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Determination of variability of apparent ileal amino acid digestibility values in barley samples for growing-finishing pigs with dual digestibility markers*

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ABSTRACT

This study was conducted to examine if the use of dual digestibility markers as well as differences in neutral-detergent fibre (NDF) contents and dietary crude protein (CP) and amino acid (AA) levels were responsible for the large variability of apparent ileal AA digestibility values in barley samples for growing-finishing pigs. Six barrows, with average initial and final body weights of 30.5 and 58.6 kg, were fitted with a simple T-cannula at the distal ileum and fed six barley-based diets at close to *ad libitum* feed intake according to a 6 × 6 Latin square design. The six diets contained 97% barley samples varying in CP and AA contents. Chromic oxide (Cr_2O_3) and acid-insoluble ash (AIA) were used as digestibility markers. Each experimental period consisted of 7 d. Ileal digesta was collected at 2-h intervals for a total of 24 h. There were large differences ($P < 0.05$) in the apparent ileal CP and AA digestibility values among the barley samples as determined by both the Cr_2O_3 and the AIA markers. The use of Cr_2O_3 and AIA markers and differences in NDF contents had no effects ($P > 0.05$) on the variability of the apparent ileal CP and AA digestibility values among the barley samples. The use of the dual digestibility markers had no effects ($P > 0.05$) on the determination of apparent ileal CP and AA digestibility values in the majority of the diets. Differences in dietary CP and AA contents were primarily responsible for the large differences ($P < 0.05$) of the apparent ile-

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al CP and AA digestibility values among the barley samples. Apparent ileal CP and AA digestibility values determined in barley samples are not reliable and should not be used in diet formulation for pigs. True ileal CP and AA digestibility values directly determined from various barley samples should be used in diet formulation for pigs.

KEY WORDS: amino acids, barley, chromic oxide (Cr_2O_3), acid-insoluble ash (AIA), pigs

INTRODUCTION

Barley is a major ingredient in pig rations in Western Canada and in some other areas of the world. This grain, together with wheat and maize, usually supplies a large proportion of the protein in rations for growing and finishing pigs.

It has been widely accepted that the determination of nutritive values of crude protein (CP) and amino acids (AA) in feed ingredients for formulation of pig diets should be conducted with the ileal rather than the faecal analysis method (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986). The apparent ileal digestibility values of AA have been determined in many barley samples, however, there is a large variability among different samples and studies (Sauer and Ozimek, 1986; Buraczewska et al., 1987). It is unclear what factors are responsible for the large differences.

Inherent factors, such as differences in neutral-detergent fibre (NDF) content, were found to be partly responsible for the variation in the apparent ileal digestibility values of AA in wheat samples (Taverner et al., 1981; Fan et al., 2001), peas (Gdala et al., 1992; Fan and Sauer, 1999) and canola meal samples (Fan et al., 1996). Furthermore, the variability of apparent ileal AA digestibility values of AA among samples of the same ingredient may also result from methodological approaches such as differences in AA contents in assay diets and the use of different methods of determination, as reported by Fan (1994), Fan et al. (1994) and Fan and Sauer (1995). An additional factor may be the use of a single digestibility marker, chromic oxide (Cr_2O_3), in combination with spot-sampling of digesta from a simple T-cannula (e.g., Köhler et al., 1990). Previous studies have shown that the use of a single marker can result in misleading ileal nutrient digestibility values in diets high in fibre content when ileal digesta samples are obtained through a simple T-cannula (e.g., Köhler et al., 1990). Therefore, it is important to clarify what the aforementioned factors are potentially causing the large differences in the apparent ileal CP and AA digestibility values among barley samples for growing-finishing pigs.

The objectives of this study were to determine if the use of different digestibility markers as well as differences in NDF contents and dietary CP and AA levels were responsible for the larger variability of the apparent ileal CP and AA digestibility values among barley samples for growing-finishing pigs.

MATERIAL AND METHODS

Animal trial procedures

Six barrows (Lacombe x Yorkshire), with an average initial body weight of 30.5 kg, were surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to De Lange et al. (1989). After surgery, the barrows were housed individually in stainless-steel metabolic crates in a temperature-controlled barn (20-22°C). During a 14-d recovery period, the barrows were fed a 16% CP grower diet (Sauer et al., 1983). Following recovery, the barrows were fed one of the six experimental diets for six experimental periods according to a 6 × 6 Latin square design. The barrows were fed twice daily, at 08.00 and 20.00 h. Feed intake was 1,600 g/d during the period 1 and increased by 160 g/d consecutively for the other five periods. Water was freely available from a low-pressure drinking nipple. At the conclusion of the experiment, the barrows, with an average final body weight of 58.6 kg, were electrically stunned before killing, bled out and dissected to determine whether cannulation had caused intestinal abnormalities.

Six barley samples selected for these studies were obtained from three barley cultivars and three different sources, namely, c.v. Hurrington, c.v. Bonanza and c.v. Argyle from Saskatchewan Wheat Pool; c.v. Bonanza and c.v. Hurrington from Alberta Wheat Pool; and c.v. Hurrington from Alberta United Grain Growers. The six barley-based diets contained 97% of the respective barley samples that provided the sole dietary CP and AA (Table 1). Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide (0.3%) and acid-insoluble ash (AIA) were used as the digestibility markers. All the ingredients were ground through a 4.8 mm mesh screen prior to diet incorporation.

Each experimental period comprised 7 d. Ileal digesta were collected for a total of 24 h: from 08.00 to 10.00 h on d 6 and every other 2 h thereafter until 08.00 h on d 7 and from 10.00 to 12.00 h on d 7 and every other 2 h thereafter until 08.00 h on d 8. Ileal digesta were collected in soft plastic tubing (length 15 cm, i.d. 2.5 cm) which was attached to the barrel of the cannula with Velcro tape. The tubing contained 10 mL of a solution of formic acid (10%, vol/vol) to minimize further microbial activity. The tubing was removed and replaced as soon as it was partially filled with digesta. Digesta were immediately frozen at -20°C following collection.

The experimental proposal, surgical procedures, and care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture and Forestry Animal Care Committee of the University of Alberta. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1980).

TABLE 1

Formulation of the experimental diets, %

Ingredients	Diets B1-B6
Barley samples[1]	97.0
Calcium carbonate	1.1
Dicalcium phosphate	0.8
Trace-mineralized salt[2]	0.5
Vitamin premix[3]	0.2
Mineral premix[4]	0.1
Chromic oxide[5]	0.3

[1] six barley samples: B1, c.v. Hurrington, B2, c.v. Bonanza and B3, c.v. Argyle from Saskatchewan Wheat Pool; B4, c.v. Bonanza and B6, c.v. Hurrington from Alberta Wheat Pool; B5, c.v. Hurrington from Alberta United Grain Growers

[2] supplied by Windsor Salt, Toronto, ON. Composition (%): NaCl, 96.5; ZnO, 0.40; $FeCO_3$, 0.16; MnO, 0.12; CuO, 0.033; $Ca(IO_3)_2$, 0.007; and CaO, 0.004

[3] the vitamin premix supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2 mg; cholecalciferol, 0.38 mg; all-rac-α-tocopherol acetate, 44.0 mg; menadione, 3.0 mg; riboflavin, 2.2 mg; niacin, 12.0 mg; d-pantothenic acid, 11.0 mg; vitamin B_{12}, 0.012 mg; choline, 550 mg; thiamine, 1.1 mg; pyridoxine, 1.1 mg; d-biotin 0.1 mg and folic acid, 0.6 mg

[4] the trace-mineral premix supplied the following minerals (mg/kg diet): $FeSO_4 \cdot H_2O$ 152 mg; $ZnCO_3$, 95.9 mg; $MnSO_4 \cdot H_2O$, 6.2 mg; $CuSO_4 \cdot 5H_2O$, 11.8 mg; KI, 0.6 mg; and Na_2SeO_3, 0.3 mg

[5] fisher Scientific, Fair Lawn, NJ

Chemical analyses

At the end of the animal trial, the digesta samples were freeze-dried, pooled within barrow and period for the same dietary treatment, ground through a 0.8-mm mesh screen and mixed before analyses. The samples of ingredients and diets were ground similarly.

Analyses for dry matter, CP and ether extract were carried out according to AOAC (1990) methods. Analysis for NDF was carried out according to principles outlined by Goering and Van Soest (1970). Chromic oxide was determined according to the procedure of Fenton and Fenton (1979) and AIA was measured according to McCarthy et al. (1974) and Van Keulen and Young (1977).

For AA analyses, with the exception of sulphur-containing AA and tryptophan, the samples were hydrolysed with 6 *M* HCl at 110°C for 24 h, derivatized as ninhydrin-positive compounds, and detected colorimetrically according to HPLC-procedures adapted from Mason et al. (1980) using an automatic AA analyzer (Beckman model 6300, Beckman Inst., Inc., Palo Alto, CA). Methionine and cysteine were determined as methionine sulphone and cysteic acid after oxidation with performic acid; the oxidation process was carried out according to AOAC (1990). The oxidized samples were then hydrolysed and analysed in the same manner as the samples that were not oxidized. All samples which received tryptophan analysis were treated separately and analysed according to the procedures of Jones et al. (1981).

Calculations and statistical analyses

The apparent ileal dry matter digestibility values in the six experimental diets and the apparent ileal digestibility values of CP and AA in the six barley samples were calculated according to equation:

$$D_D = 100\% - [(I_D \times A_F) / (I_F \times A_D)] \times 100\%$$

Where D_D is nutrient digestibility in the experimental diets (%); I_D is marker concentration in the diets (%, on as-fed basis); A_F is nutrient concentration in ileal digesta (%, on as-fed basis); I_F is marker concentration in ileal digesta (%, on as-fed basis); and A_D is nutrient concentration in the diets (%, on as-fed basis).

The digestibility values were subjected to ANOVA for a 6 × 6 Latin square design. Periods and barrows were the controlled factors in the design. Where appropriate, treatment means were compared using the Student-Newman Keuls' multiple-range test. The linear relationships between total CP and AA contents and the apparent ileal digestibility values in the barley samples were analysed. The ANOVA, the multiple comparisons, and the linear regression analyses were carried out using the GLM Procedures of SAS (1990) according to the principles described by Steel and Torrie (1980). Pearson partial correlation analyses were conducted to determine the relationships between apparent ileal digestibility values and dietary levels of CP, AA, and NDF content using the GLM procedure of SAS (1990). Variation contributed by barrows and periods was removed by creating five dummy variables for barrows and periods, respectively, and obtaining partial correlation coefficients when the dummy variables were forced into analyses (Draper and Smith, 1981). Comparison between the digestibility values, as measured by the Cr_2O_3 and AIA, was conducted by the pooled t-test (Byrkit, 1987).

RESULTS AND DISCUSSION

The barrows remained healthy and consumed their meal allowances throughout the experiment. Post-mortem examinations, carried out at the conclusion of the experiment, revealed no intestinal adhesions.

The contents of dry matter, ether extract, NDF, CP, and AA are shown in Table 2. The contents of dry matter and ether extract were relatively constant among the barley samples. However, there were large variations in the contents of NDF, CP, and AA. The NDF content ranged from 20.6 to 25.4%. The CP content ranged

TABLE 2

Chemical composition of the barley samples, % DM basis

Items	Barley samples[1]					
	B1	B2	B3	B4	B5	B6
Dry matter	91.2	90.9	91.1	89.9	91.1	90.3
Ether extract	1.5	1.3	1.2	1.3	1.4	1.5
Neutral-detergent fibre	23.6	21.9	25.4	20.6	25.4	24.4
Crude protein	8.7	12.3	9.7	16.3	10.2	13.2
Amino acids						
Indispensable						
Arg	0.42	0.88	0.42	1.26	0.93	1.15
His	0.24	0.28	0.23	0.37	0.22	0.32
Iso	0.31	0.39	0.31	0.51	0.33	0.43
Leu	0.64	0.79	0.63	1.02	0.69	0.90
Lys	0.36	0.42	0.36	0.50	0.36	0.44
Met	0.19	0.21	0.19	0.27	0.20	0.24
Phe	0.34	0.48	0.34	0.70	0.43	0.54
Thr	0.37	0.43	0.35	0.54	0.38	0.45
Trp	0.10	0.13	0.11	0.20	0.13	0.16
Val	0.47	0.58	0.48	0.75	0.49	0.61
Dispensable						
Ala	0.35	0.42	0.35	0.53	0.36	0.41
Asp	0.57	0.69	0.58	0.87	0.59	0.69
Cys	0.31	0.36	0.31	0.44	0.27	0.41
Glu	1.83	2.61	1.81	3.74	2.17	3.03
Gly	0.36	0.43	0.36	0.54	0.38	0.45
Pro	0.79	1.12	0.75	1.61	0.97	1.31
Ser	0.38	0.46	0.37	0.62	0.40	0.51
Tyr	0.12	0.15	0.12	0.23	0.15	0.16

[1] refer to footnote 1 for Table 1

from 8.7 to 16.3%. Furthermore, the variability in all AA contents was closely associated with the differences in CP content among the barley samples. Studies by Froseth and Miller (1992), with 556 barley samples, shown that barley varied greatly in AA, and NDF contents, which were mainly resulted from variety differences, the amount of fertilizer used, and environmental conditions; the latter factor also included regional differences and year of production. Furthermore, Wyatt (1993) in studies with 177 barley samples from American Pacific Northwestern region, showed a strong linear relationship between the content of CP and each of the indispensable AA. Although a smaller number of samples were involved in this study, we also observed linear relationship ($P < 0.05$) between the content of each individual AA and CP content, consistent with the aforementioned studies (Table 3).

TABLE 3

Linear relationships between amino acid (y) and crude protein (x) contents in the barley samples[1]

Items	Regression equations[2, 3]	r^2	S_{yx} [3]	*P*[4]	*P*[5]
Indispensable					
Arg	NS				
His	y = 0.0201x	0.91	0.0194	0.3334	0.0029
Iso	y = 0.0283x	0.98	0.0114	0.0914	0.0001
Leu	y = 0.0549x	0.97	0.0330	0.1000	0.0005
Lys	y = 0.1691 + 0.0203x	0.97	0.0108	0.0012	0.0003
Met	y = 0.0863 + 0.0111x	0.94	0.0085	0.0062	0.0012
Phe	y = 0.0481x	0.97	0.0264	0.1430	0.0003
Thr	y = 0.1335 + 0.0244x	0.96	0.0161	0.0123	0.0007
Trp	y = 0.0127x	0.94	0.0101	0.6274	0.0014
Val	y = 0.1140 + 0.0383x	0.99	0.0147	0.0155	0.0001
Dispensable					
Ala	y = 0.1222 + 0.0240x	0.94	0.0189	0.0280	0.0014
Asp	y = 0.0128 + 0.0005x	0.97	0.0237	0.0128	0.0005
Cys	y = 0.0213x	0.83	0.0304	0.1596	0.0120
Glu	y = 0.2674x	0.98	0.1251	0.0661	0.0002
Gly	y = 0.1307 + 0.0247x	0.99	0.0096	0.0021	0.0001
Pro	y = 0.1152x	0.96	0.0721	0.1353	0.0006
Ser	y = 0.0339x	0.97	0.0178	0.1550	0.0003
Tyr	NS				

[1] refer to Table 1 footnote '1' for the information on barley samples

[2] y = amino acid content in the barley samples (%, on dry matter basis); x = crude protein content in the barley samples (%, on dry matter basis)

[3] the regression equations were considered significant when slopes were different from zero ($P < 0.05$, n=6)

[4] standard error of estimates of the regression equations (n=6)

[5] the probability of significance for the intercepts of the regression equations

[6] the probability of significance for the slopes of the regression equations (n=6)

As the diets were formulated to include the same level (97%) of barley samples, large differences in the dietary contents of CP and AA were resulted from the different contents of these nutrients in the corresponding barley samples (Table 4). Furthermore, the directly determined contents of CP and AA in the diets were close to the calculated values based on the analysed contents in the barley samples. The contents of ether extract and NDF in the diets were calculated from the analysed values in the barley samples (Table 4).

The apparent ileal digestibility values of dry matter, CP and AA in barley samples are presented in Tables 5 and 6. There were differences ($P < 0.05$) in the apparent ileal digestibility values of dry matter among the barley diets (B5 and B6), ranging from 44.4 to 56.3% determined by using Cr_2O_3 as a digestibility

TABLE 4

Chemical composition of the experimental diets, % DM basis

Items	Diets[1]					
	B1	B2	B3	B4	B5	B6
Dry matter	94.8	94.5	94.5	93.3	94.2	93.9
Ether extract	1.5	1.2	1.1	1.3	1.3	1.5
Neutral-detergent fibre	22.0	20.5	23.8	16.8	23.8	22.7
Crude protein	8.5	11.5	9.2	15.6	9.8	12.6
Amino acids						
Indispensable						
Arg	0.38	0.85	0.39	1.24	0.64	1.09
His	0.23	0.25	0.21	0.33	0.20	0.28
Iso	0.30	0.37	0.30	0.50	0.31	0.41
Leu	0.62	0.76	0.61	1.01	0.66	0.87
Lys	0.35	0.39	0.35	0.48	0.34	0.42
Met	0.18	0.20	0.18	0.27	0.19	0.23
Phe	0.33	0.49	0.35	0.71	0.41	0.57
Thr	0.36	0.41	0.34	0.54	0.36	0.44
Trp	0.10	0.15	0.11	0.19	0.12	0.16
Val	0.46	0.56	0.47	0.73	0.48	0.61
Dispensable						
Ala	0.34	0.40	0.34	0.52	0.34	0.41
Asp	0.55	0.66	0.57	0.85	0.57	0.67
Cys	0.30	0.35	0.27	0.42	0.26	0.40
Glu	1.78	2.54	1.75	3.72	2.09	2.99
Gly	0.35	0.41	0.35	0.53	0.36	0.45
Pro	0.77	1.09	0.72	1.62	0.94	1.31
Ser	0.37	0.45	0.37	0.61	0.39	0.50
Tyr	0.12	0.17	0.16	0.30	0.18	0.24

[1] refer to footnote 1 for Table 1 for information on the barley samples

marker (Table 5). However, there were no differences ($P > 0.05$) in the apparent ileal digestibility values of dry matter among the barley diets when these were determined by using the AIA as a digestibility marker (Table 5), probably due to relatively large variation associated with the measurements.

There were differences ($P<0.05$) in the apparent ileal digestibility values of CP and all the AA among the barley samples. For CP, the difference was 28.7 and 22.9% for Cr_2O_3 and AIA methods, respectively. Of the indispensable AA, the differences ranged from 16.7% (histidine) to 55.9% (arginine) percentage units, when the digestibility values were measured with Cr_2O_3. Whereas the differences ranged from 12.3% (histidine) to 33.4% (tryptophan), when measured with AIA. Of the dispensable AA, the differences ranged from 15.2% (cysteine) to 55.4% (tyrosine) when the digestibility values were determined with Cr_2O_3, however,

TABLE 5

Comparison of variability of apparent ileal digestibility values of crude protein (CP) and amino acids (AA) in the barley samples determined by chromic oxide (Cr_2O_3) and the acid-insoluble ash (AIA) as digestibility markers. Part 1. Indispensable amino acids, %

Items	Barley samples[1]						
	B1	B2	B3	B4	B5	B6	SEM[2]
Dry matter[3]							
Cr_2O_3	52.0[ab]	52.3[ab]	44.4[c]	56.3[a]	48.4[bc]	47.9[bc]	1.67
AIA	54.3	54.2	57.4	53.7	56.6	54.9	2.80
Difference	2.3	1.9	13.0[W]	2.6	8.2[W]	7.0	3.26
CP							
Cr_2O_3	26.8[d]	49.2[ab]	34.6[c]	55.5[a]	42.5[b]	45.0[b]	2.64
AIA	30.6[b]	51.6[a]	49.8[a]	53.1[a]	51.6[a]	52.1[a]	3.78
Difference	3.8	2.4	15.2[W]	2.4	9.1	7.1	4.61
Indispensable							
Arg							
Cr_2O_3	12.4[c]	57.7[ab]	18.4c	68.3[a]	35.3[bc]	61.5[ab]	8.14
AIA	17.3[b]	59.4[a]	35.1[ab]	65.4[a]	45.1[ab]	66.9[a]	8.81
Difference	4.9	1.7	16.7	2.9	9.8	5.4	11.99
His							
Cr_2O_3	50.6[b]	59.2[a]	51.1[b]	66.4[a]	49.7[b]	60.8[a]	2.24
AIA	53.1	60.8	62.9	64.1	57.8	65.4	2.93
Difference	2.5	1.6	11.8[W]	2.3	8.1	4.6	3.69
Iso							
Cr_2O_3	32.5[d]	52.1[b]	40.8[c]	61.2[a]	44.7[cd]	51.1[b]	2.48
AIA	36.0[b]	54.3[a]	54.7[c]	58.8[a]	53.5[a]	57.0[a]	2.86
Difference	3.5	2.2	13.9[W]	2.4	8.8[W]	5.9	3.79
Leu							
Cr_2O_3	37.5[d]	55.1[b]	44.8[c]	64.4[a]	49.1[ab]	55.6[b]	2.28
AIA	41.0[b]	57.0[a]	57.9[a]	62.0[a]	57.2[a]	61.0[a]	2.81
Difference	3.5	1.9	13.1[W]	2.4	8.1[W]	5.4	3.62
Lys							
Cr_2O_3	26.4[b]	38.6[ab]	30.0[b]	47.5[a]	32.2[b]	39.7[ab]	3.26
AIA	29.9	41.0	46.5	44.2	43.0	46.2	4.36
Difference	3.5	2.4	16.5[W]	3.3	10.8	6.5	5.44
Met							
Cr_2O_3	42.8[c]	53.5[b]	51.4[b]	65.0[a]	51.3[b]	56.5[b]	2.27
AIA	45.8[b]	55.5[a]	62.8[a]	62.6[a]	59.0[a]	61.6[a]	3.08
Difference	3.0	2.0	11.4[W]	2.4	7.7	5.1	3.83

TABLE 5

Continued

Items	Barley samples[1]						
	B1	B2	B3	B4	B5	B6	SEM[2]
Phe							
Cr_2O_3	32.8[d]	61.7[ab]	49.0[c]	67.8[a]	51.6[bc]	61.3[ab]	2.98
AIA	36.9[b]	63.1[a]	61.1[a]	65.5[a]	59.3[a]	66.4[a]	2.98
Difference	4.1	1.4	12.1[W]	2.3	7.7	5.1	4.21
Thr							
Cr_2O_3	26.9[b]	37.5[b]	25.0[b]	50.5[a]	28.8[b]	36.4[b]	3.42
AIA	30.6	40.2	42.9	46.9	40.1	43.6	4.26
Difference	3.7	2.7	17.9[W]	3.6	11.3	7.2	5.46
Trp							
Cr_2O_3	19.2[c]	47.6[a]	30.0[b]	53.5[a]	37.5[b]	50.2[a]	2.82
AIA	23.0[b]	49.6[a]	45.8[a]	50.1[a]	47.3[a]	56.4[a]	4.90
Difference	3.8	2.0	15.8[W]	3.4	9.8	6.2	5.65
Val							
Cr_2O_3	41.0[d]	54.3[b]	46.8[c]	64.1[a]	48.2[c]	55.4[b]	1.93
AIA	44.0[b]	57.9[a]	59.4[a]	61.7[a]	56.4[a]	60.6[a]	2.82
Difference	3.0	3.6	12.6[W]	2.4	8.2[W]	5.2	3.42

[1] refer to footnote 1 to Table 1 for information on the barley samples

[2] standard error of the mean for the Cr_2O_3 and AIA methods, respectively (n=6). Pooled standard error for the differences between the Cr_2O_3 and the AIA methods (n=12), calculated according to Byrkit (1987)

[3] digestibility values of the experimental diets

[w] significant differences between the Cr_2O_3 and the AIA methods ($P < 0.05$, n=12)

[a-d] means within the same row followed with different letters differ ($P < 0.05$)

the differences ranged from 15.2 (cysteine) to 60.3% (proline), as determined by using AIA.

As presented in Tables 5 and 6, there were generally large experimental errors associated with the determination of apparent ileal digestibility values of CP and AA in barley samples, when measured by using Cr_2O_3 and AIA as digestibility markers. This large variation can be reflected by large values of the pooled standard errors of means, especially when the values were measured with AIA (Tables 5 and 6). It is obvious that the use of digestibility markers, i.e. Cr_2O_3 vs AIA, was not a major factor responsible for such large variability in the apparent ileal CP and AA digestibility values. Furthermore, it is noteworthy that except diets 3 and 5, there were generally no differences ($P > 0.05$) between the two digestibility marker methods in the determination of apparent ileal CP and AA digestibility values (Tables 5 and 6). The use of different digestibility markers had effects ($P < 0.05$) on the measurement of dry matter, CP and some AA digestibility values

TABLE 6

Comparison of variability of apparent ileal digestibility values (%) of crude protein (CP) and amino acids (AA) in the barley samples determined by chromic oxide (Cr_2O_3) and the acid-insoluble ash (AIA) as digestibility markers. Part 2. Dispensable amino acids, %

Items	Barley samples[1]						
	B1	B2	B3	B4	B5	B6	SEM[2]
Dispensable AA							
Ala							
Cr_2O_3	15.3[d]	32.7[b]	19.6[cd]	47.7[a]	21.9[bcd]	31.2[bc]	3.30
AIA	19.4[b]	35.5[a]	38.7[a]	44.5[a]	34.4[a]	38.6[a]	4.45
Difference	4.1	2.8	19.1[W]	3.2	12.5[W]	7.4	5.54
Asp							
Cr_2O_3	22.3[e]	39.4[cd]	28.9[de]	49.8[c]	32.0[de]	38.5[cd]	3.19
AIA	26.0[b]	41.9[a]	45.9[a]	46.7[a]	42.8[a]	45.2[a]	3.92
Difference	3.7	2.5	17.0[W]	3.1	10.8	6.7	5.05
Cys							
Cr_2O_3	58.6[d]	67.8[c]	60.3[d]	71.4[c]	56.2[d]	68.7[c]	2.28
AIA	60.6[bc]	67.8[ab]	60.3[bc]	71.4[a]	56.2[c]	68.7[ab]	2.16
Difference	2.0	0.0	0.0	0.0	0.0	0.0	3.14
Difference	3.2	1.5	17.2[W]	1.7	6.3	4.0	3.96
Gly							
Cr_2O_3	-1.1[e]	16.9[d]	1.8[de]	35.2[c]	1.5[de]	16.4[d]	4.18
AIA	-3.6[b]	20.0[ab]	22.7[ab]	31.1[a]	12.7[ab]	26.0[ab]	7.62
Difference	2.5	3.1	20.9[W]	4.1	11.2	9.6	8.69
Pro							
Cr_2O_3	40.0[d]	61.7[c]	34.8[d]	64.0[c]	40.3[d]	49.5[d]	4.03
AIA	2.3[b]	51.5[a]	46.4[a]	62.6[a]	33.1[ab]	55.4[a]	12.25
Difference	37.7	10.2	11.6	1.4	7.2	5.9	12.90
Ser							
Cr_2O_3	29.0[e]	47.0[d]	34.7[e]	57.0[c]	36.5[e]	46.2[d]	2.62
AIA	32.6[b]	49.4[a]	50.2[a]	54.1[a]	46.5[a]	52.4[a]	3.58
Difference	3.6	2.4	15.5[W]	2.9	10.0[W]	6.2	4.44
Tyr							
Cr_2O_3	1.1[e]	23.3[d]	31.5[d]	56.5[c]	25.7[d]	42.6[cd]	5.37
AIA	6.2[c]	26.8[b]	47.8[a]	53.3[a]	37.5[ab]	49.7[a]	5.55
Difference	5.1	3.5	16.3	3.2	11.8	7.1	7.72

[1] refer to footnote 1 to Table 1 for information on the barley samples

[2] standard error of the mean for the Cr_2O_3 and AIA methods, respectively (n=6). Pooled standard error for the differences between the Cr_2O_3 and the AIA methods (n=12), calculated according to Byrkit (1987)

[w] significant differences between the Cr_2O_3 and the AIA methods ($P < 0.05$, n=10)

[a-d] means within the same row followed with different letters differ ($P < 0.05$)

in diets 3 and 5, possibly due to relatively higher NDF contents in the corresponding barley samples and diets (Tables 2 and 4). Consistent with our study, studies by Van Leeuwen et al. (1996) observed no differences ($P > 0.05$) in the apparent ileal nutrient digestibility values between the Cr_2O_3 and the AIA methods. Thus, it can be concluded that the use of digestibility markers of Cr_2O_3 and AIA has little effects on the determination of apparent ileal nutrient digestibility values in barley-based diets. The use of different digestibility markers, e.g., Cr_2O_3 and AIA, and a possible separation of the distal ileal digesta into liquid and solid phases are not responsible for the large variation in the apparent ileal CP and AA digestibility values among the barley samples.

Pearson partial correlation analysis was conducted to examine if differences in NDF contents among the barley samples and diets were, in part, responsible for the large variation in apparent ileal digestibility values of CP and AA among the barley samples. The apparent ileal digestibility values of CP and AA were not correlated ($P > 0.05$) with NDF content in barley samples. These results suggest that differences in NDF content were not responsible for the variation in the apparent ileal CP and AA digestibility values among barley samples. This observation was contrary to previous studies reported for peas (Gdala et al., 1992; Fan and Sauer, 1999), canola meal samples (Fan et al., 1996) and wheat samples (Taverner et al., 1981; Fan et al., 2001). Thus, it can be concluded that differences in NDF content were not responsible for the variability of apparent ileal CP and AA digestibility values among the barley samples.

There were positive linear relationships ($P < 0.05$) between the apparent ileal digestibility values of CP and AA and contents of CP and AA (Tables 7 and 8). This suggests that a large proportion of the variation in the apparent ileal digestibility values be correlated with the differences in CP and AA contents of the barley samples or their diets. In the studies by Buraczewska et al. (1987), with barley differing in CP content, significant positive correlation was observed between the CP content and the apparent ileal digestibility values of AA. As shown by Fan et al. (1994), there were quadratic with plateau relationships between the apparent ileal digestibility values of CP and AA and their dietary contents. Before the plateau values were reached, the apparent ileal digestibility values of CP and AA were quadratically influenced by their dietary levels. As discussed by these authors (Fan et al., 1994), the effect of dietary levels of CP and AA on their apparent ileal digestibility values was of methodological nature and should be controlled in order to examine any possible effects of inherent factors. Therefore, the large variation in the apparent ileal digestibility values of CP and AA among the barley samples was largely attributed to the effect of large differences in the dietary levels of CP and AA among the barley diets.

The apparent ileal digestibility values of CP and AA in the barley samples determined in the present study were generally lower and more variable than those

TABLE 7

The linear relationships between the apparent ileal digestibility values and the contents of crude protein (CP) and amino acids (AA) in the barley samples[1] using chromic oxide as a digestibility marker

Items	Regression equations[2]	r^2	S_{yx}[3]	*P*[4]	*P*[5]
CP	y = 3.34x	0.40	10.84	0.71	0.01
Indispensable					
Arg	y = 33.50x	0.40	20.08	0.34	0.03
His	y = 24.73 + 114.13x	0.35	8.68	0.01	0.01
Iso	y = 115.27x	0.45	9.54	0.70	0.01
Leu	y = 55.55x	0.42	9.64	0.39	0.01
Lys	y = 129.62x	0.33	9.93	0.20	0.01
Met	y = 207.31	0.35	8.55	0.43	0.01
Phe	y = 17.32 +77.79x	0.39	12.40	0.04	0.01
Thr	y = 134.5x	0.40	10.86	0.07	0.01
Trp	y = 313.32x	0.51	10.61	0.63	0.01
Val	y = 71.98x	0.42	8.61	0.19	0.01
Dispensable					
Ala	y = -38.95 + 166.15x	0.41	12.84	0.01	0.01
Asp	y = 79.39x	0.44	9.68	0.10	0.01
Cys	y = 399.13x	0.58	8.56	0.20	0.03
Glu	y = 30.61 + 12.55x	0.39	11.12	0.01	0.01
Gly	y = -71.22 + 197.60x	0.36	17.23	0.01	0.01
Pro	y = 31.30x	0.23	17.71	0.21	0.01
Ser	y = 100.57x	0.43	10.52	0.66	0.01
Tyr	y = -28.13 + 375.67x	0.36	18.95	0.05	0.01

[1] refer to footnote 1 to Table 1 for the information of the barley samples
[2] y = apparent ileal CP and AA digestibility values in the barley samples (%),
x = CP and AA contents in the barley samples (%, on dry matter basis)
[3] standard error of estimate of the regression equations (n=36)
[4] the probability of significance for the intercepts of the regression equations (n=36)
[5] the probability of significance for the slopes of the regression equations (n=36)

reported previously (Sauer et al., 1977, 1981) and those compiled by Sauer and Ozimek (1986) and Knabe (1991). For an example, the apparent ileal digestibility values of lysine and threonine ranged from 26.4 to 47.5% and 25.0 to 50.5%, respectively, in these studies. The lysine and threonine digestibility values from the studies compiled by Sauer and Ozimek (1986) ranged from 64.9 to 79.0% and 64.4 to 76.0%, respectively. However, three other studies also showed relatively low apparent ileal digestibility values of lysine and threonine in barley samples (Imbeah et al., 1988; Furuya and Kaji, 1991; Fan et al., 1993). The apparent ileal lysine digestibility values were 53.3, 55.0 and 37.6%, respectively, whereas threonine digestibility values were 51.5, 51.0 and 44.2%, respectively, in these studies (Imbeah et al., 1988; Furuya and Kaji, 1991; Fan et al., 1993). The barley samples used in the studies by Imbeah et al. (1988) ranged from 9.5 to 10.0% in CP content. The more variable apparent ileal CP and AA digestibility values observed in

TABLE 8

The linear relationships between the apparent ileal digestibility values and the contents of CP and AA in the barley samples[1] using the acid-insoluble ash as a digestibility marker

Items	Regression equations[2]	r^2	S_{yx}[3]	*P*[4]	*P*[5]
CP	y = 9.02x	0.52	15.41	0.18	0.04
Indispensable					
Arg	y = 146.26 - 148.79x	0.51	29.44	0.03	0.05
His	y = 469.83x	0.49	9.65	0.26	0.05
Iso	y = 382.73x	0.60	12.43	0.09	0.02
Leu	y = 195.37x	0.53	13.86	0.14	0.04
Lys	y = 360.72x	0.45	11.89	0.14	0.07
Met	y = 1013.75x	0.43	11.71	0.16	0.08
Phe	y = 281.33x	0.53	18.63	0.18	0.04
Thr	y = 416.31x	0.45	13.70	0.13	0.08
Trp	y = -104.61 +1238.06x	0.66	13.20	0.04	0.01
Val	y = 237.85x	0.59	10.97	0.13	0.03
Dispensable					
Ala	y = 479.43x	0.55	15.05	0.06	0.03
Asp	y = 234.01x	0.58	11.96	0.06	0.03
Cys	y = 31.67 + 91.92x	0.20	11.18	0.01	0.01
Glu	y = 44.02x	0.60	13.91	0.27	0.02
Gly	y = -211.02 + 583.05x	0.60	16.65	0.03	0.02
Pro	y = -57.61 + 123.61x	0.79	10.42	0.05	0.01
Ser	y = 392.44x	0.57	13.65	0.08	0.03
Tyr	y = 1427.78x	0.40	26.27	0.11	0.09

[1] refer to footnote 1 to Table 1 for the information of the barley samples

[2] y = apparent ileal CP and AA digestibility values in the barley. The linear regression equation was not significant (P > 0.05, n=36)

[3] standard error of estimate of the regression equation

[4] the probability of significance for the intercepts of the regression equations (n=36)

[5] the probability of significance for the slopes of the regression equations (n=36)

this study in comparison with the literature results were largely due to relatively more variable CP and AA contents among the barley samples and the diets in this study. As discussed previously, the apparent ileal digestibility values of CP and AA are quadratically related to AA contents in the assay diets and the lower the AA contents are the lower the apparent ileal AA digestibility values are. Furthermore, finesse of grinding might be also responsible for the relatively low apparent ileal CP and AA digestibility values as originally demonstrated by Sauer et al. (1977) in studies with wheat. Studies by Wünsche et al. (1987) showed large differences between the apparent ileal AA digestibility values in pigs fed coarsely, medium and finely ground barley: 43.6, 54.2 and 62.0%, respectively. Barley samples used in this study and the study by Imbeah et al. (1988) were very coarsely ground, and this was again likely, in part, responsible for the relatively low apparent ileal CP and AA digestibility values observed in these two studies. Thus, relatively low CP and AA contents and coarse grinding were responsible for relatively low apparent ileal CP and AA digestibility values in the barley samples measured in this study.

As can be further implicated from this study that apparent ileal CP and AA digestibility values determined from this study and those reported in the literature are not reliable for the formulation of pig diets due to their large variability and underestimation as affected by the methodological limitations. One alternative approach is to calculate the standardized ileal digestibility of amino acids (SIDAA) as suggested by Rademacher et al. (1999) by using a basal level of the ileal endogenous CP and AA values reported in the literature. However, one should be very cautious by taking this approach, as the ileal endogenous CP and AA values can be dramatically affected by ingredient types. A recent study by Fan and Sauer (2002) has demonstrated that the ileal endogenous CP and AA values associated with barley samples are considerably higher than the average ileal endogenous CP and AA values reported in the literature. Therefore, true ileal CP and AA digestibility values directly determined from barley samples, as reported by Fan and Sauer (2002), should be used in diet formulation for pigs.

In summary, there was large variation in the apparent ileal CP and AA digestibility values among the barley samples differing in CP and AA contents. The use of digestibility markers (Cr_2O_3 and AIA) and differences in NDF contents were not responsible for such variability. Differences in CP and AA contents between the diets were primarily responsible for the large variability of apparent CP and AA digestibility values measured among barley samples. The apparent ileal CP and AA digestibility values, which are generally variable and underestimate the true digestive utilization of AA in barley, should not be used in diet formulation for pigs. True ileal CP and AA digestibility values directly determined from barley samples should be used in diet formulation for pigs.

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STRESZCZENIE

Oznaczenie zmienności w wartościach pozornej strawności jelitowej aminokwasów różnych prób jęczmienia u rosnących świń przy zastosowaniu dwóch wskaźników

Celem badań było ustalenie, czy użycie dwóch wskaźników, a także różnice w zawartości NDF i białka ogólnego (CP) oraz poziom aminokwasów (AA) w dawkach są odpowiedzialne za dużą zmienność w wartościach współczynników pozornej strawności jelitowej AA w różnych próbach jęczmienia stosowanych w żywieniu rosnących świń. Sześć wieprzków, o początkowej i końcowej wadze 30,5 i 58,6 kg, odpowiednio, z prostymi T-przetokami w końcowym odcinku jelita biodrowego, w układzie kwadratu łacińskiego (6×6), żywiono sześcioma dietami zawierającymi jęczmień (97%) i różniącymi się zawartością białka ogólnego i AA. Jako wskaźniki strawności stosowano tlenek chromu (Cr_2O_3) i popiół nierozpuszczalny w kwasie (AIA). Każdy okres doświadczalny składał się z 7 dni; treść jelitową pobierano co 2 godziny przez 24 godziny.

Stwierdzono istotne różnice ($P < 0,05$) w pozornej jelitowej strawności białka i AA pomiędzy próbami jęczmienia, przy użyciu tak Cr_2O_3 jak i AIA. Zastosowanie Cr_2O_3 i AIA oraz różnice w zawartości NDF nie miały wpływu ($P > 0,0$) na oznaczaną pozorną jelitową strawność białka ogólnego i AA w większości diet. Duże różnice ($P < 0,05$) w strawności białka i AA pomiędzy próbami jęczmienia spowodowane były głównie różnicami w zawartości tych składników w dawkach. Oznaczone wartości współczynników pozornej jelitowej strawności białka ogólnego i AA jęczmienia winny być traktowane z rezerwą i nie powinny być stosowane przy układaniu dawek dla świń; w takich przypadkach powinno się przyjmować bezpośrednio oznaczone współczynniki rzeczywistej jelitowej strawności tych składników.