Availability to pigs of amino acids in cereal grains

3. A comparison of ileal availability values with faecal, chemical and enzymic estimates

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1. Availability values for amino acids in nine cereal grains determined by faecal analyses with pigs and by the Silcock available lysine test (Roach *et al.* 1967) and an in vitro digestibility assay were compared with ileal availability values for the same grains determined with pigs by Taverner *et al.* (1981 *b*).

2. There was a significant canonical correlation of ileal and faecal availability values. On average, apparent faecal availability of the indispensable amino acids was 4·2% greater than apparent ileal availability, but the difference was up to 12·6% for threonine. Furthermore, the difference appeared to increase as the digestibility of the grain decreased.

3. Silcock available-lysine values for the cereal grains were unrelated to the lysine truly absorbed by the pig.

4. There were close relationships of ileal protein and lysine availability values on nitrogen digestibility determined by an in vitro assay using pronase enzyme.

Values for amino acid availability in grains have been determined by several different methods, often with different results. For example, Johnson *et al.* (1978) found that the availability of lysine in barley samples was greater when determined as chemically-reactive lysine (0·94) than when estimated as the true digestibility of lysine using faecal analysis in rats (0·77). Also, De Muelenaere *et al.* (1967 *a*, *b*) found that amino acid availability in cereals determined by growth assay was generally lower than that determined by faecal analysis. The discrepancy between these methods was attributed by Sauer *et al.* (1977) to the fact that dietary amino acids that remained undigested in the small intestine do not necessarily appear in the faeces, but can be degraded during fermentation in the hind gut. Thus, Sauer *et al.* (1977) found ileal availability values for amino acids in cereal grains to be generally lower than faecal availability values. Furthermore, the ileal recovery method appeared to be a more sensitive measure of amino acid availability in grains than faecal analysis.

In the present study the amino acid availability values determined by ileal analysis by Taverner *et al.* (1981 *b*) were used as a basis for evaluating other availability assays. Ileal values for five different samples of wheat and a sample each of barley, sorghum (*Sorghum vulgare* Pers.), maize and *Triticale* were compared with availability estimates determined on the same samples by faecal analysis, chemically-reactive lysine and an in vitro digestibility assay.

EXPERIMENTAL PROCEDURES

Faecal analysis

Faeces were collected for 3 d (days 9, 10, and 11) during each of the 12 d periods for which pigs were fed with each of the grain diets during which ileal digesta were also collected (Taverner *et al.* 1981 *b*). Total faecal output was collected thrice daily and stored at $-20°$ until the end of the collection period when all faeces were mixed and subsampled for

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analysis. Samples were freeze-dried and ground through a 1 mm screen before determination of nitrogen, chromic oxide and amino acids as described by Taverner *et al.* (1981 *a*).

Digestibility values were calculated by the relative concentrations of chromium in food and in faeces samples. Because of the difficulties in obtaining meaningful faecal values of endogenous amino acid output (Taverner *et al.* 1981 *a*), apparent rather than true digestibility values are presented.

Reactive lysine

The method used was that of Roach *et al.* (1967) in which the grains were first reacted with fluorodinitrobenzene (FDNB) and then subjected to acid-hydrolysis. The acid-hydrolysates were analysed for residual lysine by ion-exchange chromatography and FDNB-reactive lysine was calculated as total lysine minus residual lysine. The residual lysine content of each grain was determined at least in quadruplicate.

In vitro protein digestibility

Digestion with pronase was used to calculate the in vitro protein digestibility of each of the nine grains in this study.

Samples (1 g) of grain ground to pass through a 1 mm screen were placed in 50 ml round-bottom flasks to which was added 4 mg pronase (B grade; Calbiochem), 20 ml 0·04 M-borate buffer (pH 8·0) and three drops toluene. Maximum recovery of digested N appeared to occur after 16 h incubation at 27° (M. R. Taverner, unpublished results) in an oscillating, constant-temperature water-bath. These conditions were used for all subsequent incubations. At the end of the incubation period the undigested protein and peptides were precipitated with 10 ml sulphosalicylic acid (150 g/l) to bring the final concentration to approximately 30 g/l. The flasks were shaken for another 15–30 min and the digesta transferred to 50 ml volumetric flasks and made up to volume. Following centrifugation at 1000 ***g*** for 30 min two 10 ml portions of the supernatant fraction were taken for the determination of soluble N. Protein digestibility was determined as the ratio, soluble N : total grain N.

Statistical methods

The difference in faecal digestibility factors among the grains was tested for significance using the analyses described by Taverner *et al.* (1981 *b*).

The relationship between faecal and ileal digestibility was studied using canonical correlation analysis (Veldman, 1967; Cooley & Lohnes, 1971). This analysis was used to determine linear combinations or canonical scores of faecal digestibility factors (for example, N, lysine and threonine) that were maximally correlated with linear combinations of parameters of ileal digestibility. These correlations between canonical scores for ileal and faecal digestibility were tested by an approximate χ^2 test. When significant relationships were obtained the correlation of the canonical variates with the original variates were examined to determine the relative importance of each factor in the canonical score.

The Fortran programme CANONA (Veldman, 1967) permitted a total of twenty-one variables so that digestibilities values of N and all thirteen amino acids could not be included for both ileal and faecal analyses in one canonical analysis. Therefore, canonical correlations were determined between sets of all ileal values and each of two sub-sets of seven faecal values and also between sets of faecal values and each of two sub-sets of ileal values. Consideration of the correlation of the canonical scores with the original variables in each of the above analyses led to the choice of nine ileal variables and twelve faecal variables for an over-all canonical analysis.

RESULTS

Faecal digestibility values. Average values of apparent faecal digestibility of dry matter, N and amino acids in the five wheats and in sorghum, maize, barley and *Triticale* are presented in Tables 1, 2. Because of the difficulties encountered with blocked cannulas during the feeding of wheat no. 4, both ileal and faecal availability values for this wheat are probably unreliable and were usually omitted from the comparisons in this paper.

The average faecal availabilities of indispensable amino acids in wheats nos. 1, 5, 2 and 3 were 0·87, 0·84, 0·84 and 0·80 respectively. These values tended to be greater than those determined by ileal analysis but the same ranking of wheats was maintained. Faecal availability of threonine was considerably greater than ileal availability of threonine, as was also the case for glycine, aspartic acid and serine. However, methionine availability was less when determined by faecal analysis than by ileal analysis.

There were few differences in faecal availability of individual amino acids for sorghum, maize, and barley (average amino acid availability values 0·87, 0·86 and 0·86 respectively). There were greater increases in availability values between ileal and faecal analyses for barley and maize than for sorghum. There was also a comparatively large increase in the average availability value for amino acids in *Triticale* between ileal and faecal analysis.

The mean ($\pm$ SE) faecal availability of tryptophan in maize was $0·85 \pm 0·018$.

The important differences between ileal and faecal analysis in the relative availabilities of amino acids in each grain were the consistent decrease in the relative availability of threonine and the increase in the relative availability of methionine by ileal compared to faecal analysis.

Table 1. *Average apparent faecal digestibilities of dry matter and nitrogen, and apparent faecal amino acid availabilities in wheat samples fed to pigs*

	Wheat no.				Statistical significance of difference	Wheat no. 4		Wheat no. 5	
	1	2	3	SEM		Mean	SD	Mean	SD
Indispensable amino acids									
Arginine	$0·93^a$	$0·89^a$	$0·91^a$	0·009	NS	0·91	0·003	0·89	0·028
Isoleucine	$0·89^a$	$0·89^a$	$0·82^a$	0·014	NS	0·82	0·011	0·86	0·018
Leucine	$0·91^a$	$0·88^a$	$0·85^a$	0·009	*	0·86	0·003	0·89	0·020
Lysine	$0·79^a$	$0·75^a$	$0·71^a$	0·024	NS	0·79	0·017	0·76	0·058
Methionine	$0·85^a$	$0·86^a$	$0·78^a$	0·022	NS	0·79	0·073	0·82	0·020
Threonine	$0·86^a$	$0·85^a$	$0·79^a$	0·010	*	0·76	0·015	0·83	0·028
Valine	$0·84^a$	$0·81^a$	$0·76^a$	0·015	NS	0·82	0·018	0·86	0·020
Dispensable amino acids									
Alanine	$0·85^a$	$0·81^a$	$0·77^a$	0·017	NS	0·82	0·018	0·83	0·027
Aspartic acid	$0·82^a$	$0·78^a$	$0·70^b$	0·014	*	0·75	0·028	0·80	0·021
Glutamic acid	$0·97^a$	$0·96^a$	$0·93^b$	0·004	*	0·94	0·013	0·96	0·007
Glycine	$0·88^a$	$0·85^a$	$0·83^a$	0·010	NS	0·83	0·015	0·86	0·025
Proline	$0·97^a$	$0·96^a$	$0·94^a$	0·005	NS	0·96	0·017	0·94	0·011
Serine	$0·90^a$	$0·87^a$	$0·84^a$	0·012	NS	0·83	0·007	0·89	0·020
Mean	0·88	0·85	0·82			0·84		0·86	
N	$0·88^a$	$0·87^{ab}$	$0·83^b$	0·010	*	0·84	0·010	0·88	0·014
Dry matter	$0·86^a$	$0·86^a$	$0·86^a$	0·005	NS	0·82	0·013	0·86	0·009

NS, not significant.
a, b, Within rows, means followed by different superscripts were significantly different ($P < 0·05$).
* $P < 0·05$.

Table 2. *Average apparent faecal digestibility of dry matter and nitrogen, and apparent faecal availabilities of amino acids in sorghum* (Sorghum vulgare *Pers.*), *maize, barley and* Triticale *fed to pigs*

	Sorghum	Maize	Barley	SEM	Statistical significance of difference	*Triticale*	
						Mean	SD
Indispensable amino acids							
Arginine	0·89[ab]	0·91[a]	0·88[b]	0·002	**	0·93	0·007
Isoleucine	0·88[a]	0·86[a]	0·84[a]	0·010	NS	0·86	0·008
Leucine	0·92[a]	0·92[a]	0·87[b]	0·001	**	0·89	0·010
Lysine	0·76[a]	0·73[a]	0·82[a]	0·019	NS	0·84	0·012
Methionine	0·78[a]	0·81[b]	0·85[c]	0·006	**	0·87	0·009
Threonine	0·87[a]	0·85[a]	0·87[a]	0·008	NS	0·86	0·020
Valine	0·85[a]	0·83[a]	0·84[a]	0·010	NS	0·87	0·011
Dispensable amino acids							
Alanine	0·91[a]	0·90[a]	0·82[b]	0·012	*	0·86	0·019
Aspartic acid	0·87[a]	0·83[a]	0·83[a]	0·007	NS	0·86	0·016
Glutamic acid	0·93[a]	0·92[a]	0·95[a]	0·005	NS	0·95	0·006
Glycine	0·81[a]	0·84[a]	0·84[a]	0·015	NS	0·90	0·011
Proline	0·90[a]	0·91[a]	0·93[a]	0·008	NS	0·93	0·011
Serine	0·90[a]	0·89[a]	0·88[a]	0·006	NS	0·91	0·013
Mean	0·87	0·86	0·86			0·89	
N	0·86[a]	0·87[a]	0·87[a]	0·008	NS	0·89	0·009
Dry matter	0·91[a]	0·89[ab]	0·82[b]	0·009	*	0·86	0·008

NS, not significant.
a, *b*, Within rows, means by different superscripts were significantly different ($P < 0·05$).
* $P < 0·05$ ** $P < 0·01$.

A matrix of correlation coefficients was set up to examine the pair-wise associations of ileal and faecal availability values. When all grains except wheat no. 4 were included, faecal N digestibility was most highly correlated with ileal availability-digestibility values of serine (*r* 0·55), lysine (*r* 0·49), and valine (*r* 0·47), all being significant (*n* 32, $P < 0·01$). The faecal availability of each amino acid was often most highly correlated with its own ileal availability value; for example, lysine and threonine (*r* 0·61 and 0·63 respectively, *n* 32, $P < 0·01$). However, a more useful measure of the over-all relationship was provided by canonical analysis. There were significant canonical correlations between sets of availability values determined by ileal and faecal analysis (Table 3). Generally those amino acids most important to the canonical scores, such as alanine, leucine and lysine, were the same for ileal and faecal analysis.

FDNB-reactive lysine. The proportions of reactive to total lysine in the nine grains ranged from 0·89 to 0·94 (Table 4) but these values were not related with true availability values for lysine at the ileum. For example, there was no significant difference in the reactive lysine contents between wheats nos. 1, 2 and 3 (0·91, 0·89 and 0·90 respectively) for which the true availability values of lysine at the ileum were significantly different (0·89, 0·83 and 0·79 respectively).

In vitro digestibility. The in vitro digestibilities by pronase of the proteins in all the cereal grains are presented in Table 4. Although proteins in sorghum and maize were poorly digested by this system other grain proteins were particularly well digested (e.g. *Triticale* 0·96).

In vitro and in vivo digestibility estimates were not correlated for all the grains; however,

Table 3. *The correlation of canonical variates of ileal and faecal digestibility with the original variates of apparent amino acid availability and apparent nitrogen digestibility for wheats nos. 1, 2, 3, 5, sorghum* (Sorghum vulgare *Pers.*), *maize barley and* Triticale *fed to pigs*

Significance of canonical variate	Ileal digestibility variate	Correlation of canonical variate with original variate	Faecal digestibility variate	Correlation of canonical variate with original variate
First canonical variate	Alanine	0·69	Alanine	0·74
$P < 0{\cdot}01$	Leucine	0·58	Leucine	0·72
	Aspartic acid	0·50	Proline	−0·69
			Glutamic acid	−0·64
			Methionine	−0·45
			Aspartic acid	0·44
Second canonical variate	Serine	0·72	Valine	0·88
$P < 0{\cdot}05$	Lysine	0·71	Lysine	0·77
	Valine	0·70	Aspartic acid	0·74
	Aspartic acid	0·63	Serine	0·71
	Nitrogen	0·60	Nitrogen	0·64
	Threonine	0·43	Glycine	0·63
			Methionine	0·50
			Threonine	0·49
			Glutamic acid	0·48

Table 4. *The content of chemically-available lysine, the in vitro nitrogen digestibility and the true digestibilities at the ileum of nitrogen and lysine in cereal grains fed to pigs*

	N digestibility			Lysine availability		
	In vitro				Chemical	
Grain	Mean	SD	Ileal	Ileal	Mean	SD
Wheat no. 1	0·91	0·016	0·92	0·89	0·91	0·015
2	0·87	0·021	0·91	0·83	0·89	0·005
3	0·82	0·017	0·87	0·79	0·90	0·012
4	0·80	0·015	0·83	0·71	0·90	0·013
5	0·88	0·012	0·92	0·86	0·94	0·012
Sorghum (*Sorghum vulgare* Pers.)	0·45	0·020	0·89	0·88	0·93	0·003
Maize	0·51	0·003	0·86	0·83	0·92	0·006
Barley	0·82	0·001	0·84	0·84	0·94	0·005
Triticale	0·96	0·026	0·91	0·88	0·94	0·006

within the wheat samples the estimates were closely correlated. Excluding wheat no. 4, correlation coefficients between the average in vitro N digestibility values and the ileal N and lysine true digestibility–availability values for the other four wheats were 0·96 ($P < 0{\cdot}05$) and 0·99 ($P < 0{\cdot}01$) respectively.

Table 5. *The differences (%) between faecal and ileal values of amino acid availability in cereal grains fed to pigs (faecal-ileal values)*

	Wheat no. 1	Wheat no. 2	Wheat no. 3	Wheat no. 5	Sorghum (*Sorghum vulgare* Pers.)	Maize	Barley	*Triticale*
Indispensable amino acids								
Arginine	4·0	7·7	8·7	8·0	5·4	7·3	10·4	8·0
Isoleucine	0·2	−1·0	1·1	−0·2	−1·0	0·7	0·9	2·6
Leucine	1·1	1·5	3·5	0·3	1·2	3·0	5·0	4·1
Lysine	−2·4	−0·2	0·9	−0·9	−0·7	2·2	5·2	2·6
Methionine	−1·5	−3·4	−7·7	−4·1	−8·6	−7·4	−3·0	2·6
Threonine	8·5	8·6	12·6	7·8	5·9	11·7	10·5	12·0
Valine	−0·5	2·8	1·6	2·5	−1·5	4·1	3·2	3·8
Dispensable amino acids								
Alanine	4·0	6·9	8·5	4·0	3·0	5·1	8·4	6·6
Aspartic acid	5·8	5·3	8·3	7·2	3·2	7·2	10·3	7·2
Glutamic acid	1·0	2·1	3·0	1·9	3·0	5·4	5·0	3·4
Glycine	15·5	20·8	24·2	19·3	20·2	29·3	30·7	22·6
Proline	7·0	5·2	12·2	5·9	14·0	14·7	12·5	11·1
Serine	8·9	9·1	16·8	6·3	5·7	10·6	11·6	8·8
Mean	4·6	5·7	8·4	5·3	5·6	8·4	9·0	7·3
N	5·2	6·0	8·4	5·6	6·3	11·5	11·4	6·6
Dry matter	8·8	11·0	14·4	8·5	8·3	8·5	11·8	14·1

DISCUSSION

Comparison of availability values determined by ileal and faecal analysis

The differences between ileal and faecal values of apparent amino acid availability presented in Table 5 show that not only do these vary between grains but also to a large extent between amino acids. For some, faecal values were less than ileal, but for most, faecal values were greater. Similar observations were reported for grains by Sauer *et al.* (1977). Furthermore, the differences between faecal and ileal values were larger with the less digestible grains; for wheat, these differences were negatively correlated (r 0·96, $P < 0·01$) with ileal dry matter digestibility. This was consistent with the observations of Varnish & Carpenter (1971) and Low (1975). Sauer *et al.* (1977) suggested that this was due to greater bacterial activity in the large intestine when less digestible grains were fed. Fermentation stimulated by additional substrate available to the flora would allow more bacterial hydrolysis of undigested protein and probably a greater proportion of bacterial protein in the faeces (Mason *et al.* 1976).

Generally, the indispensable amino acids made up more than half the amino acids disappearing in the large intestine. The amino acids that disappeared to the largest extent were glycine, proline, serine and threonine, those occurring most abundantly in mucin protein (Horowitz, 1967) and endogenous ileal digesta (Taverner *et al.* 1981*a*). This illustrates the susceptibility of these proteins to bacterial hydrolysis (Hashimoto *et al.* 1963). Except for *Triticale*, there was also a net, and often large, appearance of methionine in the large intestine, which was consistent with the results reported by Cho & Bayley (1972) with pigs given soya-bean and rapeseed meals. For the indispensable amino acids, faecal analysis usually over-estimated availability, especially with threonine for which faecal values were up to 12·6% higher than ileal values. In spite of the large differences in threonine availability between ileal and faecal analysis, the correlation between these values (r 0·63) was similar to those between faecal and ileal N digestibility values (r 0·53) and between faecal and ileal lysine availability values (r 0·61), all of which were significant (n 32, $P < 0.01$). When the availability values of all amino acids were considered, there was a significant canonical correlation between faecal and ileal analysis in which lysine was among the important amino acids in the linear combinations for each method. Therefore, from a practical viewpoint, although faecal analysis may not provide an accurate value of amino acid absorption for all amino acids, variations in faecal recovery values do reflect to some extent those expected in ileal recovery.

Chemical and enzymic estimates of amino acid availability in grains

The Silcock available-lysine method of Roach *et al.* (1967) overcomes many of the analytical problems reported by Milner & Westgarth (1973) for the direct-FDNB Carpenter method with cereal grains. Furthermore, Taverner & Rayner (1975) found that Silcock available-lysine values were closely related to pig performance with soya-bean and rapeseed meals. However, in the present experiment there was no relationship between the proportion of lysine in cereal grains that was truly absorbed by the pig and that which was determined as reactive or available lysine by the Silcock method. Batterham *et al.* (1978) also found with a range of protein concentrates that Silcock available-lysine values were poorly related to truly-available lysine for pigs.

This is of particular concern because of the wide acceptance of the Silcock method as an estimate of lysine availability in feeds for pigs. It follows that there is the need to evaluate the relative efficiency of the different techniques for predicting lysine availability (Batterham *et al.* 1978).

Saunders & Kohler (1972) found that in vitro digestion of protein in wheat products with a bacterial protease (pronase) and pancreatic enzyme was closely correlated (r 0·96) with in vivo faecal digestibility of N in rats. Using the same method, Miladi *et al.* (1972) found that digestible lysine values for wheat and its mill fractions were closely correlated with rat growth. Similarly in the present experiment, in vitro digestibility values with pronase were comparable to those of Miladi *et al.* (1972) for wheat protein; the values were also closely correlated (r 0·96, n 4, $P < 0.05$) with in vivo N digestibility values. The in vitro protein digestibility values for wheat were linearly related with true availability values for lysine (r^2 0·98, $P < 0.05$), thus providing an accurate and relatively simple estimate of lysine availability in wheat.

However, when values for all the cereal grains used were included, the in vivo and in vitro N digestibility values were not closely correlated. This was due largely to the low in vitro digestibility of N in both sorghum and maize; this is probably related to the high proportion of their protein that was insoluble (Taverner *et al.* 1981 *b*).

Although the utility of ileal analysis will increase with the improvements in technique developed by workers including Braude *et al.* (1976) and Zebrowska *et al.* (1978), more routine measures of variation in protein quality of feedstuffs are required. The need to calibrate or validate such measures was demonstrated in the present study and by Batterham *et al.* (1978) when biologically-determined lysine availability values were found to be unrelated to a common (Silcock) measure of available lysine in grains and protein concentrates.

The pronase assay used in the present study and calibrated with ileal availability values offers considerable potential to broaden the scope of investigations of amino acid availability in feeds by increasing both the number and range of samples than can be studied.

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