The Relative Nutritive Value, Amino Acid Content, and Digestibility of the Proteins of Wheat Mill Fractions[1]

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ABSTRACT

The mill fractions obtained by standard milling procedures from a single sample of wheat–germ, wheat protein concentrate, red dog, bran, shorts, patent flour, and clear flour–have been analyzed for total amino acid content, and the digestibility (*in vitro*) and relative nutritive value (RNV) of the proteins have been determined. The RNV varies from a high of 80% for germ and wheat protein concentrate to a low of approximately 25% for patent and clear flour. The RNV of these products correlates highly with the lysine content of the proteins, and better still when corrections for digestibility are applied. Lysine supplementation improved the nutritional value in all products with the exception of germ and wheat protein concentrate. Supplemented patent and clear flour had RNVs between 50 and 60%. A chemical score based upon the essential amino acid content of egg protein does not appear to identify the most limiting amino acid in several of the proteins, nor predict the RNV.

There is an abundant literature related to the nutritional properties of the proteins of cereal and cereal products. Coons (1) has prepared a bibliography containing over 500 references on this subject. Much of the data, however, are fragmentary. In this paper we present a comparison of the relative nutritive value (RNV) of the proteins, as evaluated with the young rat (2,3), and the amino acid composition of seven mill fractions obtained from a single sample of wheat. The biological assays were also done after supplementation of all fractions with lysine. The effect of digestibility on the correlation of RNV with amino acid composition is described and the implications of chemical scores based upon amino acid composition of egg protein (4) are discussed.

MATERIALS AND METHODS

The various wheat fractions examined were prepared from a single sample of hard red spring wheat by standard milling procedures.[3] The protein content in all samples has been expressed as N X 6.25. Similarly, all amino acid contents are expressed as the amount of amino acid per 16 g. of nitrogen. The amino acid content has been determined by the procedures described by Kohler and Palter (5) and Knox et al. (6). Digestibility or protein availability has been determined by the *in vitro* methods utilizing digestion with pronase followed by digestion with chick pancreas acetone powder as described by Kohler et al. (7). The limited availability of energy and protein in some cereal products is in large part accounted for by the

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fact that intact aleurone cells are refractory to digestion and these cells are also not digested in the *in vitro* method. In calculating "available amino acids," it has been assumed that the biological availability of each amino acid is limited to the same degree as the "*in vitro*" digestibility of the total protein.

The biological determination of the RNV of the proteins was determined as described by Hegsted et al. (2,3,8,9). In each of the experiments, 29 groups of young male rats weighing approximately 50 g. were used. Three groups of 6 animals each received diets containing 3 different levels of lactalbumin, which is the standard protein and is assumed to be 100% utilized. Three groups of 5 animals each received diets containing 3 levels of each of the wheat fractions studied, and 1 group of 6 animals received a protein-free diet. The levels of protein provided by the diets tested are shown in Table I. Food consumption was measured and the protein intake of each animal over the 3-week experimental period was calculated. At the end of the experimental period, the animals were killed and the total body water determined by drying the carcasses at 95°C.

The RNV is defined as the slope of the dose-response curve of the protein under test divided by the slope of the dose-response curve obtained with the standard protein, lactalbumin. Body-weight gain in the 3-week experimental period and the body water of the animals at the end of the experiment have been used as measures of "response." The dose-response curves have been calculated by the computer program previously described (3). Figure 1 provides an example of the regression line obtained with the animals fed lactalbumin and that obtained with animals fed the diets in which the protein was supplied by clear flour supplemented with lysine. The slope of the lactalbumin line is 5.10 g. gain per gram of protein eaten and the slope of the second line is 2.56 g. gain per gram protein eaten. The clear flour plus lysine thus has 50% of the potency of the lactalbumin.

TABLE I. BODY WATER AND WEIGHT GAIN OF ANIMALS FED VARIOUS LEVELS AND SOURCES OF PROTEINS

Protein Source	Dietary Protein Level %	Protein Eaten g.	Body Water g.	Weight Gain g.	Protein Source	Dietary Protein Level %	Protein Eaten g.	Body Water g.	Weight Gain g.
Lactalbumin	2.49	3.3	35.1	+04.0	Bran	4.30	5.6	36.4	+00.4
	4.98	7.8	50.6	+29.0		8.60	17.7	59.8	+40.6
	7.47	14.1	68.9	+56.0		12.90	31.2	70.2	+56.8
Wheat germ	2.78	3.5	34.6	−01.6	Whole wheat	4.67	5.7	36.4	+02.6
	5.56	10.9	55.5	+33.0		9.34	14.1	44.0	+14.8
	8.33	23.8	79.4	+75.2		14.00	25.7	57.0	+35.4
Wheat protein concentrate	4.11	6.7	44.8	+17.4	Patent flour	4.03	4.7	34.2	00.0
	8.22	19.5	71.7	+64.0		8.06	10.2	37.1	+02.8
	12.33	30.3	88.6	+80.8		12.09	17.4	42.4	+12.8
Red dog	4.35	5.6	38.5	+05.2	Clear flour	4.79	5.4	33.2	−02.2
	8.70	20.4	64.8	+55.0		9.58	14.0	41.9	+12.8
	13.05	35.4	85.5	+82.0		14.36	23.6	47.0	+19.0
Shorts	4.93	5.8	31.1	+07.8	No protein	0	0	25.6	−14.8
	9.85	24.7	73.1	+67.0					
	14.78	40.0	88.6	+81.2					

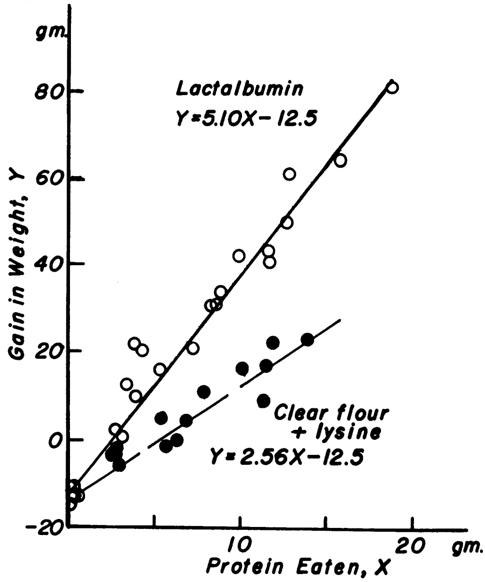

Fig. 1. Gain in weight vs. protein eaten for animals fed varying amounts of lactalbumin and clear flour supplemented with lysine. The slope of the latter line, 2.56, is approximately 50% that of the lactalbumin line, 5.10; and the RNV is thus 50%.

The diets used have also been previously described. They contained (in %): hydrogenated cottonseed oil, 9.5; cod liver oil, 0.5; salt mixture (10), 5; vitamin mixture (11), 0.5; choline chloride, 0.2. The remainder of the diet consisted of the protein source and corn starch.

The original experiments indicated that lysine was the likely limiting amino acid in most of the wheat fractions. All samples were then reassayed after addition of lysine hydrochloride to each sample in sufficient amount to raise the total lysine content of the protein in each sample to 6%.

RESULTS

Table I shows the levels of protein provided by the various diets fed and the protein intake, the mean body water, and the mean weight gain of each group of animals from which the RNV was calculated. However, as has been indicated above and demonstrated in Fig. 1, the calculations are based upon the values of individual animals rather than the three mean values shown in Table I. Table II provides similar data for the second experiment, in which the same samples were examined after the addition of lysine.

Table III presents the RNV calculated using either body water or weight gain as the measure of the response in both experiments, together with the standard error of the value presented. The mean value plus or minus 2 standard deviations is the 95% confidence limits of the determination. It may be seen that comparable values are obtained with either measure of response, and the errors are also of similar magnitude. The samples have been arranged in decreasing order of RNV, and it may

TABLE II. BODY WATER AND WEIGHT GAIN OF ANIMALS FED VARIOUS LEVELS AND SOURCES OF PROTEINS SUPPLEMENTED WITH LYSINE

Protein Source	Dietary Protein Level %	Protein Eaten g.	Body Water g.	Weight Gain g.	Protein Source	Dietary Protein Level %	Protein Eaten g.	Body Water g.	Weight Gain g.
Lactalbumin	2.49	3.5	37.5	+11.3	Bran	2.75	2.8	29.4	−05.2
	4.98	8.2	50.2	+29.2		5.50	8.4	38.4	+10.4
	7.47	13.9	66.0	+56.7		8.26	16.7	55.9	+36.8
Wheat germ	2.78	3.3	31.4	+02.2	Whole wheat	2.78	2.6	30.3	−03.8
	5.56	9.0	48.2	+24.8		5.56	6.7	38.3	+03.8
	8.33	19.0	70.4	+65.2		8.33	13.2	50.3	+25.6
Wheat protein concentrate	2.74	2.4	31.4	−03.8	Patent flour	2.78	2.4	31.0	−04.2
	5.48	7.5	43.7	+17.2		5.56	6.2	37.6	+04.2
	8.22	17.3	65.5	+52.6		8.33	12.9	49.4	+20.8
Red dog	2.78	3.0	33.9	−05.6	Clear flour	2.81	2.7	32.1	−03.8
	5.56	7.1	40.6	+08.2		5.61	6.5	37.3	+03.6
	8.33	14.5	55.0	+38.8		8.33	11.8	46.6	+17.4
Shorts	2.76	2.9	30.7	−06.0	No protein	0	0	29.9	−12.5
	5.52	8.0	42.9	+12.8					
	8.27	18.3	62.6	+49.2					

TABLE III. RELATIVE NUTRITIVE VALUE WITH AND WITHOUT LYSINE SUPPLEMENTS CALCULATED USING WEIGHT GAIN AND BODY WATER AS MEASURES OF RESPONSE

	Relative Nutritive Value[a]			
	Unsupplemented		With Lysine Supplement	
Sample	Based on weight gain	Based on body water	Based on weight gain	Based on body water
Wheat germ	80±5	79±3	80±3	80±4
Wheat protein concentrate	72±4	75±3	75±3	78±4
Red dog	60±3	60±3	68±3	72±4
Shorts	55±3	57±2	65±3	69±4
Bran	49±3	51±2	56±3	59±4
Whole wheat	36±3	39±2	57±3	63±4
Patent flour	23±4	26±3	51±3	63±5
Clear flour	25±3	27±2	50±3	61±5

[a]Relative nutritive value is expressed as percentage of the nutritive value of lactalbumin in each experiment ± the standard error. The mean value plus or minus 2 standard errors gives the 95% confidence limits of each value.

TABLE IV. AMINO ACID COMPOSITION OF WHEAT MILL FRACTIONS

Amino Acid	Clear Flour	Patent Flour	Whole Wheat	Red Dog	Bran	Shorts	Wheat Protein Concentrate	Wheat Germ
				g. amino acid/16 g. N				
Lysine	1.88	1.95	2.47	3.78	4.10	4.21	4.59	4.92
Histidine	2.07	2.10	2.11	2.48	2.74	2.57	2.49	2.38
Ammonia	4.08	4.18	3.73	2.85	2.66	2.47	2.81	2.42
Arginine	3.73	3.61	4.41	6.40	7.05	7.16	7.39	6.93
Tryptophan	1.11	1.10	1.28	1.38	1.92	1.53	1.35	1.28
Aspartic acid	3.77	3.92	4.75	6.39	7.17	7.10	7.16	7.30
Threonine	2.62	2.67	2.82	3.24	3.29	3.31	3.49	3.52
Serine	4.51	4.62	4.53	4.42	4.35	4.25	4.42	4.22
Glutamic acid	35.18	35.37	31.13	22.62	18.54	17.86	20.70	17.13
Proline	11.61	11.24	9.73	7.29	5.63	5.53	5.84	5.23
Glycine	3.41	3.39	3.91	4.82	5.72	5.40	5.15	5.39
Alanine	2.90	2.84	3.30	4.40	4.80	4.68	5.03	5.21
Valine	4.53	4.58	4.88	5.16	5.17	5.18	5.40	5.22
Isoleucine	4.08	4.30	4.01	3.83	3.63	3.61	3.83	3.68
Leucine	6.78	6.78	6.54	6.30	5.98	5.96	6.26	6.04
Tyrosine	2.93	3.11	2.89	2.69	2.66	2.66	2.78	2.58
Phenylalanine	4.99	5.08	4.73	4.29	3.91	3.81	4.02	3.78
Cystine	2.76	2.64	2.44	2.36	2.29	2.24	2.21	1.88
Methionine	1.88	1.97	1.89	1.92	1.74	1.81	2.13	1.89
% N Recovered	96.90	97.50	95.00	92.90	92.00	89.60	95.10	89.20
% N Sample	3.50	2.68	3.00	3.10	3.09	3.53	4.84	4.45

TABLE V. TOTAL PROTEIN CONTENT AND DIGESTIBILITY OF PROTEIN IN WHEAT FRACTIONS

Wheat Fraction	Protein Content	Digestibility	Essential Amino Acids		Lysine		Threonine	
			Total	Available	Total	Available	Total	Available
	%	%	% of total amino acids		g./16 g. N		g./16 g. N	
Wheat germ	25.25	91.6	43.5	39.8	4.92	4.51	3.52	3.22
Wheat protein concentrate	27.40	96.5	42.2	40.7	4.59	4.43	3.49	3.37
Red dog	17.40	94.1	40.1	37.7	3.78	3.56	3.31	3.11
Shorts	19.70	83.9	42.9	36.0	4.21	3.53	3.29	2.76
Bran	17.20	69.4	42.3	29.4	4.10	2.84	3.24	2.25
Whole wheat	16.67	91.0	28.9	26.3	2.47	2.25	2.82	2.57
Patent flour	14.39	99+	31.0	31.0	1.95	1.95	2.67	2.67
Clear flour	19.15	99+	32.1	32.1	1.88	1.88	2.62	2.62

be seen that lysine supplementation improved the nutritive value for all samples with the exception of germ and the shorts flour.

Table IV presents the amino acid content of each of the samples tested. Table V presents the percent protein in these samples (N X 6.25) and the percentage of protein apparently digestible as measured by the *in vitro* test of Kohler et al. (7). Also, for reasons that will be subsequently apparent, we have included here the content of total essential amino acids in these samples expressed as a percentage of the total amino acid content, the lysine and threonine contents, and the percentage of "available" essential amino acids, lysine and threonine. These latter figures are, of course, the total lysine or threonine content multiplied by the digestibility of the protein.

DISCUSSION

As has been discussed in recent papers (12,13,14), none of the methods currently available for the evaluation of protein quality are entirely satisfactory. The measurement of net protein utilization (NPU) and biological value (BV) have generally been thought to be the most satisfactory methods in the past, but these methods are based upon somewhat erroneous concepts (13,14). They are particularly unsatisfactory for proteins which are low in lysine. The measurement of RNV is also not ideal but more nearly approaches a satisfactory biological assay. The measurements of NPU and BV appear to overestimate the nutritive value for growth of many proteins, particularly those limiting in lysine. Thus, the RNV of patent and clear flour reported here is approximately 25% compared to lactalbumin, and is much less than the usual NPU or BV of approximately 40% reported in the literature (15).

The nutritional qualities of the proteins in wheat fractions vary widely, as has long been known. Those in wheat germ approach the values of the many animal proteins. Hegsted and Chang (2), for example, reported the RNV of casein to be 74%, and this compares to the values of approximately 80% for germ and the wheat protein concentrate. At the other extreme, as would be expected, are the patent and clear flours which yield values of approximately 25%.

According to the generally accepted theory (15,16), the nutritive value of proteins is presumed to be determined by the extent of the deficiency of the single essential amino acid present in lowest amount relative to the need. Egg protein has commonly been used as the ideal amino acid pattern since it is known to be efficiently utilized. In Table VI the chemical score for each essential amino acid is presented, this being the amount of that amino acid present in the protein expressed as a percentage of the amount in egg protein. The amino acid composition of egg protein recently published by FAO (4) has been used in these calculations. The amino acid presenting the lowest value is presumed to be "most

TABLE VI. CHEMICAL SCORE OF THE ESSENTIAL AMINO ACIDS FOUND IN WHEAT FRACTIONS

Amino Acid	Clear Flour	Patent Flour	Whole Wheat	Red Dog	Bran	Shorts	Wheat Protein Concentrate	Wheat Germ
Isoleucine	65	68	63	61	58[b]	57[b]	61[a]	59[b]
Leucine	77	77	74	71	68	68	71	69
Lysine	27[a]	28[a]	35[a]	54[a]	59	60	66	71
Methionine	56	56	53[b]	57[b]	52[a]	54[a]	63[b]	53[a]
Phenylalanine	87	89	83	75	68	67	70	66
Threonine	51[b]	52[b]	55	63	64	65	68	69
Tryptophan	75	74	86	93	129	103	91	86
Valine	66	67	71	75	75	76	79	77

[a]Most limiting amino acid.

[b]Second most limiting amino acid.

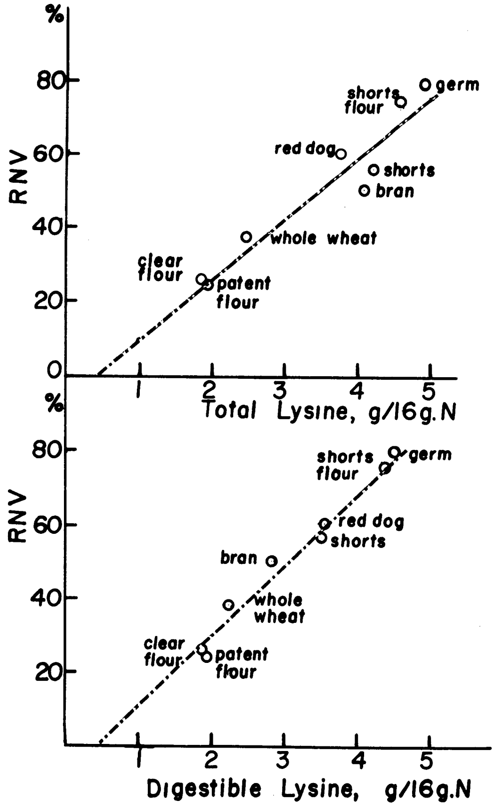

Fig. 2 (top). Data showing the close correlation between the lysine content of the various fractions and their RNV. (bottom). When the lysine content is corrected for digestibility the fit is improved, particularly for bran and shorts, which contain relatively large numbers of intact aleurone cells.

limiting" and this is indicated by superscript a; the second most limiting amino acid is identified as superscript b.

These calculations identify lysine as the most limiting amino acid in the four proteins of lowest nutritional value–clear flour, patent flour, whole wheat, and red dog–and this is confirmed by the biological tests showing a response in nutritive value when lysine is added to these proteins. For bran and shorts, methionine is identified by the chemical score as most limiting. This is unlikely, since lysine supplementation improved the nutritional value of these proteins and these proteins can be assumed to be limiting in lysine also.

Figure 2 shows that for all of the fractions tested there is a high degree of correlation between the lysine content and the RNV. Bran and shorts appear to fit rather poorly. However, when digestible or available lysine is calculated, the fit is generally improved (Fig. 2, bottom).

The second most limiting amino acid is indicated by the calculation of chemical score to be threonine for the two flours, and either isoleucine or methionine for the remainder of the samples. It appears doubtful that either isoleucine or methionine are the second most limiting amino acids since the content of these amino acids does not appear to vary sufficiently to explain the differences in RNV after lysine supplementation. There is, however, a rather high degree of correlation between the RNV after lysine supplementation and the threonine content, especially after correction for digestibility (Table V). This appears the most likely candidate as the second most limiting amino acid in all samples. However, the percentage of the total amino acids present as essential amino acids in these samples also varies approximately as the RNV after lysine supplementation (Table V). Thus, the data do not permit a decision between these two most likely possibilities.

Moran et al. (17) have reported the NPU values and other measures of protein utilization as determined with chicks in several samples of bran, germ, red dog, and shorts. Various combinations of amino acid supplements lead them to conclude that the first, second, and third limiting amino acids in these fractions were as follows: red dog–lysine, isoleucine, and methionine; bran–isoleucine, methionine, and lysine; germ–isoleucine, methionine, and tryptophan; shorts–isoleucine, methionine, and lysine. The reasons for the discrepancy between these conclusions and our own are not clear. Species' differences in amino acid requirements may be involved as well as differences in the composition of the samples tested. However, we are uncertain whether the data presented are adequate to definitely define the sequence of limiting amino acids.

The same laboratory (18,19) has evaluated the digestibility and NPU of the same samples with rats as well as several other samples of bran, shorts, and midds. Many of these samples yielded digestibility figures substantially below the values reported here for the *in vitro* digestibility test. The close correlation between "available lysine" and RNV shown in Fig. 1 at least suggests that the digestibility values are relatively satisfactory, whether the absolute values are or not.

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