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# Protein Studies.

## Plant Proteins I.—A Comparative Study of the Growth-promoting Properties of the Proteins of Peanutmeal, Sesamemeal, Coprameal, Lucernemeal, and Cottonseedmeal.

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In the course of extensive investigations at this Institute on the mineral deficiencies prevailing under natural conditions of grazing, indications pointing to a total or partial protein deficiency during certain seasons of the year have been observed. This observation, together with the fact that there exists a complete lack of reliable data on the constitution, digestibility, utilization, and nutritive values of our protein feeds in general, which seriously impair any attempt at compounding rations or supplementing protein deficient grazing on a scientific and economical basis, gave rise to the initiation at this Institute of a fairly extensive programme covering the various aspects of protein nutrition.

The problem of protein requirements, although an extremely important phase of animal nutrition, has on account of its complex nature so far defied almost every attempt at a complete and systematic solution. The method of expressing protein requirements on the basis of digestible protein, as is the popular procedure, is a survival of the times when chemical differences in the constitution of proteins were not appreciated. Nevertheless, this method of expressing protein requirements has been of immense practical value in the past and will probably continue in its popularity for the time being in lieu of a more scientific and appropriate unit of expression. However, it is fairly clear that the digestible protein conception is an expression without any physiological significance or interpretation in that it is entirely based on the difference between nitrogen intake and nitrogen evacuated in the faeces. Consequently it affords no indication as to the manner or amount of protein utilized by the animal

or the quantity necessary for maintaining the integrity of the tissues. It has also been amply demonstrated that protein feeds or mixtures of such feeds differ widely in their nutritive values, and that these differences are directly attributable to the different proportions and amounts of amino acids present in the respective protein molecules. Expressing, therefore, the requirements of protein in terms of digestibility irrespective of the differences in the amino acid constitution is not only a misrepresentation of the actual biological value of such proteins but also incongruous with the present conception on which the metabolism of proteins is based.

It appears, therefore, that an entirely new line of investigation should be followed in order to arrive at a means of expressing protein requirements, which would not only be applicable in practice but also respects the laws governing protein metabolism. Such a method has been proposed by Mitchell (1926), who recognised two distinct phases in the problem of determining the protein requirements of animals. Firstly the determination of protein requirements in terms relating to animal tissues and animal products, for the preservation and elaboration of which food protein is needed and, secondly, the determination of protein values of farm feeds and mixture of feeds to cover these requirements. According to Mitchell these two problems, although closely connected, must be investigated separately and by different means in order to arrive at a complete solution. The former may be measured by the total nitrogen content of the tissue constituents catabolized endogenously (maintenance) or by the total nitrogen content of the new tissues formed in growth and reproduction or by the total nitrogen content of the milk produced in lactation. A measurement of the latter component must consider the total protein content of the food or ration, the loss or wastage of protein in digestion and the loss or wastage of protein in the process of its conversion into tissue constituents or the constituents of body secretions. If, therefore, ultimately by this method the amount of protein utilized for the elaboration of new tissue, for instance, and on the other hand the biological value of a protein feed for growth be determined, it becomes a simple matter to state or calculate the quantity of digestible crude protein necessary to cover the protein requirements for growth. In a similar manner the protein requirements for the other forms of production may be calculated.

Thus far a general application of this method of expressing the protein requirements of animals has been rendered impossible through the lack of sufficient data. It is the intention in this programme of protein investigation to apply this method throughout and accumulate sufficient data for its practical application. In order to approach the problem systematically it was decided to concentrate investigations at first entirely on the second phase, namely, the determination of the protein values of farm feeds and mixture of feeds for the different requirements of farm animals. These investigations are first carried out with rats and later extended to include the different types of farm animals. Such a study would necessarily include: (1) a comparison of the nutritive values of the proteins of feeds or mixtures of foods; (2) the biological value of the proteins of such feeds; (3) the supplementary effect amongst their proteins; (4) the

amino acid deficiencies limiting the proper utilization of the proteins of feeds or combination of feeds. It will be appreciated that once this information is available it will be possible for practical purposes to select protein feeds or mixture of feeds of the highest biological values.

Although it is generally recognised that protein feeds differ distinctly in nutritive value, very few comparisons have actually been carried out in which food intake was controlled and the results treated statistically. The omission of these two factors in experiments of this nature detracts greatly from their significance. Mitchell and Beadles (1930), Braman (1931), Haag (1931), and Mitchell and Smuts (1932) have found the paired feeding method, developed by Mitchell (1930), very efficient in measuring the nutritive differences between protein feeds as well as their amino acid deficiencies. Braman (1931), applying this method, ascertained that cottonseedmeal is inferior in nutritive value to linseedmeal. However, their biological values were almost identical, the difference detected in the growth studies being due probably to the lower digestibility of the protein of cottonseedmeal. In pigs, Mitchell and Hamilton (1931) were able to substantiate the results of Braman and found no difference between the biological values of cottonseedmeal and linseedmeal. Nevens (1921), in comparing the proteins of cottonseedmeal and lucerne, found that the former is superior to the latter. Richardson and Green (1917) state that cottonseedmeal and flour are satisfactory sources of protein for the growth of oats. Bethke and co-workers (1928), using the method of Osborne and Mendel with rats, found no difference between the protein of linseedmeal and cottonseedmeal. With beef calves they could not detect any measurable difference when cottonseedmeal and linseedmeal were fed in combination with lucerne-hay.

Haag (1934), in a modification of the paired feeding method, found that the protein of bran was superior to that of lucerne. Morris and Wright (1933), in a determination of the relative efficiencies of protein feeds for milk production, found that decorticated earthnut cake was inferior to bloodmeal, peameal and beanmeal, but slightly better than linseedmeal. Schundt (1934) and coworkers obtained a higher nitrogen retention with yeast protein than with peanut in pigs, the nitrogen retention of peanut being higher than that of soyabeans. Daniels and Loughlin (1918) conclude that peanut contains a good quality protein. Mitchell and Villegas (1923) in their work on cocoanutmeal and soyabeans found fit to state that at 5 per cent. level there was no distinct difference in these two proteins. However, at 10 per cent. level the proteins of cocoanutmeal are slightly but distinctly less effective than the proteins of soyabeans for structural purposes in the body of the rat.

## Experimental.

Simultaneously with the determination of the biological values, comparative growth studies of the different protein feeds, based on the paired feeding method, were also conducted. Data referring to

the latter are presented in this paper. Rats were paired according to age, sex, weight and litter. Each pair received the same management and was kept under identical conditions, the only difference being the source of protein incorporated in the respective rations. Food was equated by the method of Mitchell (1930), namely, that in each pair both rats were given the same amount of food at the start and thereafter the daily amounts were regulated by the member of the pair eating the least. Rats were weighed weekly; the initial and final weights being the average weights of three consecutive days.

TABLE I.

*Composition of Rations.*

| | | | | | |
|---|---|---|---|---|---|
| Peanut Meal | 15·7 | — | — | — | — |
| Lucerne Meal | — | 57·5 | — | — | — |
| Sesame Meal | — | — | 25·0 | — | — |
| Copra Meal | — | — | — | 33·0 | — |
| Cottonseed Meal | — | — | — | — | 22·5 |
| Sucrose | 10·0 | 10·0 | 10·0 | 10·0 | 10·0 |
| Butterfat | 8·0 | 8·0 | 8·0 | 8·0 | 8·0 |
| Yeast Extract (1) | 10·0 | 10·0 | 10·0 | 10·0 | 10·0 |
| Agar (2) | 14·0 | — | — | — | — |
| Cod Liver Oil | 2·0 | 2·0 | 2·0 | 2·0 | 2·0 |
| NaCl | 1·0 | 1·0 | 1·0 | 1·0 | 1·0 |
| Salt Mixture (3) | 4·5 | 4·5 | 4·5 | 4·5 | 4·5 |
| Starch | 34·8 | 7·0 | 39·5 | 31·5 | 42·0 |
| TOTALS | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |
| Per cent. Nitrogen | 1·45 | 1·51 | 1·51 | 1·56 | 1·54 |

(1) Yeast extract was prepared according to the method of Itter S. Orent E. R., and McCallum Ev. *J.C.B.*, Vol. 108, No. 2, pp. 571–577, 1935.

(2) Agar was added to peanut ration only in the comparison with lucerne meal, in all other cases it was displaced by an equal weight of starch.

(3) A modified Osborne and Mendel Mixture described by P. B. Hawk and B. L. Oser, 1931. *Science* Vol. 74, p.369.

The composition by weight of the rations is given in Table I. Every ration was analysed for total nitrogen before the start of the experiment, in order to equalize the nitrogen content of the rations as nearly as possible either by remixing or adding more of the proteins under experiment or by making up fresh batches of food. In this manner close agreement between the nitrogen content of the different rations could be obtained. All the protein feeds except oats, which was a product prepared by a commercial company as a breakfast food, were similar to those used under practical feeding conditions except that they were put through a mill and reduced to a fine powder which facilitated mixing and ensured a more homogeneous ration.

TABLE II.

*Summary of Bodyweights, Gains, Food Records and Number of Refusals (in grms.).*

| TRIPLICATE... | 1. | | | 2. | | | 3. | | | 4. | | | 5. | | | 6. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lucerne. | Lucerne and Peanut. | Peanut. | Lucerne. | Lucerne and Peanut. | Peanut. | Lucerne. | Lucerne and Peanut. | Peanut. | Lucerne. | Lucerne and Peanut. | Peanut. | Lucerne. | Lucerne and Peanut. | Peanut. | Lucerne. | Lucerne and Peanut. | Peanut. |
| Initial Weight..... | 62·0 | 65·0 | 64·5 | 57·5 | 62·5 | 60·5 | 60·0 | 62·0 | 64·0 | 57·0 | 57·0 | 55·0 | 50·5 | 56·0 | 52·0 | 48·0 | 55·0 | 54·0 |
| Final Weight..... | 92·0 | 101·0 | 100·0 | 87·0 | 92·0 | 90·0 | 88·0 | 92·0 | 100·0 | 82·0 | 91·0 | 93·0 | 70·0 | 68·0 | 76·0 | 75·0 | 84·0 | 84·5 |
| Gains..... | 30·0 | 36·0 | 35·5 | 29·5 | 29·5 | 29·5 | 28·0 | 30·0 | 34·0 | 25·0 | 34·0 | 38·0 | 19·5 | 12·0 | 24·0 | 27·0 | 29·0 | 30·5 |
| Food Consumption | 353 | 353 | 353 | 334 | 334 | 334 | 331 | 331 | 331 | 330 | 330 | 330 | 321 | 321 | 321 | 280 | 280 | 280 |
| Refusals..... | 3 | 1 | 6 | 1 | 2 | 13 | 2 | 2 | 6 | 1 | 0 | 3 | 0 | 0 | 9 | 0 | 5 | 0 |

## Results.

In Table II is given a summary of the comparisons between lucernemeal, lucernemeal plus peanut and peanutmeal. As will be noticed the test was a modification of the paired feeding method in that triplicates instead of pairs were used. The conditions of feeding and pairing of rats were identical to those practised under paired feeding. The amount of food consumed during the experimental period was of the same magnitude for each member of the triplicate. Rats receiving the peanut ration in most cases limited the food consumption of their mates by refusing part of their daily feed when the latter was increased above a certain level. This was probably due, as Mitchell suggested, to the unpalatability of the peanutmeal.

Due to the layout of the experiment, it is statistically incorrect to compare one treatment like lucernemeal with another (peanutmeal) before the significance of the test as planned is ascertained. The mean gain in weight for the different treatments over the experimental period is 26·5 grams for lucernemeal, 28·4 grams for lucerne plus peanut, and 31·9 grams for peanutmeal. The standard error of the mean equals $\pm 2{\cdot}096$. By an analysis of the variance it is evident that the variations between treatments are by no means significantly greater than the remainder variance. Such an outcome would naturally imply that there is no reason to deduce from the data under observation, that there was a difference in response ascribable to the different treatments. When, however, the lucerne plus peanut ration is entirely ignored and lucernemeal is compared with peanutmeal the difference between these two treatments becomes significant. The mean difference ($m$) in grams is equal to $5{\cdot}417 \pm 1{\cdot}7485$ gms.; $t$ is equal to $\frac{m}{sz \text{ of mean}} = 3{\cdot}098$ and the degrees of freedom $(n-1) = 5$. Under these conditions the probability

Table III.

*A Comparison of the Weekly Gains between Peanut and Lucernemeal.*

| Weeks. | Pair 1. | Pair 2. | Pair 3. | Pair 4. | Pair 5. | Pair 6. | Totals per Week. + | − | ± |
|---|---|---|---|---|---|---|---|---|---|
| 1..... | + | + | + | + | + | + | 6 | 0 | 0 |
| 2..... | + | + | + | + | + | − | 5 | 1 | 0 |
| 3..... | + | + | + | + | − | − | 4 | 1 | 1 |
| 4..... | + | − | − | + | − | + | 3 | 3 | 0 |
| 5..... | − | − | + | − | + | ± | 2 | 3 | 1 |
| 6..... | + | − | − | + | − | ± | 3 | 3 | 0 |
| | | | | | | Total for Experiment | 23 | 11 | 2 |

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Lucerne Meal.
± Indicates equal gain.

(P) that the difference in favour of peanut is not a chance effect is 0·03. Since a value of P = 0·05 is in current biometrical practice a criterion of significance, it is obvious that peanut, by this method of comparison, is superior to lucernemeal. But in view of the actual lay-out of the experiment this significance, when judged by the entire data, must be considered accidental (Probability approximately 11 per cent.).

A comparison of weekly gains is ordinarily a less reliable and legitimate method of comparison than that of total gains, due to the fact that weekly food residues may appreciably affect the gains. The necessary conditions for statistical independence between individual values are also seriously infringed in that the weekly gains for the same pair of rats are not independent in so far as they reflect an inherent difference between the two rats. If such inherent differences exist the weekly gains are not comparable on a basis of random variation on the assumption that each rat in any one pair has, except for treatment differences, an equal chance to gain more than its mate during a particular week. Hence, due to this lack of independence in weekly gains for the same pair of rats, it is not quite correct to consider the product of the number of pairs and weeks as the total number of degrees of freedom.

Furthermore, the number of weekly gains is by no means independent of the total gains. If a particular rat of one pair gains more in weight than its weight during the experimental period, it is likely that it should have gained more in weight during the greater number of weeks. Hence the two comparisons, total gains and number of greater weekly gains, are by no means independent, but to some extent one and the same comparison. Consequently it is doubtful whether any extra information is gained by comparing weekly gains after an analysis of total gains has been made. The inclusion of comparisons between weekly gains in this paper is merely by reason of its adoption by other workers and to illustrate what was already shown by the analysis of total gains.

In a comparison of lucernemeal and peanutmeal the weekly food intake was the same for the different triplicates. This naturally gives a greater value to the statistical analysis of the weekly gains. If the number of equal weekly gains is divided equally between peanut and lucernemeal, it will be seen that out of the 35 comparisons 24 favoured peanutmeal and only 12 lucernemeal. This fact also points to a superiority of peanutmeal over lucernemeal.

In Table IV the data pertaining to the comparison of peanutmeal and sesamemeal are tabulated. Here, again, it is obvious from the number of refusals that the peanut fed rat in all but one case was the one to limit the food intake of the respective pairs. A statistical analysis of the total gains shows that the mean difference of the six pairs of rats is zero and, consequently, there is no indication whatsoever of an existing difference between the protein of peanutmeal and sesamemeal. A comparison of the weekly gains in Table V affords confirmatory evidence as to the equality of these two protein feeds. If chance alone operated, it would be expected that of the 36 comparisons the ideal outcome would be 18 for both treatments.

TABLE IV.

*Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Sesamemeal Rations (in gms.).*

| | Pair 1. | | Pair 2. | | Pair 3. | | Pair 4. | | Pair 5. | | Pair 6. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peanut Meal. | Sesame Meal. | Peanut Meal. | Sesame Meal. | Peanut Meal. | Sesame Meal. | Peanut Meal. | Sesame Meal. | Peanut Meal. | Sesame Meal. | Peanut Meal. | Sesame Meal. |
| Initial Weight..... | 85·0 | 82·0 | 77·0 | 77·0 | 74·0 | 77·0 | 87·0 | 85·0 | 94·0 | 94·0 | 70·0 | 74·0 |
| Final Weight..... | 131·0 | 129·0 | 128·0 | 124·0 | 120·0 | 121·0 | 134·0 | 129·0 | 132·0 | 137·0 | 110·0 | 117·0 |
| Gain gms..... | 46·0 | 47·0 | 51·0 | 47·0 | 46·0 | 44·0 | 47·0 | 44·0 | 38·0 | 43·0 | 40·0 | 43·0 |
| Total Food Consumption..... | 361 | 361 | 369 | 369 | 364 | 364 | 394 | 394 | 386 | 386 | 351 | 351 |
| Refusals Period..... | 3 | 1 | 1 | 3 | 6 | 0 | 4 | 0 | 2 | 0 | 5 | 0 |

When the equal gains are divided equally between peanut and sesamemeal, the respective numbers of weekly gains become 16·5 for sesamemeal and 19·5 for peanut. These figures very nearly approach the ideal outcome and therefore confirm the non-existing difference in the two treatments.

TABLE V.

*A Comparison of Weekly Gains between Peanutmeal and Sesamemeal.*

| Weeks. | Pair 1. | Pair 2. | Pair 3. | Pair 4. | Pair 5. | Pair 6. | Totals per Week. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | + | − | ± |
| 1..... | − | − | − | − | − | − | 0 | 6 | 0 |
| 2..... | + | − | + | ± | − | − | 3 | 3 | 0 |
| 3..... | − | + | ± | + | + | − | 3 | 2 | 1 |
| 4..... | + | + | ± | ± | − | ± | 3 | 1 | 2 |
| 5..... | + | − | + | − | + | + | 4 | 2 | 0 |
| 6..... | − | + | − | + | − | − | 2 | 4 | 0 |
| | | | | TOTAL EXPERIMENT..... | | | 15 | 18 | 3 |

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Sesame Meal.
± Indicates equal gain.

In a comparison of peanutmeal and coprameal in Table VI, it is fairly clear by inspecting the total gains of the different pairs, that no obvious difference exists as a result of the different treatments. The mean difference in total gain is $0{\cdot}333 \pm 1{\cdot}837$ grams, showing that the mean difference is much less than its standard error. The value of $t = 0{\cdot}164$ is insignificant and the probability that the observed difference in favour of peanutmeal is due to chance alone is approximately 89 per cent. In a comparison of the weekly gains, Table VII, the deviation of the two treatments from the ideal outcome of 18, when the equal gains are allotted proportionately to peanut and coprameal, is so small that it becomes entirely insignificant. It is, therefore, safe to conclude that coprameal and peanutmeal do not differ significantly in nutritive value.

TABLE VII.

*Comparisons of Weekly Gain between Peanutmeal and Coprameal.*

| Weeks. | Pair 1. | Pair 2. | Pair 3. | Pair 4. | Pair 5. | Pair 6. | Totals per Week. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | + | − | ± |
| 1..... | + | ± | − | + | − | − | 2 | 3 | 1 |
| 2..... | + | − | ± | − | + | − | 2 | 3 | 1 |
| 3..... | + | + | + | − | ± | − | 3 | 2 | 1 |
| 4..... | ± | + | + | − | − | + | 3 | 2 | 1 |
| 5..... | ± | + | − | − | + | − | 2 | 3 | 1 |
| 6..... | + | − | + | ± | − | + | 4 | 2 | 0 |
| | | | | TOTAL EXPERIMENT..... | | | 16 | 15 | 5 |

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Copra Meal.
± Indicates equal gain.

TABLE VI.

*Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Coprameal Rations (in gms.).*

| | Pair 1. | | Pair 2. | | Pair 3. | | Pair 4. | | Pair 5. | | Pair 6. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peanut Meal. | Copra Meal. | Peanut Meal. | Copra Meal. | Peanut Meal. | Copra Meal. | Peanut Meal. | Copra Meal. | Peanut Meal. | Copra Meal. | Peanut Meal. | Copra Meal. |
| Initial Weight | 99·0 | 99·0 | 92·0 | 93·0 | 85·0 | 85·0 | 83·0 | 84·0 | 77·0 | 77·0 | 87·0 | 80·0 |
| Final Weight | 132·0 | 126·0 | 126·0 | 127·0 | 121 | 126·0 | 118·0 | 118·0 | 126·0 | 114·0 | 112·0 | 111·0 |
| Gains gms | 33·0 | 27·0 | 34·0 | 34·0 | 36·0 | 41·0 | 35·0 | 34·0 | 49·0 | 37·0 | 25·0 | 31·0 |
| Total Food Consumption | 341 | 341 | 338 | 338 | 343 | 343 | 311 | 311 | 329 | 329 | 315 | 315 |
| Refusals | 4 | 0 | 3 | 0 | 1 | 5 | 4 | 6 | 4 | 2 | 5 | 5 |

TABLE VIII.

*Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Cottonseedmeal Rations (in gms.).*

| | Peanut Meal. | Cotton-seed Meal. | Peanut Meal. | Cotton-seed Meal. | Peanut Meal. | Cotton-seed Meal. | Peanut Meal. | Cotton-seed Meal. | Peanut Meal. | Cotton-seed Meal. | Peanut Meal. | Cotton-seed Meal. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial Weight, gms | 90·0 | 85·0 | 114·0 | 114·0 | 98·0 | 95·0 | 87·0 | 87·0 | 92·0 | 94·0 | 87·0 | 89·0 |
| Final Weight, gms | 120·0 | 122·0 | 140·0 | 149·0 | 125·0 | 135·0 | 120·0 | 117·0 | 126·0 | 135·0 | 125·0 | 131·0 |
| Gain, gms | 30·0 | 37·0 | 26·0 | 35·0 | 27·0 | 40·0 | 33·0 | 30·0 | 34·0 | 41·0 | 38·0 | 42·0 |
| Total Food Consumption | 343 | 343 | 372 | 372 | 339 | 339 | 300 | 300 | 352 | 352 | 378 | 378 |
| Refusals | 5 | 1 | 1 | 1 | 0 | 0 | 5 | 0 | 4 | 0 | 0 | 2 |

That cottonseedmeal seems to be superior to peanutmeal is indicated by the fact that five out of the six pairs of rats show a bigger total gain in favour of cottonseedmeal. Analysing the total gains in weight (Table VIII), it is found that the mean difference for the six pairs is $m = 6 \cdot 17 \pm 2 \cdot 191$ grams, and $t$ becomes equal to 2·808. The possibility, therefore, that the above difference in favour of cottonseedmeal is due to chance alone is 0·04. This probability is so small, and less than 5 per cent. which is usually taken as the limit of significance, that it may be concluded that cottonseedmeal is superior to peanutmeal. As no difference was demonstrated between peanutmeal, coprameal, and sesamemeal, it seems reasonable to deduce that cottonseedmeal is also superior to the latter two meals.

TABLE IX.

*Comparison of Weekly Gains made by Pair-mates on Peanutmeal and Cottonseedmeal.*

| Weeks. | Pair 1. | Pair 2. | Pair 3. | Pair 4. | Pair 5. | Pair 6. | Totals per Week. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | + | − | ± |
| 1..... | − | − | − | + | + | − | 2 | 4 | 0 |
| 2..... | + | ± | − | + | ± | ± | 2 | 1 | 3 |
| 3..... | ± | − | − | − | + | ± | 1 | 3 | 2 |
| 4..... | − | − | + | − | − | − | 1 | 5 | 0 |
| 5..... | − | ± | − | ± | − | − | 0 | 4 | 2 |
| 6..... | + | − | − | ± | − | − | 0 | 4 | 2 |
| TOTAL EXPERIMENT..... | | | | | | | 6 | 21 | 9 |

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Cotton-seed Meal.
± Indicates equal gain.

A comparison of the weekly gains (Table IX) shows six comparisons favouring the peanut fed rat, twenty-one favouring cottonseedmeal and nine equal gains. If the latter is divided equally amongst the peanut and cottonseed fed rats, it is found that 10·5 of the comparisons favour peanut and 25.5 favour cottonseedmeal, which means a deviation of 7·5 from the ideal outcome. The standard deviation of the frequency distribution of the outcome of 36 events, each of which may result with equal probability either one of two ways is given by the expression $\sqrt{0 \cdot 5 \times 0 \cdot 5 \times 36}$, which is equal to 3·0. The deviation of 7·5 from the ideal chance outcome is, therefore, 2·5 times the standard deviation. Interpreting this ratio from the table of values of the normal probability integral, the probability exists that chance alone would produce this deviation approximately once in a hundred trials. This comparison, therefore, definitely supports the results on total gains, namely, that cottonseedmeal is a superior protein feed to peanutmeal.

While it appears from this investigation that cottonseedmeal is a better protein feed than coprameal, peanutmeal or sesamemeal, it does not exclude the possibility that these feeds may react distinctly different in other types of farm animals. This point is illustrated by the fact that Morris and Wright report that peanutmeal is slightly superior to linseedmeal for milk production, whereas Mitchell and Hamilton, as well as Braman, found no difference between cottonseedmeal and linseedmeal for growth and maintenance of the rat and the pig. In this study again cottonseedmeal has been found to be superior to peanutmeal in the rat. By inference it seems only natural to conclude that linseedmeal should be a better protein feed for growth than peanutmeal, which has been shown to be probably not the case for milk production. The value of protein feeds can, therefore, not be estimated or assumed at random from any set of data, but must be determined with different animals for each specific purpose. This phase of work is anticipated as investigations are extended to other animals. The superiority of cottonseedmeal over the protein feeds does not imply a general application in practice without taking into account the level of feeding at which this food may be toxic to certain species of animals.

## Conclusion.

By means of the paired feeding method on rats it has been shown statistically that the protein of cottonseedmeal is superior to that of peanutmeal, while on the other hand no statistical difference could be detected between the proteins of peanutmeal and coprameal, and peanutmeal and sesamemeal. On this basis it seems reasonable to deduce that the proteins of cottonseedmeal are superior to sesame and coprameal. Evidence is presented which seems to suggest that lucernemeal is inferior to peanutmeal as a source of protein.

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