PROTEIN EFFICIENCY RATIO PITFALLS AND CAUSES OF VARIABILITY: A REVIEW

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ABSTRACT

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Presented herein is a review of the many factors, other than essential amino acid content, which can influence the results obtained with the rat protein efficiency ratio (PER) evaluations as presently conducted. Some of the causes of variation are due to the procedures specified by the Association of Official Analytical Chemists (AOAC) method, while others are associated with the types of food products now being evaluated for protein quality. The factors considered are 1) animal quality; 2) strain of rats; 3) acclimation period; 4) diet instability; 5) nutritional balancing of diets; 6) diet hydration; 7) high moisture samples; 8) diet selection; and 9) food samples with usual nutrient contents. The many potential problems emphasize the need for clearer specification for handling food samples for PER evaluations.

The protein efficiency ratio (PER) assay is one of several methods available for measuring the quality of protein in foods. The assay can be used with any actively growing animal but, as generally used, it is a 4-week assay with rats using the procedure as specified by the Association of Official Analytical Chemists (AOAC) (1). Protein quality can be defined as the ability of a protein to supply the essential amino acid requirements of the animal. The correlation of responses by different species of animals to the same proteins—particularly the response of the rat as it correlates with the human population—still needs to be defined.

The PER assay has become the major protein quality test in the food industry due to USDA (2) and FDA (3) specification of this assay for compliance with nutritional labeling and governmental food programs. This has placed severe problems on the test procedure since it has become an all-purpose assay for food products for which the AOAC test was not originally designed. Handling of products whose composition falls outside the AOAC procedures is not defined and allows considerable variability within and between laboratories. Without care in handling and interpreting these results, unrealistic and conflicting values for protein quality can be obtained. In addition, the PER assay procedure has some inherent potential for producing variable results, which will be discussed in detail later in this review.

The PER value is calculated by the equation:

$$\text{PER} = \text{grams weight gain} \div \text{grams protein consumed}$$

In all tests, a casein control group is included and the value of the test protein is reported relative to the casein group. By long history, the PER value of the test protein may be reported relative to casein corrected to a 2.5 PER.

$$\text{Corrected PER Test Protein} = \frac{\text{PER Test Protein}}{\text{PER Casein Control}} \times 2.5$$

In conducting a PER assay, the performance of the control group is as critical to the evaluation as the test treatment, since the results are reported as relative values rather than absolute values.

In any animal testing procedure, the quality of the animals used will determine the results obtained. The health of the weanling rats used will influence rat growth and, thereby, PER results and variability within the test. Animals which are stressed in shipment from the breeding source to the test location should not be used for testing. Our experience has shown that a sample of weanling rats should be examined for disease problems on arrival in the laboratory before the experiments are initiated. This is particularly critical when limited or expensive samples which would be difficult to replace are being tested.

The strain of rats used for testing does not appear to influence PER results, as shown by Morrison and Campbell (4), in comparing casein, whole egg, wheat flour, and wheat-soy flour as protein sources with two strains of rats (Table I). A similar evaluation in our laboratory comparing two strains of rats divided in five groups of ten rats each with casein protein gave mean PER values of 3.29 and 3.34, even though the mean weight gains differed by 28 g over the 28-day feeding period. Rats of the same strain and shipment should be used within each experiment to ensure reliable results. However, the strain of rats does not appear to be a factor in the variability of PER results if the rats are healthy.

The AOAC procedure (1) specifies a 3- to 7-day acclimation period in the laboratory prior to starting the experiments. In most instances, this appears to be unnecessary, but occasionally, we have obtained an effect of acclimation time on PER results. The effect of holding weanling rats 24, 72, or 144 hr on a 10% protein casein diet prior to starting the PER assay is given in Table II. Both weight gain and PER values were improved as the acclimation time increased. The effect appeared to be greater with the soybean protein test diet than with the casein control group. The starting weights indicate that the rats lost weight in the 24 and 72 hr period and gained very little between the 72 and 144 hr holding period. This suggests either a possible shipping effect on the rats or slow adaption to the laboratory facilities and diet which required a period of time to overcome before normal growth was resumed. Attempts to duplicate these observations with subsequent groups of rats have demonstrated no effect on PER values beyond the 24-hr acclimation period. The acclimation period in most studies is unnecessary except to ensure proper adaption for the occasional group of weanling rats which do not start normally.

TABLE I
Effect of Rat Strain on PER[a]

	Strain B		Strain C	
Protein Source	**Uncorrected**	**Corrected**	**Uncorrected**	**Corrected**
Casein	3.35	2.50	3.18	2.50
Dried whole egg	4.00	2.99	4.00	3.14
Wheat-soy flour	2.64	2.00	2.56	2.01
Whole wheat flour	1.44	1.07	1.50	1.18

[a]Morrison and Campbell. J. Nutr. 70: 112 (1960).

The PER diet specifies a precise nutrient content and method of substitution (Table III). This formulation applies to products which have protein content greater than 11% and fat content less than the protein content. The diet as specified by the AOAC is prone to rancidity, which can obscure the effect of essential amino acid limitation with low diet palatability and vitamin destruction. This high potential for rancidity can be attributed to the omission of an antioxidant from the formulation and the high levels of free minerals in prooxidant forms. Due to instability of the diet over a 4-week feeding period, refrigeration or freezing after preparation of the diet is absolutely essential. Most foods contain either natural or synthetic antioxidants, while the control diet contains none, which may result in the control diet being less stable than the test diet. Another potential problem is the composition of the mineral mix. In the last 20 years, a number of new trace elements have been established as necessary for rats. Specifically, selenium and chromium are omitted from the mineral mixture.

TABLE II
Effect of Acclimation Period on PER

Protein Source	Acclimation Time Prior to Initiation of Experiment, hr		
	24	72	144
Starting wt, g	64.8	55.6	58.7
Gain, g			
Casein	114	122	133
Soybean protein	77	80	92
PER			
Casein	2.96	3.03	3.26
Soybean protein	1.94	2.07	2.37
Adjusted PER			
Casein	2.5	2.5	2.5
Soybean protein	1.6	1.7	1.8

TABLE III
AOAC Diet Formulation and Adjustments[a]

Ingredient	Dietary Level %	Diet Manipulation
Protein	10	Protein from test sample
Cottonseed oil	8	Minus fat content of test sample
Salt mixture	5	Minus ash content of test sample
Cellulose	1	Minus fiber content of test sample
Water	5	Minus moisture content of test sample
Sucrose or corn starch	100	

[a]Methods of analysis, AOAC, 12th ed. (1975).

In formulating the diets, the ash content of the test sample is subtracted from the mineral mix without regard to the content of the ash or the level mineral mix replaced. Therefore, a highly imbalanced ash may replace a balanced mineral mix, which can result in a mineral deficiency or imbalance. A 3.0% minimum level of AOAC mineral mix should be maintained in all diets and the mineral mix of the control diet increased above 5%, if necessary, to equalize the diets in ash content.

The addition of water may influence the PER results obtained with some proteins. Keane *et al.* (5,6) showed an effect of water on PER values (Table IV) with the casein diet. They observed improved PER value as the water content of the diet was increased from 0 to 35%. The primary responses were obtained in the range of 0 to 15% added water, although slight increases were observed at higher water addition levels.

Similar results have been obtained by our laboratory with casein and isolated soybean protein (Table V). The casein diet had an optimum PER value at 5% added water, while the isolated soybean protein optimized at 9% added moisture. The differences may influence dietary intake. Since the level of hydration may influence the PER results, it is essential that the moisture content of the diet be

TABLE IV
Effect of Water on PER Value of Casein[a]

Water Added %	PER
0	1.84
5	2.61
10	2.66
15	2.88
20	2.94
25	2.91
30	3.02
35	3.04

[a]Keane *et al.* J. Nutrition 81: 87 (1963).

TABLE V
Effect of Water on PER

	Casein	Isolated Soybean Protein	
% Water	Unadjusted PER	Unadjusted PER	Adjusted PER
0	2.88	1.80	1.56
5	3.46	2.66	1.92
9	3.25	2.84	2.18
13	3.21	2.78	2.16
17	3.28	2.76	2.10
20	3.39	2.69	1.98
23	3.31	2.69	2.03

maintained throughout the 4-week testing period in moistureproof containers which prevent both moisture loss or pickup.

A second effect of water which must be considered is the point at which the moisture is added to the diet (7). The results of adding water to the total diet after mixing or to the protein sample before mixing are shown in Table VI. Water was added at a level of 5% of the diet to both the casein control and soy protein. In one set of treatments, water equivalent to 5% of the diet was added to the entire diet after mixing; in the other treatment, the water was added to the protein at the start of the mixing process and the remainder of the ingredients added and mixed. Hydration of both casein and soy protein test samples at the beginning of the mixing process resulted in significantly better PER values. This effect may be due to reduction in dustiness and sticky properties of the spray dried soybean isolate. The addition of the water to the protein source in the diet is a routine procedure in our laboratory.

Since dietary moisture level can influence protein quality measurement, the methods used in handling high moisture and liquid samples are critical to the PER test. The simplest way to handle these samples is to dry the sample to a reduced moisture content. This eliminates the need for freezing the diets to prevent spoilage, daily feeding of the rats and correcting the moisture losses from unconsumed food. Heating the samples is not satisfactory, since it can result in destruction of amino acids or reduced availability.

Therefore, lyophilization is the only practical method of preparing these samples. However, it must be remembered that some commercial lyophilization processes include a final heating step which may defeat the purpose of

TABLE VI
PER of Neutral Soy Protein as Affected by the Method of Adding Water to Diet[a]

Protein Source	Water Added %	4-Week Gain g	Feed Consumed[c] g	PER	Adjusted PER
Casein + water added to diet	5	123 c[b]	384 a	3.19 c	2.50
Casein + water added to protein	5	139 c	408 ab	3.40 d	2.50
Soy protein + water added to diet	5	77 a	383 a	2.02 a	1.58
Soy protein + water added to protein	5	100 b	434 b	2.31 b	1.70
sx[d]	5	5.6	16.2	0.064	

[a]Hopkins, D. T., and Steinke, F. H. J. Nutr. 106: 1438-1446.
[b]Means not showing a common letter are significantly different at $P \leq 0.05$. Duncan's multiple range test was method used.
[c]Grams, feed consumed on "as-is" basis.
[d]Standard error of the mean. Each diet was fed to 15 rats.

lyophilization. With samples of moderate moisture, where the added dietary water can be kept below 10%, the casein control diet may be adjusted up to the moisture content of the test sample diet. This reduces the possibility of modifying the protein during drying.

Diet separation and selectivity can be a major problem with some types of samples. In the process of evaluating meat products (8), we observed what appeared to be unusual PER results. The effects were apparently related to the rat's ability to select the meat particles from the remainder of the diet (Table VII). Based on the protein analysis of the unconsumed portions of the diet, whether the hamburger was fed at high moisture levels (24%) or lyophilized, the rats were able to separate the meat protein but not the casein from the remainder of the diet. The diet refusals of the casein diets were similar to the analyzed protein values of the diet, while the diet refusals of the meat diets contained less than half of the

TABLE VII
Selection of Meat by Rats from PER Test Diets[a]

Diet	Dietary Water[b] %	Analyzed Protein[c]	
		Diet %	Diet refusals %
Casein	24.2	9.8	12.7
Hamburger, raw	24.2	9.5	5.2
Casein	0.3	10.0	11.3
Hamburger, lyophilized	0.8	9.9	3.9
Casein	16.6	9.6	10.5
Hamburger, fried	16.6	9.8	4.2
Casein	0.3	10.0	11.5
Hamburger, fried, lyophilized	0.4	10.0	4.5

[a]Hopkins, D. T., Steinke, F. H., and Kolar, C. W. J. Food Sci. 41: 1426 (1976).
[b]Water added from protein source and as water.
[c]Dry matter basis.

TABLE VIII
Effect of Fat and Energy Levels on PER Value

Fat Content[a] %	ME cal/g	28-Day Gain g	PER
8	3.81	141	3.10
12	4.05	121	3.03
16	4.25	119	3.00
20	4.45	120	3.13
12	3.81[b]	140	3.15
16	3.81[b]	142	3.09

[a]Fat content varied by addition of cottonseed oil at expense of starch.
[b]Calorie level maintained by the addition of 6.14 and 11.28% of cellulose at the expense of starch.

starting protein content. Feeding the hamburger raw or cooked did not influence these results.

When evaluating the results of the PER assay, one is advised to analyze the feed refusals to ensure that the protein based on diet analysis and feed consumption is a true measure of protein intake. We have found that using a food chopper[1] as part of the final diet mixing reduces particle size and appears to reduce the rat's ability to select the dietary components.

Among the wide variety of food products to be tested, a number of types of food samples produce problems for the PER testing procedure. These include low protein, high fat, high ash, high salt, high moisture, and non-homogenous samples. In many samples, it is possible to have several of these variations simultaneously.

High fat samples are more difficult to handle than high moisture samples. While extraction of the fat is possible, the probability of the protein in the sample remaining unaffected by this procedure is small. Therefore, most samples must be fed with fat levels greater than that specified by the AOAC procedure (1). This results in an elevated calorie level as well as a high fat level. This is equivalent to evaluating the protein at a lower dietary protein content. The problem may not be with the test sample but in formulating a control diet which is comparable, will not undergo changes during storage, and will be consumed normally by the rat. Two methods of balancing the changes produced by the high fat sample are: 1) balancing the control diet to fat content of the test protein diet by adding cottonseed oil, and 2) adding fat and fiber to the diet to balance energy levels.

Both procedures appear to give similar PER results when compared in an experiment in our laboratory with casein as the protein source (Table VIII). Cottonseed oil was added at the expense of starch to give dietary fat levels of 8,

[1]Hobart Manufacturing Company, Troy, Ohio. Model 8181-D.

TABLE IX
Effect of Sucrose on PER

Protein Source	Sucrose Content	
	None	10%
Gain, g		
Casein	114	156
Soybean isolate	77	87
PER		
Casein	2.96	3.41
Soybean isolate	1.94	2.22
Adjusted PER		
Casein	2.50	2.50
Soybean isolate	1.63	1.63
	10% Sucrose	0 Sucrose
Adjusted PER	Casein control	Casein control
Soybean isolate	1.42	1.88

12, 16, and 20%, which resulted in calculated dietary metabolizable energy values of 381, 405, 425, and 445 per 100 g, respectively. Diets containing 12 and 16% dietary fat were also tested in which 6.14 and 11.28% cellulose (Solka Floc, BW 100)[2] was added at the expense of the starch to maintain the calorie content at 381 cal ME/100 g. PER values were not affected by dietary fat levels whether the energy level was maintained or allowed to increase with the fat content. These results are similar to those obtained by Hurt *et al.* (9) with 8, 16, and 24% fat levels.

The rat appears to prefer a diet with some sweet taste(10) and may consume higher quantities of the diet. This problem can occur when foods containing sucrose are compared with a casein control diet using starch as the carbohydrate source. The data in Table IX demonstrate the effect of adding 10% sucrose to the diet at the expense of corn starch. Soybean isolate has the same PER values on either 0 or 10% sucrose diets when compared with casein control diets containing similar levels of sucrose. However, if the soybean isolate is compared with the opposite control diets, *i.e.*, 0 sucrose control with 10% sucrose test diet, the adjusted PER are quite different, ranging from 1.42 to 1.88. Therefore, the carbohydrate in the test samples must also be taken into account in balancing the diets. Products such as candy and presweetened food products would be in this category. Other carbohydrates may have a negative effect on the growth and PER values if they are present in the diet at high levels. Lactose in milk and milk by-products will have a detrimental influence and make test samples appear lower in protein value than their true value. Therefore, products containing lactose must be tested with a casein control diet containing an equal quantity of lactose. Similar problems can also be expected with protein sources containing unusual carbohydrates of the mono- and polysaccharide forms (11,12) which cannot be metabolized.

Many food products contain relatively high quantities of added sodium in the form of sodium chloride, sodium salts, or sodium proteinates. These present a number of problems in the development of an assay diet and test procedure for PER. If a large quantity of the total ash content is composed of sodium or sodium chloride, this displaces a balanced mineral mixture and could produce mineral deficiencies or imbalances. The high levels of sodium or salts of sodium could result in depressed growth as they approach toxic dietary levels (13). The best solution is to balance the sodium chloride of the control diet to the level of the test diet. However, at very high levels of sodium chloride addition, we have observed in tests in our laboratory that the effect of sodium chloride may be more detrimental in the control diet than the test diet. This could be due to the sodium being bound to the protein as a sodium proteinate in the food sample with less free sodium available, or the chloride ion may be the detrimental factor rather than the sodium ion. Samples which fall into this category are processed meat, ham, and sausages, or snack foods with added salt.

In conclusion, it may be said that the PER test procedure is a relatively simple test procedure which was originally designed to evaluate high protein test products. The food samples which are now being evaluated are highly complex and present dietary problems apparently not envisioned when the test was originally developed. These problem samples include products with high

[2]Obtained from Brown and Company, Berlin, N.H.

moisture content, products with fat contents higher than the protein content, products with high unbalanced ash content, products with low protein content, and products with unusual carbohydrates. A full understanding of the test sample and its composition is essential to the development of a valid comparison. The inflexible use of the AOAC procedure without critical scientific evaluation can and will result in highly erroneous estimations of a food protein's ability to supply essential amino acids to the human population. Many of the problems discussed herein may also apply to other procedures for measuring protein quality and are not necessarily restricted to the PER assay.

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