The quality of protein in various lines of peas*

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1. The protein quality as evaluated by nitrogen incorporation efficiency (NIE) and protein efficiency ratio (PER) was determined for twenty-eight breeding lines of peas (*Pisum sativum* L.) which had been grown under similar field conditions.

2. Different lines of peas, when given as the sole source of protein to weanling rats at a 10% level in an otherwise adequate diet, varied from 18 to 78% of that of casein in their ability to support growth and nitrogen retention. There was close correlation between PER and NIE values.

3. From analysis of rat growth curves, the pea lines were separated into those that produced fairly good growth and those that barely maintained the initial body-weight. However, growth rate alone did not rank pea lines in the same order as PER or NIE.

4. Carcass protein, as a percentage of body-weight, was higher in pea-fed (20·4%) than in casein-fed rats (18·8%). This was probably associated with a difference in body fat content.

5. There was no correlation between protein quality and the protein content of the different pea lines.

Legumes are an important source of protein for the general population in developing countries and for special groups in the more affluent countries. In many areas of the world, seeds are the major source of dietary protein for most of the people. Vegetarians, in the more affluent countries, rely on legumes as one of their primary sources of proteins. Legumes are well suited for this purpose, especially in tropical climates, since in the dry state they can be stored for long periods and can be transported and prepared for consumption with a minimum amount of equipment.

Although India is the biggest consumer of legumes (FAO, 1957) with an intake per head of 60 g/d, the inhabitants of the United States and the United Kingdom consume a fairly large quantity of legumes (approximately 11 g/head). In the United States, peas are used in baby foods which, reportedly, also are consumed by the aged.

Most of the genetic work with peas has been directed towards improving their appearance, yield, resistance to disease, and freezing and canning qualities. The little work that has been done on the nutritional value of peas has been limited to increasing their nitrogen content (Pesola, 1955; Esh, De & Basu, 1959). Almost no work has been reported on the biological quality of legume proteins from different varieties of seeds.

New interest arose in improving the nutritional value of seed proteins when Mertz and his collaborators (Mertz, Bates & Nelson, 1964; Mertz, Vernon, Bates & Nelson, 1965; Mertz, Mosse, Dimler & Nelson, 1966) showed that the protein quality of maize

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could be improved to such an extent that it approached or equalled that of casein. This resulted from a threefold increase in the lysine and tryptophan content.

The study now described was conducted to evaluate the protein quality of twenty-eight lines of peas. It was hoped thereby to find peas with large quantities of high-quality protein. These lines could then be used for breeding purposes.

EXPERIMENTAL

The peas were grown at the Horticulture Research Centre, East Lansing in 1966. The strains used in this breeding programme were derived from crosses involving the varieties: Early Perfection, Miragreen, Miracle, Jade and Green Seed Perfection; foreign plant introductions 167250, 169604, 173059 and 175232; and breeding lines 56–118 and 56–184. More detailed information about the specific crosses involved in each line can be obtained from one of the authors (L. R. B.). Rainfall was adequate for growth. The peas were dried on the vine and later harvested by hand. The seeds were stored at 4° and low humidity (25–50 RH) until used. Alaska peas, a commercial variety (Gallatin Valley Seed Co., Twin Falls, Idaho, lot number 54401) grown and harvested under standard conditions of commercial seed production were used as a standard of reference.

The peas were ground in a Wiley mill until the powder passed through a 20 mesh screen. All samples were analysed for nitrogen by the Kjeldahl method (Association of Official Agricultural Chemists, 1965). The nitrogen value was multiplied by a factor of 6·25 (Hegsted, 1964) to facilitate the comparison of the nitrogenous fraction of peas with casein (vitamin-free assay protein secured from General Biochemicals, Chagrin Falls, Ohio), the reference standard in the biological assays.

For the biological assays, either the pea meal from each line or casein was added, at the expense of sucrose, to the basal diet composed of: maize oil 5 %; salt mixture 4 % (Wesson's modification of the Osborne–Mendel salt mixture; Nutritional Biochemicals Corporation, Cleveland, Ohio); vitamin mixture 2·2 % (Nutritional Biochemicals); Alpha cel (Nutritional Biochemicals) 2 %; sucrose at a level that brings the total to 100 %. Each test sample was incorporated into the ration to provide 10 % protein.

For 1 week before starting the experimental diets, weanling male Sprague Dawley rats were fed on a diet composed of: ground maize 60·7 %; soya-bean meal (50 % protein) 28 %; lucerne (17 % protein) 2 %; fish meal (60 % protein) 2·5 %; dried whey (67 % lactose) 2·5 %; limestone (38 % calcium) 1·6 %; dicalcium phosphate (18·5 % P, 22–25 % Ca) 1·75 %; iodized salt 0·5 %. Five rats were assigned to each group in such a way that the group weights did not vary by more than 3 g. Since twenty-eight lines of peas could not be assayed simultaneously, three separate experiments were performed. Casein was repeated in every experiment, and certain pea lines were assayed two or three times. Food and water were provided *ad lib*. Food intakes and weight gains were measured every week for 3 weeks.

One extra group of five rats in each assay was killed at the start of the experiment (body composition controls). The carcass (minus the gastro-intestinal contents) of each of these rats was autoclaved, homogenized and a sample analysed for nitrogen

(Mickelsen & Anderson, 1959). The remaining rats were killed at the end of the 3-week experimental period and the carcasses treated in the same manner. The nitrogen retention was calculated from the body nitrogen content of the test animals less that in the body composition controls.

Protein quality was calculated as protein efficiency ratio (PER)

$$\text{PER} = \text{weight gain (g)/g protein eaten,} \tag{1}$$

and nitrogen incorporation efficiency (NIE)

$$\text{NIE} = \frac{\text{nitrogen retained in the carcass} \times 100}{\text{nitrogen intake}}. \tag{2}$$

RESULTS AND DISCUSSION

The protein content based on nitrogen values (N × 6·25) of vine-dried peas varied from 21 to 28 % (Table 1). However, the total protein content of different strains of peas as measured by their nitrogen content may be a poor index of their protein quality. This was evident when it was observed that the protein in certain strains of peas gave PER values that were as much as five times that given by another strain which had the same nitrogen content (Table 1). Nitrogen content may not be an accurate estimate of true protein, as different strains of peas may contain different quantities of non-protein nitrogen (NPN). The NPN may contain peptides and amino acids which are nutritional equivalents of protein, as well as other nitrogen-containing compounds which cannot be utilized by monogastric animals.

The highest PER value obtained with a strain of peas was nearly 80 % of the mean value for casein. That this is not due to abnormally low values for casein is evident from the agreement of our PER values for casein with reported values (Morrison, 1964). The explanation for the similarity in PER values probably is that, although the rats fed on the best pea rations grew at only half the rate of those receiving casein, they also consumed considerably less food. The generally lower food intakes and lower weight gains of the pea-fed rats did not reflect consistent reductions in appetite (in relation to body size) since the appetite quotients calculated according to Carpenter (1953) were essentially the same for all groups (Table 1).

The PER of the same sample when assayed more than once remained fairly constant (Table 2). The SD for PER was 0·12 and for NIE was 2·12, i.e. about 7 % of the means in each instance. Similar results have been reported by other workers (Chapman, Castillo & Campbell, 1959; Campbell, 1963; Jansen, 1962). The variation was relatively high for the samples with low PER values, whereas for those with high values the deviation in PER for the same line in different experiments was surprisingly low.

NIE (Stucki & Harper, 1962) is a measure of the efficiency with which the animal converts dietary nitrogen into carcass protein. As such, its magnitude is determined by the weight gain and carcass composition of the animal. Since the ratio of weight gain to food intake is determined by PER, the important factor in differentiating NIE from PER is the percentage of protein in the carcass. The percentage of protein in the carcass of rats given diets containing certain lines of peas was significantly higher

($P < 0·05$) than that in rats given diets containing casein (Table 1). This difference suggests that the rats given these pea diets did not have as much fat in their carcasses as those fed on casein.

Table 1. *Protein quality of different pea lines as determined by biological assay with rats. The peas are arranged in decreasing order of nitrogen incorporation efficiency*

Protein source†	Experimental series‡	Protein ($N \times 6·25$) in test sample (%)	NIE§	PER§	Protein in carcass (%)	Weight gain of rats in 3 weeks (g)	Appetite quotient $\times 10^{-3}$
Casein	1, 2, 3	87·5	52·3	2·8	18·8	75·6	149
31	1	26·7	44·4	2·2	20·2	37·1	113
24	2, 3	26·3	41·2	2·0	20·1	35·2	120
4	2	25·5	39·5	2·1	18·7	39·2	123
23	2	24·8	37·7	1·8	20·0	35·8	134
15	3	26·7	37·5	1·8	20·2	34·8	133
7	1, 2, 3	27·2	36·8	1·8	20·4	42·6	152
26	3	25·1	35·7	1·7	20·2	35·2	141
17	1	27·7	34·4	1·6	20·4	32·0	139
34	1	28·5	34·3	1·8	18·5	38·2	141
20	1	23·5	33·7	1·6	20·2	27·8	125
33	2, 3	26·9	33·6	1·8	18·6	36·6	137
12	2	22·9	33·6	1·5	21·6*	37·4	167
14	2, 3	26·9	33·6	1·7	19·8	35·8	148
10	3	28·1	32·6	1·6	20·2	20·4	159
1	1	24·4	32·5	1·4	22·6*	33·8	166
28	2, 3	21·2	32·3	1·6	19·5	37·1	155
19	1	25·2	31·5	1·5	20·1	24·8	122
11	1	28·2	30·9	1·4	21·1*	34·6	169
6	3	25·4	30·5	1·4	21·8*	33·8	165
13	2	28·5	28·1	1·4	18·8	28·8	142
16	2	28·3	27·1	1·3	20·3	27·2	157
27	3	23·6	26·6	1·2	20·8	22·0	137
8	1	27·2	26·3	1·1	22·7*	33·0	207*
2	1	24·4	25·9	1·2	20·2	31·6	184
38	2	28·2	25·5	1·2	19·9	13·0	126
3	1	25·9	16·1	0·7	21·2*	23·4	248*
Alaska	1, 2, 3	22·9	13·6	0·6	21·5*	13·0	153
5	1, 2, 3	25·8	9·6	0·4	21·6*	7·7	162

* Significantly different from casein ($P < 0·05$). (Values are asterisked only in the columns for 'Protein in carcass' and 'Appetite quotient'.)

† These numbers are codes for the different strains of peas. Specific information about them can be secured from one of the authors (L. R. B.).

‡ Three separate assays were carred out, the number(s) indicate the assay in which the sample was included.

§ NIE, nitrogen incorporation efficiency; PER, protein efficiency ratio.

Both NIE and PER appeared to be equally effective measurements for evaluating the protein quality of peas. This is evident in the high correlation coefficient (r = 0·90) between them. Equally high correlations between net protein utilization and PER have been reported by Henry (1965). Thus, peas can be ranked equally effectively on the basis of PER or NIE, but not on the basis of weight gain alone (Table 1). There appears, therefore, to be no significant return from the extra work of carcass nitrogen analysis which is required for the determination of NIE but not of PER.

From rat growth curves (Fig. 1), the different pea lines can be designated as supporting (1) fairly good growth, (2) mediocre growth or (3) bare maintenance of initial weight. Rats given poor-quality diets usually showed an initial loss of weight which was regained later.

Table 2. *Variations in protein quality of pea proteins as determined by protein efficiency ratio* (PER) *and nitrogen incorporation efficiency* (NIE) *when the assays were repeated on the same sample*

Source of protein*	April 1968 Expt 1		June 1968 Expt 2		August 1968 Expt 3	
	PER	NIE	PER	NIE	PER	NIE
Casein	2·67	56·0	2·89	50·2	2·80	51·0
5	0·25	9·6	0·67	7·6	0·46	11·8
7	1·80	34·9	1·79	36·6	1·74	39·1
14	—	—	1·68	35·2	1·72	32·1
33	—	—	1·82	35·2	1·78	32·7
24	—	—	2·00	39·1	2·10	43·5

* These numbers are codes for the different strains of peas. Specific information about them can be secured from one of the authors (L. R. B.).

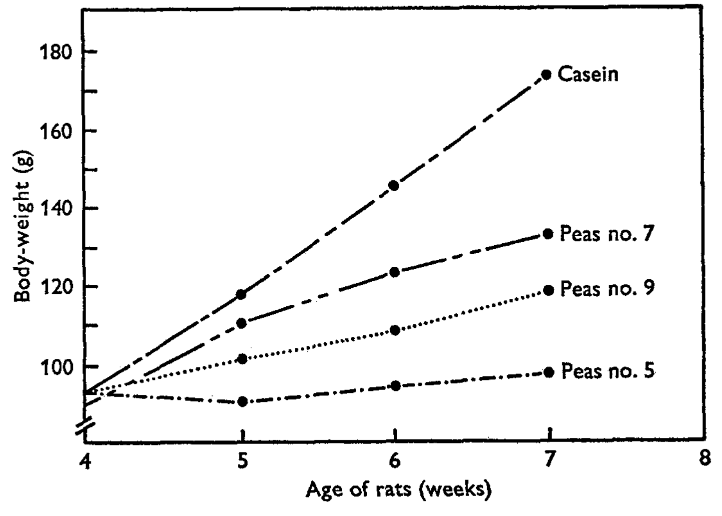

Fig. 1. Growth curves for groups of rats receiving diets containing casein or peas as the sole source of protein. The samples of peas used for this figure represented the lines of high (no. 7) medium (no. 9) and low (no. 5) protein quality.

The observation that several lines of peas (24, 15, 7, 34) contain protein of higher nutritional value than others, suggests that it may be possible to improve this character even further through appropriate breeding methods. The shortage of protein in diets of large groups of people should be a strong incentive for including the improvement of protein quality in plant breeding programmes.

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