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Protein quality in cereals and pulses

3. Bioassays with rats and chickens on sorghum (*Sorghum vulgare* Pers.), barley and field beans (*Vicia faba* L.). Influence of polyethylene glycol on digestibility on the protein in high-tannin grain

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1. Two preceding papers in this series describe the application of microbiological and other in vitro tests in the evaluation of sorghum (*Sorghum vulgare* Pers.), field beans (*Vicia faba* L.) and barley, and in assessing the influence of polyethylene glycol (PEG 4000) on the nutritional availability of the methionine. The present paper gives for comparison the results of bioassays on some of the same test samples. Net protein utilization (NPU) in rats was measured by the nitrogen balance method, and N digestibility in chickens by the ileal analysis procedure.

2. In rat tests on sorghum, N in grain of high-tannin varieties was poorly digested. Supplementation of the test diets with 0·1 g PEG 4000/g protein gave a large improvement, which was partly offset by an apparent decrease in biological value (BV). With chickens N digestibility was even lower, and was similarly improved with PEG 4000. Treatment of high-tannin grain with ammonia solution was also effective in improving N digestibility.

3. With low-tannin sorghum the amino acid digestibilities were uniformly high and were not affected by addition of PEG to the test diet. With high-tannin sorghums they were low and less uniform, and were much improved by PEG 4000.

4. With field beans, the influence of the seed-coat tannin on protein utilization was much less pronounced than with sorghum. In chickens there was a significant effect ($P < 0{\cdot}05$) of PEG 4000 on N digestibility in a high-tannin variety. With rats the effect was smaller and not significant.

5. In four samples of barley, N digestibility was high (0·87–0·96) and was not further improved by PEG 4000. The BV of a high-lysine cultivar proved marginally inferior to that of a normal variety. Possible reasons for this are discussed.

6. Over all, the results were closely consistent with those from microbiological tests with *Streptococcus zymogenes*.

We have described in an earlier paper (Ford & Hewitt, 1979*a*) the application of a modified *Streptococcus zymogenes* assay procedure in the estimation of available methionine and relative nutritional value (RNV) in rice (*Oryza sativa* L.), sorghum (*Sorghum vulgare* Pers.), barley and field beans (*Vicia faba* L.). In grain of ten varieties of sorghum the content of available methionine varied widely, from 6·3 to 17·7 g/kg protein (nitrogen × 6·25), and was highly correlated with tannin content. Similarly in field beans, the presence of tannin in the seed coat was associated with a significant reduction in the availability of methionine, though the effect was less pronounced than with sorghum. But in barleys, such small differences as were measured in the availability of methionine were not related to differences in tannin content, which was uniformly low. This was equally true for samples of barley for which other workers had reported significant differences in crude protein digestibility as measured with laying hens, and the inconsistency calls in question the value of the microbiological assays as predictive tests.

In further papers (Ford, 1977; Ford & Hewitt, 1979*b*) we described experiments in vitro on the influence of polyethylene glycol (PEG 4000) and related compounds on the protein nutritional quality in sorghum, field beans and barley. With sorghum, the presence of tannin in the testa was associated with severe depression of RNV and availability of methionine, as determined microbiologically. Addition of PEG to the test samples increased the average of

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the RNV and available-methionine values to equal those obtained for a control group of low-tannin sorghums. With seed of coloured-flowered varieties of field beans, treatment with PEG 4000 gave a smaller but consistent increase in the available methionine content, which resulted from the inactivation of tannin in the testa. Tests on twenty-three samples of barley grain showed no such beneficial effect of the PEG 4000 treatment.

We now present for comparison with these in vitro findings the results of biological tests on a selection of the samples. Ileal digestibility of the protein was measured with chickens, and net protein utilization (NPU) with rats. The effect of inclusion of PEG 4000 in the test diets on the nutritional quality was determined and, in one of the high-tannin sorghums, compared with that of pretreatment of the grain with ammonia.

EXPERIMENTAL

Test materials

Sorghum. Seed of five varieties of hybrid sorghum was examined, three being of high tannin content and three nominally tannin-free. Varieties X 3101 (high tannin) and SSK 52 (tannin-free) were obtained from the Department of Agricultural Research, Ministry of Agriculture, Botswana, through the offices of Dr C. Shorrock. Variety BR 54 (high tannin) was grown in USA and supplied by Dr J. D. Axtell. Texas No. 2 hybrid yellow sorghum (tannin-free) ('Yellow'), and a brown sorghum (high tannin) ('Brown') 'typical of shipments received from Africa and Middle East countries' (Smith *et al.* 1977), were supplied by Dr W. C. Smith. A portion of whole grain of each of these latter two varieties was treated with ammonia solution as described by Ford & Hewitt (1979*b*).

Field beans. Seed of two varieties of field beans, Throws (high tannin) and Threefold (tannin-free), was supplied for test by Dr D. A. Bond, Plant Breeding Institute (PBI), Trumpington, Cambridge.

Barley. Seed of the widely grown variety Maris Mink, and of a high-lysine cultivar HJ 191-1132, was supplied by Mr A. Rhodes, PBI. Two further samples were obtained, of the barley variety Ingrid. They were from crops grown in Sweden at widely separate geographical latitudes, and in tests with laying hens (Gohl & Thomke, 1976) they had given different values for protein digestibility that were associated with differences in their tannin content. They were provided for test by Dr S. Thomke.

All the samples were milled to pass a sieve with 0·85 mm perforations before being incorporated into the experimental diets.

Biological assays

N-balance measurements with rats. The tests were done with weanling rats, usually 4-week-old female Norwegian hooded rats from the Institute's colony; but in test R2 3-week-old rats were used, and in test R1 the rats were of the Sprague–Dawley strain. The rats were housed singly in stainless-steel cages with wire-mesh floors or, in test R1, in glass metabolism cages (Rolls *et al.* 1976).

Our usual practice is to give each of the experimental diets to each in turn of all the treatment groups (cf. Henry & Kon, 1956), but for the present investigation we adopted the simpler and quicker procedure of allocating matched groups of rats to individual test diets. The loss of precision in the measurements was compensated by replication of the assays and by increasing the numbers of rats used.

The composition of the diets is given in Table 1, and their content of the 'variable' ingredients (test foodstuff, PEG 4000, lysine hydrochloride) in Tables 2, 3, 6 and 8. The diets were given to the rats for a total of 8 d, on the first four of which each rat was offered daily a weight of diet equal to 0·1 or 0·11 of its initial body-weight. The cage floors and the

Table 1. *Composition of diets used in nitrogen-balance experiments with rats (g/kg)*

Ingredient	Egg-protein diet	Expt R1, 2	Expt R3	Expt R4	Expt R5	Expt R6, 7, 8
Dried whole egg	55	—	—	—	—	—
L-methionine	—	—	—	—	2·5	—
Potato starch	100	—	—	—	—	—
Margarine fat	100	100	25	100	100	50
Ground sugar	75	60	—	120	120	—
Rice starch	620	—	—	—	—	—
Salt mixture*		40				
Vitamin mixture*		10				
Vitamin E concentrate†		0·24				
Variable/rice starch	—	to 1 kg				

* US Pharmacopeia (1965); cyanocobalamin solution (100 μg/ml) was also added, at 0·2 ml/kg.
† Rovimix E_{25} (Roche Products, Welwyn Garden City, Herts.), containing 250 mg α-tocopheryl acetate/g.

collection trays were then washed and replaced, after first covering the bottom of each tray with Whatman grade 3 filter paper and sprinkling it with 12 ml 0·5 M-sulphuric acid. The rats were weighed and the level of feeding adjusted if necessary on the basis of this weighing, and then continued on the test diets for a further 4 d, during which faecal pellets and any leftover or spilled food were collected daily. After the balance period the rats were removed and weighed and the filter-papers transferred to jars. The cage floors and collection trays were then washed with 100 ml 0·5 M-sulphuric acid and the washings added to the filter papers. Water was later added to 500 ml and the whole homogenized in a blender jar. The collections of faeces were taken up in water and homogenized with a Silverson homogenizer (Silverson Machines Ltd, London). The homogenates corresponding to the egg diets were diluted to 50 ml and those for the 'test' diets to 200 ml.

Ileal digestibility measurements with chickens. Varnish & Carpenter (1975) developed an 'ileal digestibility' method for measuring with chickens the true digestibility of the dietary protein and of the constituent amino acids. We aimed to follow their procedure, but from experience with preliminary tests some modification proved to be necessary. In particular, we found that the method described for determining the weight of test meal to be given was difficult to follow, because our birds varied greatly in their willingness to eat the protein-free diet, some of them refusing to eat it at all. We therefore standardized the weight of test meal at 22 g/kg mean body-weight of the birds on experiment. Any birds that refused to eat or ate slowly were rejected. Ileal contents were washed out with 20 ml ice-cold physiological saline (9 g sodium chloride/l) and freeze-dried, after first carefully removing feathers that were occasionally present.

Three different kinds of chickens were used in the course of these experiments. With the sorghums, Rhode Island Red × Light Sussex from the Institute's breeding flock were used (Expts C1 and C2), and Light Sussex obtained from Houghton Poultry Research Station (Expt C3). With the beans (Expts C4–C7) and barleys (Expt C8), broiler chickens (Ross) were used. In expts C1 and C2 equal numbers of male and female chickens were allocated to each diet, and in Expts C3–C8 only male chickens were used. All the birds were 6–9 weeks old, weighing 640 g (7-week-old RIR × LS)–1646 g (8-week-old broilers) at the time of experiment.

The composition of the protein-free diet was the same as that described by Achinewhu & Hewitt (1979). With beans, the test proteins were incorporated at the expense of maize

starch, to provide 200 g protein/kg diet. An exception was Expt C7, in which the beans were tested at both 100 and 200 g protein/kg diet. With sorghums, which were much lower in protein content, the test diets contained only 72·5 or 80 g protein/kg (see Table 4). The two samples of barley variety Ingrid were tested at 80 g protein/kg diet.

In Expts C2–C8, the ileal contents from each bird were analysed individually for N and chromic oxide. In Expt C1, the birds were smaller, and so to obtain sufficient of the ileal digesta for analysis the contents from each female were combined with those from a male that had received the same diet.

Determination of amino acid digestibility. Ileal digesta collected in chick Expts C1 and C2, and faeces collected in rat Expt R3, and the corresponding diets, were analysed for amino acids. In expt C1 the freeze-dried digesta were combined to give four composite samples representing the four experimental diets. In Expt C2 the digesta from the individual birds were analysed separately, to give six replicate values for each test diet. In the rat experiment, portions of faeces containing 5 mg N were combined to give composite samples representing the different experimental groups.

Statistical analysis of the results. The results were examined by analysis of variance, and the standard errors given are based on the variation between replicates. With beans, the digestibility of the protein was measured in four experiments, Expts C4–C7 (Table 7), and for each diet the means of the results of the individual experiments are also given, together with a standard error based on the 'diets × experiments' interaction mean square. In Expt C2, in which individual results for males and females were obtained, there was no significant difference between the results given by the different sexes.

Analytical methods

Total N. Samples were digested with H_2SO_4–potassium sulphate–mercuric oxide mixture as recommended by Fleck & Munro (1965), and ammonia in the digests was determined colorimetrically with an AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants) as described by Smith & McAllan (1970).

Amino acids. Samples containing 2 mg N were heated under reflux for 24 h with 45 ml 6 M-hydrochloric acid. The amino acid composition of the hydrolysates was determined by ion-exchange chromatography essentially as described by Spackman *et al.* (1958), with an automatic amino acid analyser (LKB Ltd, Croydon).

Methionine and cystine values were based on analyses of hydrolysates prepared after treatment of the samples with performic acid (Moore, 1963).

Cr_2O_3. Cr_2O_3 was measured by the procedure of Czarnocki *et al.* (1961), modified as described by Varnish & Carpenter (1975).

Tannin. The test materials were graded by the tannins index procedure as described by Ford & Hewitt (1979*a*).

RESULTS

Experiments with sorghum

N-balance experiments with rats. In the first experiment (Expt R1) a high-tannin sorghum (X 3101) was tested, with and without PEG 4000 at 0·1 and 1·0 g/g protein. The results are given in Table 2.

The N in the test diets without PEG 4000 was poorly digested (digestibility 0·534). Supplementation of the test diet with 0·1 g PEG 4000/g protein gave a marked improvement, from 0·534 to 0·923 ($P < 0·001$), and an increase in the level of PEG 4000 to 1 g/g protein gave a further small, non-significant increase to 0·988. The large improvement in digestibility was partly offset by a decrease in biological value (BV) from 0·77 to approximately 0·59 (see p. 335), but there was a significant improvement ($P < 0·05$) in NPU. As with

Table 2. *Expt R1. True digestibility, biological value and net utilization of the protein* (NPU) *in high-tannin sorghum* (Sorghum vulgare *Pers.*) *X 3101,* measured using the rat nitrogen-balance procedure: the influence of supplementary polyethylene glycol* (*PEG* 4000)

(Values are means for three rats)

	PEG 4000 added (g/g protein)			SEM (6 df)
	0	0·1	1·0	
Digestibility	0·534	0·923	0·988	0·019
BV	0·771	0·590	0·581	0·031
NPU	0·415	0·545	0·574	0·031

* The diets contained 710 g sorghum, equivalent to 80 g protein/kg.

digestibility, the higher level of PEG 4000 gave a marginally greater improvement, though again the difference was not significant.

Table 3 summarizes the results of two further experiments (Expts R2 and R3), in each of which the nutritional quality of one low- and one high-tannin sorghum was tested in the presence and absence of supplemental PEG 4000 and lysine. Both experiments were of $2 \times 2 \times 2$ factorial design, the factors being sorghum type (low-or high-tannin), PEG 4000 supplementation (0 or 0·1 g/g protein), and lysine supplementation (0 or 4·5 g L-lysine hydrochloride/kg diet).

Digestibility of N in the low-tannin varieties SSK 52 and 'Yellow' was high (1·012 and 0·970 respectively) and that in the high-tannin varieties BR 54 and 'Brown' was comparatively low (0·658 and 0·801 respectively), but not as low as in X 3101 (Table 2). Digestibility in these high-tannin varieties decreased with increase in tannin content. Thus, for X 3101, BR 54 and 'Brown' the digestibilities were respectively 0·534, 0·658 and 0·801 and the tannin index values 1·9, 1·8 and 1·2 (Ford & Hewitt, 1979*b*).

Supplementation with PEG 4000 did not affect digestibility in the low-tannin varieties. It markedly improved digestibility in the high-tannin varieties, though not so much as to equal that in the low-tannin varieties.

In Expt R2, one rat in the group given unsupplemented high-tannin sorghum gave an exceptionally low value for BV, resulting in large variation that is indicated by separate standard errors in Table 3. Despite this the general pattern of results in Expts R2 and R3 was the same. With the low-tannin sorghums, BV and NPU were significantly increased when lysine was added, especially in Expt R2. They were not increased by PEG 4000, and indeed in Expt R2 the increase in BV resulting from lysine supplementation was smaller in presence of PEG 4000. In both experiments the BV for the unsupplemented high-tannin sorghum was higher than that for its low-tannin counterpart (Expt R3, $P < 0{\cdot}001$; Expt R2, $P > 0{\cdot}05$), but it must be emphasized at this point that these higher values for BV were in a sense artefacts and resulted from the lower level of N uptake from the comparatively indigestible high-tannin sorghums (see p. 335). As in Expt R1, supplementation with PEG reduced these anomalous high values and brought them more into line with those for the low-tannin varieties. Further addition of lysine increased the values for BV to equal those for the similarly supplemented low-tannin varieties, and it gave a marked increase in NPU. In contrast, the addition of lysine without PEG reduced the values for BV, significantly in Expt R3 ($P < 0{\cdot}05$).

N-digestibility experiments with chickens. Table 4 sets out the results of three experiments with chickens in which the ileal digestibility of sorghum was determined.

In the first experiment (Expt C1), high-tannin sorghum X 3101 was tested with and without PEG 4000 at 0·1 and 1·0 g/g protein. The results were broadly similar to those obtained

Table 3. *Expts R 2 and R 3. True digestibility, biological value* (BV) *and net utilization of the protein* (NPU) *in low- and high-tannin sorghums* (Sorghum vulgare *Pers.*), *measured using the rat nitrogen-balance procedure: the influence of supplementary lysine and polyethylene glycol* (*PEG* 4000)

(Values are means for four rats in Expt R2, and for six in Expt R3)

Sorghum type ...	Low-tannin*				High-tannin*				
PEG 4000 added (g/g protein) ...	0		0·1		0		0·1		
L-lysine hydrochloride added (g/kg diet) ...	0	4·5	0	4·5	0	4·5	0	4·5	SEM (df)
				Expt R2					
Digestibility	1·012	1·030	1·013	0·984	0·658	0·652	0·870	0·910	0·035 (24)
BV	0·540	0·758	0·574	0·691	0·698 ±0·097‡	0·680	0·623	0·675†	0·020 (20)
NPU	0·547	0·781	0·582	0·681	0·482 ±0·107‡	0·444	0·545	0·619‡	0·030 (20)
				Expt R3					
Digestibility	0·970	0·975	0·967	0·976	0·801	0·804	0·884	0·900	0·007 (40)
BV	0·658	0·714	0·666	0·733	0·736	0·689	0·675	0·734	0·013 (40)
NPU	0·638	0·697	0·644	0·716	0·591	0·554	0·596	0·661	0·014 (40)

* In Expt R2 the low-tannin sorghum (var. SSK 52) supplied 650 g/kg diet, and the high-tannin sorghum (BR 54) supplied 744 g/kg, equivalent to 80 g protein/kg. In Expt R3 the low-tannin sorghum ('Yellow') supplied 856 g/kg, and the high-tannin sorghum ('Brown') supplied 912 g/kg, equivalent to 77 g protein/kg.

† One missing value.

‡ Excluded from the analyses of variance because of variance heterogeneity.

Table 4. *Expts C1–C3. True digestibility of the protein in low- and high-tannin sorghums* (Sorghum vulgare *Pers.*), *measured using the chick ileal analysis procedure: influence of supplementary polyethylene glycol* (*PEG* 4000) *and of ammonia treatment*

(Values are means for four, six and six chickens in Expts C1, C2 and C3 respectively. In Expt C1 the ileal contents from each female were combined with those from a male chicken on the same treatment)

Expt	Sorghum†	Tannins index‡	PEG 4000 added (g/g protein) 0	0·1	1·0	Ammonia treated*	SEM (df)
C1	X 3101	1·90	0·442	0·898	0·942	—	0·022 (3)
C2	'Yellow'	0·15	0·978	0·997	—	—	0·026 (20)
	'Brown'	1·20	0·656	0·948	—	—	
C3	'Yellow'	0·15	0·973	—	—	0·890§	0·032 (24)
	'Brown'	1·20	0·661	0·905	—	0·918§	

* See p. 326.

† Diets contained 710 g X 3101 equivalent to 80 g protein, or 806 g 'Yellow' or 859 g 'Brown' equivalent to 72·5 g protein/kg.

‡ Ford & Hewitt (1979*a*).

§ Calculated from the true nitrogen content of the test diets, see p. 331.

with rats in Expt R1 (Table 2). Digestibility of the N in the unsupplemented sorghum was somewhat lower in the chicken than in the rat, and was sharply increased ($P < 0·001$) by the addition of 0·1 g PEG 4000/g protein. A further, non-significant, increase occurred when the level of PEG 4000 supplementation was increased to 1 g/g protein.

In the second experiment (Expt C2) 'Yellow' (tannin-free) and 'Brown' (high-tannin) were compared, with and without PEG 4000 supplementation. Again the digestibility values were broadly similar to those measured with rats (Table 3, Expt R3), although as with X 3101 the digestibility of N in the unsupplemented 'Brown' was lower in the chicken.

The third experiment (Expt C3) was done to test the effect of ammonia treatment of 'Yellow' and 'Brown' on the ileal digestibility of the N. The treatment increased the N content of 'Yellow' by 17·2% and of 'Brown' by 19·6%, and so complicated the interpretation of the results. The test diets were compounded on the basis that they contained the same level of N from the original grain, and any additional N from bound ammonia was discounted. If in calculating the results the N content of the ammonia-treated grain was again taken as equal to that of its untreated counterpart, it seemed that treatment of 'Brown' with ammonia resulted in a large improvement in its N digestibility, from 0·661 to 0·908. This extent of improvement was similar to that which resulted from supplementation with PEG 4000 at 0·1 g/g protein. The calculation embodies an assumption that bound ammonia was wholly digestible. However, on recalculating from the opposite assumption, that the bound ammonia was not digested, the pattern of results was much the same: ammonia treatment of 'Yellow' increased N digestibility from 0·973 to 0·991, and of 'Brown' from 0·661 to 1·029. Yet a third calculation was made, from the true N content of the test diets, and once more the general picture was unchanged (see Table 4). The differences between results calculated in these different ways were small in relation to the large differences between the effects of the ammonia treatment on the two sorghums. It is reasonable to conclude that the N digestibility of 'Brown' was low and was increased equally by PEG 4000 as by ammonia treatment, to a level not significantly different from that for 'Yellow'.

Digestibility of individual amino acids. Table 5 shows the digestibilities of individual amino acids in some of the sorghums, as measured with rats by the faecal analysis procedure, and with chickens by the ileal analysis method. With each test diet the unweighted

Table 5. *Expts R3, C1 and C2. True digestibility of amino acids in low-tannin sorghum* (Sorghum vulgare *Pers.*) (*'Yellow'*) *and in high-tannin sorghums* (*X 3101 and 'Brown'*), *measured using the rat balance and the chick ileal analysis procedures: the influence of supplementary polyethylene glycol* (*PEG* 4000)

Expt ...	R3				C1		C2		
Sorghum ...	'Yellow'		'Brown'		X 3101		'Brown'		
PEG 4000 ...	0	+	0	+	0	+	0	+	SEM (10 df)
Aspartic acid	0·97	0·97	0·86	0·92	0·49	0·93	0·70	1·00	0·028
Threonine	0·94	0·94	0·78	0·85	0·40	0·87	0·69	1·01	0·037
Serine	1·03	1·03	0·86	0·94	0·36	0·90	0·67	1·02	0·054
Glutamic acid	0·98	0·98	0·82	0·91	0·47	0·93	0·61	0·95	0·026
Proline	0·94	0·93	0·56	0·69	0·40	0·85	0·55	0·87	0·047
Glycine	0·91	0·90	0·62	0·74	0·40	0·84	0·68	0·92	0·042
Alanine	0·98	0·98	0·87	0·94	0·48	0·93	0·62	0·96	0·026
Cystine	1·02	1·00	0·72	0·85	0·01	0·74	0·56	0·96	0·083
Valine	0·96	0·96	0·82	0·89	0·42	0·91	0·65	0·96	0·036
Methionine	1·00	1·00	0·86	0·93	0·55	0·93	0·72	0·96	0·020
Isoleucine	0·97	0·96	0·86	0·90	0·42	0·92	0·63	0·98	0·033
Leucine	0·97	0·97	0·86	0·93	0·50	0·94	0·58	0·96	0·035
Tyrosine	0·96	0·94	0·83	0·91	0·44	0·94	0·60	0·98	0·030
Phenylalanine	0·98	0·98	0·86	0·93	0·60	0·95	0·65	0·99	0·031
Histidine	0·94	0·94	0·65	0·76	0·45	0·79	0·55	0·79	0·027
Lysine	0·99	1·00	0·90	0·95	0·66	0·94	0·82	0·98	0·017
Arginine	0·97	0·98	0·78	0·87	0·46	0·90	0·74	0·98	0·033
Average amino acid digestibility	0·97	0·97	0·80	0·88	0·44	0·90	0·65	0·96	—
Digestibility of nitrogen (Tables 3 and 4)	0·97	0·97	0·80	0·88	0·44	0·90	0·66	0·95	—

0, absent; +, present.

average amino acid digestibility was closely similar to that of the N. With 'Yellow', the amino acid digestibilities were uniformly high and were not affected by addition of PEG 4000 to the test diet. But with 'Brown' and X 3101 they were lower and less uniform. 'Brown' was tested both with chickens and with rats, and for all the amino acids except glycine the chicken assay values were lower, like the N digestibility (Tables 3 and 4). Addition of PEG 4000 to the high-tannin sorghums improved the digestibility of all the amino acids although, as in Expt R3, the increased values fell short of those found for 'Yellow'. In Expt C1 the very low digestibility of cystine (0·01) in X 3101 set it apart from all the other amino acids; even in the presence of PEG 4000 it was still the least digestible. In 'Brown', however, the digestibility of cystine was not so exceptionally low.

Experiments with field beans

N-balance experiments with rats. Table 6 shows the results of two experiments in which the varieties Throws (high-tannin) and Threefold (tannin-free) were compared. In the first experiment (Expt R4) all the rats that were given the test diets containing beans refused a substantial proportion (average 28·2%, range 6–53%) of their food. The resulting low and uneven level of food intake probably influenced the results of the experiment. With the rats that ate least, digestibilities tended to be low, and values for BV were markedly low and even negative in several instances. The experiment was therefore repeated (Expt R5), but with all the test diets supplemented with methionine. Food consumption during the 4 d balance period increased, to average 33·8 g *v.* 23·7 *g* in Expt R4, and 'refusals' were much lower at 8·8%.

Table 6. *Expts R4 and R5. True digestibility, biological value* (BV) *and net utilization of the protein* (NPU) *in low-tannin field beans* (Vicia faba *L.*) (*Threefold*) *and in high-tannin beans* (*Throws*), *measured using the rat nitrogen-balance procedure, and diets with and without supplementary methionine: influence of supplementary polyethylene glycol* (*PEG* 4000)

(Values are means for eight rats)

PEG-4000 added (g/g protein) ...	Threefold*		Throws†		SEM (28 df)
	0	0·1	0	0·1	
	Expt R4 (no supplementary methionine in test diets)				
Digestibility	0·936	0·927	0·905	0·930	0·0118
BV	0·251	0·257	0·230	0·281	0·044
NPU	0·238	0·239	0·210	0·263	0·042
	Expt R5 (test diets contained 2·5 g supplementary methionine/kg)				
Digestibility	0·937	0·938	0·906	0·925	0·0091
BV	0·766	0·759	0·749	0·749	0·016
NPU	0·718	0·712	0·679	0·693	0·019

* Threefold beans supplied 289 g equivalent to 80 g protein/kg.
† Throws beans supplied 316 g equivalent to 80 g protein/kg.

Table 7. *Expts C4–C7. True digestibility of the protein in low-tannin* (*Threefold*) *and high-tannin* (*Throws*) *field beans* (Vicia faba *L.*), *measured using the chick ileal analysis procedure: the influence of supplementary polyethylene glycol* (*PEG* 4000)

(Values are means for six chickens in Expts C4–C6, and for four in Expt C7)

PEG 4000 added (g/g protein) ... Expt	Threefold*		Throws†		SEM (df)
	0	0·1	0	0·1	
C4	0·891	0·861	0·829	0·884	0·018 (20)
C5	0·867	0·824	0·807	0·834‡	0·021 (19)
C6	0·844	0·875	0·843	0·866‡	0·030 (19)
C7	0·926	0·956	0·867	0·917	0·012 (12)
Mean	0·882	0·879	0·837	0·875	0·010§ (9)

* Diet contained 722 g Threefold bean meal, equivalent to 200 g protein, /kg.
† Diet contained 790 g Throws bean meal, equivalent to 200 g protein, /kg.
‡ One missing value.
§ Based on the 'diets × experiments' interaction mean square.

The results of Expt R4 must be discounted, but they were consistent with those of Expt R5 in that they showed a small increase in digestibility of Throws in presence of PEG 4000. However, analysis of variance within the assays revealed no statistically significant differences between the various test diets.

N digestibility experiments with chickens. Table 7 shows the results of four experiments with chickens, in which N digestibility was determined for the same two samples of field beans by the ileal analysis method. As in the rat experiments, there were no large differences between the different test diets, and the findings in the individual experiments were inconclusive. In all four experiments digestibility of Throws was greater in presence of PEG 4000, the average value being similar to that for the tannin-free variety Threefold. Results with Threefold were less consistent. In two experiments the N digestibilities were marginally higher in presence of PEG 4000 and in two they were lower. The least significant difference ($P = 0·05$) was 0·032 (based on the 'experiments × diets' interaction mean square from

Table 8. *Expts R6–R8. True digestibility, biological value* (BV) *and net utilization of the protein* (NPU) *in two varieties of barley, measured using the rat nitrogen-balance procedure: influence of supplementary polyethylene glycol* (*PEG* 4000)

(Values are means for six, twelve and eight rats in Expts R6, R7 and R8 respectively)

PEG 4000 added (g/g protein) ...	Maris Mink* 0	Maris Mink* 0·2	HJ 191–1132† 0	HJ 191–1132† 0·2	SEM	(df)
Expt R6						
Digestibility	0·896	0·874	0·886‡	0·883‡	0·009	(18)
BV	0·820	0·798	0·807‡	0·787‡	0·013	
NPU	0·735	0·697	0·715‡	0·695‡	0·014	
Expt R7						
Digestibility	0·863	0·863	—	—	0·007	(22)
BV	0·805	0·819	—	—	0·011	
NPU	0·695	0·707	—	—	0·011	
Expt R8						
Digestibility	0·866	0·882	0·883	0·862	0·008	(28)
BV	0·799	0·792	0·761	0·749	0·011	
NPU	0·692	0·698	0·672	0·645	0·011	

* Diet contained 842 g Maris Mink/kg, equivalent to 80 g protein.
† Diet contained 522 g HJ 191–1132/kg, equivalent to 80 g protein.
‡ One missing value.

an analysis of variance in the experiment means), and so the average increase in digestibility of Throws, from 0·837 to 0·875, was probably real.

In a further comparison within Expt C7 the digestibilities were assessed with test diets in which the beans provided only 100 g protein/kg instead of 200 g/kg. Digestibilities were significantly higher at this lower protein level ($P < 0·001$), the improvement being greatest for Throws given without PEG 4000. Thus for Throws, Throws + PEG 4000, Threefold and Threefold + PEG 4000 the values were respectively 0·962, 0·984, 0·981 and 0·976. At this lower level of protein intake the supplemental PEG 4000 clearly had little or no effect.

Experiments with barley

N-balance experiments with rats. Table 8 presents the results of three experiments on barley. In Expt R7 the conventional variety Maris Mink was given at 80 g protein/kg test diet, with and without PEG 4000 at 0·2 g/g protein. In Expts R6 and R8 Maris Mink was compared with HJ 191–1132, a high-lysine segregant, again with and without PEG 4000 in the diets.

PEG 4000 had no significant effect on digestibility in any of the three experiments, and the digestibilities of the two barleys were much the same. But in Expt R8, HJ 191–1132 was significantly inferior to Maris Mink in BV and NPU ($P < 0·01$), and there was a similar, though non-significant, trend in the results of Expt R6.

N digestibility experiment with chickens (*Expt* C8). Two samples of barley variety Ingrid were examined, from crops grown at Kävlinge in the south of Sweden and at Offer in the far north. Gohl & Thomke (1976) determined their tannin content as 1·8 and 7·5 g/kg respectively, and found in tests with laying hens that higher tannin content was associated with a significant depression in crude protein digestibility. We found no such difference in the ileal digestibilities, which for Kävlinge averaged 0·956 and for Offer 0·933. Each of the values is a mean of nine observations, and the pooled standard error of a mean was 0·10 The difference between the means was well below the 0·05 significance level.

DISCUSSION

Sorghum. Rat assays R1, R2 and R3 showed that supplementation of high-tannin sorghums with PEG 4000 gave a considerable improvement in digestibility, which was largely offset by a fall in BV. However, this fall was in a sense spurious, because estimates of BV may be widely different at different levels of protein intake (Osborne *et al.* 1919; Mitchell, 1923–4); and in testing high-tannin sorghums in the presence and absence of PEG 4000 we were in effect comparing different levels of intake of a lysine-deficient protein. Henry & Kon (1957) examined a deteriorated dried skim milk in which the lysine had been partly inactivated by the Maillard reaction, and they obtained a higher BV at 40 than at 80 g protein/kg in the test diet (0·845 *v.* 0·648). They found also that addition of lysine to the test protein gave a significant increase in the BV only at the 80 g protein/kg level. In much the same way, with high-tannin sorghums, we found that supplemental lysine increased the BV only when the digestibility of the protein had been enhanced by addition of PEG 4000 to the test diets.

With the high-tannin sorghums both the rat and the chicken assays showed considerable variation in digestibilities between different amino acids. Thus, in the rat assay R3 (Table 5) proline and glycine in the 'Brown' sorghum were less well digested than lysine and methionine. Eggum & Christensen (1975) found much the same in a study of the influence of added tannin on the digestibility of soya-bean protein in the rat: the digestibility of lysine and methionine was reduced from approximately 0·92 to 0·81, whereas that of glycine fell from 0·89 to 0·42 and of proline from 0·94 to 0·01. A notable finding in our experiments with chickens was the very low digestibility of cystine in the high-tannin sorghum X 3101 (Table 5). Unfortunately we had none of this test sample left and so we were unable to repeat the experiment.

The composition of the diets used in the tests with chickens was very different from that of a practical diet such as might be used in rearing broiler chickens. Protein was provided only by the sorghums, at 72·5 or 80 g/kg, and the question arises whether PEG 4000 would be equally effective in enhancing protein utilization in a conventional high-protein mixed ration containing a proportion of high-tannin sorghum. Further experimentation will be needed to answer this, but preliminary tests showed that, with a diet of 180 g protein/kg containing (g/kg): 300 sorghum of moderately high tannin content (29 g/kg, measured as catechin equivalent), 420 maize meal, 150 soya-bean meal and 80 fish meal, the inclusion of PEG 4000 improved food conversion efficiency (weight gain, g/food eaten, g; FCE) by 5% in a 2-week growth test with broiler chickens (Hewitt & Ford, unpublished results). Taking a value of £165/tonne for a broiler diet and the price of PEG 4000 (in December, 1978) as £565/tonne, the 5% saving on cost of food would amount to £4·60/tonne (£8·00/tonne less £3·40, the cost of PEG 4000 added at 6 kg/tonne).

Results of Expt C3 (Table 4) indicated that treatment of high-tannin sorghum with ammonia, as described by Price & Butler (1978), was fully as effective as the PEG 4000 treatment in neutralizing the tannin. It seems that both forms of treatment might prove to be highly cost-effective, but there is need for further investigation to ensure that neither has any adverse effects.

Field beans. With field beans the influence of the seed tannin on protein utilization was much less pronounced than with sorghum of similar tannin content as measured by the tannin index procedure (Ford & Hewitt, 1979*a*), and the explanation lies in the different nature of the tannin. Martin-Tanguy *et al.* (1977) isolated and examined several condensed tannins from bean testa and concluded that their growth-depressing effect seems to depend on the extent of polymerization; the species of higher molecular weight are comparatively innocuous. Even so, in experiments with laying hens given diets containing 300 g field beans/kg, these workers found a strong negative correlation betwen egg production (both

laying rate and egg weight) and content of tannin in the beans. And with chicks given mixed diets containing 500 g field beans/kg, they found a similar negative correlation ($P < 0.05$) between N digestibility and the tannin content of the beans. In a growth experiment with chicks we have examined the effect on FCE of PEG 4000 supplementation of diets containing Throws (high-tannin) and Threefold (tannin-free) at the 120 g protein/kg level. With Threefold the FCE was 0·097, and with supplementary PEG 4000 this value was little changed at 0·098. With Throws the FCE was 0·043, and it increased to 0·114 with PEG 4000 (Hewitt & Ford, unpublished results). The test diets were low in protein and severely deficient in methionine, and further experiments are in progress on the effects of PEG 4000 supplementation with more practical, mixed diets of balanced amino acid composition.

None of the experiments now reported indicated any large difference in N digestibility between Throws and Threefold, and the beneficial effect of PEG 4000 on N digestibility in Throws was of borderline significance in relation to the poor precision of biological tests. The results were closely consistent with those found in microbiological tests (Ford & Hewitt, 1979*b*), in which PEG 4000 treatment of seed of high-tannin beans increased their available methionine content by about 5%. On the whole it is clear that, if seed of coloured-flowered varieties of field beans is included at a high level in poultry diets, the tannin present in the testa may significantly depress the nutritional quality; and this effect of the tannin may be ameliorated by the addition of PEG 4000 to the diet. Further evidence for this comes from Marquardt *et al.* (1977), who showed that a water extract of field bean hulls depressed growth and efficiency of food utilization in chicks, and that the antinutritional effect was overcome by addition of polyvinylpyrollidone (PVP) to the diet. Ford (1977) showed with high-tannin sorghum that PVP was marginally more effective than PEG 4000 in increasing the biological availability of the methionine, but PEG 4000 is cheaper, and it was preferred for the present experiments because the high content of N in PVP would have complicated the comparisons of N digestibility in the different test diets.

Barley. We found with barley that the BV of the high-lysine variety HJ 191–1132 was marginally lower than that of Maris Mink, while the digestibilities of the two varieties were much the same. This was unexpected, especially in view of the finding by Johnson *et al.* (1978) that protein efficiency ratio and protein retention efficiency were significantly greater for three high-lysine varieties (including HJ 191–1132) than for Maris Mink, and N digestibility was slightly lower. The amino acid analysis of our test samples (Table 9) suggests a possible explanation for our results. Bender (1965) gave values for a 'target mixture' of essential amino acids to meet the requirements of the weanling rat given a diet containing 100 g protein/kg. Comparison with his values shows that, for lysine, Maris Mink supplied only 0·72 of the requirement whereas HJ 191–1132 supplied 0·95. In Maris Mink lysine was clearly the first limiting amino acid. But in HJ 191–1132 isoleucine and the sulphur-containing amino acids provided respectively only 0·74 and 0·73 of the requirement *v.* 0·85 and 0·83 in Maris Mink. Thus it might have been that under the conditions we used for assaying the protein nutritional quality, the higher lysine content of HJ 191–1132 was more than offset by its deficit in isoleucine or the S-amino acids.

Taking another 'target mixture', for diets containing 200 g protein/kg, recommended by the Nutrition Study Group of the Laboratory Animal Science Association (Laboratory Animal Science Association, 1969), lysine was once again the limiting amino acid in Maris Mink, providing 0·62 of the requirement, whereas in HJ 191–1132 the limiting amino acids were tyrosine + phenylalanine (0·62) and isoleucine (0·64). However, these and other estimates of amino acid requirements are imprecise, and heavily qualified with respect to the level of N in the diets and the form in which the amino acids are presented, the age and strain of the test animals, and several other variables. Clearly, our measurements of N balance with Maris Mink and HJ 191-1132, given to weanling rats as the only protein

Table 9. *Content of amino acids in barley varieties Maris Mink and HJ* 191–1132, *in relation to the requirements of the weanling rat*

Barley ...	g amino acid/kg crude protein (nitrogen × 6·25)		Amino acid content as a proportion of the requirement*	
	Maris Mink	HJ 191–1132	Maris Mink	HJ 191–1132
Lysine	37·3	49·3	0·72	0·95
Histidine	22·4	25·6	1·24	1·42
Threonine	34·5	34·7	0·84	0·85
Valine	52·2	50·9	1·04	1·02
Methionine	16·0	15·7	0·83	0·73
Cystine	22·8	18·5		
Isoleucine	36·6	31·9	0·85	0·74
Leucine	69·0	61·4	0·88	0·79
Phenylalanine	48·6	38·4	0·99	0·78
Tyrosine	18·9	17·8	—	—
Arginine	52·2	69·3	—	—
Aspartic acid	70·6	80·6	—	—
Serine	37·3	37·6	—	—
Glutamic acid	201·1	146·9	—	—
Proline	95·0	55·1	—	—
Glycine	42·9	49·8	—	—
Alanine	44·3	47·9	—	—

* The requirement was taken to be the concentrations of amino acids in the target mixture of Bender (1965) which had a biological value of 96 for 4-week-old rats given an amino acid diet containing 16 g N/kg.

source at near 'maintenance' N level, tell us very little about their protein nutritional quality as components of a high-protein mixed diet. Under these more practical conditions the higher protein content of HJ 191-1132, and the higher lysine content of the protein, should lead to economies in the use of high protein concentrate. Against this, with present high-lysine cultivars the improvement in protein composition seems to have been achieved at some expense of metabolizable energy, and Johnson *et al.* (1978) calculated that this might have resulted in a net reduction in their economic value for poultry and growing pigs.

It is not yet clear to what extent the tannin in barley may influence the nutritional quality. Gohl & Thomke (1976) examined five samples of barley of the variety Ingrid, from crops grown in Sweden at widely separate geographical latitudes. They found variation in the tannin content, measured by the reaction with ferric ammonium sulphate, that was reflected in differences in the crude protein digestibility. Similarly, Eggum & Christensen (1975) found a significant interaction between protein content, protein digestibility and tannin content. In a study of twenty-nine barleys they showed that digestibility increased with increase in protein content, and decreased with increase in tannin. In our experience with thirty-three samples of barley, representing fourteen varieties, the availability of methionine was generally high (range 0·83–1·00 of the total), like the N-digestibility values reported by Eggum & Christensen (1975) (range 0·82–0·91). But such differences as we observed were not associated with any differences in tannin content, which was uniformly low as measured by the tannins index procedure (Ford & Hewitt, 1979*a*). The same was true also for the five samples of barley variety Ingrid, portions of which Dr S. Thomke kindly supplied to us for testing. Our assays in vitro showed no correlation between the availability of methionine and the tannin content (Ford & Hewitt, 1979*a*), and we found no difference in digestibility of the N in the two samples between which Dr Thomke found the greatest differences in N digestibility and tannin content. Clearly the differences between our findings arose from the differences in the test procedures employed. Gohl & Thomke (1976) measured digestibility

Table 10. *In vivo digestibility of the protein in low- and high-tannin sorghums* (Sorghum vulgare *Pers.*) *and microbiological assay results: influence of supplementary polyethylene glycol* (*PEG* 4000, 0·1 *g/g protein*)

		True digestibility				Microbiological assay results*			
		Rat†		Chicken‡		Available:total methionine		RNV	
Sorghum	Tannin Index*	Without PEG 4000	With PEG 4000	Without PEG 4000	With PEG 4000	Without PEG	With PEG	Without PEG	With PEG
X3101	1·9	0·53	0·92	0·44	0·90	0·57	1·07	39	76
BR 54	1·8	0·66	0·89	—	—	0·47	0·89	38	84
'Brown'	1·2	0·80	0·89	0·66	0·93	0·51	0·92	43	91
SSK 52	0·22	1·02	1·00	—	—	1·14	1·19	83	83
'Yellow'	0·15	0·97	0·97	0·98	1·00	0·92	0·92	87	90

* Ford & Hewitt (1979*b*).
† From Tables 2 and 3. Values for BR 54, 'Brown', SSK 52 and 'Yellow' are averages of results for diets with and without L-lysine hydrochloride.
‡ From Table 4. Results for Expts C2 and C3 were averaged.

by the faecal analysis procedure, with laying hens given mixed diets containing equal amounts (700 g/kg) of the test barleys, which differed in N content. We used the ileal analysis procedure, with broiler chickens given isonitrogenous diets in which the barley was the only protein source. But it remains for further investigation to explain the discrepancy, and settle the question whether the tannin in barley may have any important influence on protein digestibility.

Over all, the results of these experiments support those obtained in microbiological tests with *Streptococcus zymogenes* (Ford & Hewitt, 1979*a*, *b*). Table 10 compares the results for the sorghums. The microbiological assay values for available methionine and for RNV showed clear differences between high- and low-tannin varieties, broadly similar to the differences in N digestibility revealed in the chick and rat tests. Indeed, for high-tannin samples the microbiological assays gave a true and unequivocal verdict on the nutritional quality, unlike the NPU values which were influenced by misleadingly high BV. The effect of PEG 4000 in neutralizing the influence of tannin was as pronounced in the biological tests as in the microbiological assays. With the low-tannin varieties SSK 52 and 'Yellow', protein digestibility was high, as were the availabilities of methionine and the RNV, and these results were not influenced by PEG 4000. With the high-tannin varieties X 3101, BR 54 and 'Brown', protein digestibility was low, as also were available methionine and RNV, and all three measures of protein quality were markedly increased by PEG 4000. There was some inconsistency between the microbiological and biological assays on these high-tannin varieties; the biological tests conducted in absence of PEG 4000 indicated a variation in the digestibility values with tannin content that was not apparent in the microbiological assay results. However, in view of the small number of varieties assayed biologically, it is not possible to assess the significance of this discrepancy.

With field beans the presence of tannin in seed of the coloured flowered varieties was associated with a small but significant depression in the average content of available methionine, measured microbiologically. Treatment with PEG 4000 increased the content to equal that in the seed of tannin-free varieties (Ford & Hewitt, 1979*b*). The general picture was entirely consistent with the results of the experiments now reported with rats and chickens. Similarly with barleys, the present results support the indications from microbiological tests (Ford & Hewitt, 1979*a*) that the small content of tannin had no adverse nutritional effect.

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