A PROBLEM IN STATISTICAL DESIGN FOR A PROTEIN EVALUATION EXPERIMENT

BY

LABIB ABDUN-NUR

Submitted in Partial Fulfillment for the Requirements
of the Degree Master of Science
in the Mathematics Department of the
American University of Beirut
Beirut, Lebanon,
1964

A PROBLEM IN STATISTICAL DESIGN

FOR A PROTEIN EVALUATION EXPERIMENT

LABIB ABDUN-NUR

ACKNOWLEDGEMENT

The writer wishes to express his deepest thanks and appreciation to Professor Mary Hanania Regier for her generous donation of time and for her constructive comments and wise guidance which made this work possible. He also wishes to thank Professor Z. Sabry for his care in supplying the necessary references and data.

Especial thanks are due to Miss Mona Jabbour for her diligent efforts in typing the manuscript.

ABSTRACT

The writing of this paper was initiated by an experiment that was run at the Division of Food Technology and Nutrition of the School of Agriculture at A. U. B. The purpose of the experiment was to compare the growth effects of different dietary proteins as measured by two related measures known as the P. E. R. (Protein Efficiency Ratio) and N. P. R. (Net Protein Ratio). This paper is meant to be a comment on those two measures and a suggestion of the use of other estimates obtained by different procedures for the comparison of growth effects of different dietary proteins.

Chapter I is a brief introduction to the various methods applied for dietary protein evaluation. It lays more emphasis on the so-called P. E. R. method which is discussed in more detail, together with the N. P. R. and two relevant ratio estimates, in Chapter II.

Chapter II discusses the development, uses, advantages, and handicaps of the P. E. R. measure. It compares it with the other related measure, the N. P. R., and attempts at estimating the relative efficiency of N. P. R. to P. E. R. It also introduces two ratio estimates which are shown to be easier to handle than the P. E. R. and N. P. R. both mathematically and computationally.

In Chapter III, the use of the analysis of covariance techniques for the estimation of protein effects on growth and the comparison of different protein effects is discussed in some detail. Tests for comparing different treatment effects are derived which take care of

the randomness of the concomitant variable (food intake) and of its dependence on treatment (diet) effect.

Chapter IV is a section on application and illustration. It makes use of the 4-weeks data of the experiment mentioned above to illustrate the application of the new procedures suggested in preceeding chapters to numerical data, and to make clear some points that are emphasized in the discussion.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Many workers have investigated methods by which the value of dietary protein might be determined, and various different criteria have been suggested. Nitrogen-balance studies in growth, maintenance, reproduction, fertility and lactation, growth itself, and longevity were all proposed for this purpose during the second decade of the present century. More recently, carcass analysis, chemical scores based on the amino-acid content of the protein, the ratio of creatinine nitrogen to total urinary nitrogen and changes in liver nitrogen have been related to protein quality [1,P.243ff]. All the methods that have been suggested: growth methods, nitrogen balance methods, net protein utilization and net protein retention methods, microbiological methods, chemical scoring methods, protein regeneration methods, blood amino-acid methods, etc.[2], have, directly or indirectly, aimed at evaluating the primary function of dietary protein which is : to furnish a mixture of amino-acid of the proper pattern for the synthesis of tissue proteins and for maintenance [2,P. 9].

Every one of the suggested methods has got its advantages and its handicaps. Some are believed to yield reliable results, in the sense of being accurate and not dependent on extraneous factors, but are laborious and time consuming; others are considered to be relatively simple but probably less reliable; and attempts have been made

systematically at comparing the relative merits (accuracy, exactness and reliability of estimation, and simplicity) of the different procedures [2, P. 9]. Growth methods are thought to be the simplest of all and, probably, the most suitable [2, P. 11]. J. A. Campbell [2, P. 11] reports that "if the diet contains insufficient amounts of one or more of the essential animo-acids, growth will be reduced or stopped entirely. Thus growth is a sensitive index of the supply of amino-acids and may be used to evaluate the overall effect of dietary protein."

Protein efficiency ratio.

Among the various growth methods for the evaluation of dietary protein the "protein efficiencey ratio" (P. E. R.) is probably the most widely used. This concept was introduced in 1919 by Osborne, Mendell and Ferry [3,P. 223 ff]. The P.E.R. (the gram gain in weight per gram protein consumed) was determined at several levels of the test protein and the maximum value so obtained was considered the best estimate of the value of the protein. Barnes and Bosshardt [4, P.273 ff], in a later study, found that for foods having a P.E.R. of 2.0 or greater, the maximum ratio was obtained at a dietary protein level of about 10%. Most workers, in practice, have tested protein at a single level, usually about 10%. It appears that the 10% level yields the most sensitive and valid results for assaying protein value [2,P. 15]. In 1960 Morrison and Campbell [5,P.112 ff], in a study on factors influencing the protein efficiency ratio of foods, reported that "P.E.R values varied not only

with quality and quantity of the dietary protein, but also with the duration of the experiment and the sex of the test animal." Chapman et al. [6, P.679 ff] demonstrated that the variation between animals tended to decrease over the first 4 weeks. The A. O. A. C. (Association of Official Agricultural Chemists) method uses this period for its assay, and Campbell [2, P.14] believes "that this period is the most satisfactory basis for the determination of P. E. R. values." Morrison and Campbell [5, P.112 ff], after finding differences between sexes, concluded that sexes should not be mixed or used interchangeably. Other factors which are believed to influence the P. E.R. values are : the species and age of animal, the method of feeding, and the use of an appropriate standard. Both the A. O. A. C. method and that of Chapman et al. [2, P.19] provide standard methods which do not differ much and many workers now follow those standardized procedures in the details of their assays.

For illustration, and to facilitate the understanding of the discussion that follows, a description of an experiment by which P. E. R. could be evaluated for a number of proteins is given below. The experiment was run by Campbell, Sabry and Cowan [7], at the division of Food Technology and Nutrition of the American University of Beirut.

Experimental.

140 weanling male rats of a single strain, 20 to 23 days of age, were divided, according to their body weights, into 14 groups of 10 so that one animal of each group was matched with a corresponding one from the other groups within 1 gram. 13 of the groups

were put on different diets; every group was fed one of 13 diets that differed only by the quality of their protein content. A randomized block design was used in which the rats were randomly allocated to diets and variations in their initial body weights constituted the blocks. The rats were also randomly allocated to individual cages which were kept in an air-conditioned room at 74° - 76° - F, and food and water were supplied ad libitum. Details of the preparation of the foods tested are given by Cowan et al. [8]. The experiment lasted 4 weeks. At the end of each week the amount of food intake since the start of the experiment was recorded, and the change in weight during that period was calculated for every single rat. Thus P. E. R. values were claculated at 7,14,21 and 28 days. The 14 - th group of 10 rats was fed the protein-free diet of Chapman et al. [6, P.679 ff]. This group constituted the negative control group with which each test group was matched for the determination of a related ratio, known as the "net protein ratio" (N.P.R.)[1].

The relative precision of the P. E. R. and N.P.R. ratios as measures of protein effectiveness in growth has not yet been determined, and the purpose of the above experiment was to compare the two criteria. In the chapters that follow this question is considered and an alternative measure for the effectiveness of different proteins is proposed. The 4-weeks data of the above experiment is used in application and illustration.

---

[1] see Chapter II.

CHAPTER II

THE TWO RATIO METHODS

Definition and Discussion.

On account of its simplicity of form, the protein efficiency ratio, suggested by Osborne, Mendell and Ferry in 1919, is probably the most widely used. As the name suggests, it is the ratio of gain in weight, to protein intake, both being measured in the same unit. That is,

$$\text{P. E. R.} = \frac{\text{gain on test diet}}{\text{protein intake}} \quad .$$

Thus the P. E. R. is a ratio of two dependent variables: gain in weight and protein intake. This point is emphasized by Taskar, Parthosomathy and Shentha [9, P.696 ff] in their study of the influence of food intake and duration of feeding on the evaluation of P. E. R., when they state that "unduly large variations in either of the variables would result in a lesser degree of association [between gain and intake] and give unreliable estimates of P. E. R. ... Whenever the evaluation of P. E. R. is attempted it is necessary to verify that the correlation between gain in weight and protein intake is significant. Such estimates can only be considered reliable because it is on that assumption that the values of the ratios are determined." However, in the presence of this dependence the ratio does not lend itself to any of the known standard procedures in statistical analysis,

and this may be considered one of its weaknesses. It has been subject to a variety of criticisms by workers in the biological field as well. Some such criticisms are outlined below:

(a) The assumption that the gain in weight is constant in composition is not necessarily valid. In other words, although body weight may vary with the diet, yet the percentage nitrogen in the body may remain unchanged.

Middleton et al. [10, P.865 ff] showed that the percentage of body nitrogen, after 4 weeks on test, was not greatly influenced by variations in protein content or protein quality. Bender and Doell [11, P.140 ff] demonstrated that although the amount of carcass fat may vary widely with the diet, the percentage nitrogen is remarkably constant in animals which have been on test for as long as 40 to 50 days. This objection to P. E. R. would, of course, hold against any of the so-called growth methods in which the benefits of the various proteins are measured in terms of gain in weight or growth.

(b) P. E. R. varies with food intake.

Stewart et al. [12, P.519 ff] have attributed the considerable variations in P. E. R. for individual rats within a group receiving the same protein to the difference in the amounts of food consumption. Similar results were reported by Bender and Doell [11, P.140 ff] and Bender [13, P.135 ff].

(c) P. E. R. varies with protein level.

Forbes et al. [2, P.21 ff] showed that "the quantitative relationship [relative standing] between different proteins depends on the level at which proteins are fed."

(d) Calculation of P. E. R. assumes, that all protein consumed is used for growth, whereas it is known that part of the protein is used for maintenance.

This last criticism is, probably, the most fundamental of the above criticisms. Consequently, in 1956, Bender and Doell [11, P.140 ff] described a modification of the method whereby a control group of animals fed on protein-free diet is included in the experiment and the difference between the weights of this group and the test group (instead of merely weight gain) is used in the calculation. Thus, they introduced the N.P.R. (net protein ratio) method, where N.P.R. is defined as:

$$\text{N.P.R.} = \frac{\text{gain on test diet} + \text{loss on control diet}}{\text{Protein intake on test diet}}$$

$$= \text{P.E.R.} + \frac{\text{loss on control diet}}{\text{Protein intake on test diet}},$$

where the plus sign is taken to mean the algebraic addition of the loss on the control diet. This procedure, they claimed, allows for maintenance requirements and also permits the evaluation of proteins which do not promote growth. However, the effectiveness of using a non-protein control group has not been well established, and one would question the validity of using a control group which soon becomes abnormally deficient.

N. T. Gridgeman [14, PP.36-37] has shown that the optimum ratio R of the size of the control group to that of each of the test groups in terms of the number n of test groups is:

$$R = \sqrt{n}.$$

However, in the experiment we shall consider and in most other experiments the ratio used is 1, i.e. the sample size of the control group is the same as that of any of the test groups.

Mathematical Reformulation.

We recall at this stage that one purpose of this study is to compare the precision of the two ratio methods for protein evaluation, namely the P.E.R. and N.P.R. methods. By their definition, these ratios are functions of protein intake and gain in weight, which are variables liable to chance fluctuations. The ratios themselves may, therefore, be considered as random variables, and a study of their relative precision would amount to a comparison between their variances. With reference to the experiment described at the end of Chapter I, consider now one test diet at a time, and let

y_i denote the gain in grams on the test diet for the animal in the i-th block,

z_i denote the loss in grams on control diet for the animal in the i-th block,

x_i denote the protein intake in grams on test diet for the animal in the i-th block,

n denote the size of the sample, that is, the number of blocks (the same for both test group and control group).

Using this notation, the two ratios for the animal in the i-th block are

$$\text{P. E. R.}(i) = r_i = \frac{y_i}{x_i} \qquad \text{and} \quad \text{N.P.R.}(i) = s_i = \frac{y_i+z_i}{x_i} \ .$$

In actual practice, however, the biologists utilize the information

provided by all n blocks by the simple expedient of averaging these individual ratios. In effect, therefore, the P. E. R. and N. P. R. measures reported in the literature [2, P.70 ff; 7, P.4 ff] are - in our notation -

$$\text{P. E. R.} = \bar{r}_n = \frac{1}{n} \sum^{n} r_i = \frac{1}{n} \sum^{n} \frac{y_i}{x_i} ,$$

and

$$\text{N. P. R.} = \bar{s}_n = \frac{1}{n} \sum^{n} s_i = \frac{1}{n} \sum^{n} \frac{y_i + z_i}{x_i}$$

which, clearly are not ratios at all. In order to preserve the nature of the ratios as such, one might compute, for instance,

$$\text{P. E. R.}^* = R_n = \frac{\bar{y}_n}{\bar{x}_n} \qquad \text{and} \qquad \text{N. P. R.}^* = S_n = \frac{\bar{y}_n + \bar{z}_n}{\bar{x}_n} ,$$

where $\bar{y}_n$, $\bar{x}_n$ and $\bar{z}_n$ are the arithmetic means of the observations over the n blocks. This alternative method of computation, requiring only one division per ratio, is easier to obtain. Moreover, it has the advantage of producing measures identical in form to the well-known ratio estimate [15, P.139; 16, P.112] whose properties have been considered in the statistical literature in some detail. By analogy, we might then consider our P. E. R.* as an <u>estimate</u> of some constant parameter, peculiar to the protein diet under consideration, and defined as

$$R = \frac{\bar{y}}{\bar{x}} ,$$

where $\bar{y}$ and $\bar{x}$ are the population arithmetic means corresponding to the sample means $\bar{y}_n$ and $\bar{x}_n$ respectively. We can, similarly, interpret N. P. R.* as an estimate of

$$S = \frac{\bar{y} + \bar{z}}{\bar{x}} .$$

Relative precision of R_n and S_n.

We now compare the precision of the two methods by comparing the variances of the estimates

$$R_n = \text{P. E. R.}^* \quad \text{and} \quad S_n = \text{N. P. R.}^* .$$

Following Sukhatme [15, P.140 ff] , and considering the n observations as a sample from a very large population of N possible observations, let

$$y_i = \bar{y} + e_i$$

so that

$$\bar{y}_n = \bar{y} + \bar{e}_n$$

where

$$E(\bar{e}_n) = 0 \quad \text{and} \quad E(\bar{e}_n^2) = \frac{N-n}{nN} S_y^2 = \frac{N-n}{nN} \cdot \frac{1}{N-1} \sum^{N} (y_i - \bar{y})^2 .$$

Similarly, let

$$x_i = \bar{x} + e_i'$$

so that

$$\bar{x}_n = \bar{x} + \bar{e}'_n$$

where

$$E(\bar{e}'_n) = 0 \quad \text{and} \quad E(\bar{e}'^2_n) = \frac{N-n}{nN} S_x^2 = \frac{N-n}{nN} \cdot \frac{1}{N-1} \sum^{N} (x_i - \bar{x})^2 .$$

Then the expected value of R_n is given by

$$E(R_n) = E \frac{\bar{y}}{\bar{x}} \left[1 + \frac{\bar{e}_n}{\bar{y}} - \frac{\bar{e}'_n}{\bar{x}} + \frac{\bar{e}'^2_n}{\bar{x}^2} - \frac{\bar{e}_n \bar{e}'_n}{\bar{y}\bar{x}} + \frac{\bar{e}_n \bar{e}'^2_n}{\bar{y}\bar{x}^2} - \frac{\bar{e}'^3_n}{\bar{x}^3} + \ldots \right] .$$

If the sample size is sufficiently large, we can suppose that $\left| \frac{\bar{e}'_n}{\bar{x}_n} \right| < 1$ and that the contribution of terms involving powers in

$\bar{e}_n$ and $\bar{e}'_n$ higher than the second to the value of $E(R_n)$ is negligible. The expected value of R_n can, therefore, be approximated by

$$E(R_n) = \frac{\bar{y}}{\bar{x}}\left[1 + \frac{N-n}{nN}\left(\frac{s_x^2}{\bar{x}^2} - \rho_1 \frac{s_y}{\bar{y}} \cdot \frac{s_x}{\bar{x}}\right)\right],$$

where,

$$\rho_1 = \frac{E(y_i-\bar{y})(x_i-\bar{x})}{\sqrt{E(y_i-\bar{y})^2 \cdot E(x_i-\bar{x})^2}}.$$

Similarly, we can approximate the expected value of S_n by

$$E(S_n) = \frac{\bar{y}+\bar{z}}{\bar{x}}\left[1 + \frac{N-n}{nN}\left(\frac{s_x^2}{\bar{x}^2} - \rho_2 \frac{s_{y+z}}{\bar{y}+\bar{z}} \cdot \frac{s_x}{\bar{x}}\right)\right],$$

where,

$$\rho_2 = \frac{E(y_i+z_i-\bar{y}-\bar{z})(x_i-\bar{x})}{\sqrt{E(y_i+z_i-\bar{y}-\bar{z})^2 \cdot E(x_i-\bar{x})^2}}.$$

The relative efficiency of N. P. R. to P. E. R. is given by [15, P.124] :

$$R.E. = \frac{\text{variance of P. E. R.}}{\text{variance of N. P. R.}} = \frac{V(P.E.R.)}{V(N.P.R.)}$$

$$= \frac{\frac{\bar{y}^2}{\bar{x}^2} \cdot \frac{N-n}{nN}\left(\frac{s_y^2}{\bar{y}^2} + \frac{s_x^2}{\bar{x}^2} - 2\rho_1 \frac{s_y}{\bar{y}} \cdot \frac{s_x}{\bar{x}}\right)}{\frac{(\bar{y}+\bar{z})^2}{\bar{x}^2} \cdot \frac{N-n}{nN}\left(\frac{s_{y+z}^2}{(\bar{y}+\bar{z})^2} + \frac{s_x^2}{\bar{x}^2} - 2\rho_2 \frac{s_{y+z}}{\bar{y}+\bar{z}} \cdot \frac{s_x}{\bar{x}}\right)}$$

$$= \frac{\frac{\bar{y}^2}{\bar{y}^2\bar{x}^2}\left[s_y^2\bar{x}^2 + s_x^2\bar{y}^2 - 2\bar{y}\bar{x} \cdot E(y_i-\bar{y})(x_i-\bar{x})\right]}{\frac{(\bar{y}+\bar{z})^2}{(\bar{y}+\bar{z})^2\bar{x}^2}\left[s_{y+z}^2\bar{x}^2 + s_x^2(\bar{y}+\bar{z})^2 - 2(\bar{y}+\bar{z})\bar{x} \cdot E(y_i+z_i-\bar{y}-\bar{z})(x_i-\bar{x})\right]}$$

$$= \frac{s_y^2\bar{x}^2 + s_x^2\bar{y}^2 - 2\bar{y}\bar{x} \cdot E(y_i-\bar{y})(x_i-\bar{x})}{s_y^2\bar{x}^2 + s_x^2\bar{y}^2 - 2\bar{y}\bar{x} \cdot E(y_i-\bar{y})(x_i-\bar{x}) + s_z^2\bar{x}^2 + s_x^2(2\bar{y}\bar{z}+\bar{z}^2) - 2\bar{z}\bar{x} \cdot E(y_i-\bar{y})(x_i-\bar{x})}$$

since $s_{y+z}^2 = s_y^2 + s_z^2$ because of independence between y and z.

$$\therefore \quad \text{R. E.} \leq 1 \text{ if } S_z^2 \bar{x}^2 + S_x^2(2\bar{y}\bar{z}+\bar{z}^2) - 2\bar{z}\bar{x} \cdot E(y_i-\bar{y})(x_i-\bar{x}) \geq 0,$$

i.e. if

$$S_z^2 \bar{x}^2 + S_x^2 \bar{z}^2 + 2\bar{y}\bar{z}(S_x^2 + \bar{x}^2) - 2\bar{x}\bar{z} \cdot E(x_i y_i) \geq 0.$$

$\bar{z}$ is always positive because the test period is never less than 1 week. Therefore, if $\bar{y} \geq 0$ and $E(x_i\, y_i) \leq 0$, we are certain that R. E. ≤ 1. However,

$$\bar{y} \geq 0 \quad \text{and} \quad E(x_i\, y_i) \leq 0$$

is an event which is most unlikely to happen, because $\bar{y} \geq 0$ implies that y_i is predominantly positive, and, since $x_i \geq 0$, it follows that $E(x_i\, y_i)$ is very unlikely to be negative. In fact, if we replace the above parameters by their estimates from the sample, we see that such an event never occurs because in the experiment discussed in Chapter I every diet which has $\bar{y} \geq 0$ also has $\sum x_i\, y_i \geq 0$. Thus we are never sure that R. E. ≤ 1, and whenever we are interested in testing for that we can replace parameters by their estimates and check for the above inequality.

Relative precision of $\bar{r}_n$ and $\bar{s}_n$.

Following the above procedure we can write the expected value of $\bar{r}_n$ as

$$E(\bar{r}_n) = \frac{\bar{y}}{\bar{x}}\left[1 + \frac{e_i}{\bar{y}} - \frac{e_i'}{\bar{x}} + \frac{e_i'^2}{\bar{x}^2} - \frac{e_i e_i'}{\bar{y}\,\bar{x}} + \frac{e_i e_i'^2}{\bar{y}\bar{x}^2} - \frac{e_i'^3}{\bar{x}^3} + \ldots\right],$$

with a similar expansion for the expected value of $\bar{s}_n$. Unfortunately, however, no simple approximations can be derived for these expectations no matter how large a sample we have because we are never sure

about the magnitude of $\frac{e_i'}{\bar{x}}$ and, thus, cannot make any statement about the convergence of the series in the expansion of the expected values. Thus we must seek some other procedure for comparing the variances of $\bar{r}_n$ and $\bar{s}_n$. Denoting $\frac{1}{n}\sum^{n}\frac{z_i}{x_i}$ by $\bar{d}_n$ and writing $\bar{s}_n$ as

$$\bar{S}_n = \bar{r}_n + \bar{d}_n \quad ,$$

we get

$$V(\bar{S}_n) = V(\bar{r}_n) + V(\bar{d}_n) + 2\ \mathrm{cov}(\bar{r}_n, \bar{d}_n).$$

$\bar{r}_n$ is a better estimate than $\bar{S}_n$ if

$$V(\bar{d}_n) + 2\ \mathrm{cov}\ (\bar{r}_n, \bar{d}_n) \geq 0,$$

i.e. if

$$\mathrm{cov}\ (\bar{r}_n, \bar{d}_n) \geq -\tfrac{1}{2}\, V(\bar{d}_n),$$

i.e. if

$$\mathrm{cov}\ (r_i, d_i) \geq -\tfrac{1}{2}\, V(d_i),$$

i.e. if

$$\rho(r_i, d_i) \geq -\tfrac{1}{2}\,\frac{S(d_i)}{S(r_i)}\ .$$

But this is always true if

$$-\tfrac{1}{2}\,\frac{S(d_i)}{S(r_i)} \leq -1$$

i.e. if

$$S(d_i) \geq 2\ S(r_i).$$

We conclude, therefore, that

(a) When the estimates R_n and S_n are used, P. E. R. is better than N. P. R. if

$$S_z^2\,\bar{x}^2 + S_x^2\,\bar{z}^2 + 2\bar{y}\bar{z}(S_x^2 + \bar{x}^2) \geq 2\bar{z}\bar{x}\ .\ E(y_i - \bar{y})(x_i - \bar{x}),$$

and we are certain that P. E. R. is better than N. P. R. if the mean of the y's is positive and the sum of the product of every y_i with the corresponding x_i is negative, i.e. if $\bar{y} \geq 0$ and $E(x_i\, y_i) \leq 0$, a highly improbable case.

(b) When the estimates $\bar{r}_n$ and $\bar{s}_n$ are used, P. E. R. is better than N. P. R. if the correlation between P. E. R. (i) and $\frac{z_i}{x_i}$ is greater than, or equal to, minus one half the ratio of the standard deviation of $\frac{z_i}{x_i}$ to that of P. E. R. (i). i.e. if

$$\rho(r_i, d_i) \geq -\frac{1}{2}\ \frac{S(d_i)}{S(r_i)}\ .$$

And we are certain that P. E. R. is better than N. P. R. if we know that the standard deviation of $\frac{z_i}{x_i}$ is greater than, or equal to, twice the standard deviation of P. E. R. ($_i$), i.e. if

$$S(d_i) \geq 2\ S(r_i),$$

where, clearly, to check for the above inequalities we have to replace the parameters by their respective estimates from the sample.

CHAPTER III

ANALYSIS OF COVARIANCE

Regression Model.

As we have already seen, the use of P. E. R. or N. P. R. for measuring the nutritive value or effectiveness of various proteins has its drawbacks. Hegested and Worcester, and Sherwood and Weldon, using growing rats fed on diets of constant protein content ad libitum, concluded that there was no advantage to using P. E. R. in place of gain in body weight alone [5, P.112 ff]. As pointed out in Chapter II, the P. E. R. and N. P. R. are ratios of variables which are dependent, for the protein intake has a direct effect on growth. The protein quality, besides having a direct effect on growth, can also be assumed to influence the amount of food intake which, again, will have an effect on growth. Neither the P. E. R. nor the N. P. R. ratios take that into consideration, and this is considered by the writer of this paper to be among the most serious defects of the methods. To study the effects of the different proteins after eliminating the dependence between the two variables, therefore, the following regression model is suggested:

Denote by the letter y the first variable, namely growth or gain of body weight, and by the letter x the second variable which is the amount of food intake. Then let

$$y_{ij} = \eta + t_j + b_i + B(x_{ij} - x_{..}) + d_{ij}, \qquad \begin{matrix} i = 1,2,\ldots,I \\ j = 1,2,\ldots,J \end{matrix} \qquad (A)$$

where,

y_{ij} = growth in grams of rat in i-th block (body weight) and taking j-th treatment (diet),

η = over-all mean,

t_j = effect of j-th treatment; $\sum t_j = 0$,

b_i = effect of i-th body weight; $\sum b_i = 0$,

B = regression coefficient of growth on intake,

d_{ij} = random errors which are normally distributed with means zero and variance S_y^2,

x_{ij} = food intake in grams of rat in i-th block and taking j-th treatment,

$x_{..} = \frac{1}{IJ} \sum_{i}^{I} \sum_{j}^{J} x_{ij}$.

With this model, our problem reduces to testing a hypothesis on the equality of the t_j's or obtaining their estimates.

One advantage that this model has over the P. E. R. and N. P. R. methods is that it does not have to assume that all protein consumed is used for growth. It simply attributes the growth of every rat to the fact that it belongs to a particular body weight block, and that it is taking a particular diet which has a certain fixed growth potential and which, to some extent, may be responsible for the amount of intake the rat is having and thus has its contribution to growth. However, this model does involve the usual assumptions underlying the analysis of covariance models, such as:

(i) treatment, block, and regression effects must be additive

(ii) the d_{ij}'s must be normally and independently distributed with mean zero and variance S_y^2 which is the same for all, i.e. d_{ij} is $N(0, S_y^2)$ [17-22].

Logically, these assumptions should hold true in growth problems of the type considered. However, they can also be tested for, and means of adjustment may be applied in case they are not satisfied.[1]

Another assumption which is usually made is that the covariate (x) is not affected by treatments, and that it is constant [18, P.83; 19, P.484; 20, P.284]. In the present problem, however, this assumption cannot be made since the covariate x is assumed to be related to the variable y, and it is more realistic to think of the observations on x as values taken on by a random variable, rather than as constant values, and that this variable is affected by treatments. To conform to the general theory, where the concomitant variables should be constant, it seems advisable to follow Scheffé's suggestion [21, pp. 195-6] : "If we then modify the underlying assumptions so that the distribution assumed under them - this distribution depends on the values of the concomitant variables - is conditional, given the observed values of the concomitant variables, then the distribution theory derived is also conditional, as are the significance levels, powers, and confidence coefficients. If these conditional underlying assumptions are assumed to hold for all possible values of the observations on the concomitant variables, then, regardless of the joint

---

[1] see page 19

distribution of observations on the concomitant variables, the conditional significance levels and conditional confidence coefficients are constant, and hence the same unconditionally." To conform to the general theory again, where the covariate is assumed not to be affected by treatment, we introduce a linear model for x which would take care of the dependence of x on treatments. (It will appear later, however, that our conclusions based on the original regression model, with x assumed constant, are unaffected by this additional refinement.) Therefore let

$$x_{ij} = \eta' + t'_j + b'_i + e_{ij}\ , \qquad \begin{matrix} i = 1,2,\ldots,I \\ j = 1,2,\ldots,J \end{matrix} \qquad (B)$$

where,

x_{ij} is defined as in (A) above,

η' = over-all mean

t'_j = effect of j-th treatment on intake; $\sum t'_j = 0$,

b'_i = effect of i-th block on intake; $\sum b'_i = 0$,

e_{ij} = random errors, normally and independently distributed with means zero and variances S_x^2.

Thus the linear model (A) can be rewritten as

$$\begin{aligned} y_{ij} &= \eta + t_j + b_i + B(x_{ij} - x_{..}) + d_{ij} \\ &= \eta + (t_j + B\ t'_j) + (b_i + B\ b'_i) + B(e_{ij} - e_{..}) + d_{ij} \\ &= \eta + a_i + T_j + B(e_{ij} - e_{..}) + d_{ij} \end{aligned} \qquad (C)$$

where $y_{ij}(i = 1,\ldots,I;\ j = 1,\ldots,J)$, as before, measure growth in the IJ subjects, and $T_j = t_j + Bt'_j$ $(j = 1,\ldots,J)$ measure the combined effect of the j-th treatment on growth-both directly and through its

effect on food intake.

Transformations.

In practice, before going any further, one has to test the data for the linearity, additivity and homoscedasticity assumptions implied by the model. To test for additivity one can apply Tukey's test [21, P.130 ff]. For linearity one can draw a graph of the average of growth versus the corresponding average of food intake for every treatment. To test for homogeneity of variance Bartlett's test, as well as Cochran's, can be applied [22, pp. 179-80]. If some of the above assumptions are not satisfied it will be advisable to apply a convenient transformation to the variables that would result in a new scale of measurement on which the necessary assumptions are satisfied. It sometimes happens that the square root or logarithm of the observations will more nearly satisfy the normality and homoscedasticity assumptions [22, P.182]. On the other hand, it may be found that no simple transformation of the data appears to give reasonable approximations to the assumptions. However, "no transformation may be expected to work perfectly, and it is this fact more than any other that vitiates extensive computations to fit a computationally awkward transformation [17, p.156]." Bartlett [17, p.156] has given a list of transformations that may be used under different conditions, and one may refer to it for the choice of a suitable transformation. It will be shown in a later chapter that the final estimates obtained for the 4-weeks data of the experiment described in Chapter I are insensitive to some of the above assumptions.

Treatment Effects.

It will be recalled that our purpose in making the analysis is to test for equality, and find estimates, of the J different treatment effects $t_j (j = 1,\ldots,J)$ on the amount of growth as measured by the random variables y_{ij}, and - if the null hypothesis of equality is rejected - to find out which treatments differ singificantly from which and perhaps group them into groups of almost equivalent proteins. If the usual testing procedure is used in conjunction with Model (C), it would be testing the equality of the $t_j + B\, t_j'$ rather than the equality of the treatment means t_j, which is the subject of interest. To avoid that we, therefore, start by finding estimates of the different parameters in the following manner:

1. Obtain the least squares estimates $\hat{T}_j = \hat{t}_j + \hat{B}\,\hat{t}_j'$, assuming the e_{ij} are observable, from (C),
2. Obtain the least squares estimates $\hat{t}_j'$ from (B),
3. Obtain the least squares estimate $\hat{B}$ from (A).

Then

$$\hat{t}_j = \hat{T}_j - \hat{B}\hat{t}_j' = (y_{.j}-y_{..}) - \frac{E_{yx}}{E_{xx}}(x_{.j}-x_{..}) - \frac{E_{ye}}{E_{ee}}(e_{.j}-e_{..}), \quad (D)$$

where

$$y_{.j} = \frac{1}{I}\sum_{i}^{I} y_{ij}, \qquad y_{..} = \frac{1}{IJ}\sum_{i}^{I}\sum_{j}^{J} y_{ij},$$

$$E_{yx} = \sum_{i}^{I}\sum_{j}^{J} (y_{ij}-y_{i.}-y_{.j}+y_{..})(x_{ij}-x_{i.}-x_{.j}+x_{..}),$$

$$E_{xx} = \sum_{i}\sum_{j} (x_{ij}-x_{i.}-x_{.j}+x_{..})^2,$$

with similar expressions for $y_{i.}$, $e_{i.}$, $e_{.j}$, $e_{..}$, E_{ye}, and E_{ee}.

In the above estimation procedure it is assumed that the e_{ij}

are observable, whereas in fact they are not. But since they are random variables with a well-defined distribution, we can generate an artificial set that plays the same role as the unobservable e_{ij} and satisfies the same assumptions. It is assumed that the e_{ij} are independent, all identically distributed as $N(0,S_x^2)$. If S_x^2 is known, a random sample of size IJ from the $N(0,S_x^2)$ distribution can be drawn and the elements called e_{ij}. Since S_x^2 is not known, we use an unbiased estimate of it, $\hat{S}_x^2$, obtained from the observations x_{ij} and Model (B), namely the error mean square for those observations. A random sample of e_{ij}, from $N(0,\hat{S}_x^2)$, being drawn, the estimates of the t_j's can be calculated by (D). These estimates, with the exception of the last terms $-\frac{E_{ye}}{E_{ee}}(e_{.j}-e_{..})$-, are the same as those that would have been obtained from Model (A) where the x_{ij} are constant. It follows that the only contribution effected by the introduction of Model (B) is the addition of this term $-\frac{E_{ye}}{E_{ee}}(e_{.j}-e_{..})$- to the estimate of the j-th treatment effect t_j from Model (A).

We now examine this additional term and its possible range of values. In the first place the random variable $(e_{.j}-e_{..})$ has expectation zero and variance $\frac{S_x^2(J-1)}{IJ}$. It, therefore, has a high probability of being close to zero for large sample size I. The other factor $-\frac{E_{ye}}{E_{ee}}$-, also a random variable, is of the same form as the least square estimate $\hat{B}=\frac{E_{ye}}{E_{ee}}$ of the regression parameter B in Model (A), but replaces the concomitant variables x_{ij} by the completely independent and unrelated variables e_{ij}. Its expectation is, therefore, the regression of y on e, which is zero, since y and e are - by assumption - uncorrelated. Its variance[1] is $\frac{S_y^2}{E_{ee}}$ [21, P.208] which is very small for large

[1] see page 24

sample size.

It would appear, therefore, that at least for the purpose of obtaining least square estimates of the t_j's and comparing treatment effects, the original Model (A) is adequate. This conclusion is borne out by numerical calculations, as will be demonstrated in the next chapter. This result is not surprising and, in fact, is in conformity with the remarks made by Scheffe' [21, P.198] where he says (with some changes in notation to adapt to our notation) "It is sometimes said that the analysis of covariance is valid only if the treatments do not affect the values of the concomitant variables. In the general bivariate situation described above, the treatments are at J levels, corresponding to the J populations. That the treatments do not affect the values of the concomitant variables might be interpreted to mean that for each j the distribution of the x_{ij} is the same, or, because of the other underlying assumptions, that $\mu_{x1} = \mu_{x2} = \ldots = \mu_{xJ}$, where (μ_{yj}, μ_{xj}) is the mean of the j-th bivariate population . Given this further assumption, the hypothesis tested by the analysis of covariance would then be that of the identity of the J bivariate distributions. The dictum that the analysis of covariance can be used only in this case would thus confine it to a very restricted situation. ... The general bivariate situation we have considered is a special case of the still more general situation where the concomitant variables are random variables and the underlying assumptions are satisfied conditionally, given the values of these variables. Then the analysis of covariance can be applied to get tests of hypotheses that have the correct significance level, or

interval estimates with the correct confidence coefficient, but the sense of using these tests or estimates must be considered seperately in each application."

Treatment Comparisons.

The introduction of the regression model, it will be recalled, has reduced our problem to that of finding estimates or testing hypotheses on the equality of the t_j's. Having finished with the first part of the problem we turn now to the second part, i.e. treatment comparisons.

Consider the j-th and k-th treatments. For comparing the two treatments we have seen that it is adequate to use estimates from Model (A). Thus, in testing the null hypothesis

$$H : t_j = t_k$$

i.e.

$$H : t_j - t_k = 0,$$

we shall calculate the estimate of the difference as

$$\hat{t}_j - \hat{t}_k = (y_{.j}-y_{.k}) - \frac{E_{yx}}{E_{xx}}(x_{.j}-x_{.k}). \qquad (1)$$

To test the null hypothesis H we also need to know the variance of $(\hat{t}_j-\hat{t}_k)$, because

$$\frac{\hat{t}_j - \hat{t}_k}{\text{estimate of standard deviation of } (\hat{t}_j-\hat{t}_k)}$$

has the Student t-distribution with (I-1)(J-1)-1 degrees of freedom [18, pp.86-87] .

To find the variance of $(\hat{t}_j-\hat{t}_k)$ one finds the expectation of

$\left[(\hat{t}_j-\hat{t}_k) - (t_j-t_k)\right]^2$ by first taking expectation with respect to y, keeping x fixed, and then taking expectation with respect to x. Expectation with respect to y, keeping x fixed, gives.

$$E\left[(\hat{t}_j-\hat{t}_k) - (t_j-t_k)\right]^2 = E(y_{.j}-y_{.k})^2 + E(x_{.j}-x_{.k})^2 \frac{E_{yx}^2}{E_{xx}^2} + (t_j-t_k)^2$$

$$- 2E(x_{.j}-x_{.k})(y_{.j}-y_{.k}) \frac{E_{yx}}{E_{xx}} - 2(t_j-t_k).E(y_{.j}-y_{.k})$$

$$+ 2(t_j-t_k).E(x_{.j}-x_{.k}) \frac{E_{yx}}{E_{xx}}, \qquad \text{from (1).}$$

But from (A) we get, after some algebraic manipulation,

$$E(y_{.j}-y_{.k}) = (t_j-t_k) + B(x_{.j}-x_{.k})$$

and

$$E(y_{.j}-y_{.k})^2 = (t_j-t_k)^2 + B^2(x_{.j}-x_{.k})^2 + 2B(t_j-t_k)(x_{.j}-x_{.k}) + 2\frac{S_y^2}{I}.$$

Also, with some lengthy algebraic details which we shall omit, we get

$$E(y_{.j}-y_{.k}) \frac{E_{yx}}{E_{xx}} = B(t_j-t_k) + B^2(x_{.j}-x_{.k}),$$

and

$$E\left(\frac{E_{yx}^2}{E_{xx}^2}\right) = B^2 + \frac{S_y^2}{E_{xx}}.$$

$\therefore$ $$V(\hat{t}_j-\hat{t}_k \mid x) = (t_j-t_k)^2 + B^2(x_{.j}-x_{.k})^2 + 2B(t_j-t_k)(x_{.j}-x_{.k}) + 2\frac{S_y^2}{I}$$

$$+ B^2(x_{.j}-x_{.k})^2 + \frac{S_y^2}{E_{xx}}(x_{.j}-x_{.k})^2 + (t_j-t_k)^2$$

$$- 2(x_{.j}-x_{.k})\left[B(t_j-t_k) + B^2(x_{.j}-x_{.k})\right] - 2(t_j-t_k)\left[(t_j-t_k)+B(x_{.j}-x_{.k})\right]$$

$$= 2\frac{S_y^2}{I} + \frac{S_y^2}{E_{xx}}(x_{.j}-x_{.k})^2.$$

This same formula is given without derivation by Kempthorne [17, P.102],

Cochran and Cox [18,P.87], and Federer [19, P.486] .

Now taking expectation with respect to x of this last expression, we see that the first term is unaffected. The second term, however, is a function of x and, to avoid the complication of dealing with the expectation of a ratio, we shall first replace the denominator by its expected value. By so doing we shall only be introducing a small error because when the x_{ij} are normal Fisher's fourth cumulant is zero and

$$V(\hat{S}_x^2) = \frac{2(S_x^2)^2}{(I-1)(J-1)} \quad [16, P.27],$$

which implies that, for large sample size, E_{xx} is very close to its expectation. We therefore only have to find the expected value of the numerator. From (B) we get

$$x_{.j}-x_{.k} = (t_j'-t_k') + (e_{.j}-e_{.k}),$$

$$(x_{.j}-x_{.k})^2 = (t_j'-t_k')^2 + 2(t_j'-t_k')(e_{.j}-e_{.k})+(e_{.j}-e_{.k})^2.$$

$$\therefore \quad E(x_{.j}-x_{.k})^2 = (t_j'-t_k')^2 + 0 + 2\frac{S_x^2}{I},$$

since

$$E(e_{.j}) = E(e_{.k}) = E(e_{.j})(e_{.k}) = 0, \quad \text{and} \quad E(e_{.j})^2 = \frac{S_x^2}{I}.$$

$$\therefore \quad V(\hat{t}_j-\hat{t}_k) = 2\frac{S_y^2}{I} + \frac{S_y^2}{E\{E_{xx}\}}\left[(t_j'-t_k')^2+2\frac{S_x^2}{I}\right]$$

$$= 2\frac{S_y^2}{I} + \frac{S_y^2}{(I-1)(J-1)S_x^2}\left[(t_j'-t_k')^2 + 2\frac{S_x^2}{I}\right]$$

$$= \frac{S_y^2}{S_x^2}\left[\frac{(t_j'-t_k')^2}{(I-1)(J-1)} + 2S_x^2\frac{(I-1)(J-1)+1}{I(I-1)(J-1)}\right],$$

which is estimated by

$$\frac{E_{yy}}{E_{xx}}\left[\frac{(x_{.j}-x_{.k})^2}{(I-1)(J-1)} + 2\,\frac{(I-1)(J-1)+1}{I(I-1)^2(J-1)^2}\,E_{xx}\right]$$

$$\approx \frac{E_{yy}}{E_{xx}}\left[\frac{(x_{.j}-x_{.k})^2}{(I-1)(J-1)} + 2\frac{E_{xx}}{I(I-1)(J-1)}\right]$$

$$= \frac{1}{(I-1)(J-1)}\;\frac{E_{yy}}{E_{xx}}\left[(x_{.j}-x_{.k})^2 + \frac{2}{I}\,E_{xx}\right],$$

which is the estimate of

$$2\,\frac{S_y^2}{I} + \frac{S_y^2}{E_{xx}}(x_{.j}-x_{.k})^2.$$

This again backs up the argument we had before that the fact that the x_{ij} are random does not have an appreciable effect on the estimates, and the usual analysis of covariance can thus be applied without much reservation. However, if one is keen on using estimates as given in (D), then

$$\hat{t}_j-\hat{t}_k = (y_{.j}-y_{.k}) - \frac{E_{yx}}{E_{xx}}(x_{.j}-x_{.k}) - \frac{E_{ye}}{E_{ee}}(e_{.j}-e_{.k}),$$

and by analogy with previous results and after some algebraic manipulation, we can show that

$$V(\hat{t}_j-\hat{t}_k) = \frac{2S_y^2}{I(I-1)(J-1)}\left[\frac{I}{2}\cdot\frac{S_y^2}{S_x^2}(t'_j-t'_k)^2 - \frac{2S_x^2}{(I-1)(J-1)S_y^2}B^2+(I-1)(J-1)+4\right],$$

which is estimated by

$$\frac{2E_{yy}}{I(I-1)^2(J-1)^2}\left[\frac{I}{2}\cdot\frac{E_{yy}}{E_{xx}}(x_{.j}-x_{.k})^2 - \frac{2}{(I-1)(J-1)}\cdot\frac{E_{yy}^2}{E_{xx}^2}+(I-1)(J-1)+4\right].$$

The t-Test.

Having found estimates $\hat{t}_j$ for all j and of $V(\hat{t}_j - \hat{t}_k)$ for all $j \neq k$, we arrange the $\hat{t}_j$'s in descending order, i.e. we rank them as follows:

$$\hat{t}_{r_1}, \hat{t}_{r_2}, \ldots, \hat{t}_{r_j},$$

where

$$\hat{t}_{r_1} \geq \hat{t}_{r_2} \geq \ldots \geq \hat{t}_{r_j},$$

and then we apply the t-test to test the null hypotheses

$$H: t_j - t_k = 0$$

against the alternatives

$$A: t_j > t_k .$$

we reject the hypothesis tested if

$$\frac{\hat{t}_j - \hat{t}_k}{\text{estimate of } S(\hat{t}_j - \hat{t}_k)} > t_\alpha (IJ - I - J), \qquad (E)$$

where t_α(df) indicates the α-percentile of the t-distribution with the indicated number of degrees of freedom.

One annoying feature of this test is that every comparison necessitates a separate computation of the variance of $(\hat{t}_j - \hat{t}_k)$, and that if, for example, t_{r_i} and $t_{r_{i+2}}$ are not significantly different, we cannot conclude that $t_{r_{i+1}}$ and $t_{r_{i+2}}$ are also not different. To simplify matters, Finney has suggested that an average value for all pairs of means be used [18, P.87]. However, Cochran [20, P.270] says that "this device is not recommended when the treatments produce

significant effects on x, because the variance of the difference may be substantially greater for some pairs than for others, so that the use of a single average variance becomes unsatisfactory." And since so far we have been assuming that intake is affected by treatment, we cannot recommend the use of this labor saving approximation unless it is shown that the treatments do not produce significant effects on food intake.

CHAPTER IV

APPLICATION AND ILLUSTRATION

Testing of Assumptions.

Before running the analysis of covariance on the 4-weeks data of the experiment described in Chapter I, we had to test whether the assumptions underlying the analysis of covariance were satisfied. To test for normality of the y_{ij} and the x_{ij} we drew histograms of both variables; the histograms showed some negative skewness. To test for homoscedasticity in the two variables, graphs of s_x^2 vs. $\bar{x}$ and s_y^2 vs. $\bar{y}$ were drawn, and both of these showed a linear trend between the means and the variances. It was found that the transformations $\sqrt{y_{ij} + 20}$ and $\sqrt{x_{ij}}$ improved the conditions of normality and homogeneity of variances in both variables respectively. A graph of the treatment means of the transformed y_{ij}'s vs. the treatment means of the transformed x_{ij} showed a very nice linear relationship between these new variables, which meant that the linearity of regression assumption was not violated. If there had been enough replications per treatment per block we would have applied one of the standard tests to test the additivity assumption. But in any case we shall, for the sake of the discussion, assume that additivity holds, which is not an unreasonable thing to do in growth problems.

Thus, the analysis of covariance was run on the transformed variables $\sqrt{y_{ij} + 20}$ and $\sqrt{x_{ij}}$ which, in what follows, will be denoted

by y'_{ij} and x'_{ij}, respectively.

Estimation and Hypothesis Testing.

It was found, by the analysis of variance on the x_{ij}, that intake was significantly affected by treatments. It was also found that the analysis of covariance model was not very sensitive to the normality and homoscedasticity assumptions, because the ranking of the diets with x_{ij} and y_{ij} transformed was not critically different from that with the variables untransformed. For if we look at Table 1 and Table 2 we see that the ranking is the same

Table 1. $\hat{t}_j = (y'_{.j}-y'_{..}) - \frac{E_{y'x'}}{E_{x'x'}} (x'_{.j}-x'_{..})$

Diet n^{o}.	2	12	15	16	9	17	19	6	13	11	14	18	3
$\hat{t}_j$	2.25	2.13	1.65	0.48	0.26	-0.29	-0.37	-0.50	-0.61	-0.79	-0.81	-0.84	-2.55

Table 2. $\hat{t}_j = (y_{.j}-y_{..}) - \frac{E_{yx}}{E_{xx}} (x_{.j}-x_{..})$

Diet n^{o}.	12	2	15	16	9	17	6	19	13	14	11	18	3
$\hat{t}_j$	43.82	41.52	29.66	2.46	-3.28	-8.06	-11.60	-11.90	-13.81	-14.78	-14.80	-15.10	-24.14

except for exchanges of a few adjacent pairs of estimates. After applying the t-test to each of the exchanged pairs, from Table 1, it was found that, at all practical levels of significance, the exchange is permissible since the corresponding treatments did not differ singnificantly.

For the sake of comparing results obtained from Model (A) with

those obtained from Model (C), a set of 130 random observations from $N(0, \hat{S}_x^2)$ was drawn, and estimates

$$\hat{t}_j = (y'_{.j} - y'_{..}) - \frac{E_{y'x'}}{E_{x'x'}}(x'_{.j} - x'_{..}) - \frac{E_{y'e}}{E_{ee}}(e_{.j} - e_{..})$$

were obtained as shown in Table 3.

Table 3. $\hat{t}_j = (y'_{.j} - y'_{..}) - \frac{E_{y'x'}}{E_{x'x'}}(x'_{.j} - x'_{..}) - \frac{E_{y'e}}{E_{ee}}(e_{.j} - e_{..})$

Diet nº.	2	12	15	16	9	17	19	6	13	11	14	18	3
$\hat{t}_j$	2.27	2.12	1.65	0.48	0.24	-0.30	-0.36	-0.49	-0.61	-0.80	-0.80	-0.86	-2.55

It is obvious, by comparing the estimates in Table 3 with those in Table 1, that, as was argued before, the term $\frac{E_{y'e}}{E_{ee}}(e_{.j} - e_{..})$ is negligible since the ranking of the diets is identical in both cases and the treatment effect estimates $(\hat{t}_j)$ are almost unchanged.

Now, since it was found that food intake was significantly affected by treatment, we could not apply Finney's proposition for the comparison of treatment means discussed at the end of Chapter III. Therefore test (E) was applied to compare the treatment effects. In Table 4 are listed the t values for the differences of some of the treatment estimates listed in Table 1. From this table one can have an idea about possible groups of non-significantly different treatments. For example, treatments 2, 12 and 15 form a group of equivalent treatments; treatments 16 and 9 can form another group; treatments 17, 19, 6, 13, 11, 14 and 18 stand as one group, and treatment 3 is a class by itself which is significantly lower than all the others.

Table 4. $t = \frac{\hat{t}_j - \hat{t}_k}{S(\hat{t}_j - \hat{t}_k)}$.

j,k	t	j,k	t
2,12	0.174	6,11	0.439
2,15	0.923	6,14	0.477
12,15	0.696	6,18	0.420
2,16	2.241*	6,3	2.971*
16,9	0.306	13,11	0.231
17,19	0.107	13,14	0.238
17,6	0.223	13,18	0.354
17,13	0.478	13,3	1.980*
17,11	0.575	11,14	0.030
17,14	0.553	11,18	0.067
17,18	0.809	11,3	2.444*
17,3	2.055*	14,18	0.037
19,6	0.181	14,3	2.559*
19,13	0.348	12,16	1.719*
19,11	0.618	15,17	2.771*
19,14	0.657	16,11	1.716*
19,18	0.701	16,14	1.613
19,3	2.627*	16,18	2.031*
6,13	0.131	16,3	3.223*

$t_{0.05}(106) = 1.659$[1]

---

[1] 1 degree of freedom was lost because of 1 missing observation.

* $\hat{t}_j > \hat{t}_k$ significantly

In Table 5 below, a ranking of the diets is given using the ratio estimate R_n and the transformed variables x' and y'.

Table 5. $R_n = \frac{\sum_n y_i'}{\sum_n x_i'}$

Diet no.	2	12	15	16	9	17	19	13	18	6	11	14	3
R_n	0.604	0.593	0.571	0.501	0.480	0.457	0.440	0.432	0.415	0.414	0.397	0.389	0.215

It is obvious that the ranking of the treatments in Table 5 is identical with that in Table 1 since those treatments which have been shifted around do not differ significantly, as can be seen from Table 4. This may suggest that, being very easy to compute, the ratio estimate R_n, rather than the analysis of covariance, be used for estimating protein effectiveness. This may very conveniently be done if one is only interested in having a general idea about the ranking of the treatments, in which case the ratio estimate cannot be superceded by any other estimate because it is the simplest and fastest to compute. But if one is also interested in running tests on treatment, then the ratio estimate is of no help. For, whereas the least squares estimates $\hat{t}_j$ obtained from the analysis of covariance are well-known to be normally distributed, the ratio estimates do not have a well-known distribution. Thus the t-test, which is applied to normally distributitied variables, cannot be applied to the ratio estimate unless it has been shown that it has a normal distribution. It turns out, therefore, that, although more tedious and time

consumming, the analysis of convariance is still more useful and mathematically more sound to use than the ratio estimate or the estimate the biologists use and which we have denoted by $\bar{r}_n$.

LIST OF REFERENCES

1. Rippon, W. P., A comparison of several methods for estimating the nutritive value of proteins, Brit. J. Nutrition 13, 1959.

2. Campbell, J. A., Methodology of protein evaluation, Division of Food Technology and Nutrition, A.U.B. Publication No.21,1963.

3. Osborne, T. B., Mendel, L. B., and Ferry, E. L., A method of expressing numerically the growth-promoting value of proteins, J. Biol. Chem. 37, 1919.

4. Barnes, R. H., and Bosshardt, D. K., The evaluation of Protein quality in the normal animal, Ann. N. Y. Acad. Sci. 47, 1946.

5. Morrison, A. B., and Campbell, J. A., Evaluation of protein in foods. V. Factors influencing the protein efficiency ratio of foods, J. Nutrition 70, 1960.

6. Chapman, D. G., Castillo, R., and Campbell, J. A., Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios, Can. J. Biochem. Physiol. 37, 1959.

7. Campbell, J. A. Sabry, Z. I., and Cowan, J. W., A comparison of N. P. R. and P. E. R. methods for the evaluation of protein (unpublished).

8. Sabry, Z. I. and Cowan, J. W., (unpublished data).

9. Taskar, A. D., Parthosarathy, N. R., and Shontha, C. S., The influence of food intake and duration of feeding on the evaluation of protein efficiency ration, Ind. J. Med. Res. 47, 1959.

10. Middelton, E. J., Morrison, A. B., and Campbell, J. A., Evaluatim of protein in foods. VI. Further factors influencing the protein efficiency ratio of foods, Can. J. Biochem. Physiol. 38, 1960.

11. Bender, A. E., and Doell, B. H., Biological evaluation of proteins: a new aspect, Brit. J. Nutrition 11, 1957.

12. Stewart, R. A., Hensley, G. W., and Peters Jr., F. N., Nutrititive value of protein. I., J. Nutrition 26, 1943.

13. Bender, A. E., Relation between protein efficiency and net protein utilization, Brit. J. Nutrition 10, 1956.

14. Gridgeman, N. T., Statistical considerations of the size of control groups, National Academy of Sciences - National Research Council, Washington, D. C., 1963.

15. Sukhatme, P. V., Sampling Theory of Surveys with Applications. Iowa State College press, 1958.

16. Cochran, W. G., Sampling Techniques. John Wiley and Sons, Inc., New York, N. Y., 1953.

17. Kempthorne, O., The Design and Analysis of Experiments. John Wiley and Sons, Inc., New York, N. Y., 1952.

18. Cochran, W. G., and Cox, G. M., Experimental Designs. 2d ed. John Wiley and Sons, Inc., New York, N. Y., 1957.

19. Federer, W. T., Experimental Design. The Macmillan Company, New York, 1963.

20. Cochran, W. G., Analysis of covariance: its nature and uses, Biometrics 13, 1957.

21. Scheffe, H., The Analysis of Variance. John Wiley and Sons, Inc., New York, N. Y., 1959.

22. Dixon, W. J. and Massey Jr., F. J., Introduction to Statistical Analysis. 2d ed. McGraw-Hill Book Co., Inc., New York, 1940.