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Nutritive value of lucerne-leaf proteins

Biological value of lucerne proteins and their supplementary relations to rice proteins measured by balance and rat-growth methods

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Lucerne (*Medicago sativa* L.) is essentially a forage crop, but has been eaten to a small extent by human beings (Levy & Fox, 1935; Odendaal, 1954). A cereal mixture containing 1 % of dehydrated lucerne was given for 2 years, with considerable success, in the Hospital for Sick Children in Toronto (Brown & Tisdall, 1933). Recognition of the high nutritive value of lucerne has led a number of workers to suggest its use as an ingredient in human diets (Levy & Fox, 1935; Marston, Quinlan-Watson & Dewey, 1943; Escudero & Landabure, 1943). Methods of preparing it as a vegetable dish were described by Heupke & Schöller (1942, 1943). Carotene and protein concentrates were made from lucerne by White, Weil, Naghski, Monica & William (1948). Anandswamy & Date (1956) made a lucerne concentrate which was found to be acceptable when included in sugar-coated tablets or in dehydrated soup mixtures.

The rice diet generally consumed in South India is a poor diet deficient in proteins and in certain minerals and vitamins (Aykroyd & Krishnan, 1937*a–c*). The fact that lucerne is rich in many of these nutrients, and is also a widely grown crop giving high yields, led Subrahmanyan & Sur (1949) to explore the possibility of using it as a cheap and nutritious supplement to a poor rice diet. Experiments on successive generations of albino rats showed that desiccated lucerne leaf, when given as a supplement to the poor rice diet at a 10% level, increased the rate of growth three- or four-fold.

It seemed important to ascertain how far the effect of the lucerne in accelerating growth was due to supplementary relations between rice and lucerne proteins. Few investigations have been done on leaf proteins with rats as experimental animals, compared with the large number concerned with the value of proteins in cereals, pulses and foods of animal origin. It was thought that information on lucerne-leaf proteins obtained by this means would help in planning cheap and well-balanced diets in rice-eating areas.

In the present paper, experiments on the biological value of lucerne-leaf proteins and their supplementary relations to rice proteins are described. Both the Thomas–Mitchell balance-sheet method and the rat-growth method were used. Whole lucerne-leaf powder was given throughout, since the object was to ascertain the value of the

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whole leaf and not of the extracted protein. The effect of mild autoclaving on the protein efficiency ratio was also investigated, with the idea in mind that lucerne has to be processed to make it palatable and that autoclaving might reproduce in some degree the effects of processing.

EXPERIMENTAL

Preparation of lucerne-leaf powder

Lucerne plants, $1\frac{1}{2}$–2 ft in height, were cut before flowering, thoroughly washed and then dried in a current of air at 60°. The dried leaves were separated from the stem, powdered and passed through a 40-mesh sieve. Fresh lucerne powder was prepared for each experiment. The powder used for the experiments with the balance-sheet method contained 33 % crude protein and 27·7 % true protein (insoluble in 5 % trichloroacetic acid). The powder used in the growth tests contained 30·6 % crude protein. Autoclaved lucerne was prepared by wetting lucerne powder with water, autoclaving it in steam at 2 lb pressure for $\frac{1}{2}$ h, and then drying it in a current of hot air at 60°.

Diet and feeding of animals

In the balance experiments, the ratio of lucerne protein to rice protein in the experimental diet was set at 2:3, this ratio being similar to that adopted in earlier studies on the growth-promoting effect of lucerne supplements added to the poor rice diet (Subrahmanyan & Sur, 1949). For the growth tests, two diets containing mixed proteins (lucerne protein:rice protein = 1:1 and 1:3) were used. The lower proportion of lucerne protein was chosen in this test because earlier experiments had shown that even small additions of lucerne to the poor rice diet have a marked growth-promoting effect (Sur & Subrahmanyan, 1954).

The composition of the experimental diets is shown in Tables 1 and 2. The biological value of lucerne proteins, of a mixture of lucerne and rice proteins, and of rice proteins alone, was determined in succession in that order, at the same level of protein intake, by the Thomas–Mitchell balance-sheet technique, five healthy adult male rats weighing about 150 g being used. The methods were similar to those described by Swaminathan (1937). The experimental animals were given a nitrogen-free diet in the first and the last periods. Diets containing lucerne proteins, a mixture of rice and lucerne proteins, rice proteins alone, and lucerne proteins alone, were given in the 2nd, 3rd and 4th periods respectively. Each period consisted of 7 days. Between two consecutive periods, there was a rest period of 4 days when the rats were given the stock diet. The first 3 days of each experimental period were considered as a reorientation period and urine and faeces were collected during the last 4 days. Urine was collected in bottles containing 30 ml 2·5 % (v/v) sulphuric acid, A.R., and a small amount of toluene. Faeces were washed daily with a few ml of water to remove any adhering urine, transferred to a 500 ml Kjeldahl flask and digested. The digest was made up to 250 ml and a portion taken for estimation of nitrogen by the micro-Kjeldahl method. One sample of urine was lost during the experiment and results are given for only five rats.

For the growth experiments newly weaned rats weighing about 40 g were distributed into five groups each containing six rats. Only male rats were used, since in growth experiments considerable variation due to sex difference may occur. The rats were fed *ad lib.* for a period of 6 weeks. The protein efficiency ratio was calculated as gain in weight of experimental animals per g protein intake (Osborne, Mendel & Ferry, 1919).

Table 1. *Percentage composition of diets used for the evaluation of the biological value of proteins by the balance method*

Ingredient	Nitrogen-free diet	Lucerne diet	Lucerne and rice diet	Rice diet
Lucerne-leaf flour	—	16·3	6·6	—
Rice	—	—	45·4	76·0
Coconut oil	10·0	10·0	10·0	10·0
Cane sugar	10·0	10·0	10·0	10·0
Salt mixture*	4·0	4·0	4·0	4·0
Vitaminized starch†	1·0	1·0	1·0	—
Starch (maize)	75·0	58·7	23·0	—
Nitrogen	0·049	0·899	0·865	0·858
Protein (N × 6·25)	0·31	5·62	5·41	5·36
Moisture	9·2	8·2	8·4	8·6

* Osborne & Mendel (1913).

† 1 g contained 0·25 mg thiamine hydrochloride, 0·5 mg riboflavin and 0·5 mg nicotinic acid. Equivalent quantities of vitamins were added to rice flour in the rice diet. Each rat received daily one drop of shark-liver oil containing 1500 i.u. vitamin A and 100 i.u. vitamin D per g. During the rest periods the rats were fed on a stock diet containing 6 % brewer's yeast.

Table 2. *Percentage composition of diets used for determination of the biological value of proteins by the growth method*

Ingredient	Rice diet (1)	Rice and lucerne diet (2)	Rice and lucerne diet (3)	Lucerne diet (4)	Autoclaved lucerne diet (5)
Rice (unpolished)	73·0	54·7	36·5	—	—
Lucerne-leaf flour	—	4·6	9·2	18·35	—
Autoclaved lucerne-leaf flour	—	—	—	—	18·35
Coconut oil	10·0	10·0	10·0	10·0	10·0
Cane sugar	10·0	10·0	10·0	10·0	10·0
Salt mixture*	4·0	4·0	4·0	4·0	4·0
Vitaminized starch†	3·0	3·0	3·0	3·0	3·0
Starch (maize)	—	13·7	27·3	54·65	54·65
Nitrogen	0·978	0·978	0·994	1·000	1·067
Protein (N × 6·25)	6·11	6·11	6·21	6·24	6·42
Moisture	9·0	9·0	8·75	8·20	8·0

Diet 2: rice protein: lucerne protein, 3:1.
Diet 3: rice protein: lucerne protein, 1:1.

* Osborne & Mendel (1913).

† 3 g contained extract from 5 g brewer's yeast (ethyl alcohol added to 50 % (v/v) to an aqueous extract from yeast, precipitate filtered off and liquid dried), 0·15 mg thiamine hydrochloride, 0·4 mg riboflavin and 0·4 mg nicotinic acid. Each rat received daily one drop of shark-liver oil containing 1500 i.u. vitamin A and 100 i.u. vitamin D per g.

RESULTS AND DISCUSSION

The biological value of the proteins was calculated from the formula given by Chick, Hutchinson & Jackson (1935). For calculating the endogenous urinary and faecal nitrogen for different periods, it was assumed that these values varied linearly between the first and the last periods (Mitchell, 1924). The results obtained by the balance-sheet method are presented in Table 3. The calculated biological value of the mixed proteins was compared with the experimentally obtained value by the pair-difference method. The value of the proteins in dehydrated lucerne was found to be 66, slightly higher than those (62 and 60) reported by earlier workers (Nevens, 1921; Smuts & Malan, 1938). This difference may be due to the fact that these workers used the entire plant, including both stem and leaf, as the source of protein.

Table 3. *Biological value for adult male rats of the proteins of lucerne leaf, of rice and of a mixture of the two*

Rat no.	Lucerne	Rice	Rice and lucerne: Observed	Expected†	Observed − expected
1	65·3	92·4	90·2	81·3	8·9
2	67·0	89·2	91·0	80·2	10·8
3	61·0	91·0	86·5	78·9	7·6
4	65·9	91·3	86·4	81·1	5·3
5	72·2	89·5	84·8	82·5	2·3
Mean with its standard error	66·3 ± 1·65	90·7 ± 0·92	87·8 ± 1·20	80·8 ± 0·63	6·98 ± 1·47 t = 4·76*

* Significant at the 1 % level.

† If there were no supplementary relationship between the proteins.

Mean digestibility of proteins: lucerne, 74·3 ± 0·85; rice and lucerne, 80·6 ± 0·62; rice, 88·2 ± 1·09.

The biological value of the mixed proteins (lucerne protein:rice protein, 2:3) was found to be high (88) and approached that of rice. Table 3 shows that the experimentally obtained value for the mixed proteins was invariably higher than the value calculated on the assumption that no supplementary relationship exists between them. The increase in biological value, as indicated by the pair-difference method, was not large, but was significant even at the 1% level. Hence it can be concluded that a definite supplementary relationship exists between the proteins of rice and lucerne in meeting the maintenance requirements of adult rats.

The growth studies (Table 4) showed a more pronounced supplementary relationship between rice and lucerne proteins. The protein efficiency ratio of the mixtures (2·51) was considerably higher than the ratio of either source of protein given separately. If the theoretically expected values are compared with the values obtained in the experiments, it is found that the latter exceeded the former by 34 and 54%. The difference in growth rate between the rats receiving the mixtures and those receiving rice alone was striking. Since the balance method did not reveal such marked supplementary relationships, it may be concluded that a combination of rice and lucerne proteins is more effective in fulfilling requirements for growth than requirements for maintenance.

There was no difference in the protein efficiency ratios of the two diets containing mixed proteins (Table 4, diets 2 and 3). This finding shows that the addition of even a small amount of lucerne to rice will improve protein quality—a fact which may be of practical importance, since it would be easier to include small rather than large quantities of lucerne in the rice diets of poor people.

Table 4. *Weight gain of weanling male rats on diets of lucerne, rice, or a mixture of the two, and protein efficiency ratio of the diets*

(Figures are the means of six determinations)

Diet no.	Source of protein	Body-weight: Initial (g)	Body-weight: Gain in 6 weeks (g)	Adjusted 6-week gain* (g)	Total food intake (g)	Total protein intake (g)	Protein efficiency ratio: Mean with its standard error	Protein efficiency ratio: Expected†
1	Rice	39·5	40·7	40·7	312·1	19·1	2·13 ± 0·05	—
2	Rice and lucerne, 3:1	39·2	54·1	47·1	347·9	21·3	2·51 ± 0·08	1·87
3	Rice and lucerne, 1:1	39·8	60·0	45·3	381·6	23·7	2·51 ± 0·11	1·61
4	Lucerne	39·3	18·2	27·6	257·0	16·0	1·09 ± 0·14	—
5	Lucerne, autoclaved	39·2	11·4	23·6	236·2	15·2	0·75 ± 0·08	—

A difference between protein efficiency ratios is significant at the 5 % level if it exceeds ± 0·29.

* The means in the previous column have been adjusted to a protein intake of 19·04 g by a within-group regression coefficient. A difference between adjusted growth means is significant at the 5 % level if it exceeds ± 4·6 g.

† If there were no supplementary relationship between the proteins.

The supplementary effect of lucerne is probably due to the correction of the lysine deficiency of rice proteins (Kik, 1940). Lucerne proteins contain 50 % more lysine than rice proteins (Block & Mitchell, 1946) and a small addition of lucerne will make good the deficiency of rice proteins in this respect. It is, of course, also possible that combining rice and lucerne proteins improves the relative proportion of other amino acids, and contributes to the higher protein efficiency ratio of the mixed proteins as compared with that of rice alone. In this connexion it may be noted that the nitrogen-balance experiments were done with adult rats, which have a lower lysine requirement than growing animals.

The proteins of lucerne leaf have been reported (Haag, 1934) to possess a low protein efficiency ratio (0·40 and 0·69 when given at levels of 11·0 and 11·7 % protein respectively). In my experiments, in which the protein level was 6 %, the ratio observed was higher (1·09). The growth of the rats was, however, poor.

Smuts & Marais (1938) did not find any supplementary relationship between groundnut meal and lucerne meal (the latter presumably including both leaf and stem). The experiments of Haag (1934) appear to indicate that the proteins of lucerne-leaf meal and wheat bran supplement each other.

Table 4 shows that even mild autoclaving will impair the quality of lucerne proteins.

Variations between the groups in total food intake and hence in protein intake were large. Since the protein efficiency ratio tends to rise with increasing protein intake, the results were analysed by the covariance method (Snedecor, 1946), gains in weight being adjusted for variations arising out of differences in protein intake. The results confirmed the conclusions reported above, but showed that the differences in protein ratio recorded were somewhat exaggerated by the large differences in dietary protein intake.

SUMMARY

1. The biological value of the proteins in lucerne-leaf powder and their effect in supplementing rice proteins have been studied by the Thomas–Mitchell balance-sheet method and the rat-growth method. Five male adult rats were used in the balance test and five groups of six male newly weaned rats in the growth test.

2. Lucerne-leaf proteins had a low nutritive value. The biological value and digestibility were found to be 66 and 74 respectively. The protein efficiency ratio was 1·01.

3. Both the balance method and the growth method showed a definite supplementary relationship between lucerne proteins and rice proteins, the effect being more pronounced in the growth experiments.

4. Rice and lucerne proteins together gave a mixture with a biological value of 88 and a protein efficiency ratio of 2·5.

5. A small amount of lucerne significantly increased the protein efficiency ratio of rice from 2·1 to 2·5. The addition of a larger proportion of lucerne did not lead to a further rise.

6. Mild autoclaving reduced the protein efficiency ratio of lucerne-leaf powder.

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