PROTEIN QUALITY OF FOODS. I.
Evaluation of Biological and Biochemical Parameters for Protein Quality Assessment

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ABSTRACT

Biological and biochemical parameters were evaluated to assess protein quality. Some of the parameters are already well-established such as true digestibiljty, biological value and net protein utilization; others are still being used tentatively such as total urine nitrogen (Nt), urine urea nitrogen (Nu), ratio of Nu to Nt (nu/Nt), serum urea, serum total protein and liveweight gain; one was devised in the study which is the ratio of retained nitrogen to absorbed nitrogen (Nu/nt). The efficiency of the parameters to evaluate protein quality was studied by using egg albumin, a good quality protein, and zein, a poor quality protein. Both protein sources were incorporated in the diet at different levels. The parameters used gave a clear indication of the quality of the protein studied, although they were much affected by the protein level in the diet and the total protein intake. Standard protein level and protein intake are then suggested as necessary so that the parameters can be more efficiently used as protein quality evaluators.

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KEY WORDS: Protein quality. Biological parameter. Biochemical parameter. Egg albumin. Zein. Diets. Assessment.

INTRODUCTION

Protein quality evaluation aims to determine the usefulness of a protein to support life and production. Usefulness is largely governed by the amino acid profile, the availability of the amino acid profile, the availability of the amino acids and the efficiency by which these

available amino acids are incorporated into body proteins.

The problem of protein quality evaluation can be approached from a simple chemical analysis to more complex chemical procedures using a biological system. Each approach has its advantages and disadvantages and each procedure has some limitations. The most basic measurements are obviously those of total nitrogen and true protein nitrogen, but the resulting data add little contribution to the understanding of protein quality.

Amino acid composition may be determined by either microbiological or chromatographic assay. These methods involve initial hydrolysis of protein during which several amino acids may be totally or partially destroyed. Thus, although it indicates the maximum amount of a particular amino acid present, the final analysis does not necessarily truly reflect the amino acid make-up of the original protein. The resulting amino acid profile, while serving as a valuable guide to protein quality, does not indicate the biological usefulness of the individual amino acid.

The biological approach may be divided generally into (a) those methods based upon growth, as for example, the protein efficiency ratio (PER), and (b) an even greater number based upon some aspects of nitrogen balance, as for example, biological value (BV), net protein utilization (NPU) and others. More recently, certain biochemical parameters have been suggested as indices of protein quality, i.e., blood protein level, blood urea level and number of urine metabolites.

This article presents the use of the chosen parameters to evaluate protein quality under a range of dietary conditions.

MATERIALS AND METHODS

Experimental Animals. — Three-week–old Wistar albino rats in groups of 6 were fed a standard diet (Bruce and Parkes 41B) for about 2 weeks to attain 90-120 g bodyweight before they were used in the experiment. They were then weighed and distributed individually to metabolism cages.

The animals were placed under controlled room temperature (20°c) and lighting in the small-animal laboratory of the Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne.

Diets. — The diets were formulated in such a way that if possible the only variables were quality and/or quantity of protein. The detailed formulation of the diets along with the dry matter and protein contents are shown in Tables 1 and 2. The result of the first experiment showed that the diet did not greatly affect the values of the selected parameters when albumin, or a good quality protein, was used. Therefore, a further study on protein level was made, increasing the albumin levels to 14% and 20% to find out whether such levels give a lower protein value.

Table 1. Detailed formulation (g/kg, air dry basis) and dry matter and protein composition of the albumin and zein diets fed to rats.

Constituents	Albumin (%)					Zein (%)			
	1	4	6	8	10	4	6	8	10
Maize starch	610	580	560	540	520	580	560	540	520
Cellulose	80	80	80	80	80	80	80	80	80
Glucose	160	160	160	160	160	160	160	160	160
Maize oil	100	100	100	100	100	100	100	100	100
Min-Mix (1)	20	20	20	20	20	20	20	20	
Vit-Mix (2)	20	20	20	20	20	20	20	20	20
Egg albumin	10	40	60	80	100	—	—	—	—
Zein	—	—	—	—	—	40	60	80	100
Dry matter, %	90.70	90.75	90.80	90.87	90.87	89.87	90.12	90.19	90.36
Crude Protein, % (as fed)	1.10	3.58	5.00	6.70	8.63	3.70	5.45	7.12	8.99

(1) Mineral mix. 100g containing: Starch, 7.77g: $CaCO_3$, 27.27g: KH_2PO_4, 31.82g: NaCl, 23.09g: $MgSO_4$ $7H_2O$, 9.27g: $FeSO_4$ $7H_2O$, 4.53g: $CuSO_4$ $5H_2O$, 0.14g: $MnSO_4$ $4H_2O$, 0.15g: $ZnSO_4$ $7H_20$, 0.03g: KIO_3, 0.0005g.

(2) Vitamin mix, 151.8g containing: Vitamin A, 4.5g: Vitamin D, 0.25g: alpha-tocopherol, 1.5g: Ascorbic acid, 45g: Inositol, 5g: Choline chloride, 75g: menadione, 5g: para-amine benzoic acid, 5g. Niacin, 4.5g: Riboflavin, 1.0g: Pyridoxine HCl, 20mg: Folic acid, 90mgm: Vitamin B12, 1.35 mg: (I.C.N. Pharmaceuticals Inc., Life Sciences group, Cleveland, Ohio)

Management of Feeding Trial. — The rats were offered daily with 9 g of feed which was weighed into feeding dishes and moistened with water before they were given. Water was made available to the animals at all times.

The first 7 days served as a preliminary period to enable the rats to adjust to the diet and also to allow the diet under study to replace the previous feed thus making sure that the result of the experiment was due to the diet alone.

The balance study started immediately after the preliminary period and lasted for 6 days, during which data for the calculation of true digestibility (TD), BV and NPU were taken. Later, the balance period was shortened to 5 days to accommodate another experimental period. The rats were under controlled feeding (9 g daily) during the first 5 days and were under *ad libitum* feeding during the second 5 days.

Collection of Samples. — At the end of the preliminary period, the animals were re-weighed and returned to their respective cages which were now fitted with urine and feces separators. Ten ml of 0.5 m sulphuric acid were added to each urine collector.

Chemical Analysis. — Serum and urine urea were determined using the test combination prepared by Boehringer Mannheim BmbH. Urine and fecal nitrogen were determined using the Kjeldahl method (Hamilton and Simpson, 1964).

Biological Methods. — True digestibility (TD), biological value (BV) and net protein utilization (NPU) were calculated by using the following standard formula:

$$N \text{ absorbed} = I - (F - M)$$

$$N \text{ retained} = I - (F - M) - (U - E)$$

$$TD\ (\%) = \frac{I - (F - M)}{I} \times 100$$

Table 2. Detailed formulation (g/kg, air dry basis) and dry matter and protein composition of the albumin diets fed to rats.

	ALBUMIN (%)				
Constituents	**2**	**4**	**8**	**14**	**20**
Maize starch	600	580	540	480	420
Cellulose	80	80	80	80	80
Glucose	160	160	160	160	160
Maize oil	100	100	100	100	100
Vit-Mix	20	20	20	20	20
Min-Mix	20	20	20	20	20
Egg albumin	20	40	80	140	200
Dry matter, %	91.31	91.60	92.30	91.70	92.50
Crude Protein, % (as fed)	1.71	3.36	6.25	11.30	16.15

$$BV\,(\%) = \frac{I - (F - M) - (U - E)}{I - (F - M)} \times 100$$

$$NPU = BV \times TD$$

Where:

I = mg of nitrogen intake
F = mg of nitrogen in the feces
M = mg of metabolic fecal nitrogen (MFN)
U = mg of nitrogen in the urine
E = mg of endogenous urinary nitrogen (EUN)

EUN is dependent upon metabolic body size, and this bodyweight is in kg. It is calculated by dividing the total nitrogen in the urine by the metabolic body size of each rat fed the 1 or 2% albumin.

MFN is dependent upon the amount of dry matter consumed. It is calculated by dividing the nitrogen in the feces by the dry matter consumed (g) for each rat on the 1 or 2% albumin diet.

Liveweight Gain (LWG). — The LWG is calculated by subtracting from the final liveweight the initial liveweight taken during the balance period.

RESULTS AND DISCUSSION

Albumin and Zein as Protein Sources.

Results justify the choice of egg albumin and zein as good and poor quality protein sources, respectively (Tables 3 and 4). The nitrogen of egg albumin was well-digested and absorbed while that of zein was poorly digested and absorbed. These are well-reflected on the TD values calculated from the rats fed the two diets. Also, the rats fed the albumin diets have higher nitrogen retention and lower nitrogen excretion as shown by the higher values of BV, NPU, LWG and serum protein; lower values of serum urea, Nu, Nt, Nu/Nt; and lower values of serum urea, Nu, Nt, Nu/Na as compared to the values

Table 3. True digestibility (TD), biological value (BV), the net protein utilization (NPU), urine and serum nitrogen data and liveweight gain (LWG) in rats receiving albumin and zein diets at increasing levels of protein.

Protein Source	Dietary Level (%)	TD (%)	BV	NPU	URINE Nu mgN/6 days	URINE Nt mgN/6 days	Nu/Nt	Nu/Na	SERUM Urea mg/100 cm³	SERUM Total Protein g/100cm³	LWG g/6 days
Albumin	4	99.83a	99.11a	98.92a	45.8f	118.8e	38.49	18.21d	10.87cd	4.41b	1.4b
	6	98.10a	96.67a	94.82a	76.6e	134.0e	57.16	20.83d	8.37d	5.05a	1.0b
	8	98.21a	96.97a	95.23a	53.8ef	140.8de	38.16	10.47d	9.05d	4.60b	1.8ba
	10	98.48a	93.39a	91.97a	72.0ef	174.8cd	41.14	10.63d	9.12cd	5.10a	2.6a
Zein	4	66.30cd	49.67b	33.04b	102.0d	205.0c	49.76	71.56b	13.35c	3.79e	-3.2.2d
	6	75.90b	45.72b	34.75b	160.4c	284.8b	56.28	59.77c	28.46b	3.94cd	-4.0d
	8	71.50bc	31.31c	22.28c	216.0b	396.4a	54.55	61.20cb	34.06a	4.08c	-3.6d
	10	64.66d	5.80d	3.96d	277.2a	433.2a	63.58	96.10a	30.89ab	3.81d	-2.0c
F-Value		51.51	137.55	261.23	82.91	109.02	7.82	57.56	35.04	21.07	12.84
SE		2.49	5.53	5.97	13.11	18.74	NS	5.14	2.11	.12	.49
LSD		5.01	11.17	12.05	26.49	37.85	NS	10.38	4.26	.25	.99

Means followed by a common letter are not significantly different at 5% level using DMRT.

obtained from rats fed the zein diets.

Actual values for TD and BV are slightly higher in egg albumin and slightly lower in zein when compared to the compiled data of the Food and Agriculture Organization (1970). FAO has 93.3 TD and 90.3 BV for egg albumin, and 85-90 TD and 55-62 BV for corn protein. However, as is commonly observed and as pointed out by Eggum (1973), egg protein is completely utilized at low dietary protein levels and gives a value of 100 for both TD and BV. This observation applies to the present experiment.

The dramatic fall in BV at the 10% zein level highlights the problem of assessing highly unbalanced protein. Flipot, Belzile and Brisson (1970) attributed the inferiority of zein to amino acid imbalance which has been commonly observed to result in loss of appetite. Such loss in appetite eventually results to growth failure and loss of body protein which, in turn, may account for the very low BV value.

Most of the more tentative parameters (Nu, Nt, Nu/Nt) and the devised parameter (Nu/Na) are correlated with the well-established parameter (BV) in terms of nitrogen retention. The following correlation coefficients were obtained: (a) -0.95 between BV and Nu and between BV and Nt; (b) -.77 for Nu/Nt; and (c) -0.97 for Nu/Na. The serum data also gave correlation coefficients of -0.90 and 0.80 between BV and serum urea and serum protein, respectively.

Albumin Level and Method of Feeding.

The results showed that increasing the albumin level in the diet resulted in significant ($P>0.95$) variation in the values of the parameters especially at 20% albumin level, except for TD which significantly retained its uniform values. This conforms with report of Kiriyama (1970) where rats fed with 25%

Table 4. True digestibility (TD), biological value (BV), net protein utilization (NPU), the liveweight gain (LWG) and urine and serum nitrogen data in rats receiving albumin diets at increasing levels of protein and two methods of feeding.

Manner of Feeding	Dietary Level (%)	TD (%)	BV	NPU	URINE Nu mgN/5 days	URINE Nt mgN/5 days	Nu/Nt	Nu/Na	SERUM Urea mg/100 cm^3	SERUM Total Protein g/100cm^3	LWG g/5 days
Controlled	4	97.59d	101.79a	99.41a	65.80e	93.80e	68.39d	31.46c			1.6f
	8	98.50cd	99.97ab	98.45a	62.68e	102.19e	61.38e	15.53e			5.0e
	14	99.49bc	90.80c	90.34b	161.80d	180.80d	88.71b	21.95d			13.4d
	20	98.82cd	68.85e	68.02d	435.42b	447.48b	97.32a	40.98b			14.4cd
Ad libitum	4	102.36a	96.00b	98.25a	74.17e	101.05e	71.80d	22.71d	9.53c	5.26c	18.4c
	8	101.57a	97.06ab	98.59a	99.53e	121.14de	82.62c	13.11e	12.08c	6.53b	39.6a
	14	100.20b	79.89d	80.07c	289.44c	321.62c	88.86b	27.50c	19.17b	7.17a	37.2a
	20	99.03bc	59.28f	58.72e	744.87a	753.92a	98.63a	47.31a	28.52a	6.17b	31.8b
F-Value		6.30	43.20	34.84	66.47	67.16	11.78	22.58	5.48	6.72	63.36
SE		.63	2.50	2.54	37.84	36.56	2.50	2.02	2.40	.20	2.33
LSD		1.27	5.06	5.14	76.44	73.86	5.06	4.04	5.09	.43	4.71

Means followed by a common letter are not significantly different at 5% level using DMRT.

casein diet excreted significantly high Nt, Nu and Nu/Nt. Also, a negative correlation between BV and protein content of the diet beyond 10% level was observed by some workers (Forbes, Vaughn and Yoke, 1958).

The increase in intake during *ad libitum* feeding further altered significantly ($P>0.05$) the values of the parameters. Due to the accumulated errors in the N balance, a TD of 100 was obtained. Significant increases in TD were observed at all protein levels in the diet. This is contrary to the observation of Schneider and Flatt (1975) that greatest deterioration in the feeding value of the protein was observed at the highest level (20% albumin) when the animals showed signs of general weakness and loss of appetite towards the end of the experiment. This may be explained by the report of Rechcigl (1978) on the effect of high protein where he showed that high protein intake damages the liver and the kidney due to protein overload.

A high correlation between the BV and the other parameters (Nu, Nt, Nu/Na serum urea) existed (v0.81 correlation coefficient), except in the cases of serum total protein and LWG. The very low correlations may be attributed to the fact that serum total protein and LWG are related more to the total nitrogen retained rather than to the efficiency of nitrogen retention.

The results of these experiments give valuable information on the assessment of protein quality, especially of poor protein sources. Although the parameters selected clearly indicated protein quality, still the protein level in the diet, as well as the total intake, greatly affected the values of the parameters, obscuring their value as protein quality evaluators. Therefore, a standard level should be fixed, such level selected so that there should be no excretion of extra nitrogen.

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