24-h ruminal incubation was used in 1 study, a 16-h ruminal incubation was used in 5 studies, a 14-h ruminal incubation was used in 1 study, a 12-h ruminal incubation was used in 1 study, and 8- and 12-h ruminal incubations were used in 1 study. Four studies were identified that determined RUP digestibility of DDGS using a single time point for ruminal incubation. In 3 of the 4 studies a 16-h ruminal incubation was used, and in 1 study both 8- and 12-h ruminal incubations were used. To compare estimates obtained in this experiment with those previously reported in the literature, a 16-h ruminal incubation was most appropriate. In addition, Calsamiglia and Stern (1995) determined that there was no difference in RUP digestibility measured in vitro when concentrate ingredients were ruminally incubated in situ for 12 h compared with 16 h.

After 16 h, the in situ bags were then processed according to the procedure of Gargallo et al. (2006), with some modifications. Details of this procedure are described in a companion paper (Boucher et al., 2009a). A methylcellulose wash was used to aid in the detachment of particle-associated bacteria. Whitehouse et al. (1994) reported that 79% of the particle-associated bacteria (determined by direct counting) were removed from ruminal digesta when the digesta samples were shaken in a 0.1% methylcellulose solution. Following the rinsing procedures, in situ bags were lyophilized (Labconco, Kansas City, MO) to ensure that additional heat damage was not imposed on the residual feed inside of the bags. Once lyophilized, residues were composited by sample, weighed, and ground to pass a 1-mm screen for the precision-fed cecectomized rooster assay (Aldrich et al., 1997).

Precision-Fed Cecectomized Rooster Assay

The rooster digestibility experiments were conducted from June 2006 to January 2008. Procedures for the cecectomy of roosters and experimental protocol were approved by the Institutional Animal Care and Use

Committee at the University of Illinois. The cecectomized rooster digestibility assay used in this experiment was described by Aldrich et al. (1997) and was described in detail in the companion paper (Boucher et al., 2009a). Briefly, 30 g of each feed was ground to pass a 1-mm screen and was crop-intubated to 4 cecectomized roosters. Thirty grams of DDGS RUR samples could not be intubated to the roosters because of the bulkiness of the samples; therefore, the amount of RUR sample was adjusted to the maximum amount that could be comfortably intubated, which was (average ± SD) 21.2 ± 1.7 g and 30.0 ± 0.03 g for the DDGS and FM RUR samples, respectively. Total excreta were collected for 48 h and lyophilized. Standardized feed-AA and RUP-AA digestibility were calculated.

Chemical Analysis

A portion of the RUR, feed, and excreta were ground to pass a 40-μm screen (Arthur H. Thomas Co., Philadelphia, PA) for AA analysis via cation-exchange chromatography (cIEC-HPLC) coupled with postcolumn ninhydrin derivatization and quantitation (AOAC, 2000; method 982.30; Experimental Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO). Intact feeds and RUR were also analyzed for DM, NDF, ADF, neutral detergent-insoluble CP (**NDICP**), acid detergent-insoluble CP (**ADICP**), CP, fat, NSC, starch, ash, and minerals using wet chemistry (Dairy One DHI Forage Testing Laboratory, Ithaca, NY). Details of the feed analysis procedures were reported in a companion paper (Boucher et al., 2009a). The NFC content was calculated as follows: 100 – [CP + (NDF − NDICP) + fat + ash].

Calculations and Statistical Analysis

Standardized AA digestibility for the intact feeds and standardized RUP-AA digestibility for the RUR was calculated as follows (Stein et al., 2007):

$$\text{Standardized AA or RUP-AA digestibility, \%} = \{[\text{AA intake} - (\text{AA output} + \text{basal endogenous AA})]/\text{AA intake}\} \times 100.$$

Digestibility of RUP-AA was calculated and is reported as digestibility of AA in the RUR. Indigestibility of the intact feed samples was calculated as follows:

$$\text{Indigestibility, \%} = 100 - \text{standardized digestibility, \%}.$$

Indigestibility of AA in the ruminally incubated feeds was calculated according to the equation of Prestløkken and Rise (2003):

$$\text{Indigestibility, \%} = [(100 - \text{16 h ruminal AA disappearance}) \times (100 - \text{standardized RUP-AA digestibility})]/100.$$

Data were analyzed by feed type (DDGS or FM) as a completely randomized design according to the following model:

$$Y_{ijkl} = \mu + F_i + R_{ij} + FR_{ij} + P_k + c(F)_{ijkl} + E_{ijk},$$

where Y_{ijkl} = the dependent variable; μ = overall mean; F_i = the fixed effect of the ith feed sample (i = 1,...,5); R_{ij} = the fixed effect of ruminal incubation of the ith feed sample (j = 0,1); FR_{ij} = the fixed effect of the interaction between the ith feed sample and the jth ruminal incubation; P_k = the random effect of the kth experiment (k = 1,...,4); $c(F)_{ijkl}$ = the random effect of the lth rooster with the ith feed sample, the jth ruminal incubation, and the kth experiment (l = l = 1,...,40); and E_{ijkl} = the random residual ~N (0, σ^2). The mixed procedure of SAS (SAS Institute, 2002) was used to solve the above model for each feed type. Tukey's Studentized range test was used to compare least squares means among samples. Significance was declared at $P < 0.05$ and tendencies are reported at $0.05 < P < 0.10$. The MEANS procedure of SAS (SAS Institute, 2002) was used to evaluate the difference between digestibility of individual AA and total AA. The absolute value of the difference between digestibility of individual AA and total AA within each rooster was calculated, and these values were used in the MEANS procedure. The REG procedure of SAS (SAS Institute, 2002) was used to examine the relationship between AA digestibility in feed protein and RUP when a significant difference was observed and digestibility of AA in feed protein and RUP and ADICP concentrations.

RESULTS AND DISCUSSION

Standardized digestibility estimates obtained for the RUR represent RUP-AA digestibility values, and standardized digestibility estimates obtained for the intact feeds represent feed-AA digestibility values. Therefore, throughout the remainder of this article, RUP-AA digestibility will refer to digestibility estimates of the RUR and AA digestibility will refer to digestibility estimates of the intact feeds.

DDGS

Chemical Composition and AA Profiles of Feeds and RUR. The chemical composition and concentrations (% of total) of AA in the intact and RUR DDGS samples are presented in Table 1. The concentration of CP, NDF, ADF, lignin, NDICP, and ADICP was higher in the RUR compared with the intact feeds, and the concentration of fat, NFC, starch, and ash was lower. Decreases in the concentration of NFC and starch were expected because these nutrients are more readily degraded in the rumen than fiber components. Starch, NFC, NDF, and fat concentrations were variable among the DDGS samples, and ranges in concentrations of these components were 4 to 8, 25 to 33, 31 to 38, and 11 to 13%, respectively. The wide variation in nutrient composition among DDGS samples is one of the concerns with feeding DDGS to livestock (Kleinschmit et al., 2007).

Concentrations (% of total AA) of Lys and essential AA (**EAA**) decreased and concentrations of branched-chain AA (**BCAA**) and nonessential AA (**NEAA**) increased after 16 h of ruminal incubation of DDGS. Similarly, O'Mara et al. (1997) reported a decrease in the proportion of Lys when DDGS was ruminally incubated in situ for 12 h, and Kleinschmit et al. (2007) reported slight increases in the profile of the BCAA in

Table 1. Chemical composition (% of DM) and AA profile (% of total) of samples of distillers dried grains with solubles (DDGS) before (intact feed) and after (rumen residue) 16-h ruminal incubation

	Sample[1]									
	Intact feed					Rumen residue				
Item	HDDGS[2]	DDGS1	DDGS2	DDGS3	DDGS4	HDDGS	DDGS1	DDGS2	DDGS3	DDGS4
Chemical composition (% of DM)										
CP	32.0	29.3	32.0	30.5	29.0	39.6	34.8	40.4	37.9	33.3
Total AA	27.3	30.5	29.3	29.9	26.5	38.0	33.4	42.9	42.9	41.7
NDF	38.6	37.9	30.9	31.0	30.8	60.8	58.6	50.5	53.8	56.6
ADF	24.1	16.5	16.1	16.1	16.7	37.5	25.2	28.7	29.2	26.3
Lignin	9.4	3.8	4.6	3.4	4.1	16.9	11.9	6.1	7.3	11.1
NDICP[3]	16.8	9.8	8.3	9.7	9.7	25.7	13.4	16.4	13.0	11.0
ADICP[4]	11.8	6.5	4.8	5.8	4.8	18.5	7.5	11.2	10.4	7.9
Fat	11.8	12.4	13.1	11.4	10.7	6.1	4.2	6.3	6.1	7.3
NFC[5]	29.0	25.3	28.1	31.2	33.4	18.1	14.5	18.4	13.5	12.2
Starch	3.5	4.0	4.4	7.8	5.6	3.0	2.0	2.0	2.7	2.7
Ash	5.36	4.99	4.22	5.69	5.89	1.12	1.28	0.77	1.70	1.61
Ca	0.04	0.07	0.04	0.17	0.18	0.27	0.32	0.21	0.50	0.46
P	0.78	0.89	0.77	0.80	0.81	0.16	0.06	0.10	0.09	0.09
AA[6] (% of total)										
Arg	4.9	4.1	5.3	4.8	4.7	4.5	3.4	3.4	3.7	3.9
His	3.1	2.8	3.1	3.0	3.0	2.7	2.4	2.4	2.3	2.3
Ile	4.9	4.3	4.7	4.5	4.5	4.4	4.2	4.3	4.1	4.3
Leu	14.2	13.5	13.4	13.7	13.8	14.3	15.1	14.7	15.0	14.7
Lys	2.1	2.9	4.2	3.3	3.1	1.7	1.9	2.7	2.2	2.3
Met	2.2	2.3	2.3	2.2	2.2	2.5	2.3	2.4	2.3	2.4
Phe	5.9	5.5	5.7	5.7	5.7	5.7	5.8	5.8	5.8	5.8
Thr	4.2	4.1	4.4	4.2	4.2	4.1	3.9	3.9	3.8	3.9
Trp	0.0	1.0	0.8	0.6	0.6	0.0	0.5	0.2	0.5	0.6
Val	6.3	5.7	5.8	6.0	5.7	5.7	5.3	5.2	5.0	5.1
BCAA	25.5	23.5	23.9	23.8	24.1	24.3	24.6	24.2	24.1	24.0
EAA	47.7	45.7	49.5	47.5	47.5	45.5	44.7	45.5	44.7	45.2
Ala	8.3	8.0	7.9	8.0	8.1	8.2	8.3	8.2	8.3	8.2
Asp	7.4	7.1	7.3	7.3	7.1	7.3	6.6	6.7	6.6	6.9
Cys	2.2	2.1	2.1	2.2	2.5	2.1	2.1	2.1	2.0	2.0
Glu	17.1	19.3	15.7	17.2	17.0	18.9	19.8	19.2	19.8	19.4
Pro	8.6	8.7	8.2	8.5	8.6	8.8	9.4	8.7	9.1	8.6
Ser	4.6	5.0	5.1	5.0	5.0	4.8	4.7	4.9	5.0	5.0
Tyr	4.1	4.1	4.4	4.3	4.3	4.4	4.6	4.6	4.6	4.6
NEAA	52.3	54.4	50.5	52.5	52.5	54.5	55.3	54.5	55.3	54.8

[1]Numbers following samples indicate that these samples are from different sources or batches. Heated samples were independent samples and do not correspond to another sample.

[2]H indicates the sample was heated at 140°C for 60 min.

[3]NDICP = neutral detergent-insoluble CP.

[4]ADICP = acid detergent-insoluble CP.

[5]NFC = 100 – [CP + (NDF – NDICP) + fat + ash].

[6]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

Table 2. Standardized digestibility (%) of AA in samples of distillers dried grains with solubles (DDGS) before (intact feed) and after (rumen residue) 16-h ruminal incubation determined using the precision-fed cecectomized rooster assay

	Sample[2]										
	Intact feed					Rumen residue					
AA[1]	HDDGS[3]	DDGS1	DDGS2	DDGS3	DDGS4	HDDGS	DDGS1	DDGS2	DDGS3	DDGS4	SEM
Arg	48.6^{c}	84.3^{b}	91.4^{ab}	88.0^{ab}	86.8^{ab}	45.8^{c}	89.4^{ab}	91.3^{ab}	92.7^{a}	92.0^{ab}	1.70
His	48.1^{c}	77.2^{b}	82.7^{a}	82.1^{a}	77.1^{b}	50.5^{c}	81.0^{ab}	86.4^{a}	85.4^{a}	83.0^{ab}	1.55
Ile	50.9^{e}	81.7^{d}	89.0^{abc}	84.1^{bcd}	82.9^{cd}	50.4^{e}	87.0^{abcd}	90.7^{ab}	91.7^{a}	90.6^{ab}	1.45
Leu	67.8^{d}	90.0^{c}	91.8^{abc}	90.5^{bc}	90.2^{c}	69.5^{d}	94.1^{abc}	93.5^{abc}	95.4^{a}	94.7^{ab}	0.91
Lys	10.8^{d}	57.8^{c}	78.2^{a}	66.9^{abc}	56.5^{c}	10.3^{d}	63.0^{bc}	79.5^{a}	75.8^{ab}	72.7^{ab}	2.93
Met	59.3^{d}	86.6^{b}	89.8^{ab}	87.3^{b}	87.0^{b}	69.1^{c}	92.4^{a}	93.4^{ab}	93.9^{a}	93.8^{a}	1.01
Phe	58.1^{d}	85.8^{c}	91.5^{a}	87.9^{bc}	86.7^{c}	60.9^{d}	90.4^{abc}	92.5^{ab}	93.4^{a}	93.0^{ab}	1.12
Thr	44.2^{d}	76.9^{c}	83.5^{abc}	79.9^{bc}	77.4^{c}	48.9^{d}	84.5^{abc}	87.9^{ab}	88.5^{a}	87.4^{ab}	1.77
Trp	—	90.3^{ab}	90.9^{ab}	86.7^{bc}	84.6^{bc}	—	93.8^{ab}	72.7^{c}	95.1^{ab}	101.1^{a}	3.05
Val	46.8^{e}	80.5^{d}	86.0^{abcd}	82.1^{bcd}	81.3^{cd}	46.5^{e}	88.4^{abc}	90.8^{a}	92.5^{a}	90.0^{ab}	1.68
BCAA	59.3^{d}	86.2^{c}	89.8^{abc}	87.3^{bc}	86.7^{c}	60.7^{d}	91.7^{abc}	92.4^{ab}	94.3^{a}	93.0^{a}	1.19
EAA	53.7^{e}	82.9^{d}	88.2^{abcd}	85.1^{bcd}	83.3^{cd}	56.1^{e}	88.8^{abc}	90.8^{ab}	92.1^{a}	91.0^{b}	1.30
Ala	58.6^{d}	85.4^{c}	88.7^{abc}	86.3^{bc}	85.7^{c}	63.3^{d}	91.7^{ab}	92.6^{a}	93.9^{a}	93.1^{a}	1.17
Asp	37.8^{e}	75.2^{c}	80.6^{abc}	78.7^{bc}	74.5^{c}	44.1^{e}	85.0^{ab}	87.6^{a}	88.5^{a}	87.9^{a}	1.72
Cys	59.8^{c}	78.9^{b}	84.9^{ab}	83.1^{ab}	82.2^{ab}	55.4^{c}	86.9^{ab}	88.7^{a}	90.4^{a}	90.0^{a}	2.06
Glu	57.3^{e}	88.2^{bc}	88.5^{bc}	87.7^{bc}	85.6^{c}	67.7^{d}	93.2^{ab}	93.2^{ab}	95.1^{a}	94.3^{a}	1.19
Pro	65.3^{d}	86.2^{c}	89.9^{abc}	87.8^{bc}	86.8^{bc}	68.4^{d}	91.6^{ab}	93.0^{a}	93.5^{a}	90.8^{abc}	1.03
Ser	53.6^{f}	82.9^{d}	86.6^{abcd}	84.9^{bcd}	83.8^{cd}	64.4^{e}	91.5^{ab}	91.2^{abc}	92.5^{a}	93.3^{a}	1.59
Tyr	62.0^{e}	87.0^{d}	91.2^{abcd}	87.9^{bcd}	87.4^{cd}	65.0^{e}	91.9^{abcd}	92.7^{abc}	93.3^{ab}	95.0^{a}	1.15
NEAA	56.2^{e}	84.8^{c}	87.5^{abc}	85.8^{bc}	84.1^{c}	63.0^{d}	91.3^{ab}	92.0^{a}	93.3^{a}	93.0^{a}	1.28
Total AA	55.0^{d}	83.9^{c}	87.9^{abc}	85.5^{bc}	83.7^{c}	59.9^{d}	90.3^{ab}	91.5^{ab}	92.8^{a}	91.9^{a}	1.28

[a–f]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Numbers following samples indicate that these samples are from different sources or batches. The heated sample was an independent sample and does not correspond to another sample.

[3]H indicates the sample was heated at 140°C for 60 min.

DDGS after a 12-h ruminal in situ incubation. These results collectively indicate that the AA profile of RUP differs from the AA profile of intact feed protein in DDGS samples. Two factors may contribute to the difference in the AA profile of the RUR compared with the feed: 1) AA are not degraded at the same rate in the rumen (Prestløkken and Rise, 2003; Borucki-Castro et al., 2007), and 2) microbial contamination of the RUR resulted in a different AA profile of feeds post ruminal incubation.

Standardized Digestibility of AA and RUP-AA. Standardized intestinal digestibility estimates of AA and RUP-AA of DDGS are presented in Table 2. Standardized digestibility of AA and RUP-AA differed among samples, and standardized RUP-AA digestibility was higher than standardized AA digestibility. Heating the DDGS sample at 140°C for 60 min decreased standardized digestibility of all AA and RUP-AA, particularly Lys. A decrease in Lys digestibility was expected as a result of the Maillard reaction, which can occur in feeds between the ε-amino group of Lys and reducing sugars when heated (Mauron, 1990).

Among all AA, standardized Lys digestibility was the most variable for the DDGS samples. The Lys digestibility values of the heated and 4 unheated DDGS samples were 11, 58, 78, 67, and 57%, respectively. Lysine digestibility was also lower than total AA digestibility and was generally the least digestible AA within the DDGS samples. The AA digestibility values in poultry NRC (1994) for DDGS are 65, 84, 77, 63, 72, 81, 84, 89, 75, and 88% for Lys, Met, Cys, Arg, Thr, Val, Ile, Leu, His, and Phe, respectively, which indicates that Lys digestibility in DDGS is generally lower than digestibility of the other AA. Martinez Amezcua and Parsons (2007) also reported that Lys digestibility in DDGS was substantially lower than digestibility of most AA when DDGS was crop-intubated to cecectomized roosters.

Standardized RUP-total AA digestibility of the unheated DDGS samples in the present study ranged from 90 to 93%, and the NRC (2001) RUP digestibility value for DDGS is 80%. Reported RUP digestibility estimates for DDGS vary considerably and range from 60 to 90% (Masoero et al., 1994; O'Mara et al., 1997; Kleinschmit et al., 2007; Kononoff et al., 2007). Estimates of RUP-total AA digestibility for DDGS samples reported in this experiment are higher than many literature-reported estimates. Some of the variation is likely a result of inherent differences among DDGS samples, but some of the variation might be attributed to differences in the techniques used to estimate RUP

digestibility. The wide variation of RUP digestibility for DDGS samples reported here and in the literature is a concern because feeds are not analyzed routinely for RUP digestibility. The reported variation emphasizes the need for further investigation into estimating RUP digestibility of feeds and a standardization of the protocols for the various techniques used to estimate intestinal RUP digestibility.

Only 3 digestibility estimates of individual RUP-AA in DDGS have been published (Masoero et al., 1994; O'Mara et al., 1997) and, for all estimates, the mobile bag technique (**MBT**) was used in collecting the bags from the feces. Average reported estimates for RUP-Lys and RUP-Met digestibility were (mean ± SD) 79 ± 5% and 85% ± 6%. Estimates of RUP-total AA and RUP-EAA digestibility were not reported. In the present experiment, average RUP-Lys and RUP-Met estimates for the unheated DDGS samples were (mean ± SD) 73 ± 6% and 93 ± 1%, respectively. Estimates of RUP-Lys and RUP-Met digestibility presented in this experiment agree with previously reported values. These results highlight the need to estimate Lys digestibility in DDGS samples because digestibility of Lys is lower than digestibility of total RUP and varies widely among samples.

Indigestibility of Intact and Ruminally Incubated Feeds. Indigestibility coefficients of intact and ruminally incubated DDGS samples are presented in Table 3. For all DDGS samples, indigestibility of AA was lower for ruminally incubated samples compared with intact feeds. Indigestibility of intact and ruminally incubated DDGS has not been previously measured. However, Volden and Harstad (1995) measured indigestibility of protein in intact and ruminally incubated samples of several feedstuffs including soybean meal, rapeseed meal, corn gluten meal, barley, oats, rapeseeds, FM, and lupine seeds. Of all these feeds, the authors concluded that only soybean meal and FM contain a constant protein fraction that is neither degradable in the rumen nor digestible in the small intestine. Because DDGS is a byproduct of corn, indigestibility characteristics of DDGS may be similar to those observed for other cereal grains. Prestløkken and Rise (2003) also evaluated indigestibility of individual AA in a variety of feedstuffs including barley, expanded barley, oats, expanded oats, rapeseed meal, and FM. Indigestibility of ruminally incubated samples was lower than that for intact samples, supporting the results for DDGS in the present experiment.

FM

Chemical Composition and AA Profiles of Feeds and RUR. The chemical composition and concentrations (% of total) of AA in the intact and RUR FM samples are presented in Table 4. The NDICP and ash concentrations increased in the RUR compared with the intact feed. The observed increase in NDICP concentration of the FM RUR was expected because this CP fraction is relatively resistant to ruminal degradation (Sniffen et al., 1992). The concentration of ash was also expected to increase because FM samples can contain undegradable bone fragments. The CP and fat concentrations decreased in RUR compared with intact feeds. Similar to the observations reported here, Rooke (1985) also reported that the N concentration of the 24-h RUR of FM was lower compared with intact FM, and Susmel et al. (1994) reported that CP and fat concentrations of the 14-h RUR of FM were lower than intact FM.

Concentrations (% of total AA) of AA were similar across FM type except for CFSH, which had a lower proportion of EAA and a higher proportion of NEAA than other FM samples. Unlike DDGS samples, within FM samples the AA concentration was similar for the intact feed and RUR. Susmel et al. (1994) and O'Mara et al. (1997) also reported that the AA profile of FM did not change after samples were ruminally incubated in situ for 14 and 12 h, respectively. In general, changes in the AA profiles of feedstuffs after ruminal incubation are greater for feeds that are highly degradable in the rumen and less for feedstuffs more resistant to ruminal degradation (O'Mara et al., 1997).

Standardized Digestibility of AA and RUP-AA. Standardized digestibility of AA was similar among FM types, except for CFSH, for which AA digestibility was lower (Table 5). Unlike DDGS, standardized digestibility of Lys in FM was similar to standardized digestibility of total EAA, which indicates that the Lys in FM is not damaged by heat. This is likely a result of the lack of sugars in FM because reducing sugars are needed for the Maillard reaction (Mauron, 1990).

Standardized AA digestibility estimates of all FM samples, except for CFSH, agree well with reference values for poultry (NRC, 1994). In NRC (1994), standardized digestibility of Lys and Met in FM is (mean ± SD) 88 ± 5% and 92 ± 3%, respectively. Excluding the CFSH sample, in the present experiment, standardized digestibility of Lys and Met was (mean ± SD) 86 ± 3% and 93 ± 1%, respectively. Estimates of RUP-total AA digestibility of the FM samples, except CFSH, also agree well with reference RUP digestibility values (NRC, 2001). The RUP digestibility value for both ANVY and MNHN FM in NRC (2001) is 90%. The average estimate of standardized RUP-total AA digestibility of FM samples, excluding CFSH, in the present experiment was 89%.

Table 3. Indigestibility (%) of AA in intact and ruminally incubated samples of distillers dried grains with solubles (DDGS)

	Sample[2]										
	Intact feed					Ruminally incubated					
AA[1]	HDDGS[3]	DDGS1	DDGS2	DDGS3	DDGS4	HDDGS	DDGS1	DDGS2	DDGS3	DDGS4	SEM
Arg	51.4^{a}	15.7^{c}	8.6^{def}	12.0^{cd}	13.2^{cd}	43.5^{b}	3.4^{g}	3.9^{fg}	3.5^{g}	4.9^{efg}	1.48
His	51.9^{a}	22.8^{c}	17.3^{c}	17.9^{c}	22.9^{c}	38.4^{b}	6.3^{d}	6.4^{d}	7.0^{d}	9.8^{d}	1.25
Ile	49.1^{a}	18.3^{c}	11.0^{de}	15.9^{cd}	17.1^{cd}	38.9^{b}	5.0^{e}	5.0^{e}	4.8^{e}	6.5^{e}	1.33
Leu	32.3^{a}	10.0^{c}	8.2^{cd}	9.5^{c}	9.8^{c}	26.7^{b}	2.6^{de}	4.2^{de}	3.1^{e}	4.2^{de}	0.85
Lys	89.2^{a}	42.2^{c}	21.8^{de}	33.1^{cd}	43.5^{c}	63.5^{b}	9.5^{f}	7.8^{f}	10.0^{f}	14.8^{ef}	2.41
Met	40.7^{a}	13.4^{c}	10.2^{c}	12.7^{c}	13.0^{c}	31.0^{b}	3.2^{d}	4.0^{d}	4.1^{d}	5.1^{d}	0.98
Phe	41.9^{a}	14.2^{c}	8.5^{de}	12.1^{cd}	13.3^{cd}	33.3^{b}	4.0^{e}	4.5^{e}	4.2^{e}	5.3^{e}	1.03
Thr	55.8^{a}	23.1^{c}	16.5^{c}	20.1^{c}	22.6^{c}	43.1^{b}	5.9^{d}	6.3^{d}	6.5^{d}	8.9^{d}	1.49
Trp	—	9.7^{ab}	9.1^{ab}	13.3^{a}	15.4^{a}	—	1.7^{c}	3.1^{bc}	2.6^{bc}	0.0^{c}	1.58
Val	53.2^{a}	19.5^{c}	14.0^{d}	17.9^{cd}	18.7^{cd}	42.2^{b}	4.2^{e}	4.8^{e}	4.1^{e}	6.6^{e}	1.49
BCAA	40.7^{a}	13.8^{c}	10.2^{d}	12.7^{cd}	13.3^{cd}	32.9^{a}	3.4^{e}	4.5^{e}	3.7^{e}	5.2^{e}	1.10
EAA	46.3^{a}	17.1^{c}	11.8^{cd}	14.9^{c}	16.7^{c}	36.6^{b}	4.3^{e}	4.9^{e}	4.6^{e}	6.3^{de}	1.19
Ala	41.4^{a}	14.6^{c}	11.3^{c}	13.7^{c}	14.3^{c}	31.9^{b}	3.3^{d}	4.5^{d}	4.0^{d}	5.3^{d}	1.11
Asp	62.2^{a}	24.8^{c}	19.4^{c}	21.3^{c}	25.5^{c}	48.3^{b}	5.5^{d}	6.7^{d}	6.5^{d}	8.6^{d}	1.55
Cys	40.2^{a}	21.1^{c}	15.1^{d}	16.9^{cd}	17.8^{cd}	37.1^{b}	4.9^{e}	6.7^{e}	5.3^{e}	6.0^{e}	1.71
Glu	42.7^{a}	11.8^{c}	11.5^{c}	12.3^{c}	14.4^{c}	31.3^{b}	2.8^{d}	4.9^{d}	3.5^{d}	4.8^{d}	1.15
Pro	34.7^{a}	13.8^{b}	10.1^{b}	12.2^{b}	13.2^{b}	31.9^{a}	3.0^{c}	5.0^{c}	4.7^{c}	5.0^{c}	1.08
Ser	46.4^{a}	17.1^{c}	13.4^{c}	15.1^{c}	16.2^{c}	28.4^{b}	3.6^{d}	4.4^{d}	4.2^{d}	6.8^{d}	1.33
Tyr	38.0^{a}	13.0^{c}	8.8^{cd}	12.1^{c}	12.6^{c}	32.7^{b}	3.6^{e}	4.6^{de}	4.5^{de}	4.0^{de}	1.07
NEAA	43.8^{a}	15.2^{c}	12.5^{c}	14.2^{c}	15.9^{c}	33.7^{b}	3.5^{d}	5.1^{d}	4.4^{d}	5.7^{d}	1.16
Total AA	45.0^{a}	16.0^{c}	12.1^{c}	14.5^{c}	16.3^{c}	35.1^{b}	3.8^{d}	5.0^{d}	4.5^{d}	6.0^{d}	1.18

[a–g]Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Numbers following samples indicate that these samples are from different sources or batches. The heated sample was an independent sample and does not correspond to another sample.

[3]H indicates the sample was heated at 140°C for 60 min.

Literature reported estimates of RUP-AA digestibility of FM were summarized (Masoero et al., 1994; O'Mara et al., 1997; Prestløkken and Rise, 2003; Taghizadeh et al., 2005). Average reported estimates for RUP-total AA (n = 7), RUP-total EAA (n = 5), RUP-Lys (n = 10), and RUP-Met (n = 10) digestibility in FM were (mean ± SD) 95 ± 2%, 95 ± 3%, 96 ± 3%, and 95 ± 2%, respectively. Average estimates of RUP-total AA, RUP-total EAA, RUP-Lys, and RUP-Met digestibility for the FM samples presented here were lower than average reported values and were (excluding CFSH; mean ± SD) 89 ± 2%, 90 ± 1%, 88 ± 2%, and 91 ± 1%, respectively. Most of the literature-reported estimates were obtained using the MBT with the collection of bags in the feces. Prestløkken and Rise (2003) reported a significant effect of the site of bag collection on RUP-AA digestibility of FM; fecal collection of bags resulted in higher RUP-AA digestibility estimates than ileal collection of bags. In addition, estimates of protein and AA digestibility obtained using the MBT have been reported to be higher than estimates obtained in vivo in both ruminant and swine species (Varvikko and Vanhatalo, 1990; Viljoen et al., 1997). Using the MBT, nutrients that disappear from the bags are assumed to be absorbed in the small intestine; however, this does not account for factors that affect nutrient absorption such as antinutritional factors or nutrient antagonism.

Standardized AA digestibility was similar to standardized RUP-AA digestibility for most AA within FM samples. de Boer et al. (1987) also reported that intestinal CP digestibility of intact FM samples (89%) was similar to intestinal RUP digestibility of the 12-h RUR of FM (92%) when measured in lactating cows using the MBT with the collection of bags in the feces. Therefore, because the AA profile of FM does not change after ruminal incubation and because intestinal AA and RUP-AA digestibilities are similar within FM samples, FM samples do not need to be ruminally incubated to determine RUP-AA digestibility in ruminants. This step can be eliminated, and AA digestibility values of FM samples reported in the swine and poultry literature may be adopted for ruminants.

Indigestibility of Intact and Ruminally Incubated Feeds. For ANVY, CFSH, and MNHN1 samples, indigestibility of AA was generally lower for ruminally incubated samples compared with intact samples, but for MNHN2 and PLCK, there was little difference between indigestibility of AA in intact and ruminally incubated samples (Table 6). Hvelplund et al. (1992) and Volden and Harstad (1995) reported that FM does

Table 4. Chemical composition (% of DM) and AA profile (% of total) of anchovy (ANVY), catfish (CFSH), menhaden (MNHN), and pollock (PLCK) fish meal samples before (intact feed) and after (rumen residue) 16-h in situ ruminal incubation

	Sample[1]									
	Intact feed					Rumen residue				
Item	ANVY	CFSH	MNHN1	MNHN2	PLCK	ANVY	CFSH	MNHN1	MNHN2	PLCK
Chemical composition (% of DM)										
CP	74.1	69.5	73.6	73.7	75.6	73.9	46.4	63.4	67.1	71.7
Total AA	59.8	53.1	57.0	56.1	64.2	65.9	36.0	56.3	60.6	61.6
NDICP[2]	12.4	32.8	19.4	20.4	19.2	36.7	33.9	30.5	33.4	20.1
ADICP[3]	0.8	5.5	0.6	0.6	0.8	0.5	5.0	0.5	1.0	2.1
Fat	11.9	12.3	12.7	12.4	10.4	4.6	4.8	5.4	4.0	7.7
Ash	18.71	22.66	19.57	20.21	19.00	20.54	45.32	28.39	27.05	19.16
Ca	4.33	8.32	5.18	4.95	6.48	7.22	15.69	10.17	10.01	8.35
P	2.93	3.98	3.18	2.99	3.10	3.44	7.48	4.99	4.56	3.23
AA[4] (% of total)										
Arg	6.7	8.3	7.2	7.1	7.2	6.8	8.3	7.0	7.0	7.1
His	3.5	2.5	3.3	2.8	2.6	2.7	2.4	2.6	2.5	2.5
Ile	5.2	4.5	4.8	4.9	5.0	5.1	4.2	4.9	4.9	4.8
Leu	8.9	7.7	8.4	8.5	8.8	8.9	7.5	8.6	8.5	8.8
Lys	9.4	8.0	9.3	9.1	9.1	9.1	7.3	8.8	9.0	8.6
Met	3.3	2.7	3.2	3.3	3.5	3.6	2.8	3.6	3.7	3.7
Phe	4.8	4.3	4.6	4.7	4.6	4.9	4.2	4.7	4.6	4.6
Thr	4.9	4.5	4.8	4.8	5.0	5.1	4.9	5.0	5.1	5.2
Trp	1.3	0.6	1.1	1.2	1.2	1.1	0.7	1.1	1.0	1.1
Val	6.0	5.7	5.7	5.7	5.9	5.8	5.4	5.6	5.4	5.5
BCAA	20.0	17.9	18.9	19.1	19.7	19.7	17.1	19.1	18.8	19.0
EAA	53.8	48.7	52.3	52.0	52.8	53.0	47.7	51.9	51.8	51.8
Ala	7.4	9.2	7.7	7.5	6.6	7.1	9.2	7.0	7.0	6.5
Asp	10.5	10.2	10.5	10.6	10.7	10.7	10.1	10.8	10.9	10.9
Cys	1.2	1.0	0.9	1.0	1.3	1.2	0.9	1.1	1.1	1.4
Glu	14.5	15.4	15.0	15.5	15.0	14.9	14.9	15.2	15.5	15.0
Pro	4.5	8.3	5.6	5.5	4.5	4.6	8.9	5.4	5.2	4.8
Ser	4.3	4.2	4.4	4.4	5.1	4.5	5.0	4.7	4.6	5.4
Tyr	3.8	3.0	3.5	3.6	4.2	4.1	3.4	4.0	3.9	4.3
NEAA	46.2	51.3	47.7	48.0	47.2	47.0	52.3	48.1	48.2	48.2

[1]Numbers following samples indicate that these samples are from different sources or batches.
[2]NDICP = neutral detergent-insoluble CP.
[3]ADICP = acid detergent-insoluble CP.
[4]BCAA = branched-chain AA, EAA = essential AA, NEAA = nonessential AA.

contain a constant undegradable and indigestible protein fraction, but Prestløkken and Rise (2003) reported that is not the case. However, the types of FM used in these experiments were not reported. The results of the present experiment suggest that some FM samples may contain a constant undegradable and indigestible fraction whereas other samples do not.

Volden and Harstad (1995) reported that CP indigestibility of intact FM was 2.5% of total CP, and Prestløkken and Rise (2003) reported that total AA indigestibility of intact FM was 6.2%. These values are lower than those reported in the present experiment for all FM types. The average total AA indigestibility estimate of intact FM samples in the current experiment was 10.1%, excluding CFSH. Because standardized AA digestibility estimates of FM in the current study are consistent with NRC (1994) digestibility coefficients, discrepancies between the present study and data reported by others are likely explained by differences in the technique used to estimate intestinal AA digestibility.

For ruminally incubated FM samples, Volden and Harstad (1995) reported that CP indigestibility was 3.4% and Prestløkken and Rise (2003) reported that total AA indigestibility was 4.0%. For the MNHN1 sample, the AA indigestibility estimate of 4.5% agrees well with literature-reported estimates, but for the other ruminally incubated FM samples, indigestibility of total AA was higher (6.1–16.0%). Intestinal CP and AA digestibility estimates are generally higher when the MBT is used compared with estimates obtained in vivo, which may explain the lower indigestibility estimates reported by Volden and Harstad (1995) and Prestløkken and Rise (2003).

Digestibility of Individual AA Versus Total AA

As discussed in the companion paper (Boucher et al., 2009a), it may be beneficial to assign intestinal digest-

Table 5. Standardized digestibility (%) of AA in anchovy (ANVY), catfish (CFSH), menhaden (MNHN), and pollock (PLCK) fish meal samples before (intact feed) and after (rumen residue) 16-h ruminal incubation determined using the precision-fed cecectomized rooster assay

	Sample[2]										
	Intact feed					Rumen residue					
AA[1]	ANVY	CFSH	MNHN1	MNHN2	PLCK	ANVY	CFSH	MNHN1	MNHN2	PLCK	SEM
Arg	89.7^{a}	84.7^{ab}	90.9^{a}	90.7^{a}	89.5^{a}	89.6^{a}	70.3^{c}	89.7^{a}	82.1^{b}	88.0^{ab}	1.35
His	83.7^{ab}	74.1^{c}	83.0^{ab}	84.4^{ab}	86.6^{a}	82.5^{ab}	59.4^{d}	80.3^{b}	78.6^{b}	83.3^{ab}	1.57
Ile	91.2^{a}	81.0^{b}	90.9^{a}	93.9^{a}	94.4^{a}	91.0^{a}	72.1^{c}	92.7^{ab}	91.4^{a}	92.4^{a}	0.54
Leu	91.9^{abc}	82.0^{d}	91.1^{c}	93.8^{ab}	94.8^{a}	92.0^{bc}	73.6^{e}	92.9^{a}	91.3^{ac}	92.9^{a}	1.09
Lys	84.4^{a}	72.4^{b}	84.3^{a}	85.9^{a}	90.9^{a}	87.5^{a}	63.2^{c}	88.6^{ab}	84.3^{a}	89.9^{a}	1.88
Met	92.5^{ab}	82.2^{c}	92.0^{ab}	93.3^{a}	93.6^{a}	91.1^{ab}	73.0^{d}	91.4^{a}	88.9^{b}	91.2^{ab}	0.94
Phe	89.7^{a}	80.9^{b}	90.1^{a}	92.4^{a}	93.1^{a}	89.9^{a}	71.3^{c}	90.5^{ab}	88.2^{a}	90.6^{a}	1.07
Thr	90.9^{ab}	78.0^{c}	90.4^{ab}	93.5^{ab}	93.6^{a}	91.3^{ab}	70.2^{c}	90.8^{ab}	88.0^{b}	91.6^{ab}	1.18
Trp	95.7^{a}	86.3^{b}	96.4^{a}	99.1^{a}	98.1^{a}	96.9^{a}	89.3^{b}	97.8^{abc}	98.1^{a}	98.7^{a}	1.06
Val	90.4^{a}	80.0^{b}	89.4^{a}	92.4^{a}	93.2^{a}	91.0^{a}	70.0^{c}	91.5^{abc}	88.7^{a}	91.4^{a}	1.15
BCAA	91.3^{a}	81.1^{b}	90.5^{a}	93.4^{a}	94.2^{a}	91.4^{a}	72.1^{c}	92.4^{ab}	90.6^{a}	92.4^{a}	1.00
EAA	89.4^{ab}	79.7^{c}	89.2^{ab}	91.3^{ab}	92.5^{a}	90.0^{ab}	69.8^{d}	90.5^{ab}	87.3^{b}	90.7^{ab}	1.09
Ala	89.6^{a}	79.1^{b}	89.0^{a}	89.2^{a}	91.4^{a}	88.7^{a}	65.1^{c}	87.3^{b}	79.8^{b}	88.4^{a}	1.34
Asp	87.1^{ab}	59.7^{c}	82.0^{b}	88.5^{a}	91.1^{a}	88.7^{a}	58.4^{c}	87.0^{bc}	85.3^{ab}	89.6^{a}	1.39
Cys	78.7^{b}	70.7^{c}	76.6^{b}	85.9^{ab}	89.0^{a}	79.2^{ab}	55.1^{d}	81.4^{bc}	78.9^{b}	85.0^{ab}	2.11
Glu	90.5^{ab}	76.2^{c}	89.4^{ab}	91.9^{ab}	92.6^{a}	91.1^{ab}	67.9^{d}	91.4^{a}	87.6^{b}	90.8^{ab}	1.02
Pro	84.5^{a}	78.0^{b}	85.7^{a}	86.1^{a}	87.1^{a}	84.2^{a}	63.8^{c}	83.0^{ab}	70.8^{b}	83.6^{a}	1.84
Ser	88.7^{ab}	74.5^{b}	87.7^{a}	91.4^{a}	90.5^{a}	89.5^{a}	68.0^{c}	88.9^{abc}	85.0^{a}	87.3^{a}	1.37
Tyr	90.9^{a}	80.4^{b}	91.3^{a}	93.6^{a}	93.9^{a}	91.6^{a}	76.9^{b}	93.2^{ab}	90.7^{a}	91.7^{a}	1.08
NEAA	88.6^{ab}	73.7^{c}	87.0^{ab}	90.0^{a}	91.4^{a}	89.1^{ab}	65.3^{c}	88.5^{ab}	83.9^{b}	89.0^{ab}	1.23
Total AA	89.0^{ab}	76.7^{b}	88.1^{ab}	90.7^{ab}	92.0^{a}	89.6^{ab}	67.4^{c}	89.6^{ab}	85.7^{b}	89.9^{ab}	1.14

$^{a–e}$Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Numbers following samples indicate that these samples are from different sources or batches.

Table 6. Indigestibility (%) of AA in intact and ruminally incubated anchovy (ANVY), catfish (CFSH), menhaden (MNHN), and pollock (PLCK) fish meal samples

	Sample[2]										
	Intact feed					Ruminally incubated					
AA[1]	ANVY	CFSH	MNHN1	MNHN2	PLCK	ANVY	CFSH	MNHN1	MNHN2	PLCK	SEM
Arg	10.3^{bcd}	15.3^{a}	9.1^{cd}	9.3^{cd}	10.5^{de}	6.2^{de}	14.3^{ab}	4.3^{e}	11.1^{bc}	7.6^{cde}	1.04
His	16.3^{bc}	25.9^{a}	17.0^{b}	15.6^{bc}	13.4^{b}	7.9^{ef}	17.7^{b}	6.7^{f}	12.0^{cde}	10.2^{def}	0.96
Ile	8.8^{c}	19.0^{a}	9.1^{c}	6.1^{c}	5.6^{c}	5.2^{d}	13.5^{b}	3.3^{d}	5.3^{cd}	4.6^{d}	0.83
Leu	8.1^{cd}	17.9^{a}	8.9^{c}	6.2^{cde}	5.2^{de}	4.6^{de}	12.9^{b}	3.1^{e}	5.4^{de}	4.5^{de}	0.77
Lys	15.6^{bc}	27.6^{a}	15.7^{bc}	14.1^{bcd}	9.1^{cde}	7.1^{de}	17.3^{b}	4.7^{e}	9.7^{cde}	6.2^{e}	1.59
Met	7.5^{b}	17.8^{a}	8.0^{b}	6.7^{b}	6.4^{b}	5.6^{b}	14.5^{a}	4.2^{b}	7.7^{b}	6.0^{b}	0.85
Phe	10.3^{bc}	19.1^{a}	9.9^{c}	7.6^{cd}	6.9^{cd}	6.0^{cd}	14.2^{b}	4.2^{d}	7.3^{cd}	6.1^{cd}	0.86
Thr	9.1^{cd}	22.0^{a}	9.6^{c}	6.5^{cd}	6.4^{cd}	5.3^{cd}	15.4^{b}	4.1^{d}	7.9^{cd}	5.6^{cd}	0.93
Trp	4.3^{b}	13.7^{a}	3.6^{b}	0.9^{b}	1.9^{b}	1.6^{b}	5.1^{b}	0.9^{b}	1.1^{b}	0.8^{b}	0.98
Val	9.6^{c}	20.0^{a}	10.6^{bc}	7.6^{cd}	6.8^{cd}	5.1^{d}	14.1^{b}	3.6^{d}	6.7^{cd}	5.2^{d}	0.87
BCAA	8.7^{c}	18.9^{a}	9.5^{c}	6.6^{c}	5.8^{cd}	4.9^{d}	13.5^{b}	3.3^{d}	5.8^{cd}	4.8^{d}	0.81
EAA	10.6^{c}	20.3^{a}	10.8^{bc}	8.7^{cd}	7.5^{cde}	5.7^{de}	14.6^{b}	4.1^{e}	7.9^{cde}	5.8^{de}	0.86
Ala	10.4^{cd}	20.9^{a}	11.0^{cd}	10.8^{cd}	8.6^{cde}	6.3^{de}	16.4^{ab}	5.0^{e}	11.9^{bc}	7.4^{cde}	1.00
Asp	12.9^{c}	40.3^{a}	18.0^{bc}	11.5^{cd}	8.9^{cd}	6.7^{cd}	21.0^{b}	5.7^{d}	9.4^{cd}	6.8^{cd}	1.41
Cys	21.3^{abc}	29.3^{a}	23.4^{ab}	14.1^{cd}	11.0^{d}	12.4^{cd}	24.4^{ab}	9.4^{d}	15.4^{bcd}	10.4^{d}	2.00
Glu	9.5^{c}	22.8^{a}	10.6^{c}	8.1^{cd}	7.4^{cde}	5.3^{de}	15.8^{b}	3.8^{e}	7.7^{cde}	5.9^{de}	0.90
Pro	11.3^{c}	25.5^{a}	12.3^{bc}	8.6^{c}	9.5^{c}	9.4^{c}	17.8^{b}	7.0^{c}	17.2^{b}	11.3^{c}	0.89
Ser	15.5^{b}	22.0^{a}	14.3^{bc}	13.9^{bcd}	12.9^{bcd}	6.4^{e}	16.8^{ab}	5.0^{e}	9.8^{cde}	8.7^{de}	1.12
Tyr	9.1^{bc}	19.6^{a}	8.7^{bcd}	6.4^{cde}	6.0^{cde}	5.2^{de}	12.1^{b}	3.3^{e}	6.3^{cde}	5.5^{de}	0.76
NEAA	11.4^{cd}	26.3^{a}	13.0^{bc}	10.0^{cde}	8.6^{cdef}	6.4^{ef}	17.3^{b}	5.0^{f}	10.1^{cde}	7.2^{deg}	1.01
Total AA	11.0^{c}	23.3^{a}	11.9^{bc}	9.3^{cd}	8.0^{cde}	6.1^{de}	16.0^{b}	4.5^{e}	8.9^{cd}	6.5^{de}	0.92

$^{a–g}$Least squares means within the same row without a common superscript differ ($P < 0.05$).

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Numbers following samples indicate that these samples are from different sources or batches.

Table 7. Absolute value of mean difference (mean ± SD) between standardized digestibility of individual AA and total AA in intact and ruminally incubated samples of distillers dried grains with solubles (DDGS) and fish meal (FM)

AA[1]	DDGS, intact feed (n = 20[2])	*P*-value[3]	DDGS, ruminally incubated (n = 20)	*P*-value	FM, intact feed (n = 20[4])	*P*-value	FM, ruminally incubated (n = 20)	*P*-value
Arg	3.30 ± 1.98	<0.001	3.44 ± 5.53	<0.001	3.27 ± 2.71	<0.001	3.87 ± 2.80	<0.001
His	5.74 ± 2.39	<0.001	8.02 ± 2.02	<0.001	4.93 ± 1.58	<0.001	10.99 ± 2.92	<0.001
Ile	1.95 ± 1.34	<0.001	3.17 ± 3.40	<0.001	2.99 ± 1.16	<0.001	0.41 ± 0.30	<0.001
Leu	6.86 ± 3.25	<0.001	4.17 ± 2.95	<0.001	3.43 ± 1.26	<0.001	0.76 ± 0.50	<0.001
Lys	25.18 ± 12.04	<0.001	25.00 ± 14.00	<0.001	3.86 ± 2.59	<0.001	5.12 ± 3.54	<0.001
Met	2.79 ± 1.28	<0.001	3.35 ± 3.19	<0.001	3.43 ± 1.47	<0.001	1.01 ± 0.67	<0.001
Phe	2.82 ± 0.91	<0.001	0.84 ± 0.45	<0.001	1.95 ± 1.54	<0.001	1.69 ± 0.64	<0.001
Thr	6.81 ± 2.54	<0.001	5.84 ± 3.12	<0.001	2.04 ± 0.91	<0.001	1.48 ± 1.11	<0.001
Trp	3.50 ± 3.51	<0.001	13.88 ± 13.30	<0.001	7.81 ± 2.34	<0.001	8.37 ± 5.28	<0.001
Val	3.84 ± 2.44	<0.001	3.68 ± 5.05	<0.001	1.83 ± 1.12	<0.001	1.28 ± 0.80	<0.001
BCAA	2.68 ± 0.99	<0.001	1.13 ± 0.45	<0.001	2.82 ± 1.18	<0.001	3.35 ± 1.48	<0.001
EAA	0.72 ± 0.44	<0.001	1.43 ± 1.22	<0.001	1.17 ± 1.05	<0.001	2.11 ± 0.96	<0.001
NEAA	0.64 ± 0.39	<0.001	1.18 ± 1.03	<0.001	1.21 ± 0.97	<0.001	4.61 ± 2.10	<0.001

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Five samples of DDGS each fed to 4 birds.

[3]Probability that the absolute value of the difference is greater than zero.

[4]Five samples of FM each fed to 4 birds.

ibility coefficients to individual RUP-AA within feeds in future ruminant nutrition models. To evaluate the difference between digestibility of individual AA and total AA in DDGS and FM samples, the absolute value of the difference between digestibility of individual AA and total AA in intact and ruminally incubated DDGS and FM samples was calculated (Table 7). The absolute value of the difference between digestibility of individual AA and total AA was greater than zero for all AA in intact and ruminally incubated DDGS and FM samples. The mean difference between individual and total AA for the ruminally incubated DDGS samples ranged from (mean ± SD) 1.9 ± 1.3 for Ile to 25.2 ± 12.0 for Lys. If the heat-treated sample was removed from the analysis, the difference between Lys digestibility and total AA digestibility within the ruminally incubated DDGS samples was smaller (18.9 ± 6.1; data not shown) but still different from zero. The mean difference between individual and total AA for the ruminally incubated FM samples ranged from (mean ± SD) 0.4 ± 0.3 for Ile to 11.0 ± 2.9 for His. As with the soybean meal and SoyPlus samples discussed in the companion paper (Boucher et al., 2009a), because the absolute value of the difference between digestibility of individual RUP-AA and total RUP-AA in DDGS and FM samples was greater than zero for all AA, if digestibility coefficients are assigned to individual RUP-AA within these feedstuffs, predictions of metabolizable AA supply may be improved.

Regression Analysis

Results of the regression analyses to examine the relationship between standardized AA and RUP-AA digestibility of DDGS samples and the relationship between standardized digestibility of AA and RUP-AA and ADICP concentration of all samples are presented in Table 8. The soybean meal and SoyPlus described in the companion paper (Boucher et al., 2009a) were included in this analysis. There was a direct relationship between standardized digestibility of AA and RUP-AA in DDGS samples, and these values were highly correlated (R^2 values = 0.96–0.99) for all AA except Trp. This relationship was examined because even though standardized digestibility of AA differed from digestibility of RUP-AA in the DDGS samples, if RUP-AA digestibility can be predicted from AA digestibility of the intact feeds, time and money could be saved.

There was an inverse relationship between the ADICP concentration of all intact feeds and standardized AA digestibility for all AA, and the R^2 values for this relationship ranged from 0.56 for Arg to 0.84 for Trp. An inverse relationship between the ADICP concentration of the RUR and standardized RUP-AA digestibility was also observed. The R^2 values ranged from 0.22 for Pro to 0.82 for Lys, but for most AA, the R^2 values were between 0.40 and 0.66.

In practical feeding situations, ADICP concentrations of RUR are not known. Therefore, the relationship between the ADICP concentration of the feed and standardized RUP-AA digestibility was also examined, and an inverse relationship was observed. The R^2 values generally ranged from 0.40 to 0.70, but for RUP-Lys, the R^2 value was 0.84. An inverse relationship between ADICP and intestinal CP digestibility is welldocumented (Van Soest and Mason, 1991). Although ADICP is sometimes used as an indicator of protein quality, low R^2 values between ADICP concentration and digestibility of most AA, including total AA, suggests that only about 45 to 65% of the variation in intestinal AA

Table 8. R^2 values between standardized digestibility of AA and acids in rumen-undegraded protein (RUP-AA) of distillers dried grains with solubles (DDGS) samples; acid detergent-insoluble CP (ADICP) concentrations and standardized AA and RUP-AA digestibility; and ADICP concentrations in intact feeds and standardized RUP-AA digestibility

AA[1]	Digestibility of feed and residues, DDGS[2] (n = 5)	ADICP in feeds and standardized AA digestibility of feeds (n = 16)[3]	ADICP in residues and standardized RUP-AA digestibility (n = 16)	ADICP in feeds and standardized RUP-AA digestibility (n = 16)
Arg	0.99*	0.56	0.42	0.40
His	0.99	0.76	0.46	0.54
Ile	0.98	0.78	0.65	0.67
Leu	0.99	0.74	0.52	0.58
Lys	0.96	0.82	0.82	0.84
Met	0.99	0.81	0.47	0.54
Phe	0.98	0.73	0.49	0.54
Thr	0.99	0.81	0.70	0.74
Trp	0.53	0.84	0.66	0.52
Val	0.99	0.77	0.62	0.63
BCAA	0.99	0.78	0.60	0.63
EAA	0.99	0.79	0.64	0.68
Ala	0.99	0.75	0.40	0.46
Asp	0.99	0.75	0.59	0.64
Cys	0.97	0.67	0.39	0.45
Glu	0.99	0.78	0.49	0.56
Pro	0.99	0.66	0.22	0.27
Ser	0.98	0.78	0.45	0.50
Tyr	0.97	0.76	0.61	0.63
NEAA	0.99	0.78	0.47	0.53
Total AA	0.99	0.75	0.53	0.58

[1]BCAA = branched-chain AA; EAA = essential AA; NEAA = nonessential AA.

[2]Only the R^2 values between the intact and ruminally incubated DDGS samples are presented because there was a significant difference between AA digestibility in the intact compared with the rumen-undegraded residue samples. There was no difference in AA digestibility of intact fish meal compared with AA digestibility in the rumen-undegraded residues of fish meal.

[3]n = 16. Soybean meal = 3; SoyPlus (West Central, Ralston, IA) = 3; DDGS = 5; fish meal = 5. The data for the soybean meal and SoyPlus samples are reported in a companion paper.

*All correlations presented in this table are significant ($P < 0.01$) except for the correlation between the ADICP of the residue and standardized digestibility of Pro in the residue.

digestibility within and among samples is explained by differences in ADICP concentrations. Hussein et al. (1995) reported that as the ADICP concentration of soybeans increased as a result of heat treatment, intestinal digestibility of ADICP also increased. This observation may help explain why the R^2 values between AA digestibility and ADICP were not higher.

CONCLUSIONS

Within the DDGS samples, standardized AA digestibility was lower than standardized RUP-AA digestibility, but within FM samples, standardized AA digestibility was similar to standardized RUP-AA. Therefore, FM samples do not need to be ruminally incubated before determining intestinal AA digestibility. For the DDGS samples, Lys was generally the least digestible AA, and standardized RUP-Lys digestibility varied among samples. Therefore, more accurate predictions of RUP-Lys digestibility are particularly important. Distillers dried grains with solubles and most FM samples do not contain a constant protein fraction that is neither degradable in the rumen nor digestible in the small intestine. These feeds contain a protein fraction that is indigestible in the small intestine but is partly degraded in the rumen, or digested in the small intestine after rumen incubation, or both.

Future analysis should focus on estimating digestibility of individual AA rather than digestibility of total RUP in DDGS and FM samples because the mean difference between digestibility of individual RUP-AA and total RUP-AA was greater than zero for all AA in the samples. Also, for DDGS samples, when adequate prediction equations are identified and validated, it may be possible to predict RUP-AA digestibility from the digestibility of AA in the intact feed because standardized digestibility of AA and RUP-AA was highly correlated. Although ADICP concentration may be a useful indicator of protein quality, much of the variation in AA digestibility across samples is not explained by differences in ADICP concentrations.

ACKNOWLEDGMENTS

The authors thank West Central (Ralston, IA), Adisseo (Antony, France), Novus International, Inc. (St.

Louis, MO), and Venture Milling (Salisbury, MD) for financial support of this project. We also thank the staff at the Fairchild Dairy Teaching and Research Center (Durham, NH) for feeding and caring for the cows and the following individuals at the University of New Hampshire (Durham) who helped with this experiment: Anna Pape, Holli Pinard, Heather Tucker, Nancy Whitehouse, and Carly Crawford.

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