

Protein Studies.

Plant Proteins II.—The Biological Values of Lucernemeal Sesamemeal, Peanutmeal, Coprameal, Cottonseedmeal, and Oatmeal.

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THE conception of biological values of proteins was first introduced by Thomas, who defined biological value as the number of parts of body nitrogen replaced by 100 parts of food protein. His technique, adapted to human beings, was subsequently modified and elaborated by Mitchell (1924) and Boas Fixsen and Jackson (1932) for application to rats, and forms at present the basis of measuring the biological value of proteins by the nitrogen balance sheet method. Other methods of estimating the biological or nutritive values of proteins have also been devised and applied [Osborne, Mendel and Ferry (1919) and McCollum and co-workers (1921)]. These methods, however, accept as criteria either maintenance of nitrogen equilibrium or maintenance or increase in bodyweight. Several objections, such as differences in food intake, duration of experiment, and the omission of an allowance for the maintenance quota, have been raised against the latter methods, and it has been pointed out that the significance of results obtained under these conditions is in many instances difficult to assess. In their reviews on the biological values of proteins, Mitchell and Hamilton (1929), as well as Boas Fixsen (1935), have scrutinized and fully discussed these objections.

It appears from an analysis of the methods concerned with the determination of the biological value of proteins, that the balance-sheet method is the only one which discriminates clearly between absorbed and ingested nitrogen and consequently is not influenced in any way by differences in digestibility of the proteins. The biological value obtained by this method is a measure of the maximum percentage of absorbed nitrogen utilized in anabolism and can therefore, not be vitiated by differences in food intake as is the case where increase in weight is the sole criterion. In fact, the

general tendency is to regard the biological value of each protein or each protein mixture at a definite level of intake as a constant. This assumption is based on the generally accepted theory that the body's demand for tissue synthesis involves a rather constant proportion of the different amino acids. However, different proteins may differ appreciably in their respective biological values. This fact has been amply demonstrated since the time Rubner (1897) formulated the hypothesis that all proteins were not of the same value in nutrition. This difference in biological value does not only exist between different proteins, but also in the same protein depending on the level it is fed and the purpose it is destined for in the body.

The difference in biological value amongst different proteins is directly attributable to the varied amino acid constitution of the respective protein molecules, the difference at different levels of feeding being presumably due to the excess of amino acids being drawn into the oxidative processes of the body and catabolized, while biological differences of the same protein for different functions may be determined by the needs of the body for simple nitrogenous compounds on the one hand (maintenance) and a more complete assortment of amino acids on the other hand (growth).

It seems reasonable to explain the biological differences between proteins on the basis that proteins differ chemically in their proportions and amounts of indispensable amino acids. Some proteins may lack certain of these amino acids while others may be deficient in some other or a different combination of indispensable amino acids. A deficiency of one of these amino acids may limit partially or totally the rest of the amino acids in the protein molecule for growth. When two or more of these indispensable amino acids are lacking the utilization of the rest amino acids may be further impaired. Mitchell and Smuts (1932) have shown that when maize, which is deficient in both lysine and tryptophane is supplemented with lysine only, a small improvement in growth is obtained. When Tryptophane alone is fed in conjunction with maize no improvement in growth was noticed. If, however, both lysine and tryptophane are added to a maize ration significant increase in weight resulted. The more nearly, therefore, a protein approaches in composition the average amino acid mixture for the purpose it is intended for, the higher is its biological value. A perfect protein would be one which when ingested in amounts equal to or less than the output of nitrogen on a nitrogen free diet occasions no rise in the nitrogen output, thus showing that none of the amino acids present in the protein has been wasted.

It seems very improbable that the biological value of proteins for maintenance would be the same as that for growth or milk production. According to Mitchell (1929), whose theory appears to coincide best with the known facts, the minimum endogenous nitrogen (Maintenance) does not involve the disintegration of proteins, but is related entirely to the metabolism of non-protein nitrogenous constituents of the tissues. If such is the case it can readily be appreciated that incomplete mixtures of amino acids or incomplete proteins or even ammonium salts can be partially utilized to cover

the maintenance requirement of nitrogen, but can obviously not be utilized for growth. Hence it is apparent that some proteins may exhibit higher biological values for maintenance than for growth, since amino acid deficiencies may not limit protein utilization for maintenance, but certainly does for growth. In the latter case a deficiency of one of the indispensable amino acids may block synthesis of new tissues, with the result that dianinization of the amino acids takes place and a lowered biological value is obtained.

It is, therefore, evident that representative values of protein or mixture of proteins can be obtained only when the biological value is determined for each at different levels of intake and for the different functions in the body. However, it appears fairly hopeless to separate these functions to such an extent that the biological value of the proteins used can be assessed separately, since the physiological functions, such as growth and milk production, are superimposed on maintenance. Consequently the residual amino acids, which are not used for maintaining the integrity of the tissues, such as possibly lysine and cystine may enhance the biological value for growth. Therefore it becomes necessary to relate the nitrogen retained for both maintenance and growth to the nitrogen absorbed. In this publication, biological values give a composite measure of both maintenance and growth.

The biological determinations reported in this paper are based exclusively on the nitrogen balance sheet method and consequently reference will be made only to work carried out on the same basis. Nevens (1921) working with rats obtained a biological value of 62 for lucernemeal at approximately 11 per cent. level, while Satola (1930) on lambs found a value of 56. Mitchell (1936) in comparing biological values of raw and roasted peanuts found that raw peanut was slightly superior and showed a biological value of 57.8 at an 8 per cent. level in rats in comparison to 55.7 for the roasted product. Piang (1930) obtained a value for peanut closely approximating that obtained by Mitchell, namely 59.0. However, Holdaway, Ellett and Harris (1925) assigned values of 84, 78 and 77 to peanutmeal, cottonseedmeal and soyabeanmeal respectively for lactation. On the other hand, Morris and Wright (1933) for the same function obtained a biological value of peanutmeal of 50. In the latter case peanutmeal was supplemented by appreciable amounts of oats. Hence the biological value reported for peanutmeal is actually a supplemented biological value of peanutmeal plus oats.

Nevens (1921) in his detailed study with rats on the nutritive value of cottonseedmeal obtained a biological value of 66 at a level of approximately 11 per cent. Mitchell and Hamilton (1931) in a comparison of linseedmeal and cottonseedmeal in pigs at a 9 per cent. level found the biological value of the latter to be 63. On the other hand Braman (1931) from Mitchell's laboratory reported a value of 78 for cottonseedmeal at an 8 per cent. level of feeding. In a determination of the biological values of proteins at different levels Mitchell (1924) obtained values of 79 and 65 respectively for oats at a 5 and 10 per cent. level.

EXPERIMENTAL.

Rats were used as experimental animals. All determinations were carried out according to the method of Mitchell except that only one biological value was determined on a series of six rats. The nitrogen low period was conducted either prior to or after the protein period. In several cases nitrogen low periods were run before as well as after the protein period. The data, however, did not show any significant variation and consequently it was decided to run nitrogen low periods either before or after the period of protein feeding. Six-day periods were allowed on a nitrogen low ration for rats to adjust themselves to a constant nitrogen excretion. Rats were kept for the same length of time on the protein ration before collection started. Collection periods were of seven days' duration. Urine was collected in acid, and the daily faeces digested by the usual method. The week's digests of faeces were made up to known volume and aliquots distilled for nitrogen determination. The urine collected over the period was made up to a known volume and aliquots analysed for nitrogen, correction being made in the end volume for daily addition of acid. Rations were made up so as to contain approximately 9 per cent. of protein. Analyses, in duplicate, were made of all rations after mixing and before placing them in the icebox. In all cases the nitrogen low rations were equalized as far as possible in fibre content to the protein ration. The protein feeds tested were of the same nature as that fed under practical conditions except oats, which was a prepared product put out by a commercial company as a breakfast food.

RESULTS.

The nitrogen metabolism data as well as the calculation of the biological values are given in Table 2. The standardizing periods on the Nitrogen low ration preceded the protein feeding period in all cases except in the case of coprameal where the nitrogen low period was conducted after the protein period. The purpose of the nitrogen low periods is to determine the excretion of metabolic nitrogen in faeces per gram food consumed and of the endogenous urinary nitrogen per 100 grams bodyweight. These figures are assumed to be the same under protein administration and are, therefore, applied in the calculation of biological values as factors for estimating the contribution of the body of the nitrogen appearing in the faeces and the urine.

The accepted constancy of the endogenous nitrogen is naturally an important factor in the determination of biological values. The work of Smuts (1935) has provided additional evidence in confirmation of the constancy of the endogenous nitrogen by showing that a close correlation exists between the basal heat production and the endogenous nitrogen not only within species but among different species varying greatly in size. This finding would indicate that the constancy of the endogenous nitrogen is as well established as the basal heat production. Consequently any increase or decrease of the endogenous nitrogen must be regarded as an effect of either internal or external factors, which would influence the basal metabolism in a similar manner.

With regard to the metabolic faecal nitrogen, Schneider (1935) has shown that a portion is of body origin and that this portion may make up a considerable fraction of the total metabolic faecal nitrogen below maintenance. However, for levels of food intake usually employed in the determination of biological values, this fraction becomes insignificant in comparison with that related to the dry matter consumed. Consequently no correction for the portion related to the bodyweight was attempted and the entire metabolic faecal nitrogen was related to food consumed.

The biological value of lucernemeal was determined in two cases. Although there existed a difference of 4·5 per cent. nitrogen between the two determinations, the biological values do not differ significantly. In the first instance the individual biological values varied from 55 to 66 with an average value of $60 \pm 1\cdot09$ and a coefficient of variation of 6 per cent., while in the second case the variation between individual values was from 60 to 63 with an average value of $61 \pm 1\cdot463$ and a coefficient of variation of 2·3 per cent. There is a slight tendency for a higher biological value at the lower level of nitrogen feeding, but this difference is by no means significant. These values agree fairly well with the value of 62 obtained for rats by Nevens at a 10 per cent. level. The value of 56 for lucernemeal obtained by Satola for lambs at approximately the same level is in close agreement with the values obtained on rats.

No significant difference is manifested in the biological values of Peanutmeal, Sesamemeal and Coprameal, the values being $72 \pm 1\cdot19$, $71 \pm 1\cdot03$ and $69 \pm 1\cdot15$ respectively, with coefficients of variation of 5·5 per cent., 4·8 per cent. and 5·5 per cent. The absence of differences between the biological values of these proteins agrees very well with the results obtained from growth studies conducted on these proteins in an earlier paper by Smuts (1937). Mitchell's biological value of 57 and Piang's value of 59 for peanutmeal at approximately 8 per cent. level is much lower than the value reported in this paper. Holdaway Ellett and Harris, however, obtained a biological value of 84 for peanutmeal for milk production.

The biological values of oats and cottonseedmeal are distinctly superior to that of peanutmeal, copra and sesame meal, but do not show any difference between themselves. The biological value of oats is $84 \pm 1\cdot79$ with a coefficient of variations of 1·9 per cent. and that of cottonseedmeal $81 \pm 1\cdot93$ with a variability of 2·6 per cent. The latter value agrees well with the value of 79 obtained for cottonseedmeal by Braman at an 8 per cent. level. According to the biological value obtained by Mitchell of 79 for oats at 5 per cent. level, the value reported here is higher. Since this oat product is precooked as a breakfast food, it may easily account for the higher biological value. Hayward and Steenbock (1936) found similar results with heat-treated Soyabean in comparison with the raw product.

It would appear from the respective biological values that the proteins of oatmeal and cottonseedmeal are superior to that of sesamemeal, peanutmeal and coprameal and that the latter are again

superior to that of lucernemeal. However, such differences can best be established statistically. By analysing the variance of the entire data it is found that the variance between groups is significantly greater than the variation within groups, hence there exists a real difference between groups. The standard deviation as calculated from the variance within groups and the resulting coefficients of variation are found to be S.D.=3·070 and C of V=4·32 per cent. This indicates that the variability of individual determinations within groups is extremely small and compares favourably with the variability obtained in other biological determinations (Basal metabolism). The standard error of a mean of 6 biological values is given by $3\cdot07 \div \sqrt{6}$, i.e. 1·25, and this would allow the prediction of successive average biological values, with approximately 5 per cent. probability, to fall in the range of $\pm 2\cdot50$.

When the group means are considered it is found that significance between two means can be indicated by the following differences and probability. If a difference between means of 5 and 6 determinations respectively is greater than 5·07 the probability is less than 1 per cent. On the other hand, if the difference between two means of 6 determinations each is greater than 3·60 the probability of the difference is less than 5 per cent., but when the difference is 4·83 or exceeds it the probability of the difference becomes 1 per cent. or less. On the basis of these figures Table 3 has been constructed and compares the mean differences of the biological values of the different proteins. Black type indicate a highly significant difference while the ordinary type signifies no statistical differences of the average biological values.

From this table it is clear that the proteins of oatmeal and cottonseedmeal are superior to the rest of the proteins tested but do not differ significantly between themselves. Peanutmeal, coprameal and sesamemeal must, statistically speaking, be regarded as being utilized to the same extent in metabolism and significantly better than lucernemeal.

From Table 4 it will be seen that there is very little difference between the apparent and true digestibility of peanutmeal, sesame-meal, coprameal and cottonseedmeal. On this basis it must be assumed that the rat was able to utilize more of the absorbed nitrogen derived from cottonseedmeal than from the other three sources of protein since it has a higher biological value. The apparent digestibility of oat protein is only 78·8 per cent. while if the body's contribution of faecal nitrogen be deducted the digestibility becomes 100 per cent., which would indicate that the protein of this particular oats is fully digested. The apparent and true digestibility of lucernemeal is much lower and would therefore partially account for its lower nutritive value in comparison with the other proteins.

CONCLUSIONS.

1. By means of nitrogen metabolism studies on rats the biological values were determined for oatmeal, cottonseedmeal, peanutmeal, sesamemeal, coprameal and lucernemeal. The values obtained for the respective proteins were 84, 81, 72, 71, 69, 60 and 61.

2. It is concluded on the basis of statistical analysis that the proteins of oatmeal and cottonseedmeal are equivalent in nutritive value for growth and maintenance, but superior to that of peanut, sesamemeal and coprameal. No difference was found between the proteins of peanut, sesamemeal and coprameal, which again are significantly superior to the proteins of lucernemeal.

3. The true digestibility of peanutmeal, sesamemeal, coprameal and cottonseedmeal was of about the same order, being 90, 92, 89 and 92 per cent., while that of lucernemeal was distinctly lower, namely 74 per cent. The protein of oatmeal appears to be entirely digested.

ACKNOWLEDGEMENT.

The authors are indebted to Mr. A. P. Malan, for the statistical treatment of the results.

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TABLE I.

Composition of Rations.

Ingredients.	A.	B.	C.	D.	E.	F.	G.
Peanut Meal.....	15.0	—	—	—	—	—	—
Lucerne Meal.....	—	57.5	—	—	—	—	—
Sesame Meal.....	—	—	25.0	—	—	—	—
Copra Meal.....	—	—	—	33.0	—	—	—
Cotton-seed Meal.....	—	—	—	—	22.5	—	—
Oatmeal.....	—	—	—	—	—	72.7	—
Sucrose.....	10.0	10.0	10.0	10.0	10.0	6.8	10.0
Butterfat.....	8.0	8.0	8.0	8.0	8.0	6.0	8.0
Yeast Extract (1).....	10.0	10.0	10.0	10.0	10.0	8.0	10.0
Ether Ext. Egg White	—	—	—	—	—	—	3.8
Agar (2).....	—	—	—	—	—	—	2.0
Code liver Oil.....	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NaCl.....	1.0	1.0	1.0	1.0	1.0	—	1.0
Salt Mixture (3).....	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Starch.....	49.5	7.0	39.5	31.5	42.0	—	58.7
Percent Nitrogen...	1.36	1.55	1.51	1.51	1.50	1.42	1.654

(1) Yeast extract was prepared according to the method of (Itters, Orent E.R. and McCollum E. V. (J.B.C. 108-2-571-577-1935).

(2) The agar was raised to 14 per cent. in the nitrogen low ration for the determination of the biological value of lucerne meal.

(3) A modified Osborne and Mendel salt mixture prepared by P. B. Hawk and B. L. Oser (1931 Science Vol. 74, p. 369).

TABLE 2.
Nitrogen Metabolism Data and the Calculations of the Biological Values of Different Proteins.

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	N-Low Period for LUCERNE RATIONS CONTAINING .654 PER CENT. NITROGEN.		LUCERNE RATION CONTAINING 1.55 PER CENT. NITROGEN.		PEANUT RATION CONTAINING .654 PER CENT. N.		PEANUT RATION CONTAINING 1.36 PER CENT. NITROGEN.				
				Daily Food Intake, gm.	Daily N. Intake, mgm.	Daily Fecal N., mgm.	Daily N. Intake, mgm.	Body N. in Feces, Per Day, mgm.	Food N. in Feces, Per Day, mgm.	Absorbed N., mgm.	Daily Urinary N., mgm.	Body N. in Urine, Per 100 gms.	Food N. in Urine, mgm.	Food N. Retained, mgm.
19.....	63.0	67.0	65.0	55.7	—	18.31	3.21	—	—	14.98	23.05	—	—	—
20.....	82.0	88.0	85.0	80.0	—	21.60	2.51	—	—	17.28	20.33	—	—	—
21.....	73.0	73.0	73.0	73.0	—	19.13	3.00	—	—	16.70	22.88	—	—	—
22.....	76.0	78.0	77.0	6.3	—	20.37	3.23	—	—	18.46	24.00	—	—	—
23.....	70.0	64.0	66.5	54.4	—	18.93	3.50	—	—	16.70	25.11	—	—	—
24.....	64.0	63.0	64.0	53.6	—	18.12	3.24	—	—	17.28	27.00	—	—	—
19.....	74.0	82.0	78.0	8.6	133.3	58.40	3.21	27.61	30.79	102.51	61.32	23.05	43.34	59.17
20.....	94.0	97.0	95.5	9.3	144.2	60.50	2.57	23.90	36.60	107.60	63.67	20.33	19.42	44.25
21.....	84.0	91.0	87.5	9.3	144.2	56.79	3.00	27.90	28.89	115.31	65.48	22.88	19.92	45.56
22.....	86.0	91.0	88.5	9.3	144.2	67.80	3.23	30.04	37.76	106.44	57.53	24.00	21.24	36.28
23.....	74.0	82.0	78.0	8.0	124.0	65.70	3.50	28.00	37.70	86.30	55.11	25.11	19.59	38.81
24.....	73.0	80.0	75.0	9.3	144.2	70.12	3.24	30.13	39.99	104.21	60.73	27.00	20.25	40.48
														Average,
														60

TABLE 2—(continued).

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	N-Low PERIOD FOR LUCERNE RATION CONTAINING 1-10 PER CENT. N.			Lucerne RATION CONTAINING 1-10 PER CENT. NITROGEN.			Lucerne RATION CONTAINING 1-10 PER CENT. NITROGEN.			OATMEAL RATION CONTAINING 1-42 PER CENT. NITROGEN.		
				Daily Food Intake, gm.	Daily N. Intake, mgm.	Daily Fecal N., mgm.	Body N. in Feeces, Per Day, mgm.	Food N. in Feeces, mgm.	Ab-sorbed N., mgm.	Daily Urinary N., mgm.	Per 100 gms.	Body N. in Urine, Per Day, mgm.	Food N. in Urine, mgm.	Bio-logical Value.	
8.....	52.0	52.0	52.0	52.0	4.2	—	9.81	2.09	—	12.60	24.35	—	—	—	
9.....	52.0	52.0	52.0	52.0	4.7	—	10.00	2.13	—	11.50	22.12	—	—	—	
10.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
11.....	50.0	50.0	50.0	50.0	4.9	—	8.42	1.72	—	12.60	24.00	—	—	—	
12.....	50.0	50.0	50.0	50.0	4.6	—	10.30	2.24	—	10.90	21.80	—	—	—	
13.....	50.0	50.0	50.0	50.0	5.6	—	10.42	1.86	—	7.80	15.30	—	—	—	
Average															
42.....	134.0	134.0	134.0	134.0	—	—	20.48	3.68	—	—	—	20.90	15.60	—	
43.....	111.0	105.0	108.0	—	—	—	26.70	4.05	—	—	—	22.00	20.37	—	
44.....	109.0	105.0	107.0	—	—	—	20.68	3.28	—	—	—	28.60	26.73	—	
45.....	120.0	115.0	117.5	—	—	—	16.94	2.82	—	—	—	27.72	23.59	—	
46.....	109.0	105.0	107.0	—	—	—	19.80	3.30	—	—	—	20.46	18.79	—	
47.....	127.0	125.0	126.0	—	—	—	33.44	4.18	—	—	—	23.76	18.86	—	
Average															
42.....	137.0	144.0	140.5	8.0	113.60	26.18	3.68	29.44	0.0	113.60	39.01	15.60	21.92	17.09	
43.....	115.0	122.0	118.5	8.0	113.60	23.61	4.05	32.40	0.0	113.60	44.39	20.37	24.14	20.25	
44.....	120.0	124.0	122.0	8.0	113.60	24.33	3.28	26.24	0.0	113.60	50.30	26.73	32.61	17.69	
45.....	125.0	135.0	130.0	8.0	113.60	22.59	2.82	22.56	0.03	113.57	47.23	23.59	30.67	16.56	
46.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
47.....	135.0	137.0	136.0	8.0	113.60	25.67	4.18	33.44	0.0	113.60	41.10	18.86	25.65	15.45	
Average															

TABLE 2—(continued).

TABLE 2—(continued).

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TABLE 3.
Statistical Analysis of Difference between Means.

	Oatmeal,	Cotton Seed Meal,	Peanut Meal,	Sesame Meal,	Copra Meal,	Lucerne Meal,
Oatmeal.....	—	3·57	12·73	13·90	15·23	23·20
Cotton Seed Meal.....	—	—	9·16	10·33	11·66	19·63
Peanut Meal.....	—	—	—	1·17	2·50	10·44
Sesame Meal.....	—	—	—	—	1·35	9·30
Copra Meal.....	—	—	—	—	—	7·97
Lucerne Meal	—	—	—	—	—	—

TABLE 4.
The Digestibility of the Nitrogen in Lucernemeal, Peanutmeal, Oatmeal, Sesamemeal, Cottonseedmeal and Coprameal.

Rat No.	Lucerne Meal,			Peanut Meal,			Lucerne Meal,			Oatmeal,		
	Apparent Digestibility.	True Digestibility.	Rat No.	Apparent Digestibility.	True Digestibility.	Rat No.	Apparent Digestibility.	True Digestibility.	Rat No.	Apparent Digestibility.	True Digestibility.	
19	55·3	76·9	1	76·3	94·6	8	52·5	71·5	42	77·0	100·0	
20	58·0	74·6	2	73·6	86·1	9	5·17	71·1	43	79·2	100·0	
21	60·6	79·7	3	74·5	88·0	10	—	—	44	80·3	100·0	
22	66·9	73·8	4	75·6	90·0	11	58·5	74·1	45	80·1	100·0	
23	47·8	69·6	5	80·2	96·2	12	61·3	78·0	46	—	—	
24	51·4	72·3	6	68·5	84·6	13	60·3	77·2	47	77·4	100·0	
Ave.	56·7	74·5		74·8	89·9		56·9	74·4		78·8	100·0	

Rat No.	Sesame Meal,		Rat No.	Cotton Seed Meal,		Rat No.	Copra Meal,	
	Apparent Digestibility.	True Digestibility.		Apparent Digestibility.	True Digestibility.		Apparent Digestibility.	True Digestibility.
101	79·2	96·7	1	72·0	90·9	7	66·7	90·5
102	76·2	90·1	2	71·8	86·0	8	75·1	92·2
103	74·3	92·5	3	71·6	91·7	9	70·3	86·0
104	76·5	93·0	4	73·3	95·2	10	65·7	86·8
105	72·2	86·3	5	73·3	92·2	11	72·5	88·7
106	82·2	91·4	6	75·6	94·4	12	75·1	90·8
Average	76·8	91·7		72·9	91·7		70·9	89·2